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Effect of Different Physico-chemical Parameters on Adsorption Performance of Molecularly Imprinted Polymer for Removing Aflatoxin M₁ from Cow's Milk

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ABSTRACT: In the present study, efficiency of novel molecularly imprinted polymer (MIP) as a selective adsorbent in removal of aflatoxin M_1 (AFM₁) from cow's milk was evaluated. For this purpose, effects of different physico-chemical parameters such as pH and ionic strength of the solution, agitation mode and adsorption behaviour of different adsorbents were determined by high-performance liquid chromatography. Also, the effects of adsorption on chemical composition of milk were evaluated in the terms of protein, fat, lactose and total solids. The removal efficiency of AFM₁ increased with a magnetic stirring mode and rise of pH from 3 to 7. Also, it decreased with an increment of ionic strength. The results showed that under optimized conditions (stirring at 120 rpm, solution pH = 7, contact time 100 min at 30°C) the MIPs had the highest AFM₁ removal efficiency (91.33%) and enhance its safety without any significant effects on quality of milk.

Keywords: Aflatoxin M₁; Molecularly imprinting polymer; Adsorption; Cow's milk, Ionic strength.

INTRODUCTION

Milk and dairy products have important nutritional and functional role in the human diet and provides vital constituents for humans of all ages. Nevertheless, some hazards such as chemical and microbial contaminants, natural toxins, parasites, pesticides, and drug residues, color additives, unapproved ingredients may occur in milk and dairy products which have negative effects on human health. One of the serious global concerns of dairy product consumption is their contamination to mycotoxins such as aflatoxin M_1 (AFM₁) and ochratoxin A.

Aflatoxins are natural, highly toxic, immunosuppressive, mutagenic, carcinogenic and teratogenic metabolites of certain fungi strains (Zinedine and Manes, 2009). AFM₁ is the principal metabolite of aflatoxin B_1 (AFB₁) which found especially in milk and other dairy products as a result of feeding animal with AFB1 contaminated feedstuffs (Prandini et al., 2009; Santili et al., 2015). AFM₁ contamination of dairy products is a serious health threatening problem since they are highly consumed by infants and children. Furthermore, AFM₁ is not destroyed during thermal processes such as

pasteurization and ultra-high-temperature (UHT) due to high chemical and thermal stability (Prandini et al., 2009). Therefore, the European Commission and US Food and Drug Administration set a maximum admissible level of 50 ng.kg $^{-1}$ and 500 ng.kg $^{-1}$ for AFM $_1$ in raw milk, heat-treated milk, and milk for the manufacture of other milk-based products, respectively (US FDA, 2005; European Commission, 2010). Subsequently, different approaches were studied to remove AFM₁ from dairy products such as oxidation by hydrogen-peroxide plus heat-treatment (Aman, 1992), biodegradation (Higuera-Ciapara et al., 1995; Deveci, 2007), binding of AFM₁ to adsorbents like different mineral clays (bentonite, kaolin, montmorillonitic, potassium sulphite, zeolites, smectite minerals) or activated carbons (Soha et al., 2006; Phillips et al., 2008; Di Natale et al., 2009; Carraro et al., 2014;). Although, these methods were capable of detoxifying AFM₁ in milk to safe levels, but they had adverse effects on chemical and nutritional properties of milk as they are general and non-specific adsorbents for AFM₁. In addition, the adsorbents are not renewable and safe because some of them contain contaminants such as heavy metals (Zeng, et al., 2014).

Also, complete separation of adsorbents from milk is a challenging problem. Therefore, more studies are needed to develop new methods with lower the negative effects on milk quality and maintain removal efficiency of AFM_1 .

Molecularly imprinting is a technology to synthesis polymers with well-defined artificial recognition sites which are sterically and chemically complementary to a specific target or class of target molecules (Mahkam and Poorgholy, 2011). Molecularly imprinted polymers (MIPs) have many advantages such as high selectivity, accessibility. affinity toward template. easv acquirement, good mechanical properties and high mass-transfer (Djozan et al., 2010; Ma and Shi, 2015). They have been extensively utilized for separation of hazard residues in foods (Puoci et al., 2008; Alenazi, et al., 2015; Song, et al., 2017), detection of toxins (Gadzala-Kopciuch et al., 2011; Anene, et al., 2016), solid-phase extraction (Djozan et al. 2010), destruction of pesticides (Erdem et al., 2010), stationary phase of liquid chromatography (Tamayo et al., 2005), simulated enzyme catalysis (Wei *et al.*, 2006), drug delivery (Mahkam and Poorgholy, 2011) and chemical sensors (Yola *et al.*, 2014). Nevertheless, there are a few works dealing with removing aflatoxins from foodstuffs by this approach (Wyszomirski & Prus, 2012; Szumski *et al.*, 2014; Díaz-Bao *et al.*, 2015; Semong and Batlokwa, 2017).

In the current study, we attempted to use AFM_1 MIP for reducing AFM_1 from contaminated raw milk. AFM_1 MIP was synthesized by a structural analogue of AFM_1 , 5,7-dimethoxycoumarin (DMC), as dummy template (Fig. 1) and coated on the silylated surface of stainless steel plates (SSP). The adsorption capacities of the MIPs for decontamination of AFM_1 from real milk and model solution samples were investigated. Furthermore, the effects of different physico-chemical parameters of adsorption process such as the adsorbent type, agitation mode, pH and ionic strength were evaluated. Also, the effects of MIP application on the nutritional properties of milk were investigated.



Fig. 1. Molecular structure of AFM₁ and 5,7- dimethoxycoumarin as dummy template.

MATERIALS AND METHODS

A. Reagents and chemicals

AFM1, methacrylic acid (MAA), DMC, ethylene glycol acetonitrile, dimethacrylate (EGDMA), 3-(trimethoxysilyl) propyl methacrylate (TMPM), and 2,2-azobis-2-methylpropionitrile (AIBN) were purchased from Sigma (UK) and immunoaffinity column (IAC) were supplied from (Vicam, Watertown, MA, USA). HPLC grade solvents were supplied from Merck (Darmstadt, Germany). Ultrapure water was produced by MilliQ Water System (Baunstead, Dubuque, USA). All other reagents were of analytical grade. The SSP (316L, 0.8 mm thickness) was obtained from a local ironware factory. Raw cow's milk was provided from a local farm (Tabriz, Iran).

B. Preparation of AFM₁-MIP

In this study, AFM1-MIP was synthesized on the silylated surface of SSP according to the method of Hu *et al.* (2010) with some modifications. First, the SSPs

were immersed in acetone for 30 min and then washed with methanol. Subsequently, the SSPs were soaked in alkaline oil-removing solution (50gL⁻¹ of sodium hydroxide, 20 g.L⁻¹ of sodium carbonate and 20 g.L⁻¹ of sodium phosphate tribasic in water) at 80°C for 30 min. The SSPs were treated with two oxidation solution: I: $K_2Cr_2O_7$ (20 g.L⁻¹) and sodium carbonate (2 g.L⁻¹) in water and II: H₂O₂ (5% v/v) at 60 °C for 30 min. Afterwards, the oxidized SSPs were silvlated for at least 1h by immersing into TMPM-water-methanol (1:8:1, v/v/v) at room temperature. Then, 10 mg of DMC as template, 54µL of MAA as functional monomer, 604µL of EGDMA as cross linker and 9.5 mg of AIBN were dissolved in 10 mL of acetonitrile as porogen and mixed well through ultrasonication for 5 min. Then, silvlated SSPs immersed in the solution and the sample was purged with dry nitrogen gas for 5 min to remove oxygen and sealed under nitrogen atmosphere.

Finally, the polymerization was performed in a thermostatic water bath at 60 °C for 24 h. The obtained MIPs were washed with a mixture of methanol/acetic acid solution (9:1) until no DMC was detected in the soaking solution. For the preparation of non-imprinted polymer (NIP) coated SSPs, the same procedure was applied in the absence of DMC template.

C. Effect of SSP surface characteristics on the adsorbent performance

For this purpose, MIP polymerization was performed on various types of SSP obtained by different pretreatments including:

-Cleaned bare SSP

-Oxidized SSP by K₂Cr₂O₇ and H₂O₂ solutions

- Silylated SSP

- Oxidized by $K_2 Cr_2 O_7$ and $H_2 O_2$ solutions and silylated SSP

For preparation of MIP coating, the same procedure aforementioned method was used. The prepared adsorbents were used for removing AFM1 from model solution (4mL aqueous solutions containing 2.5 μ g.L⁻¹ of AFM₁) at 30 °C for 100 min. The extraction ability of the adsorbents was monitored by measuring the residual amounts of AFM1 in solution using HPLC-FLD. The removal ratio of AFM₁ from the solution was calculated by the following equation (Yue *et al.*, 2013):

Removal efficiency (%) = $(C_o - C_t)/C_o \times 100$...(1)

Where Co and Ct represent the initial and final concentration of AFM_1 in the solution (µg $.L^{-1}$), respectively.

D. Evaluating selectivity of different adsorbents (MIP and NIP) toward AFM1

As described by Zeng *et al.* (2014), the MIPs and corresponding NIPs were separately inserted in to 4 mL AFM1 aqueous solution (1 μ g. L⁻¹) with pH=7 at 20°C. The mixture was shaken for 100 min with a speed of 120 rpm. In order to evaluate removal efficiency, the adsorbents were taken out and residual amount of AFM₁ in solution was analysed by HPLC-FDL method.

E. Effect of physico-chemical parameters on the removal efficiency of the MIP adsorbents

a) Agitation mode. In order to evaluate the effect of agitation of the sample on AFM₁ removal capacity of adsorbents, two types of MIPs (MIP I: oxidized by $K_2Cr_2O_7$ and MIP II: oxidized by H_2O_2 solution) were immersed in 4 mL of AFM₁ aqueous solution (2.5 µg. L⁻¹) at 30 °C for 100 min. The samples were treated with three different modes including: stationary, magnetic stirrer with a speed of 120 rpm and sonication in an ultrasonic bath (Elmasonic X-tra 150 H, Germany), respectively.

Finally, the initial and final concentrations of AFM_1 were measured in solution to assess the effects of the agitation methods on the removal efficiency of the MIP adsorbents.

b) pH. The effect of pH on removal efficiency was evaluated according to the method described by Farhadi *et al.* (2010) with some modifications. The MIP adsorbents were inserted into 4 mL of AFM₁ aqueous solution (2.5 μ g.L⁻¹) at 30 °C with different pH values (3, 5, 7 and 9) by adding phosphate buffer and Tris-HCl buffer. After 100 min, the adsorbents were taken out and the residual amount of AFM₁ in solution was determined to calculate the removal efficiency.

c) Ionic strength. To investigate the effect of ionic strength, AFM₁ removal efficiency was evaluated in aqueous solutions (pH=7) containing 0.5-5% (w/v) NaCl according to the method described by Hu *et al.* (2011). Briefly, the MIPs were immersed in 4 mL AFM₁ aqueous solution (2.5 μ g. L⁻¹) at 30°C for 100 min. After desired time, the removal efficiency in each sample was monitored by HPLC-FDL analysis.

F. Evaluating of removal efficiency of MIP/NIP adsorbents in spiked raw milk

The removal efficiency of the MIPs and NIPs was assessed in raw cow's milk samples spiked with AFM₁ (50 ng.kg⁻¹) (defined by the European Commission and many other countries as the maximum admissible level of AFM1 in dairy products). For this purpose, the adsorbents were submerged in the milk samples at 20°C and stirred with a speed of 120 rpm for 100 min, and then the residual amounts of AFM₁ in the samples were monitored by HPLC-FDL. To evaluate the effect of adsorption on chemical composition of milk, the protein (991.23 AOAC, 2005), fat (2000.18 AOAC, 2005), lactose (984.15 AOAC, 2005) and total solid (925.23 AOAC, 2005) contents of milk samples were determined before and after treatments.

G. Extraction and quantification of AFM1 and DMC in aqueous solution and spiked raw cow milk

Detection and quantification of AFM1in aqueous and spiked raw milk samples were carried out according to AOAC methods 970.44 (AOAC, 2005). Immunoaffinity column was used for purification and isolation of AFM₁ from milk. The Agilent 1200 series HPLC instrument consisted of a degasser, an isocratic pump a column oven thermostated at 30°C, a silica 5mm ACE 5 C₁₈, 100A°, 25 \times 4.6 mm column and a fluorescence detector model G1321A (Agilent Technologies, Palo Alto, California) and set at an excitation = 360 nm and an emission = 460 nm. (Aderden, Scotland).

DMC concentration in the samples was determined by the method of Dugo *et al.* (2012). The HPLC (BFRL HPLC SY8100, China) was used to measure the DMC in samples and standard samples. The HPLC system consisted of two LC 10 AD Vp pumps, an SPD-M10 Avp UV detector, a DGU-14A degasser, an SCL-10-Avp controller, and an OSD C₁₈, 250 × 4.6 mm, 5 μ m column (Knauer, Germany). About 20 μ L of the sample was injected and methanol-phosphoric acid 0.5% (90:10) was used as an isocratic elution mobile phase with flow-rate of 1.0 mL.min⁻¹. Data were acquired using a photodiode array detector at 326 nm.

H. Statistical analysis

In the present study, effect of different physicochemical parameters on MIP/NIP adsorbents were investigated by completely randomized design. The results were subjected to the one way ANOVA. The significance of differences between mean values was analysed by Duncan multiple range test (p 0.05) using SAS software (9.1, USA). The experiments were performed in triplicate.

RESULTS AND DISCUSSION

A. Effect of SSP characteristics on the adsorbent performance

The surface characteristics of MIP have an important role in binding capacity and specificity of adsorbent (Zeng *et al.*, 2014). Also, it was confirmed that high strength and homogenous coating of MIP on support metal surface have great influence on extraction ability (Hu *et al.*, 2011).

Effect of different surface treatments on removal efficiency is illustrated in Fig. 2. The results showed that the MIP coating on the surface of oxidized silvlated SSP could significantly increase the removal efficiency of the MIPs compared to other types of MIP. The oxidizing process created hydroxyl groups on the surface of SSP. Subsequently, the hydroxyl groups could effectively participate in the substitution reaction with methoxy groups of TMPM during silvlation process. The results also showed silvlation performed efficiently on the SSP. The plates could participate in co-polymerization process of MIP (Djozan, et al., 2010) and MIP layer was coated firmly and homogeneously on the surface of the SSP. Therefore, the MIP had significantly higher AFM₁ removal efficiency (87-90 %) compared to other ones (p 0.05).

B. Selectivity of different Adsorbents (MIP and NIP) toward AFM₁

The selectivity of adsorbents is defined as the specific interaction of adsorbent toward the adsorbate. The selectivity mainly depends on the complementarities between the surface charge of functional groups, shape and size of adsorbate and the adsorption site on the surface of MIP adsorbent (Zeng *et al.*, 2014). As illustrated in Fig. 3, the MIPs exhibited higher adsorption capacities for AFB₁ than their respective NIPs, because specific adsorption sites for the template (AFM₁) were not formed on the surface of the NIPs during the polymerization process due to the absence of the template.



Fig. 2. The effect of SSP surface characteristics on the MIP coating performance.

MIP: molecularly imprinted polymer; Suffixes I and II refer to SSP oxidized by $K_2Cr_2O_7$ and H_2O_2 solutions, respectively. (Initial concentration of AFM₁: 2.5 µg. L⁻¹; the volume of model solution: 4mL; reaction temperature: 30 °C; adsorption time: 100 min). Mean values \pm S.D (n=3). Different letters in each column indicate significant difference between samples (p 0.05).





MIP: molecularly imprinted polymer; NIP: non-imprinted polymer. Suffixes I and II refer to SSP oxidized by $K_2Cr_2O_7$ and H_2O_2 solutions, respectively. (Initial concentration of AFM₁: 1 µg. L⁻¹ in model aqueous solution (4mL) with pH=7; reaction temperature: 20 °C; adsorption time: 100 min). Mean values \pm S.D (n=3). Different letters in each column indicate significant difference between samples (p 0.05).

The highest and lowest AFM₁ removal efficiency was obtained by MIP I (90.91 %) and NIP II (30.41), respectively. Meanwhile, there was a significant difference between removal efficiency of MIP I and MIP II and their respective NIPs (p 0.05). It could be attributed to the effects of different surface oxidizing methods for MIP I and MIP II (K₂Cr₂O₇ and H₂O₂ solution, respectively). The K₂Cr₂O₇ solution created more hydroxyl groups on the surface of SSP and consequently high and appropriate silylation and MIP coating were conducted on the surface of this plate (Hu *et al.*, 2011).

C. Effect of agitation mode on adsorption capacity of the MIPs

Agitation mode has great influence on the adsorption capacity of adsorbent. Selecting the appropriate agitation mode leads to increase the separation of the adsorbate. In fact, the effective agitation can considerably facilitate adsorbate diffusion and mass transfer between solution and adsorbent (Hu *et al.*, 2011). In this study, the ability of sonication and magnetic stirring was evaluated in comparison with stationary mode.





Fig. 4. Effect of different agitation modes on AFM₁ reduction characteristic of different adsorbents. MIP: molecularly imprinted polymer; NIP: non-imprinted polymer. Suffixes I and II refer to SSP oxidized by $K_2Cr_2O_7$ and H_2O_2 solutions, respectively. (Initial concentration of AFM₁: 2.5 µg. L⁻¹; reaction temperature: 30 °C; adsorption time: 100 min). Mean values \pm S.D (n=3). Different letters in each column indicate significant difference between samples (p 0.05).

The results demonstrated that magnetic stirring method and sonication method had significantly (p 0.05) highest (83.2%) and lowest (48.3%) removal efficiency, respectively (Fig. 4). Using sonication method not only decreased the adsorption capacity of the MIPs toward AFM₁ but also it destructed the MIP coated on SSP. It could be attributed to cleaning effects of ultrasonic waves on metal surface. The results are in accordance with results of Djozan *et al.* (2010).

D. Effect of pH value on removal efficiency of adsorbents

pH is an important physicochemical parameter in controlling the adsorption process of aqueous solution through modifying the degree of ionization and surface charge of the adsorbent (Zahoor & Khan, 2014). The effect of pH on the removal efficiency of the MIP adsorbents was studied in the range of 3-9. According to results (Fig. 5), the removal efficiency of MIPs significantly increased with pH and reached to optimum value at pH=7 and then, significantly decreased (p 0.05). The lowest removal efficiency was observed in pH=9 (68.19 and 65.69 % for MIP I and MIP II, respectively). The finding are in good agreement with the results of Zahoor and Khan, (2014). Also, there was not any significant difference between MIP I and MIP II in terms of removal efficiency at different pH values (p>0.05). Regarding the chemical structure and characteristic of functional groups in MIP, pH has obviously remarkable role on adsorption efficiency of the MIPs. According to findings of Szumski et al. (2012) and Wyszomirski et al. (2014), five hydrogen bonds are likely formed between AFM1 and MAA which are essential interactions for binding. The carboxylic group of MAA participates in the formation of hydrogen bonds as donor or acceptor (Djozan et al., 2010). The group also interacts with hydrogen atoms of AFM1. As illustrated in Fig. 5, the removal efficiency of the MIPs decreased at low pH due to acidic groups of AFM1 and polymer are in their protonated form which cause electrostatic repulsion between them (Farhadi et al., 2010; Wyszomirski et al., 2014). Furthermore, at low pH condition, the carboxylic group of MAA may interact with hydronium ions instead of interaction with AFM1 due to presence of higher electrostatic force between MAA and hydronium ions. Therefore, the adsorption capacity of the MIPs decreased at low pH. Also, at alkalinity condition (pH=9), some of acidic groups in polymer structure of the MIPs were in ionic form which consequently decreased adsorption capacity of the MIPs. Finally, it could be concluded that neutral pH is optimum for removal of AFM1 by the MIPs.

E. Effect of ionic strength on removal efficiency of adsorbents

Ionic strength is a crucial parameter in adsorption process and extensively affects the electrostatic interaction between adsorbent and adsorbate. Effects of ionic strength on AFM1 removal efficiency of the MIP adsorbents are shown in Fig. 6.



Fig. 5. The effect of pH of the aqueous solution on the AFM₁ removal efficiency of prepared MIPs. MIP: molecularly imprinted polymer; Suffixes I and II refer to SSP oxidized by $K_2Cr_2O_7$ and H_2O_2 solutions, respectively. (Initial concentration of AFM₁: 2.5 µg. L⁻¹ in model aqueous solution (4mL); reaction temperature: 30 °C; adsorption time: 100 min). Mean values \pm S.D (n=3). Different letters in each column indicate significant difference between samples (p 0.05).

The results revealed that increment of NaCl amount (from 0.5 to 2.5%) increased slowly removal efficiency from 83.31 to 88.22 % When in solution increased, but further increase to 5% led to an abrupt decline of removal efficiency (68.77%). Increasing ionic strength and ion concentration in solution hindered the adsorption of AFM₁ on the MIPs because the ions accumulated near the surface of adsorbent and bound to the functional groups of polymer especially carboxylic group of MAA through electrostatic force. The association of the ions with the active sites of adsorbent

decreased the contact between the MIPs and AFM₁ molecule and consequently, decreased AFM₁ adsorption capacity of the MIPs. The results are in good agreement with previous studies dealing with MIP adsorbents (Djozan *et al.*, 2010; Hu *et al.* 2011). Hu *et al.* (2011) reported that increasing the ionic strength of solution reduced diffusion rate of adsorbate from solution to adsorbent. In contrast, Dojzan *et al.* (2010) expressed that increasing the ionic strength of solution decreased adsorbate solubility and improved its absorbency.



Fig. 6. The effect of ionic strength of the aqueous solution on the AFM₁ removal efficiency of prepared MIPs. MIP: molecularly imprinted polymer Suffixes I and II refer to SSP oxidized by $K_2Cr_2O_7$ and H_2O_2 solutions, respectively. (Initial concentration of AFM₁: 2.5 µg. L⁻¹ in model aqueous solution (4mL) with pH=7; reaction temperature: 30 °C; adsorption time: 100 min). Mean values \pm S.D (n=3). Different letters in each column indicate significant difference between samples (p 0.05).

F. Effect of AFM1 removal on milk quality

In the present study, the removal of AFM_1 from cow's milk by the MIPs and NIPs was evaluated. The results are illustrated in Table 1. As observed, the MIP I and MIP II had notable removal capacity to reduce AFM_1 in contaminated milk. However, the NIPs had

significantly lower AFM₁ removal capacity than the MIPs (p 0.05). The highest AFM₁ removal efficiency (91.33 %) was obtained by the MIP I. The results obtained for real sample (cow's milk) are completely in accordance with that of model samples.

 Table 1: AFM1 removal efficiency and the quality parameters of the raw cow's milk treated with different adsorbents.

Treatments	AFM ₁ removal efficiency (%)	Fat (%)	Protein (%)	Lactose (%)	Total Solids (%)
Control (raw cow's milk)	-	4.0±0.1 ^a	3.4±0.2 ^a	4.7±0.4 ^a	12.8±1.1 ^a
MIP I	91.31±4.71 ^a	3.9±0.1 ^a	3.2±0.3 ^a	4.6±0.1 ^a	12.6±0.9 ^a
MIP II	87.61±3.11 ^b	3.8±0.3 ^a	3.3±0.1 ^a	4.7 ± 0.2^{a}	12.5±0.6 ^a
NIP I	35.14±1.93 ^c	3.5±0.3 ^b	2.9 ± 0.2^{b}	4.3±0.3 ^b	12.1±0.5 ^b
NIP II	29.83 ± 2.47^{d}	3.6 ± 0.1^{b}	3.1±0.4 ^{ab}	4.4 ± 0.1^{b}	12.2 ± 1.0^{b}

*Mean values \pm S.D (n=3). MIP: molecularly imprinted polymer; NIP: non-imprinted polymer. Suffixes I and II refer to SSP oxidized by K₂Cr₂O₇ and H₂O₂ solutions, respectively (Initial concentration of AFM₁ in milk samples: 50 ng.kg⁻¹; reaction temperature: 30 °C; shaking speed: 120 rpm; adsorption time: 100 min). Different letters in each column indicate significant difference between samples (p 0.05).

The results of the present study suggested that the MIPs could be applied effectively for selective removal of AFM₁ from contaminated cow's milk. Similarly, Díaz-Bao et al. (2015) used magnetic powder MIP for extraction of AFM₁ from the infant formulas (1 ng kg⁻¹ of powder) and reported that removal efficiency was in the range of 43-94%. Furthermore, the amount of protein, fat, lactose and total solid was measured in treated and untreated milk samples to evaluate the effect of adsorption on milk composition and nutritional value. As seen in Table 1, the amount of protein, fat, lactose and total solid of the milk samples treated by the MIPs were not significantly different from the control sample (p>0.05). However, the significant differences were observed between the NIP treated samples and control sample (p 0.05). It could be attributed to develop the unspecific adsorption sites on the NIP surface and therefore absorb other chemical compounds in milk sample. On the contrary, chemical composition of cow's milk altered after removing AFM1 from cow's milk by bentonite clays (Carraro et al., 2014).

CONCLUSION

Occurrence of AFM₁ in dairy products is a global challenge, especially for infants and children. Therefore, all of the possible methods to enhance the safety of dairy products should be investigated. In the present study, we introduced a new adsorbent to detoxify the milk based on coating SSP with MIP layer. The Oxidized and silvlated SSP was used as supporting bed for surface coating of MIP. The prepared MIPs had higher capacity and selectivity toward AFM₁ in spiked aqueous solution compared to respective NIPs. The optimum pH, ionic strength and agitation mode for AFM₁ removal were 7, 0.5-2.5 % and stirring mode, respectively. The MIP I was most effective adsorbent for removal of AFM1 from spiked model solution and milk sample. Also, the amount of protein, fat, lactose and total solid content of milk sample didn't decrease significantly after treating with the MIP adsorption. On the basis of obtained results, it could be concluded that the MIPs can be applied as an efficient adsorbent for removal of AFM₁ from milk and improve the product safety.

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