Liquid chromatographic determination of citrinin residues in various meat products: A pioneer survey in Turkey

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ABSTRACT

Background and Aims: Occurrence of the mycotoxin citrinin could increase during Monascus purpureus fermentation in red yeast rice resulting in contamination of meat products when the red yeast rice is used as food additive. The aim of this study was to investigate extraction of citrinin from meat products by different extraction methods including LLE, QuEChERS and IAC methods.

Methods: Because of high sensitivity IAC method was selected for the extraction of citrinin in 33 meat products (salami, sausage and minced meat), then citrinin was analysed by HPLC-FLD.

Results: IAC method was linear in the range of 0.25-100 ng/g ($r^2$≥0.999) in meat products. Citrinin was found at 0.28 to 1.79 ng/g in 6 of the 19 samples (36.84%); and in one sausage sample contained citrinin at LOD level. However, citrinin was not detected in the salami and minced meat samples.

Conclusion: Our results indicated that citrinin in meat products could be an important health concern. Since no limits were set in food products, regulations should be developed concerning the presence of citrinin in other products as well as meat products, in Turkey.

Keywords: Citrinin, HPLC-FLD, Meat products

INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi due to inappropriate temperature and humidity conditions during poor cultivation and storage conditions. Until now, about 400 mycotoxins, mainly produced by Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium and Rhizopus species, have been identified (Pohland, 1993; Moss, 2002; Pal, Gizaw, Abera, Shukla, & Hazarika, 2015). Since mycotoxins cause serious harmful effects on human health and the environment, the detection of mycotoxin residues has emerged in various raw and processed food products. Even if the studies have especially focused on aflatoxins, the interest in mycotoxins including ochratoxin A, patulin, citrinin and zearalenone has increased considerably. Also, mycotoxins can cause industrial and financial losses due to color change and unwanted odor in food products (Rawat, 2015). To minimize mycotoxin-mediated diseases, it is necessary to set the maximum values and the regulations on the mycotoxin content of food products.

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Citrinin, a pyrrole derivative, is predominantly produced by *Penicillium*, *Aspergillus* and *Monascus* species, and most commonly found in corn, rice, wheat, rye, barley, peanut, bread, fruits, oilseeds (Shu & Lin, 2002; Chia-Hao, Feng-Yili, Li-Ting, Yi-Shen, & Bing-Hui, 2009). Because of the positive effects of the traditional Chinese food, red yeast rice on the digestive system and blood circulation, it is considered a valuable dietary supplement worldwide (Li, Xu, Li, & Ji, 2012; Srianta et al., 2014; Woo et al., 2014). Additionally, red yeast rice is widely used as a preservative, flavoring and food colorant, especially, in meat, fish and soybean products (Ma et al., 2000). Occurrence of citrinin could increase during *Monascus purpureus* fermentation in red yeast rice. Therefore, food processing for increasing the yield and attractiveness of the product is an important source of citrinin exposure as well as natural occurrence during agriculture.

The major adverse effects of citrinin on human health are nephrotoxicity and neurotoxicity, which can result in life-threatening consequences (Xu, Jia, Gu, & Sung, 2006). Moreover, citrinin usually occurs simultaneously with ochratoxin A, which have both hepatotoxic, immunotoxic, embryotoxic/teratogenic and carcinogenic effects, synergistically (Speijers & Speijers, 2014; Gupta, Srivastava, & Lall, 2018). Citrinin caused lipid peroxidation, mitochondrial respiratory dysfunction, and inhibition of several antioxidant enzymes, and induced micronuclei formation, aneuploidy, and chromosomal abnormalities; while it did not lead to DNA strand breaks, it did lead to oxidative DNA damage and sister chromatid exchange (ESFA, 2012).

It is well established that mycotoxin-contaminated food/feed could have substantial impacts on the economy, human health and environment. It has to be mentioned that the contamination of several foods with citrinin has been observed all over the world. Citrinin has been detected in many stored and dry foods such as cereal (Molinié, Faucet, Castegnaro, & Pföhl-Leszkowicz, 2005; Polisenska et al., 2010; ESFA, 2012), cereal-based foods (Vrabcheva, Usleber, Dietrich, & Märtlbauer, 2000; Duarte, Pena, & Lino, 2010; Zaied, Zouaoui, Bacha, & Abid, 2012; ESFA, 2012), fruit juices, medical plants, nuts (ESFA, 2012), olives and olive oil (El Adlouni, Tozlovanu, Naman, Faid, & Pfohl-Leszkowicz, 2006; Tokusoglu & Bozoglu, 2010; ESFA, 2012), tomatoes (Tölgyesi, Stroko, Tamosiunas, & Zwickel, 2015); cheese (Franco et al., 1996; ESFA, 2012), corn (Jackson, & Ciegler, 1978; Janardhana, Raveesha, & Shetty, 1999; Warth et al., 2012), beans (Petkova-Bocharova Castegnaro, Michelon, & Maru, 1991; ESFA, 2012), flour (Nishijima, 1984; Dick, Baumann, & Zimmerl, 1988), several spices (Saxena & Mehrotra, 1989; El-Kady, El-Maraghy, & Mostafa, 1995), soy (Konnonkeno & Burkin, 2008), rice (Hackbart, Prietto, Primel, Garda-Buffon, & Badiale-Furlong, 2012; Nguyen, Tzolovanu, Tran, & Pföhl-Leszkowicz, 2007), red mold rice (Li, Xu, Li, & Ji, 2005; Li, Zhou, Yang, & Ou-Yang, 2012; Childress, Gay, Zargar, & Ito, 2013; Ostry, Malir, & Ruprich, 2013; Liao, Chen, Lin, Chiueh, & Shih, 2014; Ji et al., 2015), fermented meat products (Markov et al., 2013) and feed (Talmaciuc Sandu, & Banu, 2008).

There is a regulation for citrinin regarding the maximum levels in food supplements based on *Monascus purpureus* fermented rice within the European Commission Regulation (EC) 212/2014 (EU, 212/2014); however, there is no regulation in Turkey. Hence, there seems to be an urgent need to detect citrinin residues and set maximum limits. EC regulation has set the limit of 2 ng/g for citrinin in only red yeast rice (EU, 212/2014). Therefore, we aimed to detect citrinin residues in various meat products. For this purpose, three different extraction methods were used; LLE (Liquid-Liquid Extraction), QuEChERS (Quick, Easy, Cheap, Effective, Robust and Safe) multi-residue and IAC (Immuno-Affinity Column) for the extraction of citrinin in salami, sausage, and minced meat samples. Because of its high sensitivity, the IAC method was selected for the extraction of citrinin in meat products. Then, the samples were analysed by high-performance liquid chromatography (HPLC)-fluorescence detection (FLD). The study will contribute to further investigations regarding the residue levels for citrinin in food products and the results could be considered by the regulatory affairs for the risk assessment process for citrinin.

**MATERIALS AND METHODS**

**Chemicals**

Citrinin was obtained from Sigma-Aldrich (Bellefonte, PA, USA, Cat. No: C1017) at the purity of ≥98%. A standard stock solution was prepared at a concentration of 50 µg/mL in methanol, and kept at 4 °C. Then, the HPLC standard solutions were prepared by serial dilutions at the concentration range of 0.25-100 ng/mL in the mobile phase. The mobile phase was freshly prepared by mixing water:acetonitrile: 2-propanol (65:30:5, v:v:v), and pH was adjusted to 2.95 with ortho-phosphoric acid. HPLC-grade methanol, acetonitrile, and other analytical grade reagents were purchased from Riedel-de Haën (Seelze, Germany) and Merck (Darmstadt, Germany).

**Samples**

A total of 33 meat products, including 19 branded sausages, 7 branded salami and 5 branded minced meat samples and 2 unlicensed open ground meat samples made from beef, lamb and turkey, was purchased from various supermarkets, delicatessens and butchers in Istanbul, Turkey during 2016-2018. Samples were collected as a minimum 1 kg in weight and immediately brought to the laboratory. After homogenization with IKA Ultra-Turrax disperser homogenizer (Bender&Hobein, Germany) and Merck (Darmstadt, Germany).

**Extraction of Citrinin**

**LLE (Liquid-liquid extraction)**

Briefly, 1 g of the sample was extracted with 5 mL methanol:water (80:20, v:v) using the disperser homogenizer for 5 min. After centrifuging at 4000 rpm for 15 min, the sample extract was filtered through 0.45 µm nylon syringe filter. Then, 100 µL of the aliquot was injected into high-performance liquid chromatography HPLC-FLD.

**2.3.2. QuEChERS (Quick, Easy, Cheap, Effective, Robust and Safe) multi-residue**

The QuEChERS multi-residue kit was obtained from Restek Corp. (Bellefonte, PA, USA, Cat. No: 26221), and the extraction was done by manufacturer’s instructions. Briefly, 1 g of the sample was extracted with 10 mL methanol with 0.1% acetic acid. The QuEChERS multi-residue kit was obtained from Restek Corp. (Bellefonte, PA, USA, Cat. No: 26221), and the extraction was done by manufacturer’s instructions. Briefly, 1 g of the sample was extracted with 10 mL methanol with 0.1% acetic acid.
acid water (80:20, v/v) using the disperser homogenizer for 1 min. The homogenized sample was added into the magnesium sulfate: sodium acetate (4:1) cleaning solution, shaken for 1 min, then centrifuged at 4000 rpm for 5 min. The supernatant was added into the magnesium sulfate:primary secondary amine (3:1, v/v) mixture, and shaken for 5 min, then centrifuged at 4000 rpm for 5 min. 10 mL of the supernatant was filtered through a 0.45 µm nylon syringe filter, and 40 µL of the aliquot was injected into HPLC-FLD.

**IAC (Immuno-affinity column)**

CitriTest™ immunoaffinity columns were purchased from VI-CAM (Watertown, MA, USA), and the extraction was done according to manufacturer’s instructions. Briefly, 1 g of the sample was extracted with 20 mL of methanol:water (70:30, v/v) on a shaker at 65 °C for 15 min. The supernatant was filtered through a black ribbon filter paper (Whatman, Sigma-Aldrich, Munich, Germany); and then, 1 mL of the filtrate was filtered through a 0.45 µm nylon syringe filter. The second filtrate was completely according to manufacturer’s instructions. Briefly, 1 g of the sample was injected into HPLC-FLD.

**HPLC-FLD conditions**

The chromatographic analysis was conducted using an LC-20A Shimadzu (Kyoto, Japan) high-performance liquid chromatographic system coupled to an RF-10A XL fluorescence detector. The chromatographic separation was performed using a reversed-phase Phenomenex HPLC column (C18, 250 mm x 4.6 mm, 5 µm, 100 Å, Phenomenex, Torrance, CA, USA) with a mobile phase of acetonitrile:water:2-propanol (30:65:5, v/v/v, pH 2.95) at a flow rate of 1 mL min-1. The column temperature was kept at 25 ºC. The fluorescence detection condition was at 4000 rpm for 5 min. The supernatant was filtered through a vacuum manifold (VacElut 20 Manifold, Agilent Biotech- technologies, Santa Clara, CA, USA) at a flow rate of 1 drop/second until air came through the column. The column was washed with 10 mL of 0.1% tween20 / phosphate-buffered saline and 10 mL of water, then dried under a vacuum. Citrinin was eluted with 1 mL of methanol:10 mM phosphoric acid (70:30, v/v) into a glass syringe barrel at a rate of slower than 1 drop/second. For HPLC-FLD analysis, 40 µL of the aliquot was injected.

**Method validation**

The linearity of the method was assessed using the calibration curve, established with nine levels of citrinin in the final concentrations of 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 ng/g in the meat samples. The method selectivity was validated by analysing the extracts of citrinin-free blank and the same spiked samples at 5 ng/g citrinin. The recovery experiments were performed on citrinin-free meat samples by spiking with the citrinin standard solutions to obtain the final concentrations of 1-100 ng/g for LLE, 2.5-100 ng/g for QuEChERS, and 0.25-100 ng/g for IAC. For the linearity and recovery studies, the four experiments were repeated, and each injected three times.

The method precision for the intra-day assay was assessed by analysing four independent spiked samples at five concentrations (0.25-100 ng/g) in three-replicated injections on the same day. The inter-day analysis was performed by analysing spiked samples at five concentrations (0.25-100 ng/g) in three-replicated injections on four consecutive days. The relative standard deviation of replicate results (RSDr) was used to evaluate intra- and inter-day precision. The method sensitivity was assessed by using the limit of detection (LOD; signal-to-noise ratio= 3) and the limit of quantification (LOQ; signal-to-noise ratio= 10) for citrinin. The method stability was determined with 5 ng/g citrinin-spiked samples at 25°C, 4°C and -20°C on the analysis day of 1, 3 and 10.

**RESULTS AND DISCUSSION**

**Method performance**

No interfering peak was observed at the retention time of citrinin in the meat products after LLE, QuEChERS multi-residue and IAC methods. The calibration curve was linear at 0.25-100 ng/g in the meat products with a correlation coefficient \( r^2 >0.9975 \) (Table 1). The LOD levels were calculated to be 0.17-0.33 ng/g for LLE, 0.33 -1.67 ng/g for QuEChERS multi-residue and 0.083 ng/g for IAC; and the LOQ levels were 1 ng/g for LLE, 2.5 ng/g for QuEChERS multi-residue and 0.25 ng/g for IAC.

The mean recoveries from the meat products were 64.71-87.58% for LLE (at 1-100 ng/g), 62.74-78.54% for QuEChERS multi-residue (at 2.5-100 ng/g), and 87.26-95.02% for IAC (at 0.25-100 ng/g) with a relative standard deviation (RSDr) less than 7.13% (Table 2). The inter-day repeatability recoveries

| Range (ng/g) | Calibration equation | \( r^2 \) | LOD (ng/g) |
|-------------|-----------------------|----------|------------|
| **LLE**     |                       |          |            |
| Mince meat  | Salami                | \( y = 5.7094x + 0.2787 \) | 0.9997     | 0.167      |
|             | Sausage               | \( y = 6.1928x + 1.0844 \) | 0.9997     | 0.330      |
| **QuEChERS**| **multi-residue**     |          |            |
| Mince meat  | Salami                | \( y = 5.3166x + 0.281 \) | 0.9996     | 1.670      |
|             | Sausage               | \( y = 6.4089x + 5.3636 \) | 0.9987     | 0.830      |
| **IAC**     |                       |          |            |
| Mince meat  | Salami                | \( y = 7.1135x + 4.4583 \) | 0.9995     | 0.083      |
|             | Sausage               | \( y = 6.9218x + 3.729 \) | 0.9990     | 0.083      |

**Table 1. Linearity of citrinin in the meat products.**
were mean ≥62.40%, ≥61.60%, and ≥86.60%, and the intra-day repeatability recoveries were mean ≥66.50%, ≥64.30%, and ≥87.20% for LLE (at 1-100 ng/g), QuECHERS multi-residue (at 2.5-100 ng/g), and IAC (at 0.25-100 ng/g), respectively (Table 3). EC Regulation No 519/2014 stated that for all levels of citrinin recoveries are acceptable in the range of 70-120% and the RSDr should be <22% (EU, 519/2014). According to our study, we observed good precision using the IAC method; hence, we performed the IAC method for analysing the meat samples. For the method stability studies, 5 ng/g citrinin-spiked samples were analysed at 25 °C, 4 °C and -20 °C. No change in recovery was observed after 1-3 days whereas the recovery decreased to 12±3.4% after 10 days. Consistent with our results, Markov et al. (2013) showed that the citrinin recovery was 93.5% with a good precision of RSDr 9.39% in the meat products after IAC cleanup and HPLC-FLD. In a study from Jiménez-López et al. (Jiménez-López, Llorent-Martínez, Ortega-Barrales, López et al. (2016) showed that LOQ for citrinin in red yeast rice was 10 ng/g, with an LOD of 3 ng/g and recoveries ranged from 80 to 110% after IAC cleanup and HPLC-FLD. In a study from Jiménez-López et al. (Jiménez-López, Llorent-Martínez, Ortega-Barrales, & Ruiz-Medina, 2014), developed a fluorometric flow-through optosensor using Sephadex SPC-25 as solid support for citrinin analysis in dietary supplements containing red yeast rice and obtained an LOD value of 10.5 ng/mL with RSDs lower than 3%. Application of fluorescent immunochromatographic test strips to detect citrinin in Monascus fermented food demonstrated high recoveries (86.8–113.0%) and low RSDs (1.8–15.3%) (Chen, Xu, Ma, Cui, Sun, & Zhang, 2019). In red fermented rice samples, the limit of detection was obtained as 1.0 ng/g for LC-MS/MS and 250 ng/g for HPLC-FLD (Ji et al., 2015). Data obtained in the present study indicated that the IAC extraction method was the most effective method for citrinin extraction from the meat products and had good precision. Therefore, citrinin residue analysis in various meat products was conducted with IAC method, then analysed using HPLC-FLD.

## Citrinin in meat products

The citrinin residue analysis was conducted for over a decade, and the surveys are still in progress worldwide. The physical conditions, such as climate change, affect the mycotoxin levels in food products and lead to concerns about food safety (Pa -

### Table 2. The recoveries from different meat products for LLE, QuECHERS multi-residue and IAC methods (n=4).

|          | LLE Mean recovery (%) ±SD | RSDr (%) | QuECHERS multi-residue Mean recovery (%) ±SD | RSDr (%) | IAC Mean recovery (%) ±SD | RSDr (%) |
|----------|---------------------------|----------|---------------------------------------------|----------|---------------------------|----------|
| Spiking level (ng/g) | Spiking level (ng/g) | Spiking level (ng/g) | Spiking level (ng/g) | Spiking level (ng/g) | Spiking level (ng/g) | Spiking level (ng/g) |
| Salami  | 1  66.07 ±0.04  5.63 | 2.5  66.06 ±0.12  7.13 | 5  68.17 ±0.15  4.94 | 10  76.47 ±0.75  3.91 | 0.25  91.98 ±0.01  2.56 | 0.5  91.51 ±0.02  3.25 |
|         | 2.5  72.65 ±0.07  6.86 | 5  68.80 ±0.38  3.27 | 2.5  76.47 ±0.75  3.91 | 5  94.65 ±0.03  1.92 | 5  92.55 ±0.11  4.17 | 2.5  92.62 ±0.06  2.73 |
|         | 5  79.01 ±0.08  1.96 | 10  68.80 ±0.38  3.27 | 10  76.47 ±0.75  3.91 | 10  94.01 ±0.22  1.78 | 25  95.02 ±0.35  1.62 | 25  95.02 ±0.35  1.62 |
|         | 10  86.34 ±0.39  3.52 | 25  86.93 ±0.67  1.84 | 25  86.93 ±0.67  1.84 | 25  95.02 ±0.35  1.62 | 25  95.02 ±0.35  1.62 | 25  95.02 ±0.35  1.62 |
| Sausage | 1  64.71 ±0.03  4.27 | 2.5  62.74 ±0.10  6.57 | 2.5  62.74 ±0.10  6.57 | 0.25  90.96 ±0.02  5.22 | 0.5  88.77 ±0.01  2.24 | 0.5  88.77 ±0.01  2.24 |
|         | 2.5  69.50 ±0.06  3.29 | 5  66.01 ±0.16  4.78 | 5  66.01 ±0.16  4.78 | 1  85.62 ±0.06  5.56 | 1  85.62 ±0.06  5.56 | 1  85.62 ±0.06  5.56 |
|         | 5  77.96 ±0.15  3.78 | 2.5  72.14 ±0.24  3.36 | 5  72.14 ±0.24  3.36 | 2.5  90.41 ±0.03  2.18 | 2.5  90.41 ±0.03  2.18 | 2.5  90.41 ±0.03  2.18 |
|         | 10  75.91 ±0.40  2.01 | 10  78.54 ±1.14  3.97 | 10  78.54 ±1.14  3.97 | 5  88.96 ±0.17  3.47 | 5  88.96 ±0.17  3.47 | 5  88.96 ±0.17  3.47 |
|         | 25  83.84 ±0.71  4.57 | 25  83.84 ±0.71  4.57 | 25  83.84 ±0.71  4.57 | 25  89.68 ±0.66  2.43 | 25  89.68 ±0.66  2.43 | 25  89.68 ±0.66  2.43 |
| Mince  | 1  75.10 ±0.04  5.21 | 2.5  66.12 ±0.10  6.14 | 2.5  66.12 ±0.10  6.14 | 0.25  90.41 ±0.01  3.91 | 0.5  90.41 ±0.01  3.91 | 0.5  90.41 ±0.01  3.91 |
|         | 2.5  76.41 ±0.11  5.77 | 5  65.28 ±0.22  6.68 | 5  65.28 ±0.22  6.68 | 1  92.18 ±0.01  2.73 | 1  92.18 ±0.01  2.73 | 1  92.18 ±0.01  2.73 |
|         | 5  83.56 ±0.38  4.24 | 10  71.99 ±0.18  2.48 | 10  71.99 ±0.18  2.48 | 1  92.06 ±0.03  2.24 | 1  92.06 ±0.03  2.24 | 1  92.06 ±0.03  2.24 |
|         | 10  85.04 ±0.35  2.29 | 25  72.77 ±0.89  5.60 | 25  72.77 ±0.89  5.60 | 2.5  89.63 ±0.11  2.86 | 5  87.26 ±0.07  1.93 | 10  90.38 ±0.15  1.73 |
|         | 25  87.58 ±0.76  3.49 | 25  87.58 ±0.76  3.49 | 25  87.58 ±0.76  3.49 | 25  91.46 ±0.46  1.35 | 25  91.46 ±0.46  1.35 | 25  91.46 ±0.46  1.35 |

SD: standard deviation; RSDr: Relative standard deviation of replicate results.
Table 3. Inter- and intra-day repeatability of the method for different meat products for LLE, QuEChERS and IAC methods (n=4).

**Inter-day Repeatability**

|         | LLE                           | QuEChERS multi-residue | IAC                          |
|---------|------------------------------|------------------------|------------------------------|
|         | Mean recovery (%) ±SD        | Mean recovery (%) ±SD  | Mean recovery (%) ±SD        |
|         | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     |
| Spiked  | Found (mean)                | RSDr (%)               | Spiked  | Found (mean)           | RSDr (%)                     | Spiked  | Found (mean)           | RSDr (%)                     | Spiked  | Found (mean)           | RSDr (%)                     |
| (ng/g)  | (mean)                       | (±SD)                  | (ng/g)  | (mean)                | (±SD)                       | (ng/g)  | (mean)                | (±SD)                       | (ng/g)  | (mean)                | (±SD)                       |
| 1       | 0.656                        | 65.60 ±0.03            | 4.73   | 2.5                   | 1.69                         | 67.60 ±0.13               | 7.74   | 0.5                   | 0.47                         | 94.2 ±0.02               | 4.09   |
| 5       | 3.951                        | 79.02 ±0.07            | 1.87   | 5                     | 3.54                         | 70.80 ±0.23               | 6.61   | 2.5                   | 2.35                         | 94.1 ±0.08               | 3.12   |
| 10      | 84.98                        | 84.98 ±0.35            | 4.07   | 10                    | 6.98                         | 69.80 ±0.33               | 4.76   | 5                     | 4.70                         | 94.3 ±0.11               | 2.34   |
| 25      | 22.03                        | 88.12 ±0.31            | 1.39   | 25                    | 19.45                        | 77.80 ±0.87               | 4.45   | 10                    | 9.53                         | 95.3 ±0.15               | 1.62   |

| Salami  |                              |                        |                   |                              |                        |                   |                              |                        |                   |
| Sausage |                              |                        |                   |                              |                        |                   |                              |                        |                   |
| Mince meat |                            |                        |                   |                              |                        |                   |                              |                        |                   |

|         |                              |                        |                   |                              |                        |                   |                              |                        |                   |

**Intra-day Repeatability**

|         | LLE                           | QuEChERS multi-residue | IAC                          |
|---------|------------------------------|------------------------|------------------------------|
|         | Mean recovery (%) ±SD        | Mean recovery (%) ±SD  | Mean recovery (%) ±SD        |
|         | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     | Spiked (ng/g)                | Found (mean)           | RSDr (%)                     |
| Spiked  | Found (mean)                | RSDr (%)               | Spiked  | Found (mean)           | RSDr (%)                     | Spiked  | Found (mean)           | RSDr (%)                     | Spiked  | Found (mean)           | RSDr (%)                     |
| (ng/g)  | (mean)                       | (±SD)                  | (ng/g)  | (mean)                | (±SD)                       | (ng/g)  | (mean)                | (±SD)                       | (ng/g)  | (mean)                | (±SD)                       |
| 1       | 0.665                        | 66.5 ±0.04             | 6.52   | 2.5                   | 1.61                         | 64.4 ±0.11               | 6.52   | 0.5                   | 0.44                         | 88.2 ±0.01               | 2.61   |
| 5       | 3.952                        | 79.1 ±0.08             | 2.04   | 5                     | 3.27                         | 65.4 ±0.07               | 3.26   | 2.5                   | 2.28                         | 91.2 ±0.10               | 5.22   |
| 10      | 8.54                         | 85.4 ±0.38             | 4.43   | 10                    | 6.78                         | 67.8 ±0.42               | 1.77   | 5                     | 4.56                         | 91.3 ±0.14               | 3.11   |
| 25      | 21.44                        | 85.8 ±0.49             | 2.29   | 25                    | 18.78                        | 75.1 ±0.63               | 3.35   | 10                    | 9.46                         | 94.6 ±0.18               | 1.94   |

| Salami  |                              |                        |                   |                              |                        |                   |                              |                        |                   |
| Sausage |                              |                        |                   |                              |                        |                   |                              |                        |                   |
| Mince meat |                            |                        |                   |                              |                        |                   |                              |                        |                   |

|         |                              |                        |                   |                              |                        |                   |                              |                        |                   |

SD: standard deviation; RSDr: Relative standard deviation of replicate results.
The analytical results in a total of 33 meat products (31 branded and 2 unlicensed), collected randomly from supermarkets, delicatessens and butchers in Istanbul, were shown in Table 4. Only, 6 out of the 19 (36.84%) sausage samples contained citrinin at the concentration range of 0.28-1.79 ng/g and in one sample citrinin level was found at LOD level (0.083 ng/g), whereas citrinin was not detected in seven of salami and six of mince samples. Markov et al. (2013) analysed fermented meat products (n=90) by IAC-HPLC-FLD and reported that 5.55% of the products were contaminated with citrinin at the level of LOQ (1 ng/g). There is a lack of data on citrinin levels in meat products. Therefore, when we considered the studies in red yeast rice products, Marley et al. (2016) showed that five of the nine samples containing citrinin levels exceeded the EU limit in food supplements based on rice fermented with the red yeast M. purpureus obtained from different parts of China. Accordingly, survey results from Taiwan showed that mean levels of citrinin contamination were 13.3, and 0.1 µg/g for red yeast rice (raw material), and red yeast rice processed products, respectively (Liao et al., 2014). Ji et al. (2015) observed that citrinin was found in the range of 0.14-44.24 µg/g in 10 of 12 commercial red fermented rice products.

In 2014, the citrinin concentration in red yeast rice was set at a limit of 2 ng/g by European Commission Regulation (EC) 212/2014 (EU, 212/2014). No limit was set for the other food products. Indeed, in Turkey, no limit was set even for red yeast rice regarding the citrinin residues in food products. According to our results, three of six citrinin positive meat samples contained citrinin levels of more than 1 ng/g which is 50% of the maximum limit for red yeast rice in the EU. Regarding the high consumption of fermented meat products containing red yeast rice which is the source of citrinin, the need for continuous monitoring of meat products, particularly, unbranded and produced in uncontrolled facilities is urged. Therefore, the citrinin levels of meat products as well as in other food products especially in red yeast rice products should be carefully considered. Furthermore, there is a need to set the limit of citrinin in various food products in the EU and Turkey.

**Peer-review:** Externally peer-reviewed.

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Table 4. Citrinin occurrence in total of 32 meat products as determined by IAC-HPLC-FLD.

| No. of samples | Positive (%) | LOD-LOQ (%) | Range (ng/g) | Mean ± SD |
|---------------|--------------|-------------|--------------|-----------|
| Salami        | 7            | -           | -            | -         |
| Sausage       | 19           | 6 (36.84)   | 1 (5.26)     | 0.28-1.79 | 0.867 ±0.54 |
| Mince meat    | 7            | -           | -            | -         |
| **Total**     | **33**       | **7 (21.87)** | **1 (3.12)** | **0.083-1.79** | **0.867 ±0.54** |

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