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Review

Mycotoxins in Functional Beverages: A Review

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Received: 18 June 2020; Accepted: 18 August 2020; Published: 19 August 2020

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Abstract: Consumer dietary habits have drastically changed in recent decades and functional beverages now have a strong position in the market. The majority of these beverages are produced using simple processes that use raw products, such as cereals, legumes, fruits, and nuts, among others, and these are known to be frequently contaminated with mycotoxins. This review is focused on the occurrence of these toxic compounds in plant-based milks, fruit juices, and herbal teas. The fate of the toxins during processing is discussed to establish the potential risk posed by the consumption of these kind of beverages regarding mycotoxin uptake.

Keywords: plant-based milk; fruit juices; herbal teas; aflatoxin; ochratoxin A; *Fusarium* toxins; patulin

1. Introduction

Over recent years, our society began demanding healthier products, and this has drastically changed dietary consumer habits. In this context, functional foods provide health benefits beyond the basic nutritional functions, and beverages, including plant-based milks, fruit juices, and herbal teas, are by far the most important category [1]. The main purposes of consuming these beverages are related to boosting energy; fighting ageing, fatigue, and stress; weight management; targeting specific diseases, such as hypercholesterolemia, and helping to lower glucose levels [2,3]. Their formulation supposes the presence of some bioactive ingredients with specific health benefits (i.e., vitamins, minerals, fiber, omega-3 fatty acids, flavonoids, phytosterols, probiotics, prebiotics, etc.) or the reduction in undesirable ingredients (i.e., sugar, fats, etc.) [3,4]. The determination of the real worldwide market of functional beverages is difficult to establish due to the lack of an internationally accepted definition regarding these products. However, recent reports indicated that the global annual growth rate might be near 9%, and by 2025, functional beverages are expected to represent 40% of the overall consumer demand [4].

Mycotoxins are fungal secondary metabolites that are toxic to animals and humans and are a considerable threat to food safety [5]. More than 300 mycotoxins have been described, although aflatoxins, trichothecenes, zearalenone (ZEN), fumonisins, ochratoxin A (OTA), and patulin are considered as the most important due to their high toxicity and frequent occurrence in food products [6]. These mycotoxins often contaminate the raw products used to prepare functional beverages including cereals [7,8], legumes [9–13], nuts [11,12,14], fruits [15–17], and herbs [18–20], among others. This contamination is related to the occurrence of mycotoxin-producing fungi in raw products, which, under permissive conditions, are able to synthetize these compounds at variable levels. The genus *Aspergillus* includes the most significant aflatoxin producing species (*A. flavus* and *A. parasiticus*) as well as relevant OTA producing species that are classified in sections *Nigri* and *Circumdati* (*A. carbonarius*, *A. niger*, *A. welwitschiae*, *A. westerdijkiae*, and *A. steynii*) [5]. Trichothecenes, fumonisins, and ZEN are known as *Fusarium* toxins, because their main producing species are included in this important fungal genus [21]. The presence of *Penicillium expansum* is a high concern with regard to patulin contamination primarily in fruit-derived products [22]. Table 1 indicates the most relevant mycotoxins, as well as their producing fungi and occurrence in the products used to prepare functional beverages.

Table 1. Main mycotoxins occurring in the raw products used to prepare functional beverages. Their most relevant producing fungi in these matrices are also indicated.

Mycotoxins	Fungal Species	Product
Aflatoxins (B1, B2, G1, G2)	*A. flavus* *A. parasiticus*	Cereals, nuts, legumes, herbs
Ochratoxin A	*A. carbonarius* *A. niger* *A. welwitschiae* *A. westerdijkiae* *A. steynii*	Cereals, fruits, legumes, herbs
Fumonisins (B1, B2)	*F. verticillioides* *F. proliferatum*	Cereal, legumes, herbs
Trichothecenes (DON, T-2, HT-2)	*F. graminearum*, *F. culmorum* *F. sporotrichioides* *F. langsethiae* *F. equiseti*	Cereals
Zearalenone	*F. graminearum* *F. culmorum*	Cereals, herbs
Patulin	*P. expansum*	Fruits

Considering that these raw materials are known to be contaminated by mycotoxins, the fact that these compounds might be present in the final product should be considered as they are chemically and thermally stable during food processing [6,23]. The best option to avoid mycotoxin contamination in raw materials is the maintenance of adequate conditions to prevent fungal growth. This aspect might be crucial in developing countries where good agricultural, manufacturing, and storage practices are difficult to apply, and accordingly, raw products are more contaminated by mycotoxins [24]. Due to the simple processing, the intake of natural beverages is widespread in these regions, and therefore, the population might be more exposed [25].

Despite the above, up to now, little information has been available regarding the presence of mycotoxins in functional beverages. This paper is a state-of-the-art review regarding the occurrence of mycotoxins in functional beverages. It presents a discussion of the most probable mycotoxins that might be contaminating these products when considering both the raw products and the technological processes used in their production.

2. Plant-Based Milks

Many consumers avoid dairy products due to medical reasons, such as lactose intolerance or cow's milk allergies, whereas many others demand cow's milk alternatives as a result of lifestyle choices including veganism and vegetarianism [26,27]. The benefits of these plant-based milk products include the following: do not contain cow's milk protein and cholesterol, present a low saturated fat content, and contain fibers and isoflavones [28]. They are a suitable medium for probiotic bacteria, and therefore, plant-based milks act as a source of these beneficial microorganisms for people who are not consuming dairy products [3]. Currently, great investments are made in research to modify the technological processes to maximize the quality and nutritional content of plant-based milks as well as to improve their palatability and extend their shelf life [2,28]. Considering all these aspects, it is essential to guarantee food safety related to these milk substitutes.

The most accepted classification of these plant-based milks contains five categories [2]: (i) cereal-based (i.e., oat, rice, corn, or spelt milk), (ii) legume-based (i.e., soy, peanut, lupin, or cowpea milk), (iii) nut-based (i.e., almond, coconut, hazelnut, pistachio, or walnut milk), (iv) seed-based (i.e., sesame, flax, hemp, or sunflower milk), and (v) pseudo-cereal-based (i.e., quinoa, teff, or amaranth milk).

Plant-based milks are basically water extracts of dissolved and disintegrated plant material, and therefore, their production process is quite simple [27]. The most common process to produce plant-based milk uses a wet processing method in which the material is soaked and wet milled to extract milk constituents [29]. Subsequently, the grinding waste is separated by filtering or decanting, and depending on the product, some other ingredients can be added (i.e., sugar, flavoring, oil, etc.) [26]. Thermal processing, such as conventional pasteurization or ultra-high temperature (UHT) treatments, is typically performed to inactivate spoilage or pathogenic microorganisms [27,29]. The general processing steps involved in the production of plant-based milks are indicated in Figure 1.

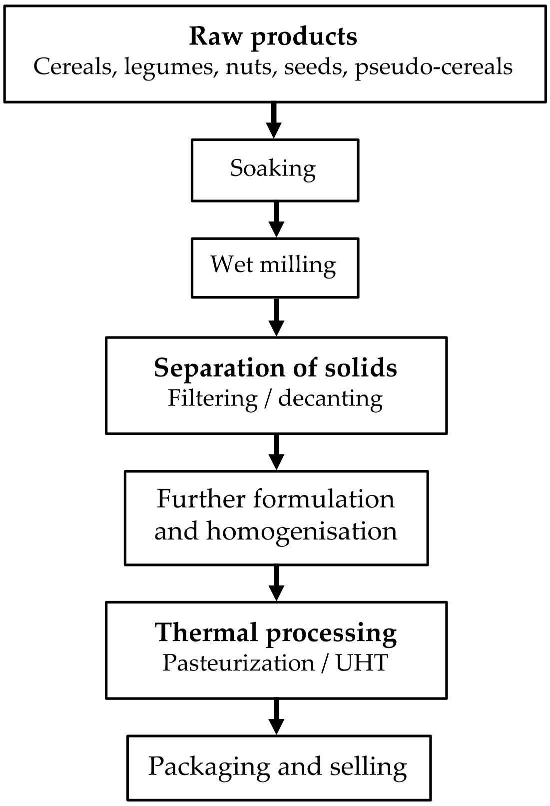

Figure 1. General manufacturing process of plant-based milks starting from raw materials.

Few reports are available regarding the presence of mycotoxins in plant-based milk; however, the reports agree that the presence of these toxins in raw products may pose a risk for the contamination of the final products. Considering the production process, mycotoxins are not drastically reduced in any of the steps, even after thermal processing to reduce microbial contamination. Mycotoxins are heat-stable molecules and some of them, including aflatoxins, OTA, ZEN, and deoxynivalenol (DON), present a decomposition temperature higher that the reached in UHT treatment [30]. Only after long periods maintained at an extremely high temperature are mycotoxin levels reduced to some extent [31]. Therefore, the presence of mycotoxins on raw products might be an indicator of a possible risk of contamination of plant-based milk.

Several works studied the occurrence of different mycotoxins in oat, soy, and rice-based milks, as they are the most consumed worldwide [32–35]. Miró-Abella et al. [32] reported, for the first time, the presence of mycotoxins in plant-based milks and found DON, OTA, ZEN, and T-2 toxin together with aflatoxins B1, B2, G1, and G2 present in certain samples, with oat-based beverages being the most frequently contaminated. The risk posed by *Fusarium* toxins in these plant-based milks were also studied by Hamed et al. [33], and DON was found in oat-based commercial drinks. The occurrence of emerging mycotoxins, such as enniatins and beauvericin, in plant-based milks was also evaluated by Arroyo-Manzanares et al. [34]. The authors reported that 75% and 12.5% of oat and soy-based milks, respectively, were contaminated by at least one of these emerging mycotoxins. All the authors mentioned above attributed the transfer of mycotoxins from raw cereals to the beverages as the most probable source of plant-based milk contamination and proposed that new research is required to study the fate of the toxins along the production processes.

Wheat milks are also susceptible of mycotoxin contamination. El-Badry [36] reported that most of wheat milk groats (80%) contained OTA at high levels. This aspect should be carefully considered as wheat milk is a product frequently used to supplement infant diets, which are especially susceptible to the toxic effects posed by mycotoxins.

Apart from the new trendy plant-based milk products commented above, a traditional tigernut milk beverage, called horchata, is widely consumed in Spain [37]. Several mycotoxins have been detected both in tigernuts and their derived beverages, including aflatoxins (B1, B2, and G2) and OTA [38,39]. However, the source of contamination was demonstrated to be related to the origin of the samples as well as to the production process applied. Rubert et al. [39] reported no contamination with mycotoxins in sterilized and concentrated horchata, whereas fresh horchata was frequently contaminated, which might be related to the ability of the fungi to produce mycotoxins directly in the beverage. The high-temperature treatment reduced the fungal viability, whereas increased sugar concentration interferes with fungal growth. On the other hand, Sebastia et al. [38] found that tigernut milk identified with the Protected Origin Designation from Valencia was safe from being contaminated by mycotoxins due to the strict controls that guarantee their quality.

Some authors suggested that tigernut milk beverages might be subjected to a fermentation process by lactic acid bacteria [37]. These microorganisms have been reported to be able to interfere with fungal development and degrade mycotoxins, and this can help to guarantee a mycotoxin-free beverage. However, extensive research is still required to select the most adequate starters to guarantee the safety of fermented tigernut milk beverages [37].

Plant-based milks are complex matrices that include high contents of proteins, carbohydrates, lipids, and fiber [28]; therefore, it is indispensable to optimize the analytical procedures, mainly the sample pretreatments and clean-up steps to avoid the matrix effects that might interfere in quantification by analytical instruments [40]. Due to the increasing importance of plant-based milks, several authors developed new detection methods to detect different mycotoxins in these products by applying different pretreatment methods to avoid matrix effects. These methods are based on salting out assisted liquid–liquid extraction in the case of *Fusarium* toxin determination [33,34], dispersive liquid–liquid microextraction for aflatoxins [35], or QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction in the case of a multidetermination of 11 mycotoxins [32]. In the case of mycotoxin determinations in tigernut milk, a matrix solid phase dispersion was adapted to eliminate lipidic interferences [38,39].

3. Fruit Juices

Fruit juices offer an image of fresh and healthy products to consumers that are seeking alternatives with a high content in nutrients and bioactive components (i.e., vitamin A, vitamin C, minerals, polyphenols, dietary fiber, etc.) [41,42]. Fruit juices are an optimal media to promote the growth of probiotic bacteria [1,4].

It is important to highlight that juice production is quite simple, and these products usually consist of different types of squeezed, blended, or shaken fruits without any further processing. It is well known that mycotoxins might be transferred to the juice if rotten fruits are not removed during processing [42]. Therefore, it is essential to prevent mycotoxin production in unprocessed fruit in order to avoid juice contamination. Different methods proposed to avoid fungal proliferation in fruits, including the application of good agricultural and storage practices, the application of antifungal agents, and the use of genetically engineered products, have been described as useful to prevent mycotoxin contamination of fruits [17]. Children are the major consumers of juice and are also the most susceptible to their toxic effects, which drastically increases the social concern regarding this issue [16,43]. Pallarés et al. [44] demonstrated a high dietary exposure through fruit juices in children that reached considerable percentages of the tolerably daily intake for certain mycotoxins.

Traditionally, the most relevant toxins occurring in juices are patulin and OTA, frequently found in apple and grape juices, respectively, and review articles on this topic are available [15,45,46]. Regarding the presence of other mycotoxins, aflatoxins and *Alternaria* toxins have been also reported in different kinds of juices, and their presence should not be disregarded [43]. Consumers are demanding new tastes and textures, which has opened a new market of juices from non-conventional fruits or even multi-fruit products. These aspects highlight the importance of new studies on the occurrence of mycotoxins in juices including the possible co-occurrence of several mycotoxins. Pallarés et al. [44] recently reported a common multicontamination of fruit juices and found co-occurrence up to seven different mycotoxins in some cases.

Although there are relevant problems to human health, only a few regulations are available worldwide to establish the maximum levels of mycotoxins in juices. Since 2006, the European Union limits the patulin content in fruit juices (50 μg/kg) and OTA in grape juices (2 μg/kg) [47]. The same legislations regarding OTA and patulin are applied in Brazil, but in this country, the levels of the latter toxin are only regulated in apple juices [48]. China also allows maximum levels of 50 μg/kg of patulin in fruit and vegetable juices [49].

The presence of *Alternaria* spp. is frequently detected in fruits and vegetables, and this ubiquitous genus is considered one of the main spoilage fungi of these products [43]. Little is known regarding the natural occurrence of *Alternaria* toxins in fruit juices. However, many authors have indicated that these toxins are of interest from the human health viewpoint, as they have been reported in a variety of juices prepared from apples, grapes, different berries, and prunes [15]. Escrivá et al. [50] reported that *Alternaria* toxins are highly stable in fruit juices and are able to remain stable for long periods, which makes very difficult to decontaminate these products. The European Food and Safety Authority (EFSA) highlighted the importance of *Alternaria* toxins due to increasing dietary exposure and encouraged the scientific community to generate more analytical data on these mycotoxins in relevant food commodities, including fruit juices [51]. However, no regulations regarding the maximum levels of *Alternaria* toxins have yet been established.

As mentioned above, the contamination of apple juice by patulin is frequently reported; however, recent studies have also detected this toxin in juices from pears, grapes, sour cherries, black currants, oranges, pineapples, and passion fruits, among others [45]. Considerably high levels of patulin were found by Iqbal et al. [52] in juices and smoothies prepared from a mix of fruits and marketed in Pakistan. Hussain et al. [53] also reported high levels of patulin in mango and orange juices in Pakistan, which confirms the need for strict regulations due to the serious hazard for local consumers in this country.

The most significant mycotoxin contaminating grape juice is OTA, mainly due to the high levels of contamination of *Aspergillus* section *Nigri* on grapes [46,54]. However, these fungi were also reported as capable of producing fumonisin B2, and currently, this toxin is considered as an important emerging risk in grape products [55]. Despite the widespread ability of *Aspergillus* section *Nigri* to produce fumonisin B2, it is necessary to evaluate, in detail, its natural occurrence in grape juices [46]. In addition to OTA, *Alternaria* toxins and patulin are a cause of concern in grape juices [54].

The consumption of new, different, and trendy types of fruits has drastically increased in the last few years. As a consequence, the occurrence of mycotoxins in these products and their derivatives has raised the interest of many researchers who described the emergence of new toxins not previously described in juices. Juan et al. [56] studied the occurrence of different mycotoxins in different berry juices. The authors reported that the most significant risk in this product is posed by the presence of aflatoxins and *Alternaria* toxins (57% and 71% contaminated samples, respectively). Moreover, 26% of the samples exceeded the maximum legal limits established for the sum of aflatoxins in dried fruits in Europe (4–10 µg/kg). Carballo et al. [57] described a high occurrence of *Fusarium* HT-2 toxins in juices from mixed fruits at mean levels of 22.38 µg/L. Sugarcane fruit has been recently pointed out as a significant risk for consumers due to high contamination levels of both aflatoxin B1 and fumonisin B1 [58].

Different chemical, physical, and biological detoxification strategies have been proposed to reduce the presence of mycotoxins in juices [17]. For example, clarification with active carbon has been used widely to remove patulin in fruit juices in many countries [59]. However, the use of these methods are restricted due to possible losses in organoleptic or nutritional properties, their high cost, and safety problems due to their toxic properties [16]. In this context, the most promising results were obtained by the use of biological methods, and several detoxifying agents were reported to be an ecofriendly alternative to remove mycotoxins from juices due to their effectiveness at a reasonable cost [17,45]. Different microorganism are able to reduce OTA or patulin levels to some extent, although the most significant results in juices were obtained using bacteria or yeasts that efficiently adsorb the toxins to their cell wall or produce enzymes to degrade them [45,59,60]. Although several potential biological detoxification agents have been proposed, many studies are still necessary before their commercial application in juices [59]. Farbo et al. [61] designed a method to eliminate OTA from grape juices by mixing immobilized *Candida intermedia* yeast cells into magnetic calcium alginate spheres. The results obtained are promising, because the authors were able to apply this method in a prototype bioreactor reaching a rate of 57% OTA removal after flowing the juice through a glass chromatography column packed with beads [61].

4. Herbal Teas

Herbal beverages, commonly known as teas, are a popular global beverage and are preferred due to their beneficial properties that may enhance the overall health status from different perspectives [62]. These beverages are known to improve the antioxidant status and to be the source of important natural bioactive compounds, such as carotenoids, phenolic acids, flavonoids, and alkaloids, among others [63]. The presence of mycotoxins in herbal teas should be carefully studied, and the risk posed by these toxins might not be disregarded since, to our knowledge, no maximum levels are currently established in any country.

The majority of studies have been focused on the occurrence of mycotoxins in the raw herbs and aflatoxins are by far the most frequently occurring mycotoxins in tea and herbs worldwide. Many of these studies reported samples contaminated by extremely high mycotoxin levels, which indicates that it is essential to monitor their presence in these commodities, as they might pose a risk to consumer health. Duarte et al. [64] analyzed a high number or teas and herbs marketed in Portugal and reported that 65% of the samples were contaminated by aflatoxins at high levels between 2.78 and 28.15 µg/kg. A similar approach carried out in Morocco detected a high contamination of these toxins in tea (58% of contaminated samples) with extremely high levels of total aflatoxins up to 116.2 µg/kg [65].

Aflatoxins are also frequently found in black tea samples marketed in Iran and Pakistan, where 27.5% and 78.3% samples were found to be contaminated, respectively [66,67]. Tosun et al. [68] studied the presence of the four most relevant aflatoxins separately in local tea samples in Turkey and reported the occurrence of aflatoxins B1, B2, G1, and G2 in 54%, 29%, 71%, and 46% of the samples, respectively, at maximum levels of the total aflatoxins at 34.18 µg/kg. Viswanath et al. [69] reported a high occurrence of aflatoxigenic fungi on black tea samples obtained from local markets in India, although they only found one positive determination for aflatoxin B1, but it was at high levels (19.2 µg/kg).

The studies on the presence of other mycotoxins in raw herbs has also been of interest, and up to now, ZEN, fumonisins, enniatins, and DON have been detected at different levels. The natural occurrence of fumonisin B1 was reported by Martins et al. [70] both in black tea and other herbs for infusions marketed in Portugal, and this toxin was detected in a 65% of the samples at highly variable levels. Duarte et al. [64] also found an important number of tea and herbs samples (62%) contaminated with ZEN in Portugal, and in most cases, this co-occurred with aflatoxins, which should be considered due to the possible synergistic effects. In an integrated study on the occurrence of mycotoxins in dry tea samples, Reinholds et al. [71] were able to detect at least one mycotoxin in 90% of the analyzed samples. Enniatin B and DON were the most frequently detected followed by OTA and aflatoxin B1, although the highest levels of contamination were found in the case of ZEN (up to 90.7 μg/kg).

Some recent reports focused on the occurrence of mycotoxins directly in tea beverages after the production process. Pallarés et al. [72] optimized a protocol to simultaneously detect 16 different mycotoxins in tea beverages (black, red, green, and green mint). The authors reported the presence of aflatoxins B2, G1, and G2 as well as *Fusarium* toxins 15ADON and enniatin B. This protocol was also applied to established contamination levels of other types of herbal tea beverages, including anise, chamomile, linden, pennyroyal mint, thyme, valerian, and horsetail, and the same mycotoxins were detected at variable levels [73].

As mentioned before, mycotoxins are heat-stable molecules, and they are unlikely to be affected by the boiling temperatures used in the tea-making process [30]. The effect of the tea-making process on the transfer of mycotoxins to the final product has been also studied by different authors. Around 30% of aflatoxins present in tea leaves are known to remain in the beverage after boiling and filtration [69]. Similarly, OTA transfer from black tea to the beverage was found to be around 40% and the transference seemed to be affected by the contact time between the tea and water [74]. Similar results were previously obtained by Malir et al. [75], and they established the OTA transfer from black tea to the beverage at 35%. These authors highlighted the importance of pH in the amount of OTA extracted and reported a low transfer rate when the pH of the beverage decreased.

Monbaliú et al. [76] hypothesized that fumonisin B1 might be easily transferred from tea leaves to drinkable products due to the high hydrophilic properties. However, they could not demonstrate the extraction of this toxin during tea processing, likely due to the low fumonisin B1 levels of the raw material used in their experiments. In a recently published work, Reinholds et al. [71] also reported an efficient transfer of 32% and 100% of DON and ZEN from dry tea to the infusion. On the contrary, other authors reported a reduced mycotoxin content or even a complete elimination after the infusion process and indicated that only a small fraction migrated from the plant to the beverage [77]. However, the results obtained seemed to be related to the initial mycotoxin concentrations, because the toxins that were detected at the highest levels were able to remain in the final infusion.

Other tea preparations are becoming trendy among consumers, and therefore, it is interesting to assess the presence of mycotoxins after the making process. Ismail et al. [67] demonstrated that more than 50% of aflatoxins present in black tea leaves become dissolved and become part of the Chai tea beverage likely due to the high-water solubility of these toxins. Iha et al. [78] demonstrated a transfer of approximately 30–40% of aflatoxins and 20–30% of OTA in ginger tea beverages from tea bags and the mycotoxin extraction was significantly increased at higher boiling temperatures near 100 °C.

To our knowledge, no regulations are available regarding the maximum levels of mycotoxins in herbs and teas intended for the preparation of infusions for human consumption.

5. Conclusions

Our society is notably changing its dietary habits, and in this respect, the consumption of functional beverages has drastically increased in the last few years due to consumer awareness toward healthy food. Mycotoxins are widely present in the raw products used to prepare most of these functional beverages, although the studies on the occurrence of these toxic compounds in the final products are still scarce. Many of the most relevant mycotoxins regarding food safety, including aflatoxins, OTA, patulin, and *Fusarium* toxins have been detected at variable levels in functional beverages. The data available up to now indicated that the processing methods were not able to significantly affect the mycotoxin levels. It would be interesting to study the possible application of new preservation methods opposed to heat-based ones to eliminate mycotoxins without interfering with the beverage properties.

More research is needed to establish the real risk posed by the intake of functional beverages in order to improve the current regulations regarding the maximum limits of mycotoxins in these products.

Author Contributions: J.G.-S. drafted and corrected the manuscript; B.P. supported the drafting and revised the manuscript; C.V. revised and corrected the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Spanish Ministry of Science and Innovation, grant number RTI2018-097593-B-C21.

Conflicts of Interest: The authors declare no conflict of interest.

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