pagepress

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Presence of aflatoxins B and G and ochratoxin A in artisanal dried fruit ice cream produced in the province of Messina, Italy

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Abstract

This study aims to investigate and identify the presence of aflatoxins B and G, as well as ochratoxin A, in pistachio and hazelnut ice cream samples produced at the artisanal level in the province of Messina, Italy. This research is motivated by concerns regarding the sourcing of raw materials and ingredients, such as pure nut paste, used in artisanal ice cream production. It is hypothesized that these ingredients may contain higher levels of contaminants compared to those used at the industrial production level, where the control over raw materials is generally more stringent. Considering that approximately 10% pure paste is used in the preparation of artisanal ice cream, the concentrations of pure pistachio and hazelnut pastes were calculated by adjusting the results obtained from the analysis of the ice cream for a dilution factor of 1:10. According to Regulation (EU) No. 2023/915, 36% of both pistachio and hazelnut pure pastes were found potentially to be non-compliant with aflatoxins and ochratoxin A. The results substantiate the hypothesis regarding the critical importance of sourcing pure nuts at the artisanal level. They underscore the necessity of supplier validation within this production segment, indicating that ice cream can also pose a risk of consumer exposure to aflatoxins and ochratoxin A.

Key words: mycotoxins, Regulation (EC) 2023/915, pistachio paste, hazelnut paste, dilution factor.

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Introduction

Ice cream is one of the world's most popular foods, whose global market has grown steadily in recent years, from $57 billion in 2017 to nearly $75 billion in 2024 (Bedford, 2022; Nalbone *et al*., 2022). According to market analysis, pistachio is the favorite ice cream flavor among consumers, followed by chocolate and hazelnut (Ansa, 2023). This is in line with the general increase in the consumption of nuts and nut products in recent decades, as these products are known to contain several health-promoting compounds, including macronutrients, micronutrients, fat-soluble bioactives, fiber, and water-soluble vitamins (Gervasi *et al*., 2021). In this regard, scientific evidence suggests that the regular intake of nuts (20-30 g/day, according to the Food-Based Dietary Guidelines recommendations for nuts and seeds) can provide several health benefits (European Commission, 2024). In particular, pistachios contain at least 15 essential micronutrients, as recognized by the U.S. Food and Drug Administration (FDA), supplying over 10% of the daily recommended value with a single serving (28.5 g) (FDA, 2016; Mandalari *et al*., 2021). Despite these nutritional qualities, it is well known that the consumption of nut products is generally associated with the potential assumption of mycotoxins, harmful substances produced by the metabolism of various molds which frequently contaminate nuts and produce these toxins during prolonged storage (Łozowicka *et al*., 2024). There are several types of mycotoxins for human health, such as aflatoxins (AFs) B1, B2, G1, G2 and ochratoxin A (OTA), generally produced by molds of the *Aspergillus* and *Penicillium* genus, which contaminate various substrates such as nuts, cereals, oilseeds, cocoa beans, coffee, and spices (El-Sayed *et al*., 2022). AF M (hydroxylated products of AF B) are less harmful than other ones and have been extensively investigated in milk and dairy products (Muaz *et al*., 2022). Mycotoxin danger resides in their chronic toxicity, because the continuous ingestion of small doses of toxins induces accumulation damage (Janik *et al*., 2021). In this regard, mycotoxins are genotoxic, carcinogenic, immunotoxic, mutagenic, nephrotoxic, and teratogenic (Ülger *et al*., 2020). As previously mentioned, there are several potential sources of exposure to mycotoxins. However, a recent European Food Safety Authority scientific opinion on the "Risk assessment of aflatoxins in food" provides a deeper understanding of the role of nuts as a source of AFs (EFSA CONTAM, 2020). It highlights that the highest mean concentrations of AF B1 and AF T (the sum of B and G AFs) are found in the food category 'legumes, nuts and oilseeds' (especially pistachios, peanuts, and 'other seeds'). Therefore, it is presumable that also the derived products, such as several kinds of sweets or ice cream containing a relevant concentration of nuts, could represent an important source of AFs. Despite this, as far as ice cream is concerned, we are not aware of any investigations into the matter except in the case of AF M, whose presence in ice cream is widely investigated (EFSA CONTAM, 2020; Muaz *et al*., 2022). For these reasons, the present study aimed to evaluate the presence and concentration of AF B, G and

OTA in artisanal pistachio and hazelnut ice cream produced in the city of Messina, to verify if the procurement of pistachio and hazelnut paste, used for the processing of ice cream, could be critical for the artisanal production. In this regard, the mycotoxin limits in nuts and their derived products are precisely regulated by Regulation (EU) 2023/915 (European Commission, 2023), and it is unlikely that the utilization of non-compliant products in the industrial ice cream production, where the analytical control of raw materials is normally planned. Our hypothesis was the potential distribution of non-compliant nut paste to the artisanal producers who are unable to punctually verify these parameters.

Materials and Methods

For the current research, 50 samples of artisanal pistachio ice cream (25) and hazelnut ice cream (25) were collected from parlors in the province of Messina. The samples were analyzed twice. Specifically, 20±0.1 g of each sample was transferred into a beaker with 4 g of sodium chloride, and 100 mL of a methanol/water extraction mixture was added. After homogenization with an ultra-turrax and paper filtration, the sample was diluted in phosphate buffered solution, refiltered through glass microfiber, and purified using a specific immuno affinity column (Aflastar and Ochrastar, Romer Labs, Getzersdorf, Austria) according to the manufacturer's instructions. Reverse phase chromatographic analysis of purified extracts was conducted by a High-Performance Liquid Chromatography Nexera LC-40 (Shimadzu Italia, Milan, Italy) equipped with a fluorescence detector RF-20A XS and a column Kinetex C18 5µm, 250×4.6 mm. The operating conditions for Fs analysis were as follows: i) mobile phase: water/methanol/acetonitrile (*v*/*v*) in a ratio of 6:3:1; ii) flow rate: 1 mL/min; iii) oven temperature: 50°C; iv) cell temperature: 25°C; v) injection volume: 8 µL; vi) detention: ʎ Ex:365 nm - ʎ Em:455 nm; for OTA, analysis was performed as follows: i) mobile phase: acetonitrile\acetic acid 2% (*v*/*v*) in a ratio of 43.5:56.5; ii) flow rate: 1 mL/min; *iii*) oven temperature: 25°C; iv) cell temperature: 25°C; v) injection volume: 20 µL; vi) detention: ʎ Ex:333 nm - ʎ Em:460 nm. Mycotoxin concentrations were calculated against calibration curves obtained by the analysis of a mix of AFs B1, B2, G1, and G2 (Restek, Pennsylvania, USA) or OTA (Romer Labs, Getzersdorf, Austria) appropriately diluted in the range of 0.03-10 ppb, in methanol and methanol/glacial acetic acid 98:2, respectively.

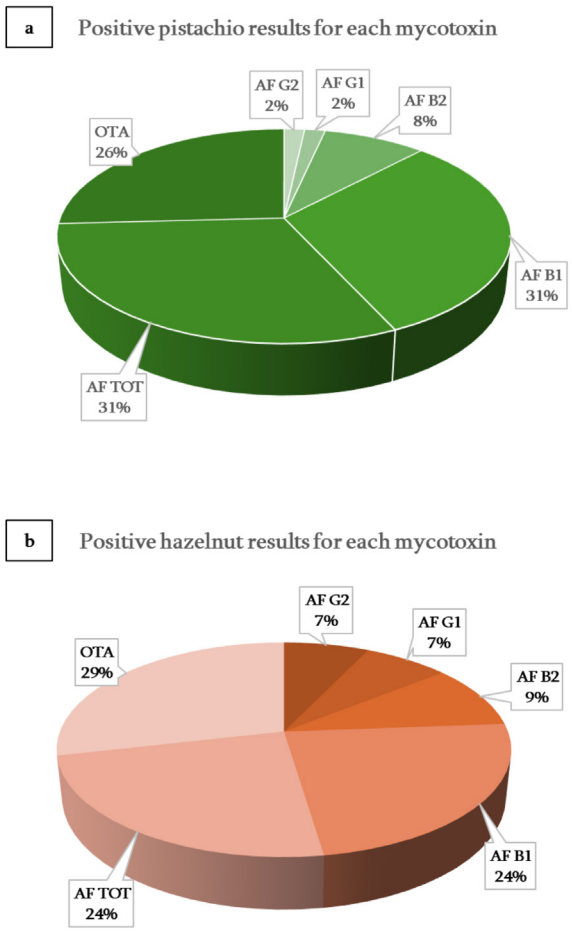

Figure 1. Percentage of positive samples for each mycotoxin in pistachio (**a**) and hazelnut (**b**) ice cream samples. AF, aflatoxin; OTA, ochratoxin A.

Figure 2. Distribution of samples by concentration classes for aflatoxin B1 (**a**), total aflatoxin (**b**) and ochratoxin (**c**) in pistachio (green bars) and hazelnut (brown bars) ice cream.

Results and Discussion

The percentage of pistachio ice cream samples testing positive [≥ limit of quantification (LOQ)=0.1 µg/kg] was 84%. Specifically, AF contamination was detected in 18 samples (72%), while OTA was found in 15 samples (60%). Additionally, 11 samples (44%) exhibited the simultaneous presence of both AFs and OTA. For hazelnut ice cream, the percentage of positive samples (≥LOQ=0.1 µg/kg) was 64%. AF contamination was detected in 10 samples (40%), while OTA was present in 12 samples (48%). Furthermore, 5 samples (20%) showed the simultaneous presence of AFs and OTA. Figure 1 displays the percentage of positive samples for each mycotoxin in pistachio (Figure 1a) and hazelnut (Figure 1b) ice cream samples. Figure 2 shows the distribution of samples by mycotoxin concentration classes in ppb. Considering that Regulation (EC) 2023/915 (from now on "Regulation") sets limits of 8 µg/kg for AF B1, 10 µg/kg for total AFs, and 5 µg/kg for OTA in pistachios and their processed products, and for hazelnuts and their processed products, the limits are 5 µg/kg for AFB1 and OTA and 10 µg/kg for total AF s, all samples complied with the law currently in force. However, according to the Regulation, these limits apply to processed products consisting of at least 80% of tree nuts. For derivatives where the nut content is diluted beyond this percentage, Article 3 of the Regulation specifies that the 'dilution factor' must be considered. It is well known that the percentage of pure nut paste used in the artisanal production of ice cream is about 10%. Therefore, our results could be reinterpreted in light of this dilution factor. Particularly, considering Figure 2, the third class of mycotoxin concentration (>0.8 ppb, or >0.5 for hazelnut samples of AF1; >1 ppb for total AF; >0.5 for OTA) represents the percentage of samples not complying with limits of the Regulation. Beyond this consideration, which cannot be deemed entirely reliable since the dilution factor applicable to each analyzed sample is uncertain, our findings lead us to suspect that in the production of artisanal ice cream, it is plausible that a significant percentage of cases involve the use of pure paste not in compliance with the limits set by European Union regulations. These findings complement other research on the hygienic characteristics of ice cream (Nalbone *et al*., 2022) and raise important considerations regarding the food safety of its consumption.

Conclusions

Controlling the mycotoxin concentration of products in the market can be quite challenging, especially within smaller distribution channels. This issue is particularly evident in the field of artisanal ice cream, where the raw ingredients used may not be held to the same strict regulations as those used in industrial ice cream production. As a result, the oversight of these ingredients can vary significantly, impacting the overall quality and safety of the final product. The results of this study reveal that compliance with current legislation regarding raw materials, such as nut pastes, is often inadequate among small-scale producers. This study highlights the need to draw attention to these issues to advocate for enhanced regulatory measures during both the market entry phase of raw materials and the self-regulatory processes employed by small producers. Addressing these issues is crucial to ensure product integrity and safety for the consumer.

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