Research paper

Clay minerals as adsorbents of aflatoxin M₁ from contaminated milk and effects on milk quality

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ABSTRACT

The capacity of clays to remove or attenuate the contamination of aflatoxin M₁, in bovine milk was studied, while considering the effects of these clays on the nutritional properties of the milk (in terms of protein, fat and lactose). To make the experimental results transferable to practical use, only clays available from the market were tested, and a very simple clay–milk interaction procedure was used. A preliminary test was developed to compare the adsorption behaviour of two clays, a kaolin and a bentonite, at variable clay–milk ratios and to trace the variations in the protein, fat and lactose contents. Then, only bentonites were selected for additional study. The detoxification capacity of the bentonites used was variable but still very efficient: contaminated bovine milk (up to approximately 80 ng/L) was purified to safe levels (50 ng/L for adults and 25 ng/L for lactants) with moderate alteration of the nutritional properties of the milk. Moreover, the amount of bentonite residue in the purified milk was very low (0.4%). The kaolin was less adsorptive than the bentonite but still able to decrease the aflatoxin M₁ within legal limits and was better at maintaining the nutritional properties of the milk. The protein is more sensitive to clay adsorption than are fat and lactose, and there was a decrease in protein in the treated milk with increases in the ratio of clay to milk. Among the studied samples, a saponite-rich bentonite showed the highest adsorbent capacity, in agreement with theoretical considerations regarding higher cell surface areas (available for AFM₁) and higher surface hydrophobicity of saponite. Analyses of isothermal adsorption in water using the two more effective bentonites were also performed. The data collected indicate an effective and safe use of clays in the detoxification of milk (and dairy products) contaminated with aflatoxin M₁.

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1. Introduction

Aflatoxins are secondary metabolites of moulds and are the strongest animal carcinogen (Squire, 1981). Aflatoxin B₁ is the most toxic to humans and animals. Its metabolite aflatoxin M₁ appears in milk and milk products as a direct result of the animal ingesting feed contaminated with aflatoxin B₁ (EFSA, 2004). The social–environmental concerns regarding aflatoxin began after the first large-scale case of animal contamination (Blount, 1961), and it was clear that a substantial amount of food was affected (Rustom, 1997). The chemical and thermal stability of aflatoxins has further increased the concern and led to testing many methods to reduce the aflatoxin content in foods (Prandini et al., 2009). One of the most promising approaches to minimise the adverse effect of aflatoxin in food is to exploit the binding affinity of aflatoxin to smectite minerals (Phillips et al., 2008 and references therein). Ample literature is available, primarily in the field of animal nutrition and in vitro aqueous experiments (see the reviews by Dixon et al., 2008; Jaynes and Zartman, 2011; Phillips et al., 2008 and references therein). A further step was the use of smectite in human nutrition; in particular, the effectiveness of a particular smectite as an agent for sequestering aflatoxin was found (Afriyie-Gyawu et al., 2008a; Phillips et al., 2008; Wang et al., 2008), and its safety for humans after ingestion was studied (Afriyie-Gyawu et al., 2008b, Wang et al., 2005).

The experimental data indicating the efficiency of smectite as a detoxification agent are also supported by theoretical models of aflatoxin B₁ adsorption (Deng and Szczesna, 2011; Deng et al., 2010, 2012; Phillips et al., 1995). Nevertheless, the development of a model to predict the adsorption of aflatoxin B₁ from smectites is further complicated by the crystal-chemical variability within the smectite group (Mulder et al., 2008; Tenorio Arvide et al., 2008). Several factors that affect the adsorption maximum have been identified: the amount of smectite in the clay; cation exchange capacity; hydrated radius of the interlayer cation; particle size distribution; specific surface area; FTIR evidence of...
Fe and/or Mg in the smectite structure; extreme pH; and organic carbon. None of these factors alone can be considered a reliable proxy of afatoxin B₁ adsorption. Experimental tests are the best approach, at least in the study of afatoxin adsorption in vitro. Additional conditions should be considered for the in vivo practice (Jaynes et al., 2007).

Most of the published studies concern afatoxin B₁ (produced by moulds). However, human exposure to afatoxin M₁ (a metabolite of B₁) is also very threatening, as it primarily occurs through the consumption of milk and other dairy products (IARC, 2002), representing a substantial risk, due to their importance as a foodstuff for adults and especially children (Anfossi et al., 2012; Prandini et al., 2009). Despite this concern, only a few studies have focused on the detoxification of milk contaminated with afatoxin M₁. Chemical treatments of contaminated milk, e.g., by acidification (Deveci, 2007; Higuera-Ciapara et al., 1995) and oxidation (Aman, 1992) were developed, but Applebaum et al. (2007) stated the superior efficiency of a Wyoming bentonite as opposed to potassium sulphite in eliminating afatoxin M₁. Moreover, Soha et al. (2006) experimented with three different bentonites and underlined the advantages of using silicates to detoxify contaminated milk instead of thermal and chemical treatments. Di Natale et al. (2009) compared the binding capacity of various adsorbents (a montmorillonitic bentonite, activated carbons and zeolites) on heavily contaminated milk and distinguished between very efficient (the bentonite and certain carbons) as opposed to others. A montmorillonite modified by an organic polyelectrolyte (poly-diallyldimethylammonium chloride) was also proposed for processing afatoxin-contaminated fluids, but without specific data on milk (Huebner and Phillips, 2003).

In contrast to most of the literature, which refers to afatoxin B₁ (AFB₁) in water, the aim of this study was to test the efficiency of clays (several bentonites and a kaolin) as effective binding agents of afatoxin M₁ (AFM₁) found in contaminated milk. The clays were selected because of their easy accessibility (all of them are available on the market) and to account for the variable crystal-chemical features of smectites. In addition, the effects of clays on the nutritional properties of milk were considered. Isothermal adsorption experiments were performed to complete the characterisation of the smectite–AFM₁ interaction.

2. Materials and methods

A preliminary test was performed to compare the effects of a kaolin and a bentonite at various clay–milk ratios. A second experiment was planned using bentonites because the kaolin was less effective in the adsorption of AFM₁.

2.1. Preliminary tests

Preliminary tests were developed using bovine milk from a small farm near Potenza (Italy) and commercial clays: a bentonite (here labelled Bent1) and a kaolin (labelled Kaolin1), already characterised by Bonina et al. (2007). Different amounts of AFM₁ were added to samples of the raw milk, resulting in the following concentrations (ng/L) of AFM₁: 12.0, 43.6, 56.8, and 77.8. Forty millilitres of each of these four samples of contaminated milk received different amounts of each clay: 1.2, and 4 g. The dispersions were shaken for 24 h; after sedimentation (12 h at 20 °C), the milk samples were separated, and the AFM₁ amounts were measured by HPLC–FLD (high performance liquid chromatography with postcolumn fluorescence derivatisation) using an Agilent Technologies 1100.

To check the nutritional parameters of the milk, the amounts of fat, protein, and lactose were measured by infrared spectroscopy both before and after the treatments using a MilkoScan FT 6000 spectrophotometer.

2.2. Bentonite selection

Several bentonites were collected on the international market to characterise their composition. All of the samples were characterised using a series of techniques, e.g., X-ray diffraction (XRD), X-ray fluorescence (XRF), Fourier transform infrared (FTIR) analysis and thermogravimetric and differential thermal analysis (TG–DTA). A subset of six bentonites were selected for the tests of AFM₁ adsorption (three samples from Italian commercial offices: Bent2, Bent3 and Bent9; one from Turkey: Bent6; one from Greece: Bent10 and one from USA: Bent11). Various criteria were taken into account in selecting the most suitable materials: (i) the abundance of smectite, based on the XRD and TG results; (ii) the wide crystal-chemical spectrum of the smectites, based on the XRD data (primarily the d₄₀₀ value) and XRF results, and (iii) the presence of both Fe–OH–Al and Mg–OH–Al bending vibrations in the FTIR spectra.

Samples Bent2 and Bent3 are commercial products for the clarification of wine, due to their efficiency in binding wine proteins (Ferreira et al., 2002).

2.3. XRD analyses of bentonites

The samples were pulverised by hand, and the powders were pressed using frosted glass. A Philips X’change system, Cu tube and secondary monochromator were used.

Semi-quantitative estimates of the mineral constituents of the bulk bentonites were developed using XRD, following two different approaches: the reference intensity ratio of selected peak areas (Barahona Fernandez, 1974) and a full pattern fitting (Srodon et al., 2001), using the database by Eberl (2003).

2.4. Chemical analyses

The chemical analyses of fused discs (1:10 rock–Li tetraborate ratio) of the bentonites were obtained via X-ray fluorescence using a Philips PW2400 spectrometer equipped with a Rh tube. Several international geologic standards were used for instrument calibration. The loss on ignition (LOI) was measured after heating the sample to 110 °C and 980 °C. Permanganate titration was used to evaluate the concentration of divalent Fe.

2.5. Fourier transform infrared (FTIR)

A Thermo Nicolet spectrometer (NEXUS 670 FTIR) was used to acquire the FTIR spectra of the bentonites in transmission mode from 400 to 4000 cm⁻¹. The pellets were prepared by mixing 2.5 mg of bentonite and 400 mg of anhydrous KBr and carefully homogenising the mixture in an agate mortar.

2.6. Thermogravimetric and differential thermogravimetric analyses

Thermogravimetric and differential thermogravimetric data were obtained using a prototypal Le Chatelier instrument (IGG-CNR, Padua). A type S thermocouple (Pt–10% Rh/Pt) placed inside an electric furnace provided the sample and furnace measurements. The heating rate was 10 °C/min in air starting from room temperature.

The percentages of cis- and trans-vacant arrangements of diocthedral smectites were determined by curve fitting the differential thermal signal at temperatures below and above 600 °C (Wolters and Emmerich, 2007).

2.7. Adsorption of AFM₁ in milk by bentonite samples

Six types of bentonites were selected for the test of adsorption of AFM₁. Afatoxin M₁ (Supelco/Sigma-Aldrich) was used as the milk contaminant. The bentonites were Ca²⁺-homoisohedral to avoid the influences of different chemical interlayers, and as such each bentonite sample was treated three times with a 0.1 M CaCl₂·6H₂O aqueous solution.
AFM₁-free raw bovine milk from a farm in Potenza province (Italy) was selected. The levels of fat, protein, and lactose were determined using infrared spectroscopy. To contaminate the milk, 75 ng/L of AFM₁ was added. This concentration was chosen because 50 ng/L represents the legal limit in the European Union (European Commission, 2010) and in many other countries (Mohammadi, 2011).

The adsorption experiments were performed twice: 0.5 g of each Ca²⁺-homoionised bentonite was added to 20 mL of contaminated milk (the lowest clay–milk ratio used in the preliminary test).

The dispersions were shaken for 24 h; after sedimentation (12 h at 20 °C), the milk samples were separated and analysed. The amounts of AFM₁ were measured using HPLC–FLD, and the quantities of fat, protein, and lactose were measured using infrared spectroscopy.

2.8. Isotherms

To obtain a more complete picture of the adsorption capacities of the two more effective bentonite samples, isothermal adsorption procedures were performed in water.

Two dispersions containing the two types of bentonite were prepared by adding 15 mg of clay to 30 mL of distilled water. Separate water–acetonitrile solutions containing AFM₁ at concentrations of 0.0, 0.4, 1.6, 6.0 and 8.0 mg/L were prepared. Then, 0.4 mL of the bentonite dispersions was added to 7.5 mL of the AFM₁ solutions. These procedures were performed in duplicate.

After 24 h of shaking, the samples were centrifuged at 10000 rpm for 15 min, and the amounts of the toxin in the supernatants were determined by measuring the AFM₁ absorbance at 360 nm (in water–acetonitrile solvent) with UV/visible spectrophotometry (Pharmacia Biotech Ultrospec 4000 spectrophotometer). The adsorbed AFM₁ was then calculated as the difference between the initial and final concentrations in the supernatants.

For the standard curve, the AFM₁ solution concentrations at 0.0, 0.4, 1.6, 3.2, 6.0, and 8.0 mg/L were measured. The values of absorbance were then derived from the standard curve. The supernatant concentrations of the AFM₁ were calculated and represented in accordance with the Langmuir equation:

\[
C_{eq}/q = K + C_{eq}/Q_{max}
\]

where \(K\) is a constant, \(C_{eq}\) is the concentration at equilibrium, \(q\) is the amount of AFM₁ adsorbed, and \(Q_{max}\) is the maximum adsorption capacity.

2.9. Bentonite quantification in milk

Four dispersions were prepared to determine the bentonite content in the discarded sediment (and in the treated milk, by difference). Three dispersions contained the same types and amounts of milk and bentonite, and the fourth dispersion was carried by milk alone. The following procedures were the same for the four dispersions: the only difference was in the content of the fourth dispersion, where bentonite is not present.

For the bentonite-containing dispersions, 1 g of bentonite was added to 40 mL of milk in three beakers. The four dispersions were shaken for 24 h and then left to settle for 12 h at 20 °C. The supernatants were removed, and the discarded sediments were collected and heated at 380 °C for 6 h. These sediments were weighed and crushed, and precise amounts of an internal standard (corundum NIST 676) were added to the four sediments and to a single sample of bentonite. In particular, five solid mixtures with 22.4% of the internal standard were obtained. These mixtures were analysed using X-ray microdiffraction (μXRD). A Rigaku D-max Rapid microdiffractometer was used, equipped with an image plate detector, a flat graphite monochromator, and a CCD camera for the positioning of the sample under the X-ray beam. The analyses were performed at 3° of fixed omega angle and a 360° rotation range of the phi angle, using CuKα radiation, power of 1.20 kW (40 kV and 30 mA), a 100-μm collimator beam diameter, and a time of 2 h. The μXRD data were collected as two-dimensional images and converted to 2θ-θ profiles using the Rigaku R-Axis Display software.

The bentonite content in the discarded sediments was measured following the procedure described by Chipera and Bish (2002) and using the μXRD traces obtained from the bentonite and from the discarded sediments (with and without bentonite), all with 22.4% corundum.

3. Results

3.1. Preliminary tests

The sequestration of AFM₁ from the milk was strictly dependent on the clay used, as sample Bent1 was always more efficient than sample Kaolin1. Only at the lowest AFM₁ contamination (12 ng/L) did the two clays exhibit comparable results, with a very high level of purification. As expected, stronger effects were seen by increasing the clay–milk ratio, but the kaolin seemed to reach a maximum of adsorbed AFM₁ (approximately 31 ng/L remaining in the milk, from an initial level of 78 ng/L), whereas the bentonite always displayed a trend toward the complete elimination of AFM₁ by increasing the amount of clay added to the milk (Fig. 1).

![Fig. 1](image-url) Concentrations of AFM₁ adsorbed by samples Bent1 and Kaolin1, in comparison with the initial values in milk (preliminary test). Suffixes A, B and C refer to 1, 2, and 4 g of clay, respectively, added to 40 ml of milk. Initial concentrations of AFM₁ in milk: a) 12.0 ng/L, b) 43.7 ng/L, c) 56.8 ng/L and d) 77.8 ng/L.
The nutritional properties of the milk were reported in terms of fat, protein and lactose concentrations (Fig. 2). The initial content of fat was 3.1%, and this level decreased after the addition of the two types of clays, Bent1 (approximately 2.4%) and Kaolin1 (approximately 2.7%), but regardless of the clay–milk ratio.

In contrast, the protein content was sensitive to the amount of clay in the milk dispersion and to the type of clay. The protein decreased regularly down to 1.8% in the case of Bent1 and to 2.4% in the case of Kaolin1, at the maximum clay–milk ratio (0.1).

A different situation was observed with regard to the lactose. The lactose content appeared to increase slightly with the addition of clay. This phenomenon was considered an effect of instrumental interferences under similar laboratory and instrumental conditions (Soha et al., 2006).

3.2. Adsorption of AFM1 in milk by bentonite samples

The mineralogical and chemical features of the six bentonites selected for the adsorption test are reported in Tables 1 and 2. All of these bentonites are very rich in smectite, and one sample (Bent9) is characterised by a higher amount of opaline silica than in the others. According to the d001 values and chemical analyses, the smectites that characterise the samples cover a relatively wide range of crystal-chemical composition. Sample Bent11 contains a typical trioctahedral smectite (saponite), and a ferruginous smectite is present in sample Bent10, whereas dioctahedral smectites are present in the other four samples. The SiO2–Al2O3 ratio of the dioctahedral smectite samples (taking into account the associated minerals) suggests that montmorillonite terms prevail and that sample Bent3 has the highest beidellitic component (its trans-vacant component is also relatively high, as observed by Wolters et al., 2009, in certain beidellitic materials).

Table 3 presents the AFM1 amounts remaining in the milk after the treatments with the six types of bentonites. The AFM1 was adsorbed by the six bentonites at rates of 70–100% (Table 3), and samples Bent9 (with dioctahedral smectite) and Bent11 (with trioctahedral smectite) displayed the higher AFM1 adsorption capacities. These two samples were selected to verify their ability to adsorb AFM1 in water solutions.

According to the values shown in Table 3, the milk seems to maintain its nutritional quality after the treatments with the six bentonites: the amounts of fat, protein, and lactose in the samples of treated milk do not differ significantly from those in the untreated milk.

The amount of bentonite that remains in the milk after settling was measured in triplicate in sample Bent11, which represents the most efficient adsorbent among the tested materials. The treated milk contains approximately 0.4% Bent11 in dispersion (average of the 3 experiments: 0.42%, standard deviation: 0.12).

3.3. Adsorption of AFM1 from aqueous solution

The Langmuir equations plotted as Ceq/q versus Ceq allow for calculating the Qmax by the angular coefficients of the two straight lines.
obtained ($R^2 = 0.81$ and 0.93 for samples Bent9 and Bent11, respectively; Fig. 3). Samples Bent9 and Bent11 exhibited maximum rates of adsorption of 0.018 mol/kg and 0.079 mol/kg, respectively. These results are similar to those obtained by Kannewischer et al. (2006) for the sample displaying the lowest capacity for adsorbing AFM1. The adsorption data of the studied samples indicated relatively low percentages of toxin adsorbed from the solution that contained AFM1, in the range of mg/L: 1 to 3% using Bent9 and 3 to 6% using Bent11. This observation indicates the more effective absorption capacity of Bent11 compared to Bent9.

4. Discussion

The feasibility of removing AFM1 from milk is achieved using the selected bentonites, and, based on these results, the addition of bentonites to milk represents a suitable tool for improving the quality of food materials such as milk (and likely also other dairy products). Table 3 shows that no less than 65% of the AFM1 was removed from all of the samples. The differences in the AFM1-binding capacity of the six bentonites is not surprising because a wide variation in the maximum AFM1-adsorption capacity (up to 100-fold) had already been observed in a systematic study of several bentonites (Kannewischer et al., 2006). Even if there have been many advances in explaining the mechanism behind such wide variability, certain points still deserve additional study (Deng et al., 2012). According to Deng et al. (2012), this choice was likely proper because divalent cations are able to compensate for the charge imbalance using fewer atoms than monovalent cations, and thus aflatoxin is better able to occupy the empty spaces between the hydrated cations inside the interlayer spacings. After the adsorption procedures, sample Bent11 (saponite-rich bentonite) was observed to be more than four times more effective than the other samples. This behaviour of sample Bent11 was due to several factors, such as the low amount of mineral phases other than smectites and the higher value of the b cell parameter of its smectite, as shown by the (060) reflection (Table 1). The wider cell area implies a lower surface coverage of the Ca$^{2+}$ hydrated ions than in the other samples. An additional favourable feature of the saponite is the location of isomorphous substitution in the tetrahedral sites, which leads to higher hydrophobic interaction of oxygen atoms along the clay surfaces (Rana et al., 2009). This hydrophobicity may enhance the capacity to adsorb aflatoxin (Deng et al., 2012). For this reason, such a peculiar characteristic of saponite should be explored in more detail, also considering that no other data are available regarding aflatoxin adsorption by saponite.

Nevertheless, the adsorption capacity of the dioctahedral smectites can be chemically improved by exploiting their vacancies in the octahedral sheet (Jaynes and Zartman, 2011): an increase in the occupancies in this sheet would involve a decrease in the layer charge and an increase in the interlayer spaces available for the aflatoxin.

As a practical tool in milk detoxification, bentonites are more promising than kaolin, being better adsorbents of AFM1 (Fig. 1) and AFB1 (Gallo et al., 2010; Marroquin-Cardona et al., 2009). However, in milk with low levels of AFM1, kaolins may also be suitable for milk detoxification, especially considering that sample Kaolin1 reduced the protein content less than did sample Bent1. In particular, at low clay–milk ratios, the decrease in protein after the addition of bentonite was small (preliminary test) or negligible (six selected bentonites), in agreement with previous studies (Applebaum and Marth, 1982; Di Natale et al., 2009; Soha et al., 2006). Nevertheless, the subtraction of protein from the milk linearly increased with the amount of bentonite, up to approximately 40% of the initial protein content, whereas at the same clay–milk ratio, Kaolin1 extracted only approximately 20% of the protein (Fig. 2). The sequestration of proteins by clays has been studied at a great deal (Lagaly et al., 2006; Theng, 2012), and it has been found that

![Table 2](https://example.com/table2.png)

**Table 2** Chemical analyses of the selected bentonites (n.d. = not determined).

<table>
<thead>
<tr>
<th></th>
<th>Bent2</th>
<th>Bent3</th>
<th>Bent6</th>
<th>Bent9</th>
<th>Bent10</th>
<th>Bent11</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO2 %</td>
<td>47.07</td>
<td>51.16</td>
<td>49.06</td>
<td>55.74</td>
<td>46.93</td>
<td>47.67</td>
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<tr>
<td>TiO2</td>
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<td>0.21</td>
<td>0.19</td>
<td>0.49</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>Al2O3</td>
<td>15.63</td>
<td>20.32</td>
<td>16.84</td>
<td>14.17</td>
<td>5.82</td>
<td>3.16</td>
</tr>
<tr>
<td>Fe$_{2}$O$_3$tot</td>
<td>4.95</td>
<td>2.33</td>
<td>1.71</td>
<td>4.96</td>
<td>14.87</td>
<td>0.30</td>
</tr>
<tr>
<td>MnO</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
<td>0.06</td>
<td>0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>MgO</td>
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<td>2.45</td>
<td>3.47</td>
<td>8.74</td>
<td>25.45</td>
</tr>
<tr>
<td>CaO</td>
<td>3.35</td>
<td>1.54</td>
<td>5.07</td>
<td>2.47</td>
<td>0.81</td>
<td>0.64</td>
</tr>
<tr>
<td>Na$_2$O</td>
<td>2.39</td>
<td>3.52</td>
<td>0.87</td>
<td>0.82</td>
<td>0.11</td>
<td>0.30</td>
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<tr>
<td>K$_2$O</td>
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<td>0.89</td>
<td>0.76</td>
<td>1.01</td>
<td>0.43</td>
<td>0.15</td>
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<tr>
<td>P$_2$O$_5$</td>
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<td>0.04</td>
<td>0.05</td>
<td>0.12</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>LOI &lt; 110 °C</td>
<td>11.48</td>
<td>11.16</td>
<td>11.91</td>
<td>9.93</td>
<td>12.08</td>
<td>12.56</td>
</tr>
<tr>
<td>LOI &gt; 110 °C</td>
<td>8.75</td>
<td>6.37</td>
<td>10.79</td>
<td>6.79</td>
<td>9.00</td>
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<td>Total</td>
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<td>100.04</td>
<td>99.28</td>
<td>99.91</td>
</tr>
<tr>
<td>Fe$^{3+}$/Fe$^{2+}$</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3.4</td>
<td>5.5</td>
<td>27.7</td>
<td>0.1</td>
</tr>
</tbody>
</table>
this sequestration is facilitated at low pH, when proteins are protonated and easily adsorbed. This feature is exploited for decontamination of food materials such as wine (Blade and Boulton, 1988) and is also documented for casein (Mills and Creamer, 1971), the principal protein in milk (Coultrate, 1989). In the case of milk, its circumneutral pH limits the protein adsorption of clays, but certain protein loss is difficult to avoid because edge adsorption is common (Lagaly et al., 2006). The preliminary test with Bent 1 indicated only a slight decrease in the amount of protein (approximately 13 ± 4%), which was not observed in the successive experiments with the six bentonites at a clay–milk ratio (1:40). The commercial Bent 1 was not Ca₂⁺–homoionised and contained important amounts of Na⁺ and K⁺ (Bonina et al., 2007).

The chemical variability of the smectite interlayer in sample Bent 1 appears to have produced the greatest degree of interaction with the milk proteins. In contrast, the similar calcium-based chemistry of milk and smectite interlayers of Ca₂⁺–homoionised bentonites appear to reduce the interactions and/or adsorption processes between the proteins and clays. Unlike protein, the adsorption of fat is not of concern because the fat adsorption was low with the clays used (Table 3, Fig. 2) and, eventually, is a possible commercial target. Other bentonites also displayed no affinity for milk fat (Soha et al., 2006), in agreement with the hydrophilic character of smectite surfaces (Schoonheydt and Johnston, 2006). In addition, the lactose content did not change significantly after the addition of bentonite or kaolin to the milk, a trend that was also found in earlier studies (Di Natale et al., 2009; Soha et al., 2006).

Whether the nutritional properties remain unchanged or are modified only slightly with respect to a decrease in the protein, the addition of clay results in a certain amount of clay remaining in the treated milk, even if most of the clay will settle to the bottom (discarded sediment). The presence of bentonite in food is mentioned in USA regulations (FDA, 2012): these regulations list bentonite as a GRAS (generally recognised as safe) component that can be used as a “direct human food ingredient”. No maximum values are defined, but “good manufacturing practice” is required (i.e., no significant residue in foods). Also the rules of the European Union mention bentonite and kaolin for use in foodstuff, up to 5% (EC, 1995), and these same maximum values have been confirmed more recently (EFSA, 2008), in consideration of the safety of aluminium from dietary intake. Another possible reference is the Codex Alimentarius (WHO-FAO, 2011), which mentions the presence of several clay silicates, but only in milk powders, cream powders and other dairy products, up to 1%. No defined limits are presented for blends of skimmed milk and vegetable fat in powdered form, but good manufacturing practices are mentioned. The experimental results (approximately 0.4% bentonite in the treated milk) matches both the USA and EU rules regarding food and, at levels above these regulatory limits, the safety of bentonite and kaolin for human consumption is generally accepted if the toxicon trace elements, adsorbed species and harmful minerals are at safe levels (Carretero et al., 2006; López-Galindo et al., 2007; Plumlee et al., 2006). More specific studies regarding the safety of bentonite ingestion for humans indicated that 3 g/day of a particular smectite can be considered safe (Wang et al., 2005), even with chronic exposure (Afrifiyie-Gyawu et al., 2008a, b).

The six selected bentonites were subjected to Ca exchange to facilitate the comparison of various adsorbents and also (i) to avoid the presence of other exchangeable ions (possibly hazardous), (ii) to make the exchangeable fraction fully compatible with the milk (Ca is the major inorganic element in milk on an equivalent basis, USDA, 2012) and (iii) to enhance the capacity to adsorb aflatoxin. Other cations that may be more effective than Ca, such as Sr and Ba (Deng et al., 2012), are not essential elements (Lindh, 2005; WHO, 1996), and precautions suggest the avoidance of these elements. In particular, Sr can interfere with bone mineralisation, and excess Ba is associated with disorders of the digestive and respiratory tracts, inhibition of bone mineralisation and disorders of the cardiovascular system (Kabata Pendas and Mukherjee, 2007).

Clays can play (at least) a dual role against aflatoxin contamination because they are able to sequester AFM₁ from gastrointestinal media and can also adsorb AFM₁ from milk that has already been produced. These two toxin molecules are very similar but not identical, and the maximum adsorption capacity of smectites may differ with regard to the two aflatoxins. The comparison is problematic for two reasons: too few data are available with regard to AFM₁ (isothermal adsorption experiments are reported in this work for the first time), and AFB₁ exhibits high variability in its Qmax values (Kannewischer et al., 2006; Mulder et al., 2008). The addition of an OH group at carbon-4 (labelled carbon-7 in Deng et al., 2010) in AFM₁ with respect to AFB₁ is sufficient to produce different profiles of their reaction rates with DNA fragments (Marien et al., 1987). This behaviour is expected because this OH group neighbours the carbon-8–carbon-9 double-bond (labelled carbon-10–carbon-11 in Deng et al., 2010), the putative active site of the molecule (Cathey et al., 1994; Paniel et al., 2010). This OH group is not present in AFB₁, which is the only difference between the two aflatoxins. Nevertheless, its influence on the adsorption by clay minerals is not clear. It appears that this OH group may not be protonated and links smectites by stronger ionic bonds. Otherwise, if this OH group is involved in an intramolecular hydrogen bond with the neighbouring oxygen-10 (following the schematic representation by Paniel et al., 2010), this site may decrease its reactivity. Therefore, the interactions between AFM₁ and smectites may be similar to those in the model proposed by Deng et al. (2010) for AFB₁. According to such a model, AFB₁ is bound to the exchangeable cations in smectite, in accordance with the lower capacity of kaolinite to adsorb AFM₁ measured in this study. In any case, the AFM₁ distribution in milk is heterogeneous, and a significant amount is bound to the casein (Mohammadi, 2011): this feature makes more complex the mechanism of adsorption by clay minerals.

5. Conclusions

The interactions between the milk and the six selected bentonites and the preliminary tests using a different bentonite and a kaolin indicate the effectiveness of bentonite in removing AFM₁. The significance of these results was confirmed by the amounts of protein, fat, and lactose in the milk samples after the adsorption procedures: the amounts of these organic nutrients did not decrease significantly compared to the initial samples, and the nutritional properties of the milk were maintained using low clay–milk ratios. Kaolin was less effective than the bentonites (based on the experimental data and the literature regarding AFB₁) but was still capable of detoxifying the contaminated milk (up to approximately 80 ng/L), even when using a small amount of kaolin (2.4% in the initial milk dispersion).

All of these results indicate new possibilities in milk purification based on the ability of clays to adsorb AFM₁, whereas most of the literature addresses the use of clay minerals to sequester AFB₁ in foodstuff (Dixon et al., 2008). Another finding is the higher affinity of AFM₁ for the saponite-rich bentonite compared to all of the other clay samples. Considering the wide variations in bentonite adsorption of AFB₁ (Kannewischer et al., 2006; Mulder et al., 2008), the new results regarding AFM₁ cannot be generalised, but the theoretical superiority of tetrahedrally substituted smectites, as hypothesised by Deng et al. (2012) based on the results by Liu et al. (2012), should be taken into consideration.

The presence of mycotoxins in foods is an emerging problem worldwide (Kabak et al., 2006; Lizárraga-Paulín et al., 2011) and is related to climate change (Prandini et al., 2009) and population weakness (Williams et al., 2004). All possible tools to achieve the safety of food materials should be developed, and the adsorption of contaminants by clay minerals has certain advantages, such as ease of use, mechanical separation rather than chemical or biochemical transformation of the toxin and its efficacy in producing products safe for human consumption.


