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Effect of Calcination on the Structure of Montmorillonite and Its Adsorption of Aflatoxin B_1

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ABSTRACT

Aflatoxins are secondary metabolites primarily produced by *Aspergillus flavus* and *A. parasiticus*. Among them, aflatoxin B_1 (AFB_1) has garnered the most attention due to its toxigenic, mutagenic, and carcinogenic properties. Researchers worldwide have explored various strategies to mitigate the presence of these hazardous toxins. A practical approach to reducing AFB_1 contamination is to add a clay, such as montmorillonite (MMT), as a toxin binder to animal feeds. Once MMT in the feed enters an animal's gastrointestinal tract, it adsorbs AFB_1, reducing its toxicity and allowing the toxin to be excreted in feces, ultimately reducing the toxicity of AFB_1 in the feed. To further enhance MMT's adsorption of AFB_1, heat modification of MMT is required. This heating process at temperatures of 300, 500, 700, and 900 °C, which is also known as calcination, alters the adsorption characteristics of AFB_1. To assess the effect of calcination on the adsorption capacity of MMT to AFB_1, modified and raw MMT samples were mixed with AFB_1 at 100 ppb under constant agitation and incubated at 30 °C for 2 h at three different pH levels (pH 2.5, 5.2, and 6.6). High-performance liquid chromatography (HPLC) was utilized to determine the concentration of unbound AFB_1. The results show that the MMT calcined at 300 °C exhibited the highest AFB_1 adsorption, with binding capacities at pH levels of 2.5, 5.5, and 6.6 of 79.19%, 92.16%, and 91.55%, respectively. In contrast, raw MMT exhibited a lower adsorption of 66.46%. The improved adsorption after calcination is probably due to an increase in reactive surface area, an improved porosity, and favorable electrostatic conditions. Characterization of the MMT using SEM, FTIR, XRD, and XRF confirmed the structural and surface modifications that occurred upon calcination. However, a high calcination temperature of 900 °C reduced adsorption capacity, which could be attributed to both structural degradation and decreased cation-exchange capacity. This study contributes to new areas by examining the interplay between pH and calcination temperature in AFB_1 binding by MMT.

Keywords: *Aflatoxin B1, Toxin binder, Montmorillonite, Grain corn*

1. INTRODUCTION

Aflatoxins are major food and feed safety threats globally because they have a combination of both high toxicity and the potential to cause cancer. Two primary fungal producers of these toxic compounds are *Aspergillus flavus* and *A. parasiticus*, which are prevalent in warm, humid agricultural areas, particularly in Malaysia. The variations of aflatoxins are numerous, but AFB_1, AFG_1, AFB_2, and AFG_2 are the four most dangerous [1]. Of these, the International Agency for Research on Cancer [2] has classified AFB_1 as a Group 1 carcinogen because it exhibits hepatotoxic and mutagenic effects and can suppress the immune system. Unfortunately, food processing does not destroy AFB_1, which means that humans and animals can be exposed to this toxin at every stage of its production.

To mitigate toxicity and the prevalence of aflatoxin, a range of detoxification strategies has been explored to combat AFB_1, including physical, chemical, and biological methods. Adsorbent materials commonly referred to as toxin binders have emerged as one of the most widely recognized post-harvest interventions, primarily in livestock production systems [3]. The mechanism involves toxin binders binding to AFB_1 in the gastrointestinal (GI) tract, where the toxin is adsorbed, its bioavailability is significantly reduced, and its systemic absorption is prevented; it is eventually excreted in the feces. The mechanism of adsorption involves the formation of non-covalent interactions, including hydrogen bonding, van der Waals forces, and ionic interactions, between the binder surface and the toxin molecule [4].

Among the numerous binders, clay minerals are considered a promising intervention candidate due to their effectiveness and safety. Among the clay minerals, aluminosilicate clays, such as montmorillonite (MMT), have been shown to have high capacities for binding AFB_1. MMT

is a layered 2:1 smectite clay composed of an octahedral alumina sheet sandwiched between two sheets of tetrahedral silica. MMT exhibits a high surface area and cation exchange capacity. These characteristics are essential for its adsorption behavior. Numerous studies have examined raw and modified forms of MMT to optimize binding. In thermal treatment, where calcination is the primary method, MMT is exposed to high temperatures, leading to structural changes, including the removal of interlayer water and partial dihydroxylation, which affect porosity, total surface area, and surface chemistry [3, 5]. All of these may increase its affinity for AFB_1. A study by Johnson et al. [6] demonstrated that calcination of MMT enhances binding to aflatoxin G_1 in vivo.

However, a disadvantage of thermal modifications is that, at higher temperatures, they can break down the MMT structure, resulting in reduced AFB_1 binding. Thus, determining the optimal calcination temperature range for maximum binding is crucial. Since studies investigating the sorption of AFB_1 onto calcined MMT across various calcination and pH conditions are notably scarce, this study aims to evaluate the adsorption performance of thermally modified MMT under varying temperatures and pH values.

2. METHODOLOGY

2.1 Calcination Process

Montmorillonite and AFB_1 were purchased from Sigma-Aldrich. Methanol, ethanol, and acetonitrile were HPLC-grade, also from Sigma-Aldrich. Other chemicals and reagents were of analytical grade.

A modified MMT was prepared by calcining 5 g of MMT in a digital muffle furnace (WiseTherm, Korea) at different temperatures: 300 °C, 500 °C, 700 °C, and 900 °C for 3 h. After calcination, the modified MMT was ground and sieved into a fine powder with a particle size of approximately 100 µm.

2.2 MMT-AFB_1 Adsorption Capacity Test

The adsorption capacity of AFB_1 was assessed using in vitro experiments. Each of the modified MMT and raw MMT samples was separately mixed with AFB_1 at a concentration of 100 ppb, using an adsorbent dosage of 3.0 mg/mL. The mixtures were incubated at 30 °C for 2 h with constant agitation at three different pH levels (2.5, 5.2, and 6.6), which represent the gastrointestinal (GI) tract environment in monogastric animals. After incubation, the mixtures were centrifuged at 4,000 rpm for 30 min, and the supernatant, containing unbound AFB_1, was analyzed using high-performance liquid chromatography (HPLC).

The percentage of adsorption rate was calculated using the following equation:

$$\% \text{ binding capacity } 100 \times (Ci - Cf)/Ci$$

where;
Ci is the initial concentration of AFB_1
Cf is the concentration of unbound AFB_1 after the incubation period

2.3 Characterization of Modified Montmorillonite

Characterization of raw and modified MMT was conducted using Scanning Electron Microscopy (SEM) using Hitachi SU8600 (Japan), Fourier Transform Infrared (FTIR) Spectrometer using Perkin Elmer (USA), Geochemical analysis was performed using Rigaku ZSX Primus IV X-ray fluorescence (XRF) spectrometer (Japan), and Mineralogical variation of the samples was ascertained using D8 Advance Bruker X-ray diffraction (XRD) machine (Germany).

2.4 Statistical Analysis

Due to the small sample size (n = 4 per group) and the non-normality of the percentage data, the Kruskal–Wallis test was used to compare differences across groups. Dunn's *post hoc* test with Bonferroni correction was applied for multiple comparisons where applicable, with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 MMT-AFB_1 Adsorption Capacity Test

The adsorption capacity of raw MMT for AFB_1 at three different pH levels (2.5, 5.2, and 6.6) is shown in Figure 1. These pH ranges simulate conditions in the chicken stomach and intestines [7]. The results indicated that the adsorption capacity of raw MMT showed no significant differences across all pH values studied. On the other hand, after being calcined at 300 and 500 °C, the modified MMT exhibited a significantly greater ability to adsorb AFB_1 ($p < 0.05$) than the raw MMT. Specifically, the binding capacities at pH 2.5, 5.5, and 6.6 for the calcined temperature of 300 °C were 79.19, 92.16, and 91.55%, respectively, compared to 66.46% for raw MMT, while for the calcined temperature of 500 °C, they were 77.77, 89.55, and 89.96%, respectively, compared to 66.46% for raw MMT (Figure 1).

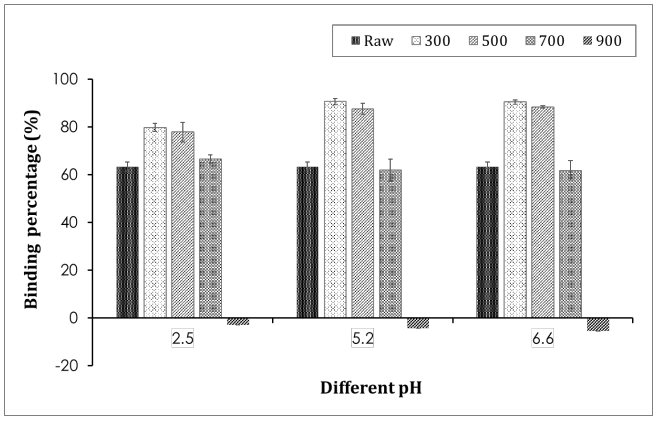

Figure 1. Comparison of raw and modified MMT on the AFB_1 adsorption capacity percentage at different pH levels.

The increase in adsorption at calcination temperatures of 300 and 500 °C across all pH values studied could be attributed to enhanced porosity, an increased number of reactive sites, and favorable surface chemistry. This finding was consistent with the general observation that thermal treatments increase pore size and moderately decrease the cation exchange capacity (CEC) without compromising the clay structure. Similar effects were observed for palygorskite and bentonite [8]. The peak adsorption capacity at pH 5.2–6.6 for the calcined temperature range of 300 to 500 °C was possibly the result of increased environmental pH conditions, coupled with thermal treatment that enhanced van der Waals forces and hydrogen bonding interactions between the AFB_1's aromatic moieties and silicate surfaces, which may have enhanced adsorption, and was also observed on a modified MMT in *in vitro* and *in silico* studies [9].

However, increasing the calcination temperature from 300 °C to 700 °C resulted in significantly lower adsorption across all pH levels studied, with no significant differences compared to raw MMT. Increasing the calcination temperature further to 900 °C resulted in a pronounced decrease in AFB1 adsorption by MMT, likely because the layered structure was completely collapsed during dihydroxylation [10]. This phenomenon was also observed in smectite clay minerals, where exposure to very high temperatures adversely affects adsorption functionality [11].

3.2 Characterization of Modified MMT

3.2.1 Morphological analysis

The previous results demonstrated that modifying MMT via calcination enhances its adsorption of AFB_1. This modification has probably created sufficient space between the layers, increasing interaction with the low-polarity AFB_1 and thereby increasing adsorption. Figure 2(a) shows the surface morphology of raw MMT, and Figure 2(b) shows the changes that occur after calcination at 300 °C.

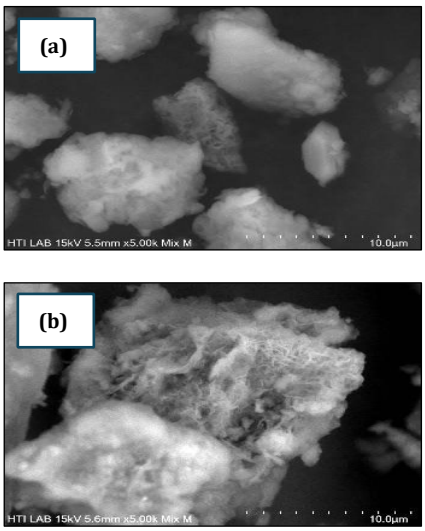

Figure 2. Morphology of (a) raw MMT and (b) calcined MMT.

Analysis of the raw MMT morphology in Figure 2(a) indicates that MMT exhibits a smooth surface morphology, which is characterized by the presence of distinct plates and a lamellar structure. On the other hand, the morphology of the modified MMT at the calcination temperature of 300 °C exhibits a more open structure compared to the raw MMT (Figure 2(b)). It was also observed that the modified MMT exhibits expanded pores, which are postulated to enhance surface accessibility for AFB_1 adsorption.

3.2.2 Surface Functional Groups Analysis

FTIR spectra of the surface functional group analysis of raw and modified MMT calcined at various temperatures (300, 500, 700, and 900 °C) are shown in Figure 3.

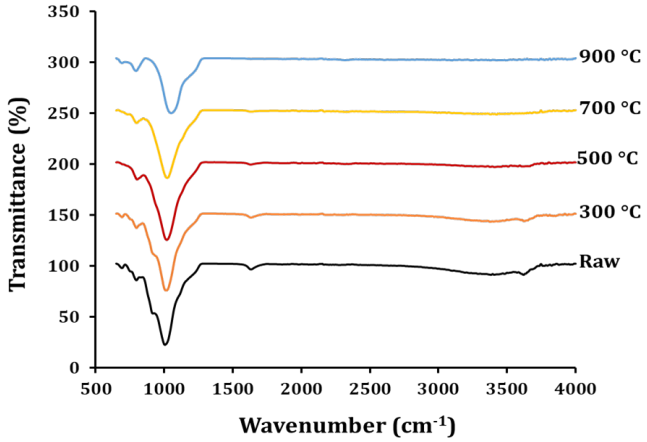

Figure 3. FTIR spectra of raw and modified MMT calcined at different temperatures (300, 500, 700, and 900 °C).

The spectra reveal a broad absorption band around 3620 cm ^{1} that is characteristic of O–H stretching vibrations (Figure 4).

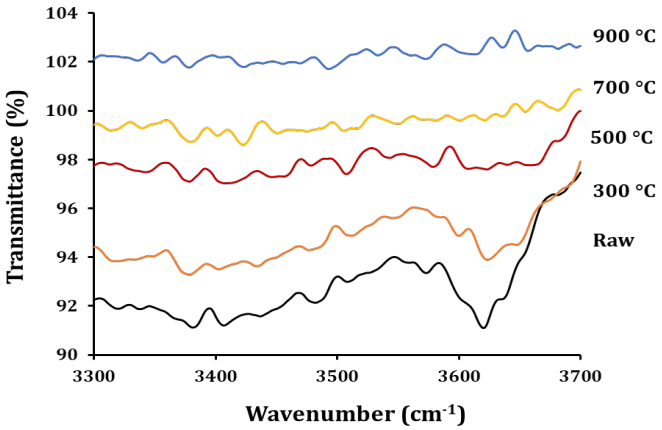

Figure 4. FTIR spectra of raw and modified MMT in the wavenumber range of 3300 to 3700 cm^{-1}.

This band range is typically associated with hydroxyl groups and adsorbed water, and in the raw MMT, this peak was prominent. This prominent peak indicates a high degree of hydration in the MMT structure. It is postulated that increasing the calcination temperature leads to a gradual decrease in the intensity of this peak. This decrease reflects the progressive dehydroxylation of the surface and removal of physically adsorbed water. Substantial dehydration and collapse of the MMT surface hydroxyl networks were observed at the highest temperature studied, 900°C, where this band was significantly diminished [11].

The distinct peak observed at 1632.0 cm^{-1} is due to the bending vibration of molecular water (H–O–H) (Figure 5). At relatively low calcination temperatures, this peak was observed in both the raw and calcined MMT, suggesting retention of MMT's water content. At higher calcination temperatures, water removal via thermal desorption likely occurs, as this peak was significantly diminished, especially above 500°C. At the highest calcination temperature of 900°C, this peak was completely absent, further emphasizing the efficient elimination of moisture at high temperature [12].

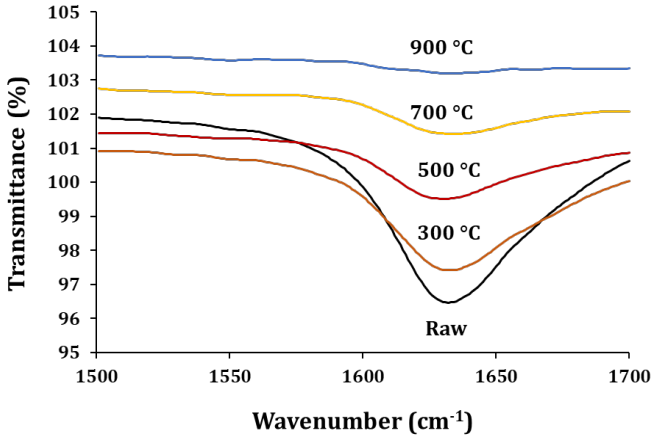

Figure 5. FTIR spectra of raw and modified MMT in the wavenumber range of 1500 to 1700 cm^{-1}.

The bands around 910 to 1000 cm^{-1} correspond to the asymmetric stretching vibrations of Si–O–Si bonds (Figure 6). This stretching indicates the presence of silicate or silica-based structures of MMT. At low calcination temperatures, a more amorphous silicate framework is likely to form, as reflected in the poorly defined, relatively broad bands.

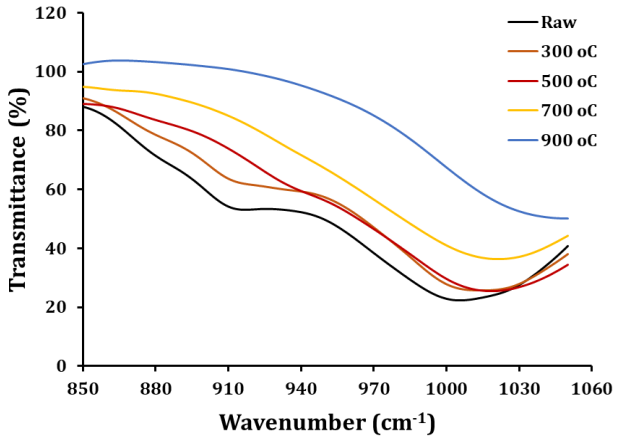

Figure 6. FTIR spectra of raw and modified MMT in the range of wavenumbers from 850 to 1060 cm^{-1}.

As the calcination temperatures increased, particularly at 700°C and 900°C, these bands became sharper and more intense. This change probably reflects an increase in polymerization and structural reorganization of the silicate network, with enhanced crystallinity or densification of the silica matrix due to the heat-induced structural evolution of MMT [13].

The thermal behavior of the studied material was further evaluated by FTIR spectroscopy, providing valuable insights, including a systematic reduction in hydroxyl and water-related bands. Furthermore, the enhancement of silicate stretching vibrations was observed, all of which confirm the occurrence of dehydration, dehydroxylation, and reorganization processes in the silicate network.

3.2.3 Crystal structure and geochemical analysis

The raw and modified MMT calcined at 300, 500, 700, and 900 ℃ were further analyzed geochemically using an XRF spectrometer. The analyses show the presence of oxides, which include SiO_2, Al_2O_3, Fe_2O_3, MgO, CaO, K_2O, and Na_2O (Table 1).

Table 1 Chemical composition (%) of raw and modified MMT calcined at different temperatures (300, 500, 700, and 900 °C) through XRF analysis

Sample	RAW MMT	CALCINATION TEMPERATURES (°C) OF MMT			
		300	500	700	900
Na_2O	0.26	0.28	bdl	0.30	0.33
MgO	1.49	1.47	0.39	1.44	1.46
Al_2O_3	20.10	19.80	11.40	20.00	20.10
SiO_2	69.70	68.70	61.50	69.20	69.50
P_2O_5	0.05	0.05	1.77	0.05	0.05
SO_3	0.01	0.02	bdl	0.01	0.01
K_2O	2.47	2.71	5.46	2.60	2.48
CaO	0.45	0.51	1.82	0.47	0.45
TiO_2	0.74	0.89	bdl	0.79	0.75
MnO	0.02	0.02	bdl	0.01	bdl
Fe_2O_3	4.62	5.38	15.60	4.99	4.69
Co_2O_3	bdl	bdl	bdl	bdl	bdl
ZnO	0.01	bdl	bdl	0.01	0.01
Ga_2O_3	0.01	0.01	bdl	0.01	0.01
As_2O_3	bdl	bdl	0.00	bdl	bdl
Rb_2O	0.01	0.02	0.07	0.02	0.01
SrO	0.01	0.01	bdl	0.01	0.01
Y_2O_3	bdl	bdl	bdl	bdl	0.01
ZrO_2	0.07	0.07	0.23	0.07	0.06
Nb_2O_5	0.01	0.01	bdl	0.01	0.01
BaO	0.05	0.00	bd	0.06	0.00
Total	100.09	99.95	98.24	100.03	99.92

Note: (bdl=Below detection limit).

These oxides, which constitute about 1% of the total composition of the clay minerals, influence the chemical and crystal structure of MMT [14]. Of these, the primary building blocks of the clay lattice are SiO_2 and Al_2O_3, with the interlayer or substitutional sites occupied by minor oxides such as Fe_2O_3, MgO, CaO, K_2O, and Na_2O. In the interlayer region of the MMT, the oxides affect characteristics such as plasticity and cation exchange capacity. In the raw, untreated MMT, SiO_2 (69.70%) and Al_2O_3 (20.10%) dominated the oxide composition, with minor amounts of Fe_2O_3 (4.62%), K_2O (2.47%), MgO (1.49%), and trace amounts of other oxides.

The oxide percentages in the calcined MMT varied from 300 to 900 °C, with changes in composition, including increases, decreases, and disappearance of some oxides. At a calcination temperature of roughly 500 °C, dehydration of MMT was observed, in which MMT loses structural water and hydroxyl groups are broken down, all of which lead to changes in the concentrations of Al_2O_3 and SiO_2. These changes may affect the composition of other oxides bound within the MMT structure [15], creating new mineral and amorphous phases.

Thermal treatment causes significant changes in iron oxides, which can oxidize or concentrate when heated. The distribution of potassium and calcium compounds may also change upon heating or crystallize differently. Calcination of MMT at 500 °C resulted in the apparent loss of TiO_2 and some trace elements, likely due to their volatilization or migration. Spikes in P2O5, Fe2O3, and K2O offset the loss of TiO2 and some trace elements. These phenomena may be caused by the removal of structural water or the loss of volatile components, thereby concentrating the remaining oxides.

At the calcination temperatures of 300, 700, and 900 °C, the percentages of SiO_2 and Al_2O_3 were found to remain steady in raw MMT and in MMT. However, their levels fell to 61.50% and 11.40%, respectively, after calcination at 500 °C. Compositional changes at 500 °C indicate dehydroxylation, collapse of the clay structure, and the formation of new mineral phases [16]. At the calcination temperature of 500 °C, the MMT's Fe_2O_3 composition increased to 15.60%, which was then returned to the baseline level at higher calcination temperatures. As other elements are volatilized or iron oxides crystallized, iron thus became more concentrated [17]. The concentration of other components, including MgO, CaO, K_2O, and Na_2O, is also altered by calcination. These alterations were consistent with the migration or volatilization of interlayer cations during dehydroxylation.

The XRD analysis also revealed that raw MMT is a multiphase material composed of layered silicates characteristic of illite and kaolinite, including MMT. The presence of quartz as a persistent contaminant originates from ancient rocks (Figure 7).

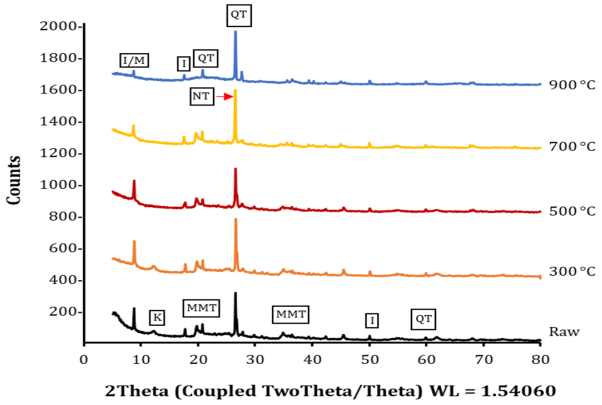

Figure 7. XRD pattern of raw and modified MMT calcined at different temperatures (300, 500, 700, and 900 °C). (I/M: Illite/Muscovite, K: Kaolinite, MMT: Montmorillonite, O: Quartz. NT: Nontronite.

Muscovite and illite were found to have large basal reflections at 9° 2θ, while kaolinite shows a peak at 12.3° 2θ. MMT's broad basal reflection appears at lower angles, and these phases upon calcination at 300 °C were found to remain intact. However, at temperatures of 500 °C and above, a sharp decline in the intensities of the MMT and kaolinite peaks was observed, which may indicate dehydroxylation and partial structural collapse.

This peak transition may enhance surface reactivity, as observed in another study on aflatoxin adsorption by smectite clay [18]. This is specifically so at temperatures ranging from 700 to 900 °C. A result of thermally induced phase transformations is the formation of Fe-rich smectites, including nontronite. In other studies, an increase in affinity for aflatoxin by these iron-bearing phases may be due to their expanded interlayer spacing and increased surface charge [19, 20]. Muscovite appears to emerge at temperatures of≥500 °C, particularly at 900 °C. This prominence is likely due to the transformation of illite or chlorite. However, its impact on adsorption was less significant.

The Rietveld mineral quantification results for the raw and calcined MMT show the relative amounts (weight percent) of the various crystalline phases, such as MMT, kaolinite, and illite, present in the samples (Table 2). Quartz has excellent thermal resistance and is generally stable across a wide temperature range, demonstrating its inertness when heated. The mineral chlorite occurs at lower calcination temperatures (300 and 500 °C), indicating that at higher temperatures it decomposes or transforms.

Muscovite was absent in raw or lower-temperature MMT calcination but was observed at 500 °C, and its presence increased significantly at 900 °C. It is known that phyllosilicates, at high temperatures, which include illite or chlorite, are transformed into muscovite. At 700 °C, Fe-Al rearrangement during high-temperature treatment produces nontronite, an Fe-rich smectite, which emerges as the primary phase. Nontronite was not observed at 900 °C, which is likely due to further transformation or recrystallization.

Table 2 Rietveld mineral quantification of raw and calcined MMT

Sample	RAW MMT	CALCINATION TEMPERATURES (°C)			
		300	500	700	900
Quartz	31.80	44.90	30.10	31.50	30.90
Illite	38.90	34.80	26.80	5.40	20.00
Kaolinite	12.10	6.70	3.60	3.60	3.10
Mmt	17.20	8.80	6.80	5.30	4.20
Chlorite	nd	4.80	1.80	nd	nd
Muscovite	nd	nd	30.90	nd	41.80
Nontronite	nd	nd	nd	54.20	nd

Note: (bdl=Below detection limit).

The calcination of MMT resulted in the degradation of less active phases and the formation of more adsorptive ones, including nontronite and altered smectites. This process enhances MMT's potential for aflatoxin remediation [19, 20].

4. CONCLUSIONS

In this study, the thermal treatment of MMT at different pH values enhances AFB1 adsorption through a combination of structural and surface chemical changes. In this study, a moderate calcination of MMT at 300–500 °C was found to be optimal for AFB_1 binding. This optimal range yields an optimal combinatorial process that includes increased surface porosity, preservation of structural integrity, and, most importantly, favorable charge interactions. In addition, the most effective pH range for AFB_1 binding was 5.2 to 6.6, which provides optimal electrostatic conditions for toxin binding. However, an impairment of the clay's adsorption capacity across all pH values was observed at the higher thermal treatment temperature of 900 °C. This impairment is suggested to be caused by the process of dehydroxylation, where a collapse of the layered structure occurs, leading to an alteration of surface charge properties, as supported by XRD and XRF analyses. The findings in this study suggest an important need to fine-tune the thermal parameters of MMT to increase adsorption. This study fills a significant gap in the literature by providing a systematic assessment of the effects of pH and calcination on AFB_1 sorption. The outcomes of this study contribute to a better design of optimized clay-based mycotoxin binders for feed applications.

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