JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Lutein from Ozone-Treated Corn Retains Antimutagenic Properties

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The present study was conducted to determine the influence of an ozonation process on lutein and protein in clean and contaminated corns. This study aimed to determine the levels of lutein and protein in corn before and after ozonation and to verify the antimutagenic potential of the extracted lutein against aflatoxin using the Ames test. The lutein content was analyzed by high-performance liquid chromatography. Nitrogen analysis and sodium dodecyl sulfate−polyacrylamide gel electrophoresis were used to analyze protein. Clean ozone-treated corn had a total lutein content of 28.36 μg/g, which was higher than that of 22.75 μg/g in the untreated clean corn. However, the lutein content was 11.69 μg/g in the ozone-treated contaminated corn, which was lower than that of 16.42 μg/g in the untreated contaminated corn. In both corn samples, the protein content of ozone-treated corn was lower than that of untreated corn, indicating that protein could be destroyed by the ozonation process, which may influence the nutritious value of the corn. Lutein extracts alone showed no mutagenic potential against *Samonella typhimurium* tester strains TA100. Lutein extracts from corn inhibited the mutagenicity of AFB1 in a dose−response manner more efficiently than lutein standard. Lutein extracts from different corn samples had similar antimutagenic potentials against AFB1, so the ozone treatment did not affect the antimutagenic potentials of lutein extracts.

KEYWORDS: Corn; ozonation; lutein; antimutagenic

INTRODUCTION

Corn is currently the third most planted field crop after wheat and rice. The bulk of corn production occurs in the United States, People's Republic of China, and Brazil, which together account for 73% of the annual global production of 589.4 million tons (*1*). In Louisiana, corn ranks fourth after sugar cane, cotton, and rice as an agricultural commodity. Corn is a very important commodity not only to the United States but also to the whole world. However, in most warm and humid regions, the corn crop is highly susceptible to fungal invasion and aflatoxin production. Current estimates show that in 1998, 25% of corn fields in Louisiana were rejected or never harvested due to suspected aflatoxins contamination. Moreover, the presence of aflatoxins in food and feeds poses serious problems in human and animal health. AB1 is the most potent of four naturally occurring aflatoxins. Because of health and economic problems, the poison has been the focus of considerable research since its discovery (*2*).

To limit human exposure to aflatoxins, prevention and control programs are constantly being studied and evaluated to get more efficient and safer methods. There are several kinds of methods in decontamination such as physical, chemical, or biological methods. The chemical methods are currently the most practical approaches to inactivate aflatoxins. Ozone treatment is one method that has been studied. It is a less expensive, nonchemical waste-producing alternative to other treatments. Ozone is unstable and converts to oxygen in the material to which it is applied. Ozone, a powerful oxidizing agent, reacts across the 8,9-double bond of the furan ring (*3*). Ozone is able to reduce aflatoxin in cottonseed meal and peanut meal (*4, 5*). It has been reported that 91% of the total aflatoxins were destroyed in 22% moisture cottonseed after 2 h, while the reduction in the peanut meal was only 78% after exposure to ozone for 1 h. McKenzie et al. (*2*) have reported that aflatoxins in corn could be reduced by 95% after being treated with 14 wt % ozone for 92 h. Prudente and King (*6*) have observed a 92% degradation of aflatoxin by ozonation.

Corn is a rich source of flavonoids, polyphenols, and carotenoids (*7*). The occurrence of these antioxidants not only decreases pest infestation (*8*) but also directly reduces aflatoxin levels in the grains (*9*). Flavonoids, carotenoids, and polyphenols mitigate the toxic and/or mutagenic effects of aflatoxin (*10*−*14*). Pure α-carotene and lutein, both of which occur in corn, reduced the mutagenic effect of aflatoxin to 2% that of a control (*11*).

Although ozonation has been proven to be an effective method for decontamination of aflatoxin in corn for animal use, its suitability and acceptability have yet to be evaluated. This study therefore set out to isolate and identify lutein, to verify that lutein extracts from corn have antimutagenic effects against

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10.1021/jf801562v CCC: $40.75
Published on Web 08/06/2008

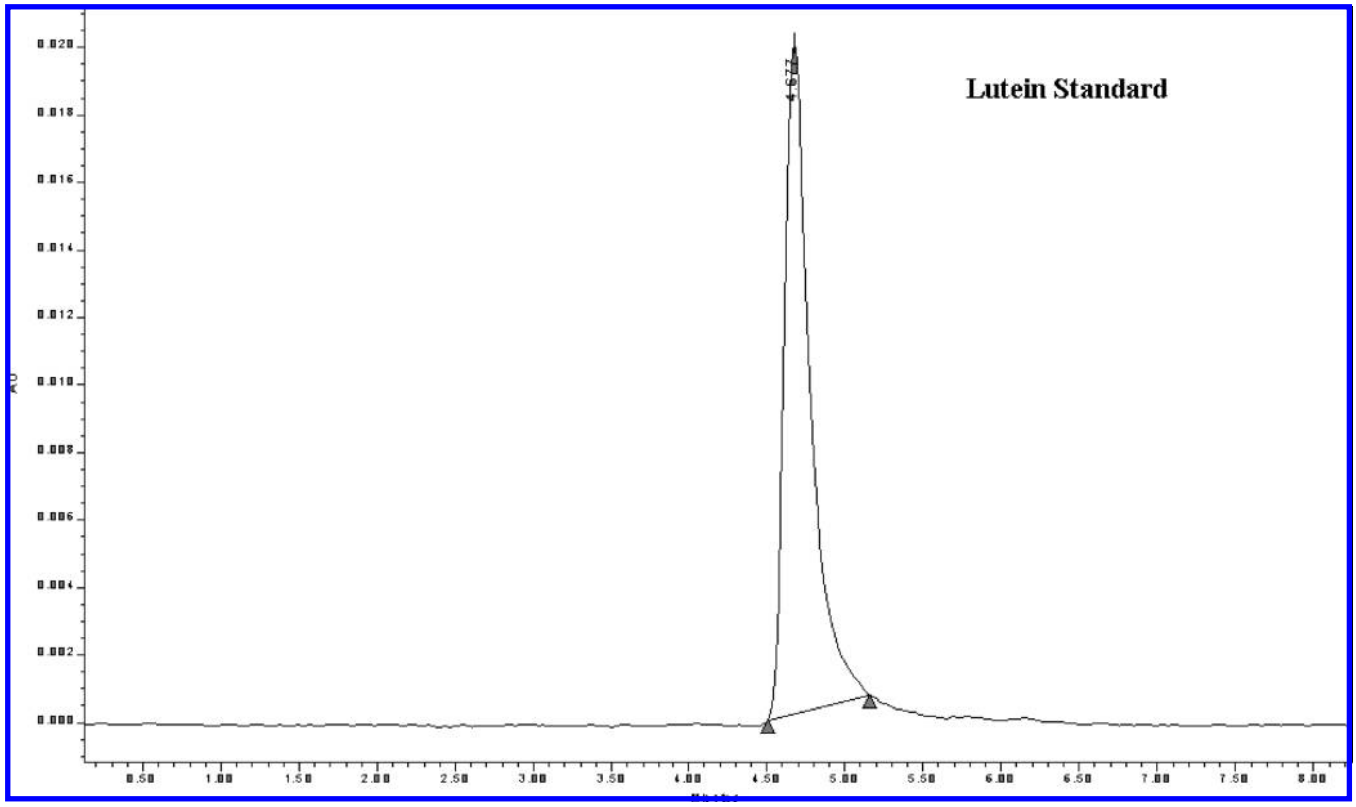

Figure 1. Chromatogram of lutein standard.

aflatoxin, and to determine the effects of ozonation on lutein levels in clean and contaminated corns. This study also aimed to determine the effect of the ozone process on the protein composition of corn.

MATERIALS AND METHODS

Chemicals. Ethanol, potassium hydroxide, hexane, acetone [high-performance liquid chromatography (HPLC) grade], petroleum ether, and methanol (HPLC grade) were obtained from Fisher (Fairlawn, NJ). Ampicillin, D-biotin, magnesium sulfate, sodium ammonium phosphate, citric acid monohydrate, L-histidine, tetracycline, magnesium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, β-nicotinamide adenine dinucleotide phosphate (NADP, sodium salt), glucose-6-phosphate, glucose, sodium chloride, potassium chloride, lutein standard, pure aflatoxin standard, and butylated hydroxy toluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoretic gels (4−12% Bis-Tris gels), lithium dodecyl sulfate sample buffer, molecular weight marker, acetic acid, running buffer, and staining solutions were obtained from Invitrogen (Carlsbad, CA). Bacto agar was obtained from Difco Laboratories (Detroit, MI). Oxoid nutrient broth no. 2 was sourced from Unipath Ltd. (Basingstoke, Hampshire, England). Rat liver postmitochondrial supernatant (S9 mix) was purchased from Molecular Toxicology Inc. (Boone, NC). Bacterial tester stain TA100 was kindly provided by Dr. Bruce Ames (UC Davis, CA).

Ozone Treatment of Corn Samples. Corn samples were kindly provided by Dr. Kenneth S. McKenzie of Lynntech, Inc. (College Station, TX). The samples were treated at Lynntech, Inc. as follows. Ten kilograms each of corn sample with and without aflatoxin contamination was treated with ozone. The corn sample was placed into a 30 gallon polyethylene reactor with a false bottom. A 25.4−38.1 cm headspace was allowed to achieve even ozone dispersion through the corn. The reactor lid was fitted with 1/4" Teflon bulkheads. Ozone gas, 10−12 wt %, was flowed in through the top at approximately 2 L/min. A 2.5 L/min vacuum was placed at the bottom. All corn samples were treated for 96 h at 12−15 h intervals with mixing occurring every 30 h. The treatment protocol included ozone-treated clean corn (A), untreated clean corn (B), ozone-treated naturally contaminated corn (C), and untreated naturally contaminated corn (D).

Corn Sample Preparation. Ten kilograms of corn sample from each treatment was ground using a Romer Hammer Mill to produce three subsamples that were further ground using a Brinkmann mill to pass a no. 20 mesh sieve. Samples were transferred to clean plastic bags, labeled, and stored at 4 °C until further analysis. Aflatoxin determination in samples was carried out using the AOAC-approved multifunctional column (Mycosep) method (*15*). Untreated and ozone-treated clean corn contained less than 2 ppb AFB_1. AFB_1 in contaminated corn was 587 ppb and that in ozone-treated contaminated corn was 47.7 ppb, which is below the levels required for animal feed.

Extraction of Lutein. Lutein extraction was a modification of the procedure of Moros et al. (*16*). Triplicate ground corn samples, 20 g of each treatment type, were each placed in 500 mL Erlenmeyer flasks, and 120 mL of 0.1% (w/v) BHT−EtOH solution was added to each flask. The flasks were sealed with screw caps and placed in a 75 °C water bath for 5 min. The flasks were then removed from the water bath, and 4 mL of 80% KOH was added to each flask. Samples were then shaken for 2 min and returned to the water bath for 10 min until saponification occurred. After the samples were saponified, the flasks were immediately placed into an ice bath to cool, and then, 60 mL of cold deionized water was placed into each flask, followed by 30 mL of hexane, followed by shaking. Then, the sample solutions were centrifuged at 2500 rpm for 10 min. The top hexane layer was removed with a Pasteur pipet and added to a separate 250 mL Erlenmeyer flask. The hexane extraction was repeated until the top layer was colorless. All hexane extracts were combined in the same flask. The hexane was evaporated in a nitrogen stream passed into the flask until dry. The residue was then solubilized in 5 mL of mobile phase (methanol/acetone 90:10) and stored at −20 °C for HPLC analysis.

HPLC Method. The analytical HPLC system consisted of a reversed phase Supelco (Bellefonte, PA) Discovery C18 column (i.d. 3 mm × 25 cm), a Waters 2690 separation module, a 996 photodiode array detector, and a Millennium chromatography manager. A guard column (4 mm × 23 mm) containing the same packing materials as the C-18 column was installed ahead of the C18 column. The mobile phase was a mixture of methanol and acetone at a ratio of 90:10. The flow rate was isocratic at 1.0 mL/min. The injection volume of all samples was 20 μL. The detector was set at 456 nm. The analyses were performed

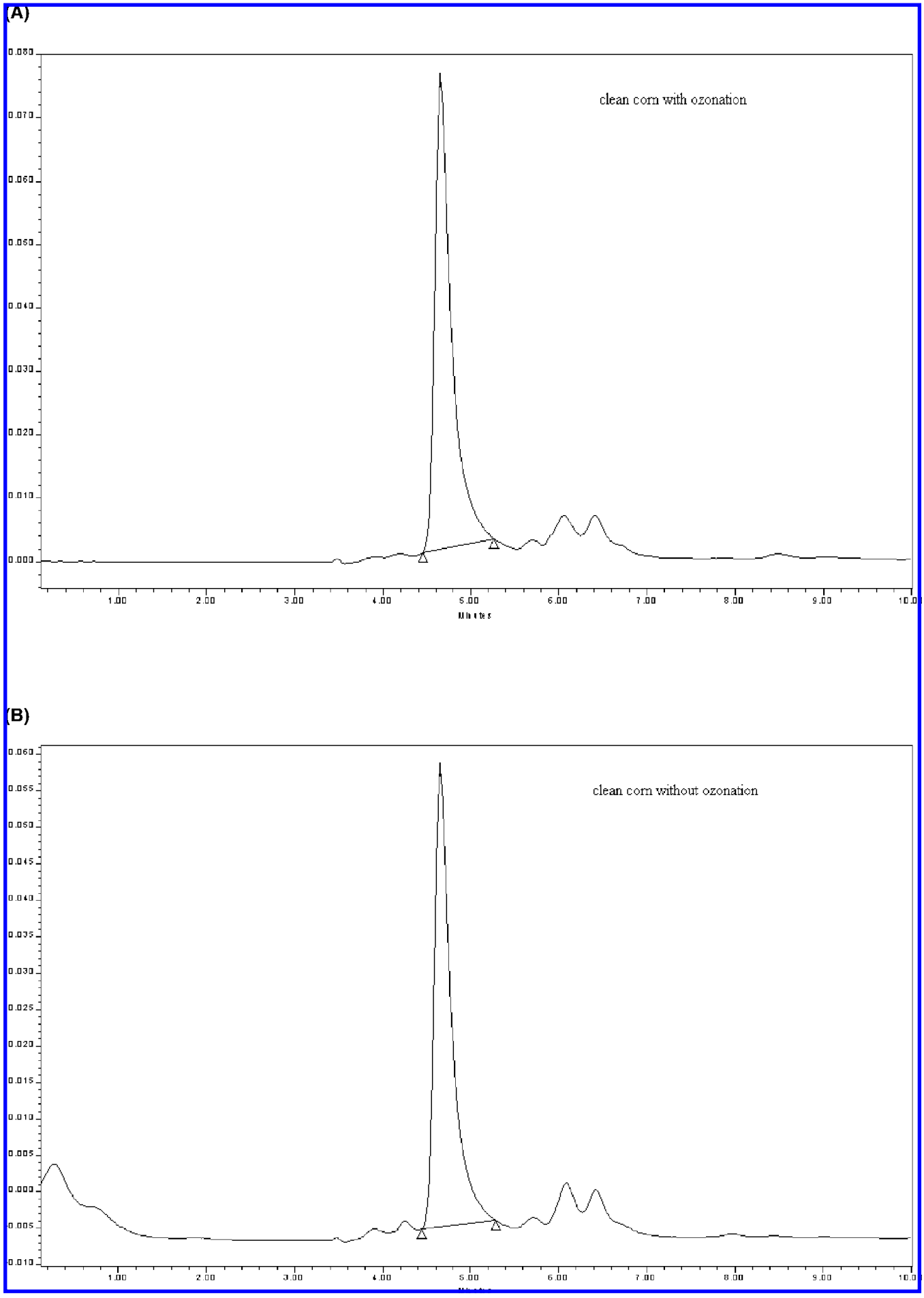

Figure 2. Continued.

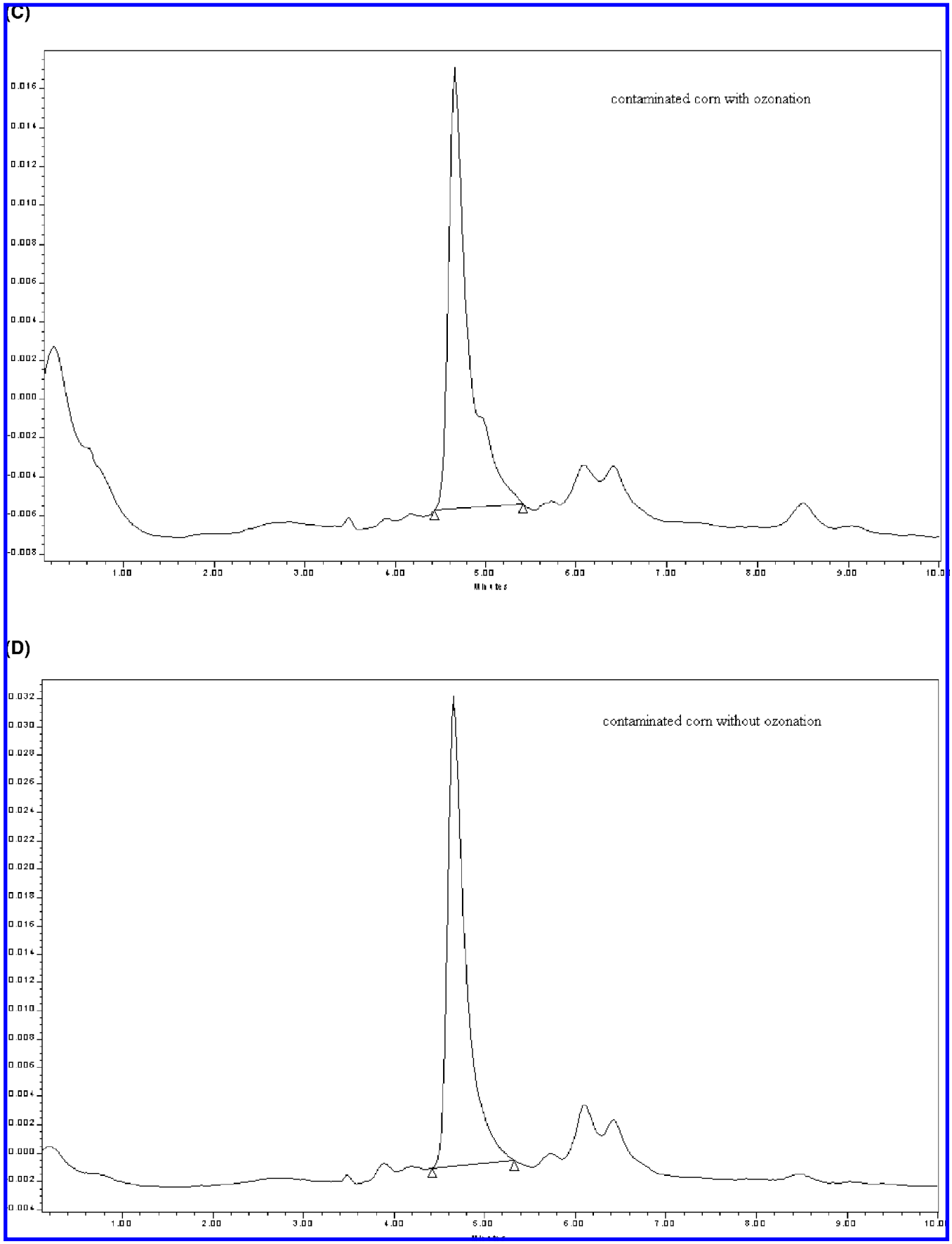

Figure 2. (A) Chromatogram of lutein extract in the clean corn with ozonation, (B) chromatogram of lutein extract in the clean corn without ozonation, (C) chromatogram of lutein extract in the contaminated corn with ozonation, and (D) chromatogram of lutein extract in the contaminated corn without ozonation.

Table 1. Lutein (μg/g) and Protein (%) Contents of Different Corns[a]

sample	lutein content (μg/g corn)	protein content (%)
A	28.36 ± 0.35	10.56
B	22.75 ± 0.11	12.16
C	11.69 ± 0.12	8.85
D	16.42 ± 0.19	12.04

[a] Sample key: A, clean corn with ozonation; B, clean corn without ozonation; C, contaminated corn with ozonation; and D, contaminated corn without ozonation.

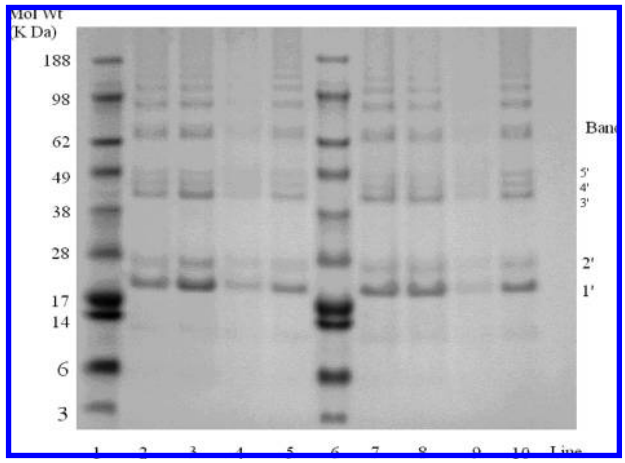

Figure 3. SDS-PAGE of protein extracted from corn. Lanes 1 and 6, molecular weight standards; lanes 2 and 7, clean corn with ozonation; lanes 3 and 8, clean corn without ozonation; lanes 4 and 9, contaminated corn with ozonation; and lanes 5 and 10, contaminated corn without ozonation.

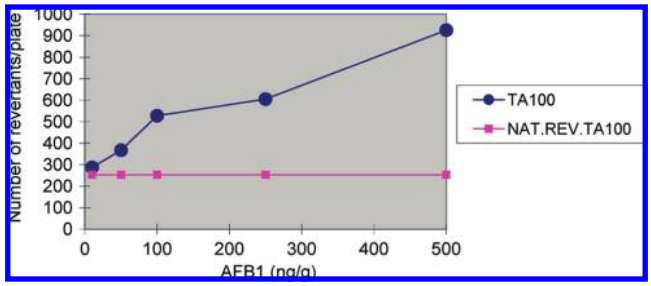

Figure 4. Standard curve for pure AFB1 using *Salmonella typhimurium* tester strains TA100 with metabolic activation (values are means of three replicates).

Table 2. Number of Revertants of Lutein Standard Control without AFB1

concentration (μg/plate)	no. of revertants
0	251 ± 11
0.02	247 ± 15
0.2	258 ± 10
0.8	261 ± 13
2	243 ± 8
10	264 ± 12

in triplicate. The contents of lutein in the corn were calculated by comparing the peak area with that of standard lutein using a standard curve.

Protein Analysis. *Extraction of Protein from Corn.* Corn flour (200 g) was defatted by extraction with 500 mL of petroleum ether at 21 °C overnight in a 1000 mL Erlenmeyer flask. The defatted flour was air-dried under a hood, extracted with stirring with 1000 mL of 70% ethanol containing 0.5 M NaCl in water for 4 h at 21 °C, and refrigerated until equilibrated to 4 °C. Then, the mixture was centrifuged at 4000 rmp for 10 min at 4 °C. The supernatant was decanted into a container. The ethanol was removed under vacuum by rotary evaporation, and the protein solution was lyophilized. The protein concentration in the powder was determined by nitrogen analysis (N × 6.25) (2410 Nitrogen Analyzer, Perkin-Elmer, Shelton, CT). All assays for each treatment sample were done in triplicate.

Electrophoresis of Corn Protein. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was carried out following Invitrogen protocol (Carlsbad, CA). Lyophilized corn protein extract powder at 1 mg/mL was dissolved in sample buffer. Ten microliters of the protein sample was added to 25 μL of sample buffer and 65 μL of deionized distilled water following instructions from the gel's manufacturer. Electrophoretic separation was carried out using a Mini-VE electrophoresis unit (Amersham Pharmacia Biotech, Piscataway, NJ). The gel was stained using Novex Colloidal Blue. Samples were run in duplicate.

Evaluation of Antimutagenicity of Lutein Extracts. The antimutagenicity of lutein extracts was tested using the standard plate incorporation *Salmonella*/microsomal mutagenicity assay as described by Maron and Ames (*17*) and Prudente and King (*6*). A single colony was picked from an ampicillin master plate and placed in 40 mL of sterile nutrient broth in an Erlenmeyer flask. The flask was lightly capped to allow airflow and placed in a gyratory water bath, set at 200−250 rpm and 37 °C, for 12−14 h. In this test, TA100 test strain was used. After incubation, growth was confirmed by checking the turbidity using a spectrophotometer at 650 nm. Sterile Oxoid Broth no. 2 was used as a blank. Absorbance readings in the range of 0.75−0.85 A indicated an optimal cell density of $1-2 \times 10^9$ bacterial cell/mL.

S9 mix was prepared just before commencement of the test. All equipment and solutions were sterilized, and all operations were conducted under a laminar flow hood. Before preparing the S9 mix, lutein extracts were dried with a stream of nitrogen, reconstituted in dimethyl sulfoxide (DMSO), and diluted (5, 25, and 625 times). Lutein standard was also solubilized in DMSO (0, 0.002, 0.02, 0.08, 2, and 10 μg/plate). The concentrations of AFB1 in DMSO used in each plate for the AFB1 standards were 10, 50, 100, 250, and 500 (ng/plate). During the assay, the S9 mix was kept on ice. AFB1 (500 ng) was combined with 0.2 mL of histidine/biotin solution, 0.1 mL of TA100, 0.1 mL of lutein standard/extracts, and 0.5 mL S9 mix with 2 mL of soft top agar. The mixtures were vortexed and poured onto a minimal glucose agar plate and incubated at 37 °C for 48 h. The number of revertants was counted and was compared against natural revertants and AFB1 standard. All assays were done in triplicate.

Statistical Analysis. Each control and treatment group was replicated three times. Student's *t* test procedure (Excel Data Analysis, Microsoft Inc., Seattle, WA) was used to compare the levels of lutein in the treated and untreated corn. In the Ames test, the statistical significance of the differences between the lutein standard and the lutein extract was also determined using Student's *t* test. Significant difference among means was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Lutein Determination. The lutein standard elution profile with the C18 column and reverse-phase chromatography is shown in **Figure 1**. The retention time was about 4.7 min for lutein. Lakshminarayana et al. (*18*) and Li et al. (*19*) reported that the retention time of lutein standard using a C18 column and a similar mobile phase was about 4.5 min. **Figure 2** shows chromatograms of lutein extracts of the different corn samples. The peaks were well-separated by the C18 column. According to Moros et al. (*16*) and Lakshminarayana et al. (*18*), the next two peaks may be zeaxanthin and chlorophyll, respectively.

Table 1 shows the content of lutein in the different corn samples. The content of lutein in the treated clean corn was higher than that of lutein in untreated clean corn. The amount of lutein extracted from clean corn significantly increased ($p < 0.001$) by 24.6% after ozonation, while the amount of lutein in the contaminated corn significantly decreased ($p < 0.001$) from 16.42 to 11.69 μg/g after ozonation.

Moros et al. (*16*) found an average lutein content of 14.68 μg/g in corn, which was lower than our result of clean corn

Table 3. Number of Revertants of Lutein Extracts in Control without AFB1 at Each Concentration Tested[a]

	first dilution		second dilution		third dilution	
sample	no. of revertants	concn (μg/plate)	no. of revertants	concn (μg/plate)	no. of revertants	concn (μg/plate)
A	249 ± 7	5.7	254 ± 12	1.14	249 ± 9	0.23
B	262 ± 13	4.5	257 ± 12	0.90	243 ± 19	0.18
C	248 ± 14	2.3	247 ± 11	0.46	262 ± 11	0.092
D	243 ± 17	3.2	258 ± 8	0.64	249 ± 10	0.128

[a] Sample key: A, clean corn with ozonation; B, clean corn without ozonation; C, contaminated corn with ozonation; and D, contaminated corn without ozonation.

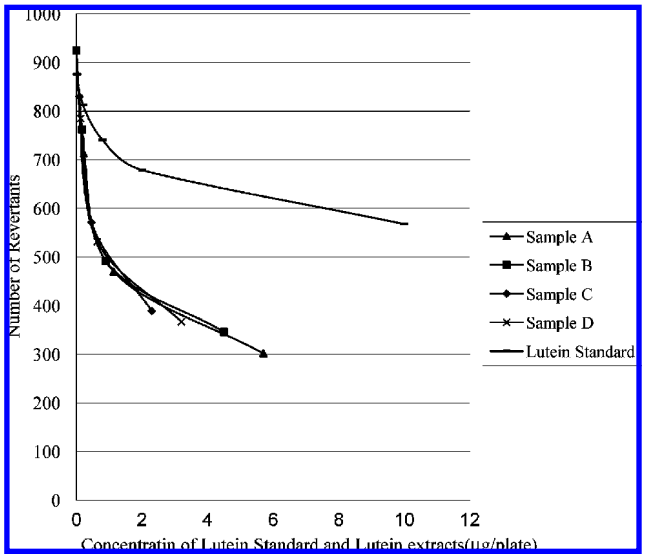

Figure 5. Antimutagenic effect of lutein standard and lutein extracts against AFB1 (500 ng/g) in the following samples: A, clean corn with ozonation; B, clean corn without ozonation; C, contaminated corn with ozonation; and D, contaminated corn without ozonation.

Table 4. Antimutagenic Potency of Lutein Standard Against AFB1 (500 ng/Plate) in TA100

concentration (μg/plate)	no. of revertants	% inhibition
0	925 ± 23	0.0
0.02	876 ± 34	5.3
0.2	813 ± 45	12.1
0.8	741 ± 25	19.9
2	679 ± 39	26.6
10	568 ± 50	38.6

without ozonation. When the extraction step was repeated five times, the amount of xanthophylls was 22.81 μg/g (*15*). The reason for the greater amount of lutein in the ozone-treated corn in our study may be that lutein is bound to other compounds such as fatty acids, protein, and starch or trapped in the corn solid. Because of ozonation, lutein may have been released from those compounds. Lutein ester is one kind of these bound products (*20*). KOH was used to enrich free lutein from lutein ester by saponification. In the research of Moros et al. (*16*), the content of lutein in whole corn was compared with corn gluten meal. As a result, the total xanthophylls concentration was 145.91 ± 2.06 μg/g corn gluten meal, about 7.2 times higher than whole corn assayed under similar conditions. Moreover, the protein content of gluten meal is about 60% (dry basis) as compared to 7.6% protein in whole corn, about 7.9 times higher. These results suggest that the xanthophylls are probably bound to a protein, probably zein. It was pointed out that if hexane was used to remove fat from the corn, about 85% of the xanthophylls remained in the corn (*16*). Fifteen percent of xanthophylls may be lutein ester in the oil, while the rest of the lutein may interact with zein by hydrophobic bonds.

Zein, which is ethanol-soluble, is classified as α-, β-,γ-, and δ-zein on the basis of differences in solubility and sequence (*21*). The structure of zein in the corn is the key point of combination with lutein. There are 56.7% α-helix, 7.1% β-sheets, and 8.2% coil, with 28% of the structure not determined in α-zein (*22*). The model reported by Argos et al. (*23*) indicated that the repetitive sequence of the zein-forming α-helix is highly hydrophobic, that is, rich in leucine, and also includes phenylalanine and tyrosine. Thus, it is reasonable to speculate that such a hydrophobic α-helix region in zein has a high affinity for lutein molecules. When corn was treated with ozone, the ozone possibly destroyed zein so as to release lutein. However, in the contaminated corn, the amount of lutein in the treated corn was less than that in the untreated corn. It might be that because of aflatoxin contamination, zein was already degraded, making lutein more accessible to ozone in the contaminated corn.

Protein Analysis. Table 1 shows the content of protein in different corn samples. The content of protein in the treated corn was lower than in untreated corn. In the clean corn, the protein content decreased by 1.6% after ozone treatment. In the contaminated corn, protein content decreased by 3.2%. Corn contains 70−75% starch, 5% lipids (triglycerides), and 11% protein by weight (*24*). The protein content of our sample was similar to that of the literature. Zein comprises 50% of the total protein component in the mature seed. These results suggest that ozone can destroy the protein.

Figure 3 shows the result of SDS-PAGE analysis of the proteins, which shows two bands at approximately 22 and 26 kDa. Cabra et al. (*22*) found that the SDS-PAGE results usually divide α-zein into two groups based on their migration (Z19 and Z22). However, the apparent molecular mass of the peptides was often different in the various reports because of the use of different gel systems, standard proteins, and corn varieties. Apparent molecular masses of 18−24 kDa for Z19 and 21−26 kDa for Z22 have been reported (*25*). In fact, α-zein is a mixture of a large number of proteins. Wilson (*25*) showed at least 15 components in α-zein by RP-HPLC serial analysis. There were no changes in protein type between samples (**Figure 3**). Some bands were lighter when the corn was treated with ozone, indicating that the concentration of that protein was lower. The contaminated corn protein may have been more susceptible to ozone degradation. Bands for ozonated contaminated corn sample were the lightest, and the concentration of protein, which was 8.85% in the treated contaminated corn, was the lowest. Bands for ozonated clean corn were lighter, and the protein content in the treated clean corn was between the amount for untreated and the amount for treated contaminated corns (**Table 1**).

Evaluation of Antimutagenicity of Lutein. The antimutagenic potential of lutein extracted from corn was evaluated to determine the potential risk that the ozonation process might have on the final product. **Figure 4** shows the dose−response curve for AFB1 standard. Qin and Huang (*26*) reported that

Table 5. Antimutagenic Potency of Lutein Extracts Against AFB1 (500 ng/Plate) in TA100 (see **Table 3** for Concentrations Tested)[a]

	first dilution		second dilution		third dilution	
sample	no. of revertants	% inhibition	no. of revertants	% inhibition	no. of revertants	% inhibition
A	302 ± 13	67.4	470 ± 7	49.2	713 ± 12	22.9
B	346 ± 20	62.6	492 ± 4	46.8	762 ± 21	17.6
C	389 ± 10	57.9	571 ± 14	38.3	830 ± 9	10.3
D	367 ± 11	60.3	532 ± 20	42.5	785 ± 13	15.1

[a] Sample key: A, clean corn with ozonation; B, clean corn without ozonation; C, contaminated corn with ozonation; and D, contaminated corn without ozonation.

with a concentration of 500 ng AFB1/plate in TA98, the mutagenic potency was 1117 revertants/plate. On the other hand, Bhattacharya et al. (*27*) found that with a concentration of 400 ng AFB1/plate in TA100, the mutagenic potency was 2386 ± 158 revertants/plate, 2.5 times more as compared with our results. Prudente and King (*6*) observed that with a concentration of 500 ng AFB1/plate, the mutagenic potency was about 900 revertants/plate, which is similar to our result 925 revertants/plate.

Lutein standard and lutein extracts were investigated for mutagenic potential (**Tables 2** and **3**). The number of revertants for lutein standards at the concentrations tested was similar to the negative control (natural revertants) (**Table 2**). Although some of the lutein extracts (**Table 3**) had a slightly higher number of revertants than the natural of revertants, they were still close to 253 ± 23. The Ames test showed that purified lutein and lutein extracts from ozonated corn do not induce mutagenicity in TA100 using the plate incorporation method. Kruger (*28*) investigated two formulations of purified lutein, encapsulated beadlet containing 10% purified lutein and non-encapsulated purified lutein. For both samples and for all five tester strains, the number of revertants was not increased. Our findings are consistent with a number of previous studies demonstrating the absence of any mutagenic effect of lutein using the Ames test in *S. triphimurium* strains (*11, 12, 29*).

The antimutagenic effect of lutein standard and lutein extracts on AFB1 mutagenicity is shown in **Figure 5**. The number of revertants and percent inhibitions of mutgenicity by AFB1 using lutein standard and lutein extracts are summarized in **Tables 4** and **5**. Lutein standard and lutein extracts inhibited AFB1 (500 ng/plate) mutagenicity in a dose−response manner. Lutein extracts were more efficient than that of lutein standard, as shown in **Figure 5**. At the concentration of 0.2 μg lutein standard/plate, the inhibition was 12.1%, while at similar or lower concentrations of lutein extracts, the inhibitions of A, B, C, and D were 22.9, 17.6, 10.3, and 15.1%, respectively (**Table 5**). Gonzalez de Mejia et al. (*12*) found similar results with lutein extracts from marigold using tester strain YG1024, where at the concentration of 0.002 equiv μg lutein/plate, the inhibition was 11 and 55% for purified lutein and lutein extracts. The result suggests that the lutein extracts have a mixture of antimutagenic agents that may have a synergistic effect against AFB1 mutagenicity. Statistical analysis of our results showed that the number of revertants of lutein standard was significantly higher than that of lutein extract ($P \leq 0.001$). However, the number of revertants among lutein extracts was not significantly different at $P \leq 0.1$. Lutein extracts from different corn treatments had a similar antimutagenic potential (**Figure 5**). Ozone did not affect the antimutagenic potential of lutein but did affect lutein levels in corn.

Some studies were done on the mechanism of lutein against AFB1 mutagenicity. Gonzalez de Mejia et al. (*11*) observed a modest inhibition (31% at 10 μg lutein/plate) on AFB1 mutagenicity in a preincubation study on the DNA repair system of tester strain YG1024, which is a derivative of TA98. When the bacteria were incubated with lutein and S9 first, the percent inhibition of 10 μg lutein/plate was 71%. A new absorption peak was detected at 378 nm when lutein and AFB1 were incubated together (*11*). The result indicated that lutein can inhibit AFB1 mutagenicity by forming a complex between lutein and AFB1, therefore limiting the bioavailability of AFB1. In studies conducted by Cardador-Martinez et al. (*13*), it was observed that the greatest inhibitory effect of phenolic compounds present in beans occurred when the phenolic extract (PE) was incubated with AFB1, independent of the first or second incubation in a two-stage incubation protocol. This suggested that PE could interact directly and nonenzymatically with the proximate and/or ultimate mutagen (AFB1 8, 9 - expoxide) or form a complex between the phenolic compounds and the AFB1, thereby reducing the bioavailability of AFB1. Mechanistic studies suggest that chlorophyllin can act as an "interceptor molecule" through the formation of tight molecular complexes with carcinogens such as AFB1 (*30*). Thus, chlorophyllin may diminish the bioavailability of AFB1.

The mechanism of lutein against AFB1 mutagenicity is most probably the result of a combination of the following reasons: (i) Lutein may interact directly and nonenzymically with the proximate and/or ultimate mutagen(s); (ii) formation of a complex may occur between lutein and AFB1; and (iii) lutein may also affect the metabolic activation of AFB1 by S9 and the expression of AFB1 modified *Samonella* DNA (*11*).

Results of the study indicated that the ozone process did change the level of lutein in the corn. In clean corn, the content of lutein in treated corn was higher than that of lutein in untreated corn. On the contrary, the ozone process decreased the lutein content in the contaminated corn. A protein analysis study showed that ozone could destroy protein, which in turn may affect the nutritional quality of the corn. Lutein standard and lutein extracts showed no mutagenic potential when tested against *S. typhimurium* tester stain TA100. The lutein extracts from corn had a stronger effect on the mutagenicity of AFB1 than lutein standard, perhaps due to lutein extracts having a mixture of antimutagenicity agents that could have a synergistic effect on AFB1 mutagenicity. Lutein extracts from different corn samples had similar antimutagenic potentials, and ozone can not affect the antimutagenic potential of lutein but can affect the levels of lutein in corn. In conclusion, results from the present studies demonstrate that ozone can destroy some antimutagenic compounds and protein as well as aflatoxins, which might affect the nutritional quality of commodities.

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Received for review May 19, 2008. Revised manuscript received June 24, 2008. Accepted July 3, 2008. This research was funded by the Louisiana Soybean and Grain Research and Promotion Board, the Louisiana Board of Regents, and the Louisiana Agriculture Experiment Station.

JF801562V