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Original research

Employing calcined and non-calcined aluminum-magnesium layered double hydroxides for detoxification of solution and nonalcoholic beer from aflatoxins B_1 and G_1

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ABSTRACT

Detoxification of solution and beer from aflatoxins B_1 and G_1 using layered double hydroxides is the focus of this study. The aluminum-magnesium layered double hydroxide (Al-Mg LDH) is selected in its calcined and non-calcined forms to evaluate the influence of calcination on detoxification. They were produced using the co-precipitation method and used in contaminated solutions and beer. Characteristics of the adopted adsorbents were studied by XRD, FTIR and SEM methods. Effects of quantity of the adsorbents, exposure time, and initial content of aflatoxin were investigated. Moreover, effects of the adsorbents on the properties of beer were studied. The non-calcined form of Al-Mg LDH was not able to adsorb aflatoxins B_1 and G_1 considerably. On the opposite, its calcined form exhibited more than 90% adsorption just in less than one hour in solutions and in less than 4 hours in the beer samples. The reason is pointed to the fact that calcination of Al-Mg LDH considerably enhanced characteristics of the adsorbent. It almost doubled surface area of the material, increased its total pore volume over 54%, and added to its pore size over 35%.

Keywords: Adsorption; Al-Mg LDH; Co-precipitation; FTIR; Surface area

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1. Introduction

Aflatoxins are a group of secondary fungal metabolites belonging to mycotoxins. They are produced by fungi of the *Aspergillus* genus, particularly *A. flavus*, *A. parasiticus* and *A. nomius*. They develop naturally in food products and cause a wide array of toxic effects in human and animals (Ismail et al., 2018). There are more than 20 types of aflatoxin molecules, although the most prominent are aflatoxins B_1, B_2, G_1, G_2, M_1. Aflatoxin B_1 (AFB_1) and the mixture of aflatoxins B, G and M are classified by the International Agency for Research on Cancer as group 1 carcinogens for their acute toxicity on liver and kidney in both human and animals (World Health Organization, 2002). AFB_1, as the most potent aflatoxin, is followed by AFG_1, AFB_2 and AFG_2 in order of toxicity. Not only their toxicity but also their stability under most conditions has induced significant health risks and financial loss.

The EU has set the strictest standards, which establishes that any product for direct human consumption cannot be marketed with a concentration of AFB_1 and total aflatoxins greater than 2 mg/kg and 4 mg/kg, respectively (European Standards, 2020). Likewise, US regulations have specified the maximum acceptable limit for aflatoxins at 20 mg/kg (Wu, 2006).

The basic structures of aflatoxins are difuran ring and coumarin, which mainly account for their toxicity (Heathcote et al., 1978). Different approaches have been tested to remove or degrade the aflatoxins in foods, and the most prominent of these methods can be categorized into physical, chemical, and biological methods. This is while the adsorption procedure is categorized as a practical solution for decontamination regarding problems corresponding to safety issues, possible loss in nutritional quality and limited efficacy and incurred costs.

The chemical and thermal stability of aflatoxins has resulted in testing many approaches to decrease the aflatoxin content in foods (Prandini et al. 2009; Carraro et al. 2014). The major part of the

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existing research works in the field of adsorption is on animal nutrition and in vitro aqueous experiments. In several studies where sequestering agents were evaluated in vivo, their ability to protect animals from the effects of dietary AFB_1 was established. Activated carbons and montmorillonite clays effectively bind AFB_1 in vitro (Galvano et al., 2001; Huwig, 2001).

Elimination of aflatoxins from animal feed by transforming them into bentonite and hydrated sodium calcium aluminum silicate (HSCAS) has been examined in the feed industry (Phillips et al., 1995). The binding affinity of aflatoxin to smectite minerals has been identified as one of the promising procedures to minimize the negative effects of aflatoxin in food (Phillips et al., 2008) and its safety for humans after ingestion (Wang et al., 2005; Afriyie-Gyawu et al., 2008). In a review paper, Vila-Donat et al. (2018) considered various adsorbents including aluminosilicate, bentonite, zeolite, diatomite, sepiolite, yeast cell wall, lactic acid bacteria, micronized fibers, activated carbon, organoaluminosilicates or modified clays, polymers such as cholestyramine, and divinylbenzene-styrene.

In a report by Jaynes (2012), cholestyramine, other anion exchange resins, chitosan, bentonite and layered double hydroxides (LDHs) were distinguished to be potential additives to bind fumonisins from water and from aqueous corn meal. According to a study by Hsu (2018), fumonisin B_1 (FB_1) adsorption by Al-Mg LDHs is primarily due to the electrostatic interactions between the anionic part of FB_1 and the positive charge sites on LDHs. Anion exchange of NO_3^- and CO_3^{2-} by FB_1 is recognized to be the governing adsorption mechanism. However, increase in the amount of anionic FB_1 by increasing pH of the solution cannot increase the adsorption capacity as the anionic FB_1 and OH- compete for adsorbing site.

Matusik and Deng (2020) synthesized the LDH materials by co-precipitation and examined their use for FB_1 removal. Characterization of LDH was performed by XRD, FTIR, and XPS. For obtaining insight into the removal mechanisms, chemical analysis was utilized. A higher adsorption capacity was attained with the Al-Mg LDH samples and the removal efficiency showed no difference between Cl and NO_3 intercalated LDH. At lower layer charges of Mg/Al, the adsorption capacity displayed improvement as a result of the lower content of bonded carbonates and the increase of non-polar sites which resulted in matching between the adsorption domains of LDH with FB_1.

In a review paper, Matusik (2021) described the advantages of using LDH for adsorption of aflatoxins. In addition, applications of LDH in adsorption processes, catalyze, electrochemistry, chemistry of polymers, biomedicine, and wastewater process were evaluated. The progressive trend of research works on this adsorbent was also mentioned. It was highlighted that use of suitable amounts of LDH in the adsorption process can result in lowering of the production costs.

Johnston et al. (2021) reviewed the advantages of utilizing LDH for detoxification of organic toxins from aqueous media. Its high capacity of ion exchange and ability to be adjusted by changing the metal components, anions, or the preparation procedure were highlighted. Moreover, effects of the structure and physicochemical characteristics of LDH on its adsorption capacity and its ability to detoxify organic pollutants from aqueous matrix were explored. Significance of properties of metal layers, inter-layer anions and material properties of LDH on its adsorption capacity were also enumerated.

Adsorption is known to be a surface characteristic. The material candidate for adsorption should have a large surface area, maximum number of active sites and high porosity. For adsorption, layered double hydroxides are promising layered materials due to their special characteristics including ease of synthesis, uniqueness of structure, uniform distribution of various metal cations in the brucite layer, their surface hydroxyl groups, intercalated anions with interlayer spaces, and high chemical and thermal stability. Layered double hydroxides have been applied essentially for omitting toxic metals, ions, organic dyes, and organic materials from water and atmosphere (Chaara et al., 2010; Yang et al., 2014; Pourfaraj et al., 2017; Peng et al., 2015), and for water remediation from industrial pollution (Zubair et al., 2017; Extremera, 2012), and for removing antibiotics from aqueous media (Abdel Moaty et al., 2019).

Taking into account the unique properties of LDH in material absorbance, it is opted for investigating adsorption of AFB_1 and AFG_1 in the containing solutions and in non-alcoholic beer in this study.

2. Material and Methods

The non-calcined and calcined aluminum-magnesium layered double hydroxides were examined for their ability to remove aflatoxins B_1 and G_1. They were produces using the co-precipitation method. The prepared adsorbents were tested in a series of preliminary experiments. After determining the optimum adsorbent, its characteristics were explored and its capacity in detoxifying the contaminated solution and beer matrix was investigated.

2.1. *Preparation of the layered double hydroxides*

The co-precipitation method was used for synthesizing the layered double hydroxides at room temperature. Along with vigorously stirring, a solution containing 0.75 M of $Mg(NO_3)_2.6H_2O$ and 0.25 M of $AlNO_3.9H_2O$ was added at a rate of 1.0 mL/min to a solution of NaOH (2 M) and Na_2CO_3 (0.2 M). The produced slurry was treated at 80℃ for 24 h and then centrifuged and washed with deionized water until pH of the supernatant stabilized at 7.0. Then, the obtained solids were dried at 80℃ for 24 h. Hereafter, the sample is called Al-Mg LDH.

2.2. *LDH calcination*

Calcination of Al-Mg LDH was conducted by heating for three hours in a furnace at 500℃. Before performing the analysis and using in adsorption experiments, both of the calcined and non-calcined LDHs were ground and passed through an 80-mesh sieve.

2.3. *Preliminary adsorption experiments*

Aiming at selecting a suitable LDH, Al-Mg LDH and its calcined type were considered in a series of initial tests. In these experiments, 0.1 g of the mentioned adsorbents was exposed to adsorb 15 ppb of AFB_1 and AFG_1, in separate tests, at the times of 0, 10, 20, 30, 60, 120, and 180 min. The batch experiment was utilized for the adsorption process. Each adsorbent was added to the centrifuge tubes containing solutions of the considered aflatoxin. The dispersions were shaken with constant agitation at the room temperature in a vortex for the mentioned prescribed times. After centrifugation, the supernatants were separated and the amounts of

AFB_1 and AFG_1 were measured at each time in their respective experiments by HPLC-FLD, as explained in Sec. 2.9. The calcined Al-Mg LDH was picked up as the superior adsorbent in terms of effectiveness and efficiency for this study. It is explained further in the Results section. For this reason, rest of the study proceeded by taking up the Al-Mg LDH adsorbent.

2.4. *Characterization of Al-Mg LDH*

For gaining the Fourier transform infrared (FT-IR) spectra of Al-Mg LDH and its calcined type, a Tensor 27 Bruker (Bruker Optics Ltd., Coventry, UK) was utilized in transmission mode from 400 to 4000 cm^{-1}. The pellets were prepared by admixture of 1 mg of LDH and 100 mg of anhydrous KBr and carefully homogenizing the mixture in an agate mortar.

The crystalline structures of the dried Al-Mg LDH and its calcined type were studied using X-ray diffraction (XRD) by a Phillips/PANalytical X´pert Pro-MPD powder. The diffractometer was operated at 40 kV and 40 mA utilizing CuKα radiation source and SolX energy dispersive detector with a step size of 0.05° 2θ and a dwell time of 5 s per step at 1.54 angstrom.

Before and after adsorption of AFB_1 and AFG_1, morphologies of the calcined Al-Mg LDH were assessed by scanning electron microscopy (SEM). The thoroughly dried samples were coated by a thin gold layer being 10 nm in thickness, and then were captured with an electronic microscope (Philips XL 30, Eindhoven, the Netherlands). The magnification of SEM images was 125× and 500×.

2.5. *Distribution of particle sizes*

Measurement of particle sizes was conducted by employing the method of static scattering of laser light (SLS) based on the light pattern scattered on the detector. This pattern is a set of scattering patterns produced by each particle. This method is applicable for particles larger than 1 μm. It was performed in this study by the Mastersizer 2000/Malvern panalytical technologies/United Kingdom apparatus. Because of the nature of the sample and impossibility of making a suspension, sample particles were scattered in the air in powder form and were measured.

2.6. *Adsorption of AFB_1 and AFG_1 in solutions by the calcined Al-Mg LDH*

AFB_1 and AFG_1 (Supelco/Sigma-Aldrich) were utilized separately as the solution contaminants. Solutions of 5, 10, 15, and 50 ppb AFB_1, and similarly of AFG_1, were respectively exposed to 0.01, 0.1, 0.5 and 1g of the calcined Al-Mg LDH in different time durations, being 10, 20, 30, 60, 120, 180, and 1440 min. The batch experiments were used for the aflatoxin adsorption processes. In each run, the specific amount of Al-Mg LDH was added to 15ml centrifuge tubes containing solutions of the mentioned amounts of each aflatoxin. The adsorption experiments were conducted with constant agitation at the room temperature in a vortex. The parameters of adsorption considered in this work included the initial concentration of the aflatoxin, the amount of adsorbent, and the exposure time. The adsorbent was separated using centrifugation after adsorption. The supernatant was subsequently used for analysis using HPLC as described in Sec. 2.9.

The removal efficiency (*RE*) or the adsorption rate of each of the considered aflatoxins was calculated using Eq. (1):

$$RE(\%) = \frac{C_0 - C}{C_0} \times 100 \qquad (1)$$

where $RE(\%)$ represents the removal percentage of the aflatoxin, and C_0 and C are the concentration of aflatoxin before and after adsorption, respectively.

2.7. *Adsorption of AFB_1 and AFG_1 in non-alcoholic beer samples*

The calcined Al-Mg LDH was also utilized to determine the adsorption level of AFB_1 and AFG_1 in non-alcoholic beer samples. The beer samples were spiked with each mentioned aflatoxin at 10 and 20 ppb. These values of concentration can be compared against the allowable limit of 5 ppb in the country of research (ISIRI, 2011). Adsorbent amounts of 0.5 and 1g were added separately to 30 mL of the contaminated beer. The dispersions were shaken for 1, 2, 3, and 24 h. After centrifugation, the upper part of the beer samples was separated and analyzed. The aflatoxin amounts were determined using HPLC equipped with fluorescence detector (HPLC–FLD) as explained afterwards in Sec. 2.9.

2.8. *Qualitative characteristics of the beer samples*

The values of the initial sugar, total sugar, saccharose, brix, dry matter, and total ash were measured using Lactostar, Funke Gerber model. For contaminating the beer samples, 0.05, 0.1, 0.2, 0.4 and 0.8 ppb of the aflatoxins was added in successive tests as the allowable limit for AFB_1 and AFG_1 in the country of research is 5 ppb (ISIRI, 2011). In addition, two different amounts of the adsorbent equal to 0.5 and 1g were considered to assess the rate of adsorption. Exposure was allowed to last 24 h and the beer was separated afterwards by centrifugation to study its properties.

2.9. *Quantification of the aflatoxins by HPLC*

The ISIRI (2011) procedure was followed to measure the aflatoxin content of the beer samples by the HPLC-fluorescence detector. The beer acquired after centrifugation and separation of LDH by taking the upper part and applying an extra filtration, was utilized for analysis. First, 20 mL of phosphate-buffered saline (PBS) and then 20 mL of beer was passed through an immunoaffinity column for clean-up and purification. Immunoaffinity columns were purchased from Vicam Company (Watertown, MA, USA). The pH of the added PBS was 7.4. The aflatoxin was eluted with 2500 μL of acetonitrile, followed by 0.4 mL of ultra-pure water. The resulting solution was completely dried under a gentle stream of N_2. The residue was dissolved using 1 mL of the mobile phase (deionized water, acetonitrile, and methanol [60:20:20, V/V]). For each detection, 100μL of this solution was injected into the HPLC system.

HPLC (Dionex model 1100, San Diego, U.S.A.), accommodated with a C18 ODS2 column (reversed phase) and a fluorescence detector, was utilized to determine the quantity of aflatoxin. The excitation and emission wave lengths of the fluorescence detector were set at 360 and 440 nm, respectively. Five standard solutions of each aflatoxin (0.05, 0.1, 0.25, 0.5, and 0.75 ppb) were injected into the HPLC to attain a standard calibration curve. Then, the samples were passed through a 46 microns filter one by one and a volume of 100 μL of each sample

was injected into the HPLC. To measure the quantity of each toxin, the peak areas were computed and compared with the related standard calibration curve. Limit of detection and limit of quantification were respectively 0.01 and 0.03 ppb. The retention time was about 7 min and recovery percentages were above 98%.

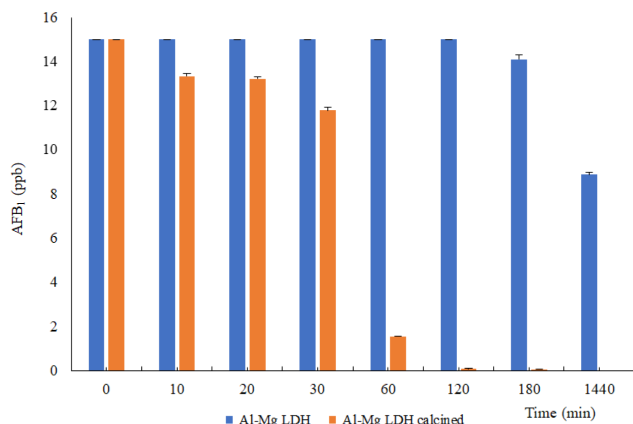

Fig. 1. Adsorption of an initial amount of 15 ppb of AFB_1 by 0.1g of Al-Mg LDH and its calcined type.

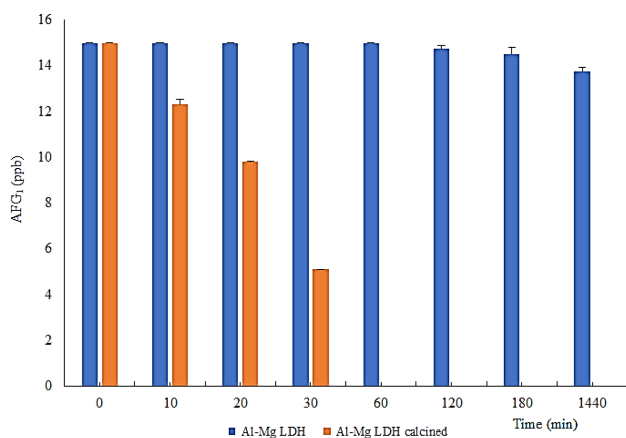

Fig. 2. Adsorption of an initial amount of 15 ppb of AFG_1 by 0.1g of Al-Mg LDH and its calcined type.

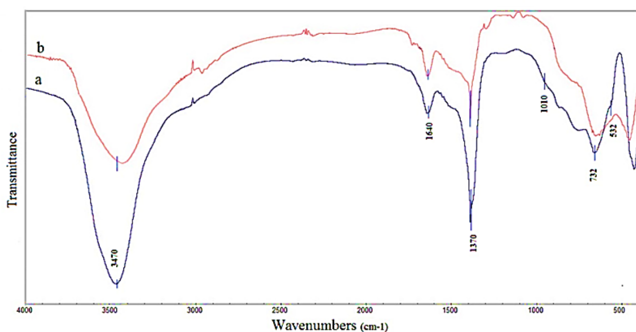

Fig. 3. FT-IR spectra of: (a) Al-Mg LDH, (b) calcined Al-Mg LDH.

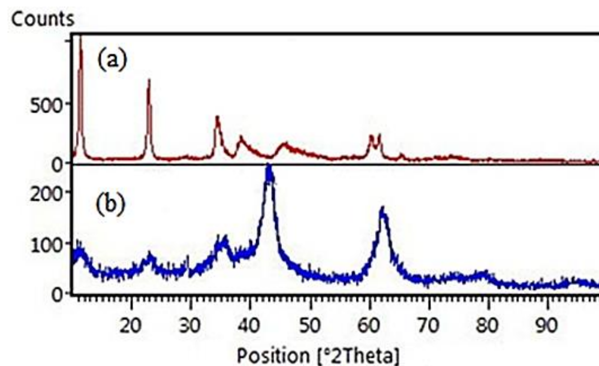

Fig. 4. XRD patterns: a) non-calcined Al-Mg LDH, b) calcined Al-Mg LDH.

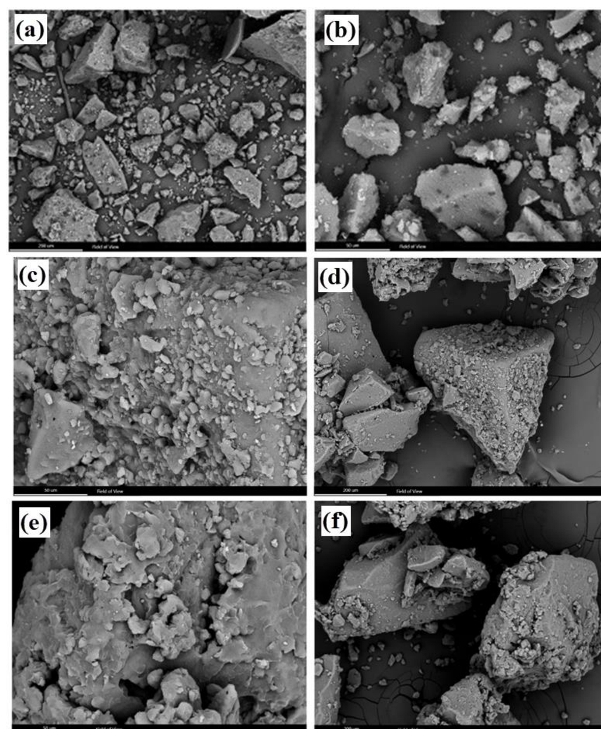

Fig. 5. SEM images of: (a) and (b) calcined Al-Mg LDH before aflatoxin adsorption; (c) and (d) calcined Al-Mg LDH after AFB_1 adsorption; (e) and (f) calcined Al-Mg LDH after AFG_1 adsorption. Figures (a), (c) and (e) are in 50µm view with 500× magnification and Figures (b), (d) and (f) are in 200 µm field of view and 125× magnification. The detector mode is SE.

3. Results

3.1. Preliminary tests

For selecting the appropriate adsorbent, two different adsorbents including calcined and non-calcined types of Al-Mg LDH were examined. Value of the adsorbent was taken to be 0.1 g in all of the experiments of this section, where 15 ppb of aflatoxin was used unanimously. Variation of aflatoxin content with time was determined.

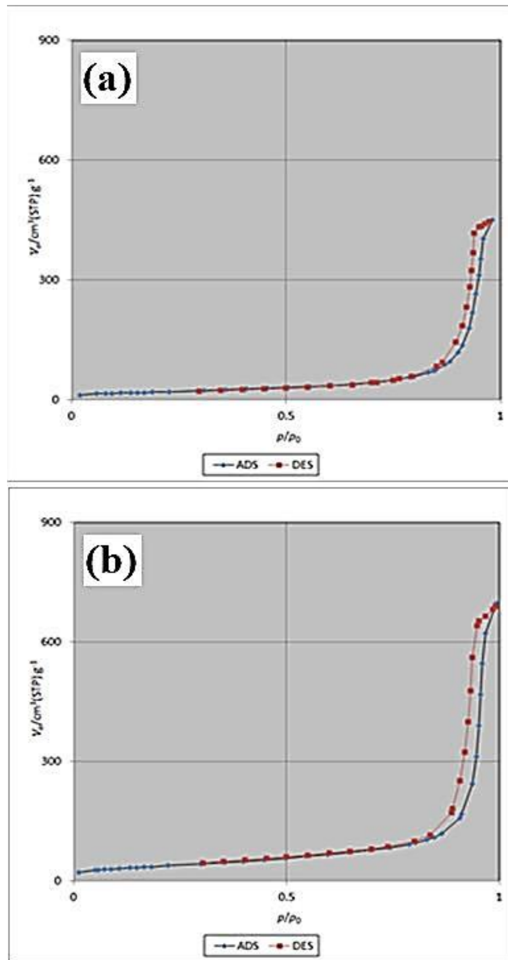

Fig. 6. Adsorption/desorption isotherms of: (a) Al-Mg LDH, and (b) calcined Al-Mg LDH.

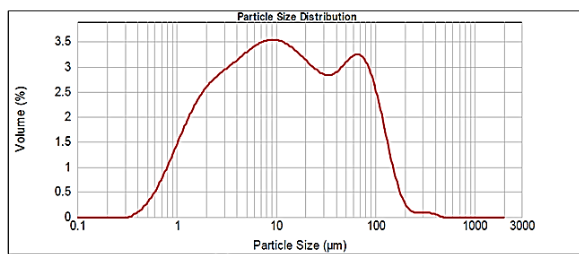

Fig. 7. Results of the particle size distribution experiments.

3.1.1. Adsorption of AFB_1

As shown in Fig. 1, the non-calcined type of Al-Mg LDH had adsorbed only 6% of AFB_1 in the first 3 hours. After lapsing 24 h, the *RE* was observed to be only 41% in the non-calcined Al-Mg LDH sample. These results clearly show that before calcination, the considered layered double hydroxide did not have the required ability to adsorb the aflatoxins even after an extended time of exposure.

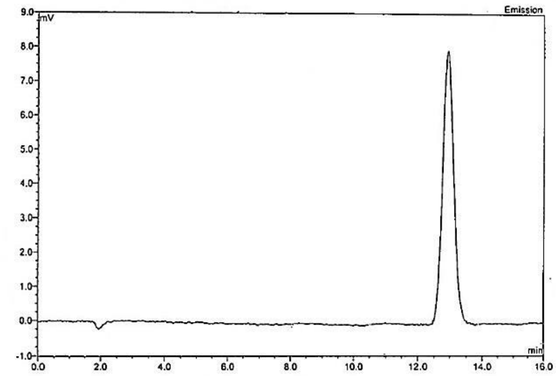

Fig. 8. Sample chromatogram in solution for AFB_1.

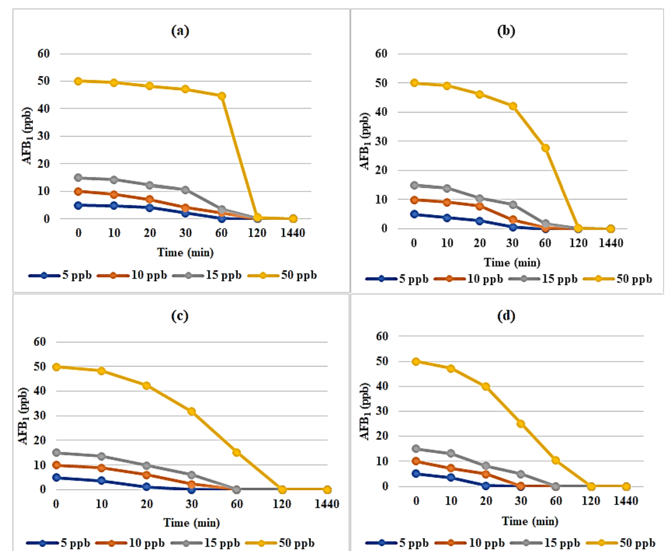

Fig. 9. Variation of AFB_1 remained at different exposure times according to the initial value of AFB_1 with the quantity of the calcined Al-Mg LDH equal to: (a) 0.01 g, (b) 0.1 g, (c) 0.5 g, (d) 1 g.

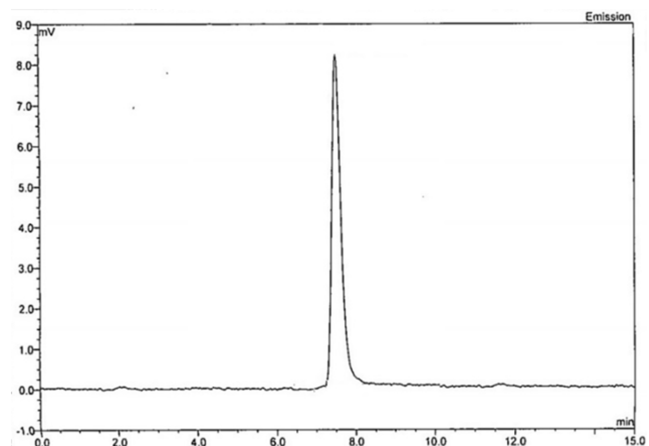

Fig. 10. Sample chromatogram in solution for AFG_1.

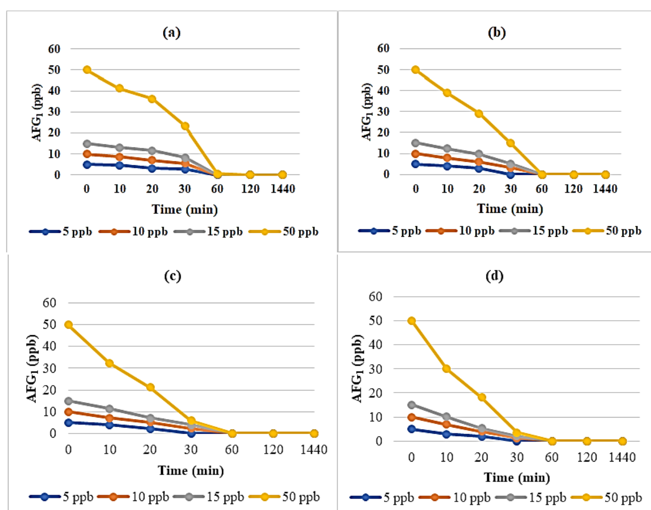

Fig. 11. Variation of AFG_1 remained at different exposure times according to the initial value of AFG_1 with the quantity of the calcined Al-Mg LDH equal to: (a) 0.01 g, (b) 0.1 g, (c) 0.5 g, (d) 1 g.

When using the calcined adsorbent, results of adsorption showed that the calcined Al-Mg LDH adsorbed about 90% of 15 ppb of AFB_1, being equivalent to 3 times the allowable quantity, in less than one hour and about 100% in 3 hours of contact. Results of this test confirmed the clear superiority of the calcined Al-Mg LDH over its non-calcined type as the preferable adsorbent for this study.

3.1.2. *Adsorption of AFG_1*

Results of the preliminary tests of AFG_1 adsorption are shown in Fig. 2. It is seen that after 3 h of exposure, the non-calcined Al-Mg LDH adsorbent had adsorbed only 3.2% of AFG_1. Nonetheless, after 24 h of exposure, the *RE* was only 8.25% in the Al-Mg LDH sample. Use of the calcined form of the adsorbent is observed to make a large difference in the adsorption process for this aflatoxin too. In Fig. 2 it is observed that the calcined Al-Mg LDH exhibited a full adsorption in less than one hour. The results of this test are another confirming reason for selecting the calcined Al-Mg LDH as the preferred adsorbent in this study over its non-calcined type.

3.2. *Characterization of Al-Mg LDHs*

The FT-IR spectra of Al-Mg LDH prior to and after calcination are shown in Fig. 3.

As seen in Fig. 3, all bands have displayed comparable features. The strong and broad bands in the range of 3474–3525 cm^{-1} are referred to extension of O-H groups and distortion vibration mode of the hydroxide basal layer or the interlayer molecule (Aisawa et al., 2002). The shoulder around 1627–1641 cm^{-1} can be related to the deformation mode of interlayer water molecules (Kang et al., 2013). The extensive adsorption around 1363–1377 cm^{-1} corresponds to the antisymmetric ν3 mode of the interlayer carbonate anions, revealing the fact that the LDH phase consists of some CO_3^{2-} (Xu & Lu, 2005). It is also witnessed that the bands at 3474–3525 cm^{-1} and 1363–1377 cm^{-1} in the calcined LDH have become smaller due to loss of H_2O and CO_2, respectively, in the calcined type.

The band at 1010–1012 cm^{-1} may be related to the distortion mode of Al-OH (Kloprogge & Frost, 1999). In addition, the bands at 732 and 532 cm^{-1} originated from the translation modes of the hydroxyl groups were essentially influenced by the trivalent aluminum (Kloprogge & Frost, 1999). The band at 642–663 cm^{-1} can be ascribed to the M-OH extension and M-O-M' flexural oscillation due to the diverse cations in the hydrotalcite layer (Choudary et al., 2002; Ni et al., 2008).

Crystal assembly of Al-Mg LDH precursor powders is distinguished by the XRD analysis. Fig. 4 illustrates the XRD patterns of the Al-Mg LDH precursors before calcination. All patterns exhibit peaks around 2θ = 11, 23, 34, and 60°, corresponding to (003), (006), (012), (110), and (1013) reflections of non-calcined LDH. In addition, the patterns also reveal that the sharp and strong intensities of the peaks can be associated with the good crystallinity of LDHs.

The results display the fact that the temperature of 500℃ is the most appropriate choice for calcination of the catalysts to gain calcined LDH because of loss of crystal water and CO_2 released from CO_3^{2-}. Such losses can add to the surface area of the existing LDH material and make it to become an efficient adsorbent. After calcination, the LDH samples exhibit peaks having the low property of the poor crystalline hydrotalcite phase.

SEM images obtained for the LDH prior to and after aflatoxin adsorption are displayed in Fig. 5.

The calcined Al-Mg LDH precursor shows an irregular surface with low porosity where few aggregates are observed (Figs. 5a,b) while it presents a high porous surface after AFB_1 and AFG_1 adsorption (Figs. 5c-f). The latter images display aggregates on surface due to the presence of adsorbed species.

Results of the surface area and pore size analysis are illustrated in Fig. 6.

Absorption-desorption isotherms confirm the fact that Al-Mg LDH and its calcined type display a type IV isotherm and H3 hysteresis loop. This is a common sign for plate-like particles and slit-shaped pores, based on the IUPAC categorization. Calcination of Al-Mg LDH has resulted in augmentation of the surface area from 69.367 to 136.04 m^2g^{-1} and the total pore from 0.69 to 1.0647 cm^3g^{-1}. The pore size varied from 16.29 nm to 22.07 nm after calcination. Increase of the surface area and pore size provides for a much stronger adsorption capacity. This is consistent with the witnessed adsorption capacity of the calcined Al-Mg LDH in comparison with the non-calcined adsorbent.

Results of the particle size distribution tests are shown in Fig. 7.

This figure shows that about 90% of particles possess sizes less than 82 microns and 50% of particles are smaller than 11 μm, while only 10% of particles are sized less than 1.6 microns.

3.3. *Adsorption from solution*

3.3.1. *AFB_1*

Referring to the explanation given in Sec. 2.6, a sample chromatogram of AFB_1 in solution is shown in Fig. 8.

In this phase of the research, performance of the calcined Al-Mg LDH as the selected adsorbent and effect of the parameters including the initial amount of aflatoxin, value of the utilized adsorbent, and the exposure time were explored. Fig. 9 displays how adsorption of AFB_1 changes during the exposure time as a

function of the initial amount of aflatoxin and value of the adsorbent.

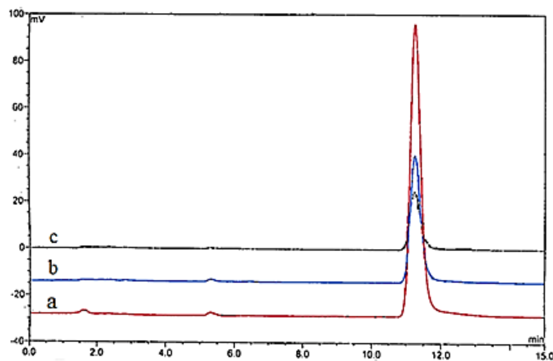

Fig. 12. Sample chromatograms of beer: (a) After spiking with AFB_1, (b) after detoxification with 0.5 g adsorbent, (c) after detoxification with 1 g adsorbent.

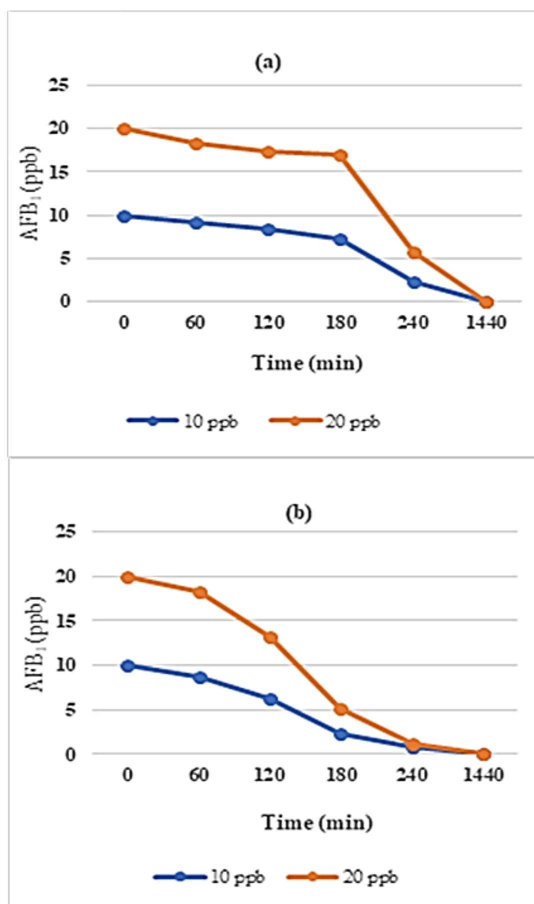

Fig. 13. AFB_1 remained in beer at different times against the initial amount of AFB_1 and value of the calcined Al-Mg LDH equal to: (a) 0.5 g, (b) 1 g.

As shown in Fig. 9, use of various amounts of the adsorbent from 0.01 to 1 g in solutions containing 5 to 50 ppb of AFB_1 has been evaluated. When 0.01 g of the adsorbent is utilized, full adsorption happens in less than two hours for the solutions containing 5 and 10 ppb of AFB_1, while for higher amounts of aflatoxin about 99% adsorption takes place in the same time. The maximum required time for complete adsorption with 0.01 g of the adsorbent and the highest amount of aflatoxin was less than 24 h.

When amount of the adsorbent was increased to 0.1 g, the removal efficiency (*RE*) was enhanced and more than 99.6% of the highest concentration of AFB_1, being 50 ppb, was adsorbed in less than two hours. In the same time, when using 0.5 g of the adsorbent, for the values of aflatoxin being 5, 10, and 15 ppb, full adsorption and for 50 ppb of AFB1, 99.8% adsorption was observed. When the adsorbent amount was increased to 1 g, the adsorption was completed in less than two hours for every amount of aflatoxin.

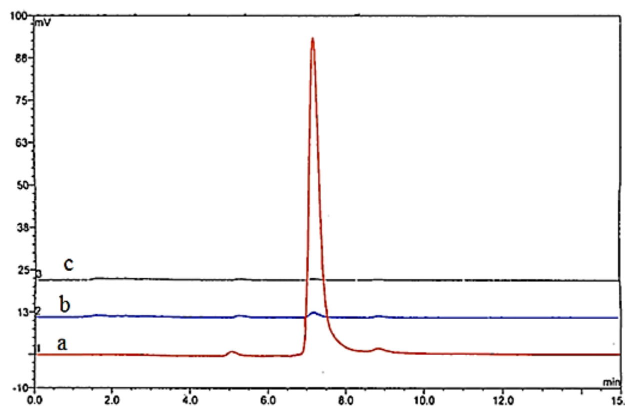

Fig. 14. Sample chromatograms of beer: (a) After spiking with AFG_1, (b) after detoxification with 0.5 g adsorbent, (c) after detoxification with 1 g adsorbent.

3.3.2. *AFG_1*

Referring to Sec. 2.6, a sample chromatogram of AFG_1 in solution is shown in Fig. 10.

Fig. 11 shows variation of adsorption level of AFG_1 in different exposure times corresponding to the initial value of aflatoxin and the adsorbent amount.

As seen in Fig. 11, use of 0.01 to 1 g of the adsorbent in solutions containing 5 to 50 ppb of AFG_1 has been explored. In the case of using 0.01 g of the adsorbent, for the solutions containing 5, 10, and 15 ppb of AFG_1 full adsorption occurs in less than one hour, while for the higher amount of aflatoxin about 98.8% adsorption happens in the same time. The adsorption process is completed in any case in less than 2 h. When the quantity of adsorbent is increased to 0.1 g and higher, adsorption is completed for all of the studied amounts of AFG_1 in less than 1 h.

3.4. *Adsorption from non-alcoholic beer*

3.4.1. *AFB_1*

Referring to the explanation given in Sec. 2.7, sample chromatograms of beer before and after spiking and after aflatoxin adsorption are shown in Fig. 12.

Amounts of the adsorbent equal to 0.5 and 1 g were utilized for examining adsorption of AFB_1 from beer. Moreover, values of the initial aflatoxin quantity were taken to be 10 and 20 ppb.

Fig. 13 displays change of the remained AFB_1 at different times of exposure in beer after its treating with various amounts of the calcined Al-Mg LDH and the initial quantity of the aflatoxin.

As displayed in Fig. 13, when using 0.5 g of the adsorbent, the adsorption process proceeded quite slowly in the initial three hours. Afterwards, the adsorption rate increased considerably. This is while when the adsorbent amount was doubled and increased to 1 g, the adsorption rate was high enough from the beginning but it increased with time and reached to its highest value after lapse of two hours.

When the adsorbent amount was equal to 0.5 g, after 4 hours the adsorption amount was 77 and 72% for the aflatoxin values of 10 and 20 ppb, respectively. The adsorption was completed in longer enough times afterwards. The results show that use of 1 g adsorbent against 10 and 20 ppb of AFB_1 has resulted in increase of the adsorption rate and 92 and 94% adsorption has taken place in 4 hours for the mentioned values of aflatoxin, respectively. Letting the exposure time to last longer enough, has resulted in full adsorption for the same cases.

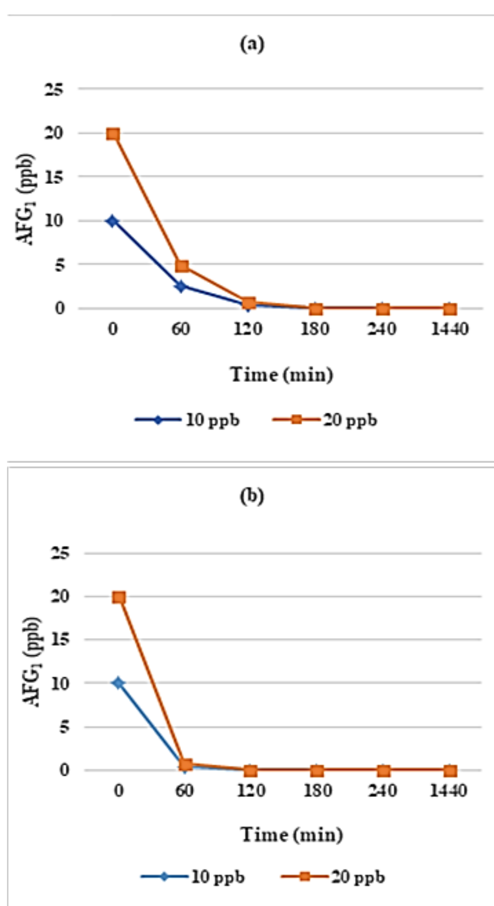

Fig. 15. AFG_1 remained in beer at different exposure times against the initial amount of AFG_1 and value of the calcined Al-Mg LDH equal to: (a) 0.5 g, (b) 1 g.

3.4.2. AFG_1

Referring to Sec. 2.7, sample chromatograms of beer before and after spiking and after aflatoxin adsorption are shown in Fig. 14.

Adsorbent quantities equal to 0.5 and 1 g were used for exploring AFG_1 adsorption from beer. The initial values of aflatoxin were selected to be 10 and 20 ppb.

Fig. 15 exhibits variation of the remained AFG_1 at various exposure times in beer under different amounts of the initial quantity of AFG_1 and various values of the calcined Al-Mg LDH.

Fig. 15 shows that for the case of using 0.5 g of the adsorbent, the remained amount of AFG_1 decreased quickly in the first hour and its adsorption rate reached the value of 96% in 2 h for both of the aflatoxin amounts of 10 and 20 ppb, and to 100% adsorption in later times. In comparison, when doubling the adsorbent amount and increasing its value to 1 g, the adsorption process was fast from the beginning and the adsorption rate reached to more than 95% in just one hour. In this case, for 10 and 20 ppb of AFG_1, the adsorption rate reached to 96.3% and 95.9%, respectively. In the same case, full adsorption was witnessed to happen in 2 h unanimously.

3.5. Evaluation of the beer characteristics after being exposed to the calcined Al-Mg LDH

Quality of beer was evaluated after being exposed to the calcined Al-Mg LDH for 24 h. Values of initial sugar, total sugar, saccharose, brix, dry matter, and total ash were determined and none of them was found to vary considerably in the presence of AFB_1 or AFG_1. Table 1 lists the results of the quality tests of beer.

4. Discussion

Reduction of AFB_1 and AFG_1 from contaminated solutions and beer by using Al-Mg LDH adsorbent was investigated. It was revealed that desired adsorption by the non-calcined type of the mentioned LDH was not possible as it was only 41% at maximum for AFB_1. For adsorption of AFG_1, the value was 8.25%. This is while the calcined type of Al-Mg LDH proceeded in rational time duration with complete adsorption of the toxins in the solution and beer by using appropriate amounts of the adsorbent. This shows a significant difference in adsorption capacity of the calcined and non-calcined types of the adsorbent.

Actually, the adsorption mechanism of various toxic contaminants in LDH-containing hybrids basically depends on the type of the hybridizing material and pollutant. In general, physical adsorption, hydroxide precipitation, anion-metal complexes, electrostatic interaction, pi-pi interactions and chemical bonding are the common mechanisms involved in the adsorption process of LDH containing hybrids (Zubair et al., 2017). As mentioned by Faraji et al. (2021), possible interactions of LDHs include ion-exchange mechanism, Van Der Waals linkages, and hydrogen bonding. In the case of AFB_1 and AFG_1, Van Der Waals linkages and hydrogen bonding seem to be the prevailing mechanisms.

Possible mechanisms of aflatoxin adsorption have been investigated in various research works. The aflatoxin molecule is a difurano coumarin that is considered to be an almost non-polar molecule.

Table 1. Results of the quality tests of beer in presence of AFB_1 and AFG_1 before and after exposure to the calcined Al-Mg LDH, displaying mean values of each parameter.

Sample	Initial sugar (%)	Total sugar (%)	Saccharose (%)	Brix	Dry matter (%)	Total ash (%)
Beer	1.629	3.090	1.340	4.840	5.206	0.104
Beer + 0.5 g calcined Al-Mg LDH	1.635	3.110	1.320	4.890	5.213	0.111
Beer + 0.5 g calcined Al-Mg LDH + 10 ppb B_1	1.633	3.080	1.330	4.870	5.233	0.108
Beer + 0.5 g calcined Al-Mg LDH + 10 ppb G_1	1.625	3.060	1.330	4.850	5.207	0.106

In many papers, ability of binding of aflatoxin to clayey adsorbents in the form of surface adsorption and reaction with calcium in the interlayer zone has been recognized to be essentially responsible for adsorption (Dakovic et al., 2008). In the mentioned study, a number of models has been also presented for describing the aflatoxin adsorption mechanism.

The first model, known as electron giver-taker, explains that carbonil groups of aflatoxin molecule having a relatively positive charge based on electron sharing are adsorbed to the negative charge surface of smectite.

In relation to the second model, which is called the selective chemical adsorption, Philips et al. (1995) state that tensile bands of aflatoxin carbonil disappear between 1700 and 1750 cm^{-1} when reacting with the novasil bentonite and two new peaks are formed between 1400 and 1600 cm^{-1}. For interpreting this phenomenon, they distinguished the fact that variation and transformation of tensile bands was due to collation of two groups of aflatoxin carbonil with metals having empty d-orbitals. It is very interesting that disappearance of the mentioned tensile bands has also occurred identically in the current research, as observed in Sec. 3.2 in FT-IR studies (Fig. 3) that is probably due to a similar reaction.

The third model is the hydrogenic binding and its formation through the furan ring. In this model, development of two hydrogenic bindings between aflatoxin and edges of montmorillonite is recognized to be the main mechanism of the chemical adsorption. For describing the adsorption mechanism of the current research, such a model is very much probable because possibility of binding of the oxygen molecules of the coumarin part to OH in LDH is high. Moreover, in this model, epoxidation of furan while adsorbing aflatoxin is known to be responsible for adsorption of the toxin on smectite (Abdel Moaty et al., 2019; Hsu, 2018).

Other possible models for binding are based on similar organic materials containing oxygen. Accordingly, carbonil groups in asters, amids, ketons, and aldeids, are known to be adsorbed through bipolar-ionic reactions as well as reaction between transferrable ions and carbonil groups with clay minerals (Jaynes, 2012; Matusik & Deng, 2020). In general, at high moisture hydrogenic binding between carbonil oxygens and the hydrated shell is possible. This is while binding in the form of bipolar-ionic reactions or transfer of cation with carbonil oxygen occurs at dry conditions.

The adsorption has been known also to be because of the carbonil group and the cations existing between the layers (Dakovic et al., 2012). Deng et al. (2010) explained a multiple-process mechanism including bonding between two groups of carbonils of aflatoxin containing two carbons having positive charges to the surface of adsorbent being negative in charge. They mentioned also possibility of formation of hydrogenic bands between carbonil aflatoxin groups and the hydrated shell of the adsorbents. In addition, co-ordinance between the exchangeable ions and the two oxygens existing in the coumarin ring or the carbonil groups through bi-polar ionic bonds or co-ordinance with ions have been explained as possible mechanisms. According to Nones et al. (2017), development of collated complexes of the zinc ions existing in the adsorbents with the aflatoxins is possible. On the other hand, Vila-Donat et al. (2018) discussed the possibility of surface adsorption at surface or at the interlayer space and also collation or reaction of aflatoxin with the middle layer cations, especially those of calcium. Effectiveness of di-carbil system in this regard and feasibility of bonding with the zinc cation substituted by calcium has been mentioned by Zhao et al. (2018).

In general, in dry condition, bonding of aflatoxin can be due to bi-polar ionic reactions and co-ordinance between ions exchangeable with the carbonil group. In wet condition, the aflatoxin molecule bonds to the adsorbent by hydrogenic connections between oxygens of the carbonil group and moisture of the surface layer. Considering the structure of layered double hydroxides, it seems that surface adsorption and reaction between the existing cations and the carbonil groups of the aflatoxins is the prevailing adsorption mechanism. Hydrogenic connection can also be effective in this regard.

Results of the surface area and pore size analysis in this research revealed the important fact that calcination of Al-Mg LDH almost doubled the surface area of the material, augmented its total pore volume over 54%, and increased its pore size over 35%. Enhancement of the mentioned characteristics should be a clear reason for the fact that the calcined Al-Mg LDH benefits from a much larger adsorption capacity than the non-calcined Al-Mg LDH.

Another important feature of Al-Mg LDH is the fact that no considerable reduction of the beer nutrients was observed even after 24 h of exposure to various quantities of the adsorbent. No inclination was witnessed for adsorption of the nutrients including the initial sugar, total sugar, saccharose, brix, dry matter, and total ash.

5. Conclusion

In this study ability of LDH for adsorbing AFB_1 and AFG_1 was investigated. During the course of the study, the Al-Mg LDH adsorbent was examined in its non-calcined and calcined forms. The XRD, FTIR, SEM, and BET methods were used to identify the characteristics of the studied adsorbents. Isothermal adsorption was also performed to identify variation of the surface area and pore size of the adsorbent in its non-calcined and calcined forms. Findings of the study can be summarized as follows: 1) For 10 ppb and larger amounts of AFB_1 in the solution, the adsorption level reached to about 99% percent in 2 hours and to 100% in longer times. For AFG_1 the process was much faster. With the same amount of the adsorbent, as much as 50 ppb of the aflatoxin was

fully adsorbed in less than 2 h. 2) By increasing the amount of the calcined Al-Mg LDH to 0.1 g, 0.5 g, and then 1 g, it was possible to complete the adsorption process of AFB_1 and AFG_1 in less than 2 h and one hour in solution, respectively. It was true even for aflatoxin contents as large as 50 ppb. 3) In the matrix of beer, adsorption was generally slower. The adsorption process proceeded slowly in the first three hours and then accelerated considerably. At 4 h, 77 and 72% of AFB_1 was adsorbed in beer samples containing 10 and 20 ppb of AFB_1, respectively. For AFG_1, the process was much faster as at least 96% of the aflatoxin was adsorbed for all the cases considered in less than 2 h. 4) When using a larger amount of 1 g of the calcined Al-Mg LDH in beer samples containing 10 and 20 ppb of aflatoxin, the adsorption level reached to higher values. It was 94 and 92% respectively for AFB_1 in 4 hours and 96.3 and 95.9%, respectively, for AFG_1 in one hour. 5) Amounts of the beer nutrients were determined before and after exposure to the calcined Al-Mg LDH for 24 h. None of the nutrients was found to change considerably. 6) Determination of the absorption-desorption isotherms showed that calcination of Al-Mg LDH considerably enhanced characteristics of the adsorbent. It doubled the surface area, increased the total pore volume over 54%, and added to the pore size of the adsorbent over 35%. It was revealed to be the reason why aflatoxin adsorption was quicker and higher by the calcined form of the Al-Mg LDH. Summing up, this research work profoundly established the calcined Al-Mg LDH as a very effective and efficient adsorbent for adsorption of AFB_1 and AFG_1 from beer. Significantly, it prepared the way for exploring other LDH compounds for adsorption of various aflatoxin materials from food products, in future research works.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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