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Short Communication

Comparative Analysis of Aflatoxin M1 in Marketed Butter by ELISA and HPLC

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Abstract

The aim of this study was to examine the presence of aflatoxin M1 (AFM1) in butter samples marketed in the Black Sea Region of Turkey with two complementary analytical methods to improve accuracy. A total of 40 samples were analysed by enzyme-linked immuno absorbent assay (ELISA) as the screening test and high performance liquid chromatography (HPLC) with fluorescence detection (FLD) after immunoaffinity column (IAC) clean-up as the confirmatory method. Results indicated that butter samples in the Black Sea region were of good quality with respect to AFM1, with no contamination detected. Furthermore, the accurate and sensitive IAC/HPLC-FLD method was confirmed as being appropriate for the detection of AFM1 in butter.

Keywords: *Butter, Aflatoxin M1, Black Sea, Immunoaffinity column, HPLC, ELISA*

Tereyağlarında Aflatoksin M1 Düzeylerinin ELISA ve HPLC ile Karşılaştırmalı Analizi

Özet

Bu çalışmada Karadeniz bölgesinde tüketime sunulan tereyağlarında iki farklı analitik yöntem karşılaştırarak aflatoksin M1 varlığının araştırılması amaçlandı. Bu amaçla, toplam 40 tereyağı numunesi ELISA testi ile tarandı ve ardından doğrulayıcı bir yöntem olannumunelerin temizleme aşamasında immunoafinite kolonların kullanıldığı floresan detektörlü yüksek performanslı sıvı kromatografi (IAC/HPLC-FLD) ile analizleri yapıldı. Yapılan çalışma ile herhangi bir tereyağı numunesinde AFM1 tespit edilmemiş olup, Karadeniz bölgesinde üretilen tereyağlarının AFM1 açısından iyi kalitede olduğu sonucuna varıldı. Ayrıca, elde edilen sonuçlara göre tereyağında AFM1 tespitinde kullanılan IAC/HPLC-FLD metodunun doğrulayıcı ve hassas bir yöntem olduğu ortaya konulmuştur.

Anahtar sözcükler: *Tereyağı, Aflatoksin M1, Karadeniz, İmmunoafinite kolon, HPLC, ELISA*

INTRODUCTION

Aflatoxin M1 (AFM1) is a hydroxylated metabolite of aflatoxin B1 (AFB1) that can be found in the milk of dairy cattle fed with AFB1-contaminated feeds [1].

The stability of AFM1 determines its persistence in foodstuffs such as butter, yogurt, cheese, cream and ice cream [2]. However, this toxin is not inactivated by the thermal processing (pasteurization and ultra-high-temperature (UHT) treatment) used in the dairy industry [3]. As milk and milk products are an important sources of nutrients, the contamination of these products with AFM1 is a potential risk for human health worldwide [4]. However, due to their low concentration in foods and feedstuff, analytical methods for detection and quantification of aflatoxins have to be specific, sensitive, and simple to carry out [5]. For the qualitative, quantitative and accurate determination of mycotoxin levels in food and feed products, the methods include thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) with flourescence (FLD) or diode array detector (DAD), gas chromatography coupled with mass spectrometry (GC-MS) or electron capture detection (GC-ECD), liquid

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chromatography-tandem mass spectrometry (LC-MS/MS) enzyme-lynked immunosorbent assays (ELISAs) and a combination of immunoaffinity column (IAC) techniques [6]. In general, fast and easy-to-use ELISA based aflatoxin screening kits are commercially available for all major types of aflatoxins. However, cross reactivity with related mycotoxins is a drawback of this method [7]. Therefore, quantification is predominantly done with HPLC-FLD due to the excellent native fluorescence activity of aflatoxins. In addition, IAC clean-up has shown great potential to increase method specificity and sensitivity [8]. The objective of this study was to investigate the occurrence of AFM1 in butter in the Black Sea Region of Turkey by screening with ELISA, followed by the comfirmatory method, HPLC-FLD.

MATERIAL and METHODS

Reagents and Materials

AFM1 analytical standard solution, phosphate-buffered saline (PBS) and immunoaffinity columns (IAC) were sourced from R-Biopharm Rhône Ltd. (Glasgow, Scotland). Acetonitrile and methanol were of HPLC grade (Sigma-Aldrich Co., USA). Whatman filter paper (No. 4) and Whatman 934-AH glass microfiber filter were from GE Healtcare (Buckinghamshire, UK).

Sampling

A total of 40 butter samples were purchased from supermarkets in the East, Middle and West Black Sea Regions of Turkey. They were produced in October-November, 2009 (first period) or May-June, 2010 (second period).

ELISA Procedures

The samples were prepared according to the application note, Ridascreen® Aflatoxin M1 (Art. No. R1121, R-Biopharm AG, Darmstadt, Germany) and analysed as described in the instructions of the AgraQuant® Aflatoxin M1 Sensitive kit (COKAQ7100, Romer Labs® Inc., U.S.A).

IAC/HPLC procedures

Aflatoksin M1 levels were determined in the butter samples by the method described in Sakuma et al.[9].

RESULTS

AFM1 was not detected in any of butter samples collected from the Black Sea region of Turkey. Mean recovery of AFM1 spiked into butter at 0.5 µg/kg was 84%. The limit of detection (LOD) was 0.023 ng/g and the limit of quantification (LOQ) was 0.077 ng/g. The HPLC standard calibration curve was linear over the range of concentrations of AFM1 injected and the R-squared (R^2) value was 0.99. The method exhibited great accuracy and reproducibility for detecting AFM1 in butter.

The HPLC chromatogram of the AFM1 spiked sample of butter is shown in *Fig. 1*. There are no interferences in the section where AFM1 was eluted.

DISCUSSION

In Turkey, many surveys have examined AFM1 content in milk products but only a few have been done with butter, especially with HPLC-FLD. Aydemir Atasever et al.[10] investigated AFM1 levels with ELISA in 80 butter samples obtained from supermarkets between September 2007 and September 2009 in Erzurum, Turkey, and reported that 66 samples were contaminated with AFM1 ranging from 10 to 121 ng/kg and 13 samples exceeded the maximum legal limit. Tekinşen and Uçar [11] were scanned AFM1 by ELISA method using with IAC in 92 butter and 100 cream cheese samples obtained from retail outlets in five major cities of Turkey. They found 100% of the butter samples and 99% of the cream cheese samples were contaminated with AFM1, ranging from 10 to 7.000 ng/kg and from 0 to 4.100 ng/kg, respectively. In another study, 223 samples of dairy products (27 samples of butter) marketed in Ankara, Turkey during September 2002-September 2003, were analysed for AFM1, total aflatoxin and AFB1 with ELISA. The AFM1 contamination rate was 90.58% in dairy products, including 92.6% in butter [12]. Var and Kabak [4] investigated AFM1 by ELISA in 70 dairy products (10 butter samples) purchased in different supermarkets in Adana, Turkey. They detected AFM1 in three of 10 butter samples. In addition, Tosun and Ayyildiz [13] studied AFM1 levels with ELISA in organic milk and dairy products marketed in Turkey between February 2010 and February

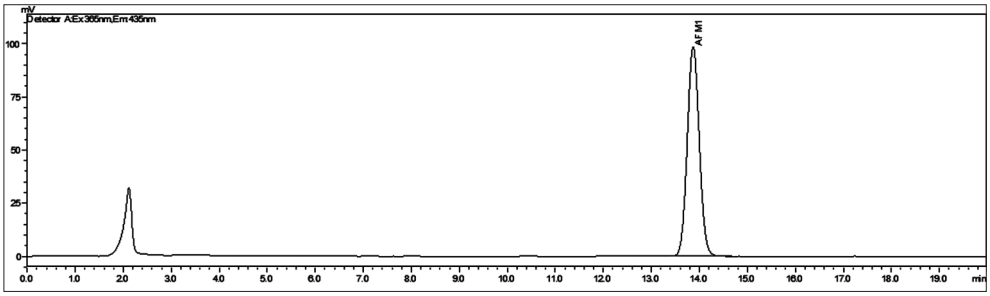


Fig1. HPLC-FLD chromatogram of spiked butter sample
Şekil 1. Tereyağı geri kazanım çalışmasına ait HPLC-FLD kromatogramı

2011. No AFM1 contamination was detected in organic butter samples.

During butter processing, the protein membrane around fat globules is broken down and the serum phase is separated. Due to the chemical structure of AFM1 and its affinity for casein, it adsorbs on this fraction of protein. Therefore, cream contains less AFM1 than milk and butter contains less AFM1 than cream [14]. As a result of the associated effects of these factors, less AFM1 occurs in the lipid phase (butter and cream) because it is concentrated in the serum phase and protein fraction. This data may be promote to our results. However, it is probable that good manufacturing practices and good storage prevented butter samples from getting mouldy.

Most of the current analytical techniques used for detection and quantification of aflatoxins involve sampling, extraction and clean-up, followed by an appropriate detection method, depending on the level of precision required of the result [7].

ELISA (test kits) are often used to screen and quantify mycotoxins as a rapid and practical method [15] but the drawbacks of the method are cross reactivity and matrix dependence, which often result in extreme over-estimation [16]. Therefore, comfirmatory methods such as LC, GC, LC/MS, GC/MS are needed for a sensitivity and accuracy. In the present study, the HPLC-FLD method used with an immunoaffinity column was highly advantageous, including rapidity, when used specifically for determination of the level of AFM1 in butter. Moreover, we achieved lower LODs and LOQs than several studies [17,18] by using the method described by Sakuma et al.[9].

In conclusion, the results of our survey showed that butter collected in a specific period from Black Sea Region was of excellent quality with respect to AFM1 contamination because none was detected. However AFM1 levels in dairy products such as butter can vary from season to season and year to year because they are related to levels of aflatoxins in feed. Therefore, regular surveillance studies are necessary to compile datum for a more comprehensive evaluation of AFM1 contamination in dairy products. The IAC/HPLC-FLD method that we applied to butter could be used more broadly as a sensitive and accurate detection method for AFM1 detection. Moreover, the systematic use of new analytical techniques to measure AFM1 levels with great precision can help to improve public health standards.

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REFERENCES

1. Sugiyama K, Hiraoka H, Sugita-Konishi Y: Aflatoxin M1 contamination in raw bulk milk and the presence of aflatoxin B1 in corn supplied to dairy cattle in Japan. *J Food Hyg Soc Jpn*,49, 352-355, 2008. DOI: 10.3358/shokueishi.49.352

2. Anfossi L, Baggiani C, Giovannoli C, Giraudi G: Occurrence of aflatoxin M1 in dairy products. **In,** Torres-Pacheco I (Ed): Aflatoxins - Detection, Measurement and Control. 1-20, Intech, Rijeka, 2011. DOI: 10.5772/22724

3. Fallah AA, Rahnama M, Jafari T, Saei-Dehkordi SS: Seasonal variation of aflatoxin M1 contamination in industrial and traditional Iranian dairy products. *Food Control*, 22, 1653-1656, 2011. DOI: 10.1016/j.foodcont.2011.03.024

4. Var I, Kabak B: Detection of aflatoxin M1 in milk and dairy products consumed in Adana, Turkey. *Int J Dairy Technol*, 62, 15-18, 2009. DOI: 10.1111/j.1471-0307.2008.00440.x

5. Wacoo AP, Wendiro D, Vuzi PC, Hawumba JF: Methods for detection of aflatoxins in agricultural food crops. *J Appl Chem*, 1-15, 2014. DOI: 10.1155/2014/706291

6. Bhat R, Rai RV, Karim AA: Mycotoxins in food and feed: Present status and future concerns. *Compr Rev Food Sci*, 9, 57-81, 2010. DOI: 10.1111/j.1541-4337.2009.00094.x

7. Yao H, Hruska Z, Diana Di Mavungu J: Developments in detection and determination of aflatoxins. *World Mycotoxin J*, 8, 181-191, 2015. DOI: 10.3920/WMJ2014.1797

8. Awad WA, Ghareeb K, Böhm J: Occurrence, health risks and methods of analysis for aflatoxins and ochratoxin A. *J Vet Anim Sci*, 2, 1-10, 2012.

9. Sakuma H, Kamata Y, Sugita-Konishi Y, Kawakami H: Method for determination of aflatoxin M1 in cheese and butter by HPLC using an immunoaffnity column. *Food Hyg Safe Sci*, 52, 220-225, 2011.DOI:10.3358/shokueishi.52.220

10. Aydemir Atasever M, Atasever M, Özturan K, Urçar S: Determination of aflatoxin M1 level in butter samples consumed in Erzurum, Turkey. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-A): S159-S162, 2010. DOI: 10.9775/kvfd.2010.2671

11. Tekinşen KK, Uçar G: Aflatoxin M1 levels in butter and cream cheese consumed in Turkey. *Food Control*, 19, 27-30, 2008. DOI:10.1016/j.foodcont.2007.01.003

12. Aycicek, H, Aksoy A, Saygi S: Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. *Food Control*, 16, 263-266, 2005. DOI:10.1016/j.foodcont.2004.03.004

13. Tosun H, Ayyildiz T: Occurence of aflatoxin M1 in organic dairy products. *Qual Assur Safe of Crop*, 5, 215-219, 2013. DOI:10.3920/QAS2012.0147

14. Bakirci I: A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. *Food Control*, 12, 47-51, 2001 DOI:10.1016/S0956-7135(00)00020-7

15. Krska R, Schubert-Ullrich P, Molinelli A, Sulyok M, Macdonald S, Crews C: Mycotoxin analysis: An update. *Food Addit Contam*, 25, 152-163, 2008. DOI: 10.1080/02652030701765723

16. Krska R, Welzig E, Berthiller F, Molinelli A, Mizaikoff B: Advances in the analysis of mycotoxins and its quality assurance. *Food Addit Contam*, 22, 345-353, 2005. DOI: 10.1080/02652030500070192

17. Herzallah SM: Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chem*, 114, 1141-1146, 2009. DOI: 10.1016/j.foodchem.2008.10.077

18. Hussain I, Anwar J, Asi MR, Munawar MA, Kashif M: Aflatoxin M1 contamination in milk from five dairy species in Pakistan. *Food Control*, 21, 122-124, 2010. DOI: 10.1016/j.foodcont.2008.12.004