Evaluation of mycotoxins and heavy metals pollution in some types of noodles in local markets

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Abstract. This study was conducted to estimation mycotoxins and some heavy metals in some types of noodles in Salah Al-din governorate markets. In this study 6 species of Aspergillus were detected which were A. flavus, A. parasiticus, A. fumigatus, A. terreus, A. ochraceus and A. niger and 5 genus of fungi were Fusarium verticillioides, Penicillium, Mucor, Alternaria and Rhizopus isolated on the three types of media used. The results show presence of the three types of mycotoxins in all samples. The concentrations of the aflatoxin B1 ranged between 1.145 - 5.041 ppm. Concentrations of fumonisin between 8.498 - 19.496 ppm. Concentrations of ochratoxin A ranged between 3.960 - 52.543 ppm. The results also show that the concentration of copper and cadmium was at (0.50 - 1.23) (0.05 - 0.31) ppm respectively, while the concentration of lead was less than 0.1 ppm in all samples.

Keywords. Noodles, Aflatoxin, Ochratoxin, Fumonisin, Fungi, Heavy metals.

1. Introduction

Instant noodles is one of breakfast and staple food item of East Asian countries, whose consumption is gradually increasing day by day worldwide. Noodles, being a poor source of proteins due to use of refined flour in its production [1]. Worldwide, China ranks first in the consumption of noodles followed by Indonesia, Japan, and Vietnam [2]. [3] listed the ingredients of Instant Noodles (product) Neoguri Udon (Seafood & Spicy) consist of, noodles, potato starch, wheat flour, palm oil and salt, Dried solids packet, Mussel, green onion, carrot. Powder soup packet, salt, monosodium glutamate, glucose sugar, Soy sauce powder, Spices, corn flour, caramel color. Mycotoxins can be produced at the growth and storage of crops [4]. Aflatoxins considered toxic secondary metabolites of fungal origin contaminate agricultural crops before, during and after harvest [5]. Aflatoxins are a poisonous, carcinogenic of mycotoxins that are produced by the fungi Aspergillus flavus and Aspergillus parasiticus; there are 20 different types of aflatoxins identified until now, four groups of aflatoxins B1, B2, G1 and G2 [6]. Aflatoxins are toxic products by fungal metabolism, associated with serious health consequences and substantial economic losses for agriculture, livestock and poultry sectors in the developing countries [7]. One hundred wheat product samples (50 instant noodle and 50 bread samples) were collected from supermarkets in Bangkok, Thailand aflatoxin B1 contamination in these products 1 ng / g [8]. In another study no contamination with Afla B1 was detected in wheat flour in
Italy [9]. A broad spectrum of *Fusarium* species causes diseases in plants and produces important mycotoxins like trichothecenes, zearalenone and fumonisins, which are the major threats to animals and humans [10]. Fumonisins B1 (FB1) is a mycotoxin produced in some grains (mainly corn) by *Fusarium* species such as *Fusarium verticillioides* and *Fusarium proliferatum* [11]. Fumonisins B1 include many types FB1, FB2, and FB3, occurred with the highest frequency, whereas FB1 found at the highest concentrations [12]. Fumonisins cause health effects in the liver and kidney, the health effects of fumonisins in humans was limited [13]. Fumonisins contamination most commonly in the cereals (rice, barley, wheat, maize, oat, rye and millet) [14, 15]. Ochratoxin (OTA) has been to be nephrotoxic, hepatotoxic, and immunotoxic in various animals and was classified by the International Agency for Research on Cancer as possibly carcinogenic to humans [16]. The mode of action is not clear discussed [17, 18]. *Aspergillus ochraceus* known to produce OTA and OTB, is a contaminant in many foods including wheat (grains and berries) and cereals [19]. Ochratoxin A content in the corn and wheat samples ranging 0-25.90 ng/g [20]. Concentration of OTA in raw wheat grain including (durum wheat, spelt, and emmer) should contain at maximum 5 μg/kg [21]. Heavy metals on the earth’s crust are significantly contaminated to environment by mining and processing metal, Human activity such as pesticide and herbicide also occurred heavy metals in environment [22]. Studies on heavy metals considered important from public point, where the attention has drawn to necessity of measuring the accumulation of heavy metals, those metals which serious hazards to human [23]. The study aimed to detect the presence of fungi, mycotoxins and the levels of Cd, Pb and Cu in instant noodle types (packets) that manufactured in some different countries which may effect on human consumption.

**2. Materials and Methods**

**2.1 Collection of samples**

Ten types of noodles samples (curry with vegetables, spring vegetables, cream of mushrooms, chicken cream, maggi mushroom cream, bechamel mix, chicken with noodles, creamy vegetables, pasta flavored chicken, lentil soup) were collected from Salah al-din Governorate markets with three replicates and it was saved until the estimate.

**2.2 Isolate and identification of fungi**

The fungal isolates were depending on classification key ([24, 25, 26] by observing growing on (CYA) Czapek Yeast Capek extract Agar, and Rose Bengal agar and PDA media, and the physical characteristics of the mycelia such as features (shape, size, color, and hyphae) by the compound microscope contain a digital camera and using a lactophenol cotton blue stained on slide mounted with small parts of mycelium.

**2.3 Quantification of aflatoxin B1, fumonisin B1 and ochra A by ELISA**

The mentioned method was followed by a company Shenzhen Lvshiyuan Biotechnology Co. Ltd. China prepared for the materials and solutions used in extraction and determining the aflatoxin B1, fumonisins B1 and ochratoxin A using the ELISA method [27]. This method depends on mixing a specific amount of the complex of each poison-enzyme with a specific amount of the sample to be examined and mixed well and added to the titration plate covered with antibody surface. After incubation for a period of 15 minutes at room temperature, competition is between the toxin in the sample and the toxin associated with the enzyme with these bodies. Then the substance subject to the enzyme is added to the reaction solution and the reaction is stopped when the blue turns yellow. The optical intensity of the color of each slide was read using the spectrometer of the ELISA plates Bio_Tek Korean origin at a wave length 450 nm, in the central laboratory of Tikrit University.
2.4 Determination of heavy metals

Determination of heavy metal in 10 duplicate Noodles samples are analysis by the Atomic Absorption type ELCO [28]. A known volume of noodles samples was transferred to electric oven for drying and Muffle Furnace USA at 600 °C to weight stable. Ashed taken up in 5 ml of 5 % HNO₃ and filtration by Whatman No. 1. The contents of Cd, Cu and Pb were direct determined by Atomic Absorption Spectrophotometer type Shimadzo-6200 (Japan) for each heavy metals copper, cadmium and lead to be determined and measured by placing the sample in a transparent liquid form.

3. Results and Discussion

3.1 Occurrence of fungi in noodles

In this study 6 species variety of Aspergillus were detected such as A. flavus, A. parasiticus, A. fumigatus, A. terreus, A. ochraceus and A. niger and 5 genus of fungi were isolated on the three types of media used, other genus Mucor spp., Fusarium verticillioides, Penicillium spp., Rhizopus spp. and Alternaria spp. (Table 1). A. flavus, A. parasiticus, A. ochraceus and Fusarium verticillioides found in all samples, other genus were isolate from some samples. Numerous studies have shown that fungi generating toxins, especially Aspergillus considered one of the main pollutants in foods and fungi A. flavus and A. niger are the most isolated species [29].

Table 1. Number of Fungi isolates in some types of noodles.

| No. | Fungi                  | Isolates No. |
|-----|-----------------------|--------------|
| 1   | A. flavus             | 15           |
| 2   | A. parasiticus        | 12           |
| 3   | A. fumigatus          | 5            |
| 4   | A. terreus            | 4            |
| 5   | A. ochraceus          | 11           |
| 6   | A. niger              | 8            |
| 7   | Mucor spp.            | 3            |
| 8   | Fusarium verticillioides | 15        |
| 9   | Penicillium spp.      | 6            |
| 10  | Rhizopus spp.         | 3            |
| 11  | Alternaria spp.       | 2            |

The current results coincided with [30] isolate and identify fungi from food resources (Indian rice, rice, popcorn, pasta, pistachios and noodles), showed to 577 isolates of fungal genera and species, A. flavus, A. niger, Alternaria, Penicillium, Aspergillus terreus, Rhizopus and Aspergillus prasiticus are 249, 149, 91, 43, 28, 10, and 7 isolates, respectively. One of the most common types is found in food sources A. niger, A. ochraceus, A. terreus, A. flavus, A. fumigatus, A. parasiticus and A. versicolor [31]. The reason for the emergence of fungal in foodstuffs is that the species possesses the ability to secrete a large number of enzymes that analyze foodstuffs that are used in nutrition and growth as well as increasing its spreading capacity, especially since some of its types can grow in a low moisture content as well as the relative density of the boards that produce them [32].

3.2 Concentrations of mycotoxins in noodles samples

Figures 1, 2 and 3 show the concentrations of the toxins of aflatoxin B1, fumonisin B1, and ochra A in some types of noodles in Salah El-Din Governorate markets. The results show the presence of the three types of mycotoxins in all samples. The concentrations of the aflatoxin B1, fumonisin B1 and ochratoxin A between (1.145 - 5.041) (8.498 - 19.496) (3.960 - 52.543) ppm respectively. This may
be due to infection with molds that produce toxins in the field or during storage, and provide the appropriate conditions for the production of these types of mycotoxins [33].

Figure 1. Concentration of aflatoxin B1 in some types of noodles

Figure 2. Concentration of fumonisin B1 in some types of noodles.

The highest of aflatoxin concentrations of Afla B1, Afla B2, Afla G1 are 65.77, 19.27, 1.02 µg/kg respectively. For the cereal food category, CBS11 recorded the quantities of Afla B1, Afla B2, Afla G1 and Afla G2 at 35.46, 4.92, 3.39 and 0.32 µg/kg respectively, the pasta category, PS1 recorded the highest quantities of 0.94 and 0.85 µg/kg for Afla B1 and Afla B2 respectively [5]. The highest fumonisin B1 value 1.46 and 0.46 mg/kg was measured in 64 maize flour and corn flakes samples respectively [34], [35] showed positive OTA levels ranging from 0.6–3.4 in wheat flour samples µg/kg. OTA was detected in forty samples contain corn flour, corn flakes, wheat flour, bread and biscuits samples was 0 - 25.90 ng /g, the greatest level was measured at 360 ng /g of ochratoxin A in biscuit sample [20]. And that these results obtained were higher than the allowable limit by the European Union, which is 2 µg / kg of aflatoxins, also the concentrations of fumonisin B1, the permissible limit ranges between 200 - 800 ppb, according to the standard specifications set by the Codex Committee [36].
FB1 toxicity affects mainly the liver organ, which is characterized by apoptotic, necrosis, and regeneration [37]. A recent study has found that FB1 can cause changes in the tissues of liver, lung, and kidney such as apoptosis and necrosis, leading to infiltration of inflammatory cells which were observed in these organs [38]. As well as for other agricultural crops, such as wheat, rice, field pistachios, and soybeans, where many studies have recorded high rates of pollution of these crops from aflatoxins [39]. The JECFA (FAO)/(WHO) has set the provisional acceptable weekly intake of OTA at the rate of 100 ng / kg and 14 ng / kg of body weight per day [40].

3.3 Concentration of heavy metals in noodles samples

Figure 4, 5 and 6 showed that the concentration of heavy metal in some types of noodles. The results indicate to the concentration of Copper and Cadmium at (0.50 - 1.23) (0.05 - 0.31) ppm respectively. While the concentration of Lead less than 0.1 ppm in all samples. These results was accepted with [41] reported the concentrations of Cd, Pb and Cu in Iranian noodles at 0.469, 2.797 and 1.563 mg/kg and in imported noodles 0.62, 3.07 and 11.866 mg/kg respectively. Also accepted with [42] found the magnitude of heavy metals as Pb Ca Ni and Ar (1.17-1.67), (0.53-0.82), (0.27-0.43), (0.17-0.41) mg/kg respectively contamination in noodles samples. The concentration levels of heavy metals Tin, Cadmium, Zinc, Manganese, Iron, and Cobalt in six type of noodles, i.e., Maggi, Knorr, Shan shoop, Shinramayun, Indomie, and Mama observed at (3.3–9.7), (0.066–0.59), (6.2–11.4), (2.4–6.3), (4.8–17.5), and (0.39–0.74) mg / kg respectively [43].
The concentration of lead, Cr, cadmium and aluminium in Iranian instant noodle at 1.21, 0.08, 0.03 and 9.15 mg/kg and in imported instant noodle at 1.00, 0.07, 0.04 and 15.90 mg/kg, respectively [44]. [45] reported that cadmium concentration determined in some foods and beverages in Japan follows cereals, seasoning and canned in syrup at (0.004 - 0.380) (0.01 - 0.06) (0.01) ug/ g respectively. [46] reported that the conventional agriculture was contaminated by lead, the possible sources of Pb in wheat include irrigation with contaminated water, metal based pesticides, industrial emissions, transportation and method of harvesting and storage. According to [47, 48] weekly intake of metals from sources not exceed 0.05 and 0.075 mg/kg body weight for Pb and Cd respectively, it accumulation in the skeleton and cause renal tubular damage and may give rise to kidney damage, Cd is among the most heavy metals and is particularly toxic. [49] Showed that changes were recorded in various parameters of oxidative damage, while the accumulation of metals in tissues with disturbances of hematological and biochemical parameters, it was observed levels of heavy metals in tissues had different pattern after mixture and single exposure.

4. Conclusion

The current study represents for detecting mycotoxins AFB1, OTA, FB1 by ELISA. Isolate and identify fungi and determination of heavy metals in some noodles samples. The results show to the all samples of noodles were contamination with aflatoxin B1, ochratoxin A and fumonisyn. Fungi A.
flavus, A. parasiticus, A. fumigatus, A. terreus, A. ochraceus and A. niger, other genus Mucor, Fusarium verticillioides, Penicillium, Rhizopus and Alternaria. Heavy metals copper, cadmium and lead.

5. References

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