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Ochratoxin A in grapes and wine

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Abstract

The mycotoxin ochratoxin A is a potent nephrotoxin and a possible human carcinogen. It occurs in a variety of plant products, including wine, grape juice and dried vine fruits. Several surveys have shown that the range of ochratoxin A contents detected in wine produced in Europe varied between 0.01 and 3.4 $\mu g\,l^{-1}$. Both incidence and concentration of the toxin were higher in wines from southern regions and increased in the order white < rosè < red. In Italy, field trials were conducted in 1999 and 2000 to study fungi associated with grapes and their ability to produce ochratoxin. *Aspergillus* and/or *Penicillium* strains were present on grapes, starting from setting in a few vineyards. The highest level of grape colonisation was found at early veraison in 1999 and at ripening in 2000. In both years, 95% of strains belonged to the genus *Aspergillus. Aspergillus niger* aggregate was dominant, with about 50% of the ochratoxin-positive strains identified as *A carbonarius*. Other authors have confirmed the relevance of these fungi and underlined the contribution of *A. carbonarius* to the ochratoxin contamination of wine. This species is very invasive and colonises and penetrates berries, even without skin damage. It emerges that temperature, rain and relative humidity are the main factors that influence ochratoxin production in grapes.

Introduction

The mycotoxin ochratoxin A (OTA) is a potent nephrotoxin to all animal species tested, with the exception of mature ruminants. OTA is carcinogenic to rodents and possesses teratogenic, immunotoxic and possibly neurotoxic and genotoxic properties. Furthermore, it has been implicated as a factor in the human disease Balkan Endemic Nephropathy and the development of urinary tract tumours in humans. In 1993, IARC classified OTA as a possible human carcinogen (group 2B).

The worldwide occurrence of OTA contamination of raw agricultural products has been amply documented. It has been found in a variety of plant products such as cereals, coffee beans, beans and pulses (Kuiper-Goodman and Scott, 1989; Micco et al., 1989; Pohland et al., 1992; Stegen et al., 1997; Jørgensen, 1998). OTA has also been detected in beverages such as beer, wine and grape juice (Majerus and Otteneder, 1996; Zimmerli and Dick, 1996).

Ochratoxin A in grape and grape derivatives

Several surveys in different European countries, in Morocco, Japan and Australia confirmed the frequent presence of OTA on grape products and wine (MAFF, 1997; Ueno, 1998; Burdaspal and Legarda, 1999; MAFF, 1999; Cholmakov-Bodechtel et al., 2000; Festas et al., 2000; Gareis et al., 2000; Majerus et al., 2000; Otteneder and Majerus, 2000; Stockley, 2000; Tateo et al., 2000; Filali et al, 2001; Markaki et al., 2001; Pietri et al., 2001). The range of OTA content in wine produced in Europe varied between 0.01 and 3.4 $\mu g\,l^{-1}$. Values higher than 0.5 $\mu g\,l^{-1}$ were reported by several European authors (Burdaspal and Legarda, 1999; Majerus et al., 2000; Markaki et al., 2001; Pietri et al., 2001), and also from Morocco (Filali et al., 2001). Only Ospital et al., (1998) in France and Festas et al. (2000) in Portugal, reported lower levels or absence of OTA, at least in certain good quality wines. A few samples of dessert wine contained between 1 and

3.9 μg l^{-1} (Burdaspal and Legarda, 1999; Pietri et al., 2001).

Grape juice, especially from red grapes (Cholmakov-Bodechtel et al., 2000) has been highlighted as an important source of OTA in children's diets. Values reported are between 1.16 and 2.32 μg l^{-1} (Majerus et al., 2000; Filali et al., 2001). The highest OTA content, among grape and its derivatives, was measured in dried vine fruits (MAFF, 1997) with more than 40 μg kg^{-1} (MacDonald et al., 1999; MAFF, 1999); OTA was also found in vinegar, especially balsamic vinegar (Markaki et al., 2001), although the level was low (0.2 μg l^{-1}). The high number of reports published during the last 5 years give evidence of the problems related to the presence of OTA in grapes and its processed products for their contribution to the total daily intake, both in adults and children. The interest in this subject is also confirmed by the efforts devoted to develop precise and accurate analytical methods for this toxin (Zimmerli and Dick, 1996a; Tateo et al., 1999; Festas et al., 2000; Visconti et al., 2000; Markaki et al., 2001; Soleas et al., 2001).

All the studies cited are primarily aimed at quantifying OTA, while little information is available about the origin of the toxin. A gradient was observed through regions and wine colour; both OTA incidence and concentration were higher in products from southern regions and increased in the order white < rosè < red (Majerus and Otteneder, 1996; Ospital et al., 1998; Otteneder and Majerus, 2000; Markaki et al., 2001; Pietri et al., 2001). According to Majerus and Otteneder (1996), the latter gradient may be connected with longer mash standing. Zimmerli and Dick (1996) suggest that OTA found in wines of southern European origin is probably formed after the harvest of the grapes, but prior to the alcoholic fermentation. These provisional conclusions were followed by further studies that more carefully considered the fungi responsible for the synthesis of this toxin and their presence in field and post-harvest.

Fungi responsible of the presence of OTA in grape

Fungi responsible of the presence of OTA have been studied especially on cereals, where *Penicillium verrucosum* and *Aspergillus ochraceus* (formerly known as *Aspergillus alutaceus*) are considered the main producers. Until 1998, they were also believed to be responsible for the production of the toxin in grapes (Ospital et al., 1998), but OTA-producing *A. carbonarius* and *A. niger* were identified in dried vine fruits in 1999 (Codex Alimentarius Commission, 1999).

In Italy, field trials were conducted in 1999 and 2000, to study fungi associated with grapes and their ability to produce OTA in two grape-growing areas in the north and in the south of the country. Different farming systems, representative of their own area, were taken into account. Ten bunches were collected from each vineyard at two growth stages in 1999 (early veraison and ripening) and at four growth stages in 2000 (setting, berries increase, early veraison and ripening). They were sub-sampled to isolate *Aspergillus* and *Penicillium* spp. Fungal isolates were purified, characterised and stored on suitable media; afterwards, they were tested for OTA production.

Aspergillus and/or *Penicillium* strains were present on grapes, starting from setting, in a few vineyards. One month later, these fungi were detected in more vineyards, and at early veraison and ripening they were present in all vineyards. The highest level of colonisation was found at early veraison in 1999 and at ripening in 2000 (Battilani et al., 2000, 2001a). These observations are relevant, both because these genera are usually considered post-harvest moulds and because they were all isolated from berries which showed no symptoms. In 1999 and in 2000, 95% of strains belonged to the genus *Aspergillus*. The identification was carried out for some representative strains and, according to these observations, *A. niger* aggregate was dominant, with about 50% of the OTA-positive strains identified as *A. carbonarius*. *Aspergillus fumigatus* and *P. pinophilum* were also identified among the OTA producers (Battilani et al., 2000, 2001a). Other authors have confirmed the relevance of these fungi. In fact, in a study on table grapes carried out in South America, *Aspergillus* section *nigri* moulds were frequently isolated during grape storage and *A. niger* var. *niger* and *A. carbonarius* were identified as potential OTA producers (Da Rocha et al., 2000). In other studies carried out in Spain, Abarca et al. (2001, 2001a) isolated black *Aspergilli* from dried vine fruit and Cabanes et al. (2001) underlined the contribution of *A. carbonarius* to the OTA contamination of wine. In contrast, a specific research programme conducted in Portugal on mycotoxin production by fungi isolated from grapes, pointed out the absence of OTA-producing fungi (Abrunhosa et al., 2001).

OTA content in grapes

The same 10 bunches, collected in each of the nine vineyards, were randomly sampled (Battilani et al., 2000, 2001a) and the berries were analysed for OTA content by HPLC. OTA was found in significant quantities only in 1999 in southern vineyards, starting from veraison: the concentration had increased at harvesting. Substantial differences in OTA content were observed between vineyards, even when the cultivar was the same, and they had been trained in a similar way. Differences in fungicide treatments in the different vineyards were acknowledged, both in number of sprays and in active ingredients used, but the OTA content in grape did not show any clear relationship with these factors. Cultural factors have not shown a clear effect on OTA content in grape, the most remarkable factors being the year and the geographic area (Battilani et al., 2001b). In conclusion, it emerges that temperature (north–south difference), rain and relative humidity (1999–2000 difference) are the main factors that influence OTA production in grapes.

Behaviour of OTA-producing fungi on grape berries

Knowledge of the infection cycle of *Aspergillus* and *Penicillium* is scarce and completely absent as far as grapes are concerned. Preliminary information was obtained by Battilani et al. (2001). Experimental trials were conducted to determine how different OTA-producing *Aspergillus* and *Penicillium* strains colonise grapes. This research consisted of a field and an *in vitro* trial. The former investigated the occurrence of the toxin on the skin and pulp of naturally-infected berries picked in September 1999. The latter tested the ability of strains of *A. carbonarius*, *A. fumigatus* and *P. pinophilum* to colonise, penetrate and develop inside intact and artificially damaged berries. The results showed that even if the skin of grape berries seems to be the more frequently contaminated tissue, OTA was also present in the pulp. The fungal species involved determine the pattern of toxin contamination through berry tissues. In fact, as shown by artificial inoculation, berries were susceptible to OTA-producing fungi early in their growth stage, at least starting from one month after setting, but the amount of fungal growth depended on the species involved. *A. carbonarius* was very invasive; it colonised and penetrated berries, even without skin damage. In contrast, *A. fumigatus* only colonised the pulp in damaged berries and *P. pinophilum* apparently never grew inside berries.

The amount of OTA produced was clearly influenced by the status of the berries and especially that of the fungus. The high content detected in damaged berries inoculated with *A. carbonarius* (30 $\mu g\,kg^{-1}$ produced in 7 days, about 1000 times greater than produced by *A. fumigatus* under the same conditions), is related not only to the intrinsic toxigenic characteristics of the strain, but also to its aggressiveness. In fact, the difference in OTA production by the fungi in liquid medium was not very different (26,910, 20,226 and 16,028 $ng\,kg^{-1}$, respectively, for *A. carbonarius, A. fumigatus* and *P. pinophilum*; Battilani et al., unpublished data).

Conclusions

The need of further research on factors influencing growth and OTA synthesis on grape of OTA-producing fungi is evident. It will allow preventative actions, able to reduce OTA in grape to the minimum possible level to be defined. Up to now, only corrective actions have been studied. Satisfactory results have been obtained using additives able to adsorb OTA, even if with adverse effects on the colour and polyphenol indexes of the wine (Dumeau and Trione, 2000). While waiting for further information, which could be useful to reduce the risk of the presence of OTA and its derivatives in grapes and wine, some encouraging news has come from Punam Jeswal and Jeswal (1998) on antidotal action of grape juice on the negative hepatorenal effects of OTA. They observed that concurrent administration of berry and leaf juice of the common grape to mice, together with OTA, significantly reduced the hepatic and renal damage caused by ingestion of the mycotoxin. In addition, none of these animals showed the formation of hepatocarcinoma, whereas 25% of the animals receiving only OTA developed well-differentiated renal carcinoma and hepatic lesions.

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