OCHRATOXIN A IN WINES: CURRENT KNOWLEDGE

SECOND PART: MYCOTOXINS AND WINE

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Toxins produced by moulds

Certain moulds can produce toxins. These mycotoxins are secondary metabolites, which are often produced in minute quantities, they are toxic for humans and can contaminate numerous foodstuffs. The moulds are either present in the soil, on plants or in storage buildings.

Several moulds can develop on grapes. Besides *Botrytis cinerea*, which is responsible for grey rot, *Alternaria*, *Clasdosporium*, *Fusarium*, *Aspergillus* and *Penicillium* species can also be encountered.

The presence of the mycotoxin Ochratoxin A has been detected in wines, grape juices and raisins in the mid-nineties. Analyses conducted in Denmark and Finland in 1997 and 1998 have shown that wines could contain significant amounts of Ochratoxin A. It could also be found in cereals, beer, coffee and cocoa.

Since then, numerous studies including those conducted by ICV have allowed to better understand the importance of Ochratoxin A, the factors leading to its formation and prevention strategies.

Ochratoxin A: a mycotoxin present in wines

Ochratoxin A is synthesized by *Aspergillus* and *Penicillium* species. First observations had shown that *Aspergillus* was more common in Southern vineyards and *Penicillium* in Northern vineyards.

Recent studies led to the identification of the predominant Ochratoxin A producing moulds present on grapes: *Aspergillus carbonarius*, which is by far the species with the highest Ochratoxin A production potential, and *Aspergillus niger*, whose activity is lower (cp. boxed text). On raisins, *Aspergillus ochraceus* and *Aspergillus foetidus* are found.

In 2001, 373 grape samples of Carignan, Syrah, Sauvignon, and Muscat varieties were taken at bunch closure, veraison or harvest. The incidence of mould infection increased with grape development. Among the moulds, approximately 10% produced Ochratoxin A at veraison and 47% at maturity.
In 96% of the cases, the Ochratoxin A producing moulds were identified as *Aspergillus* species (95% *A. carbonarius* and 1 % *A. niger*) and in 4% as *Penicillium* species.

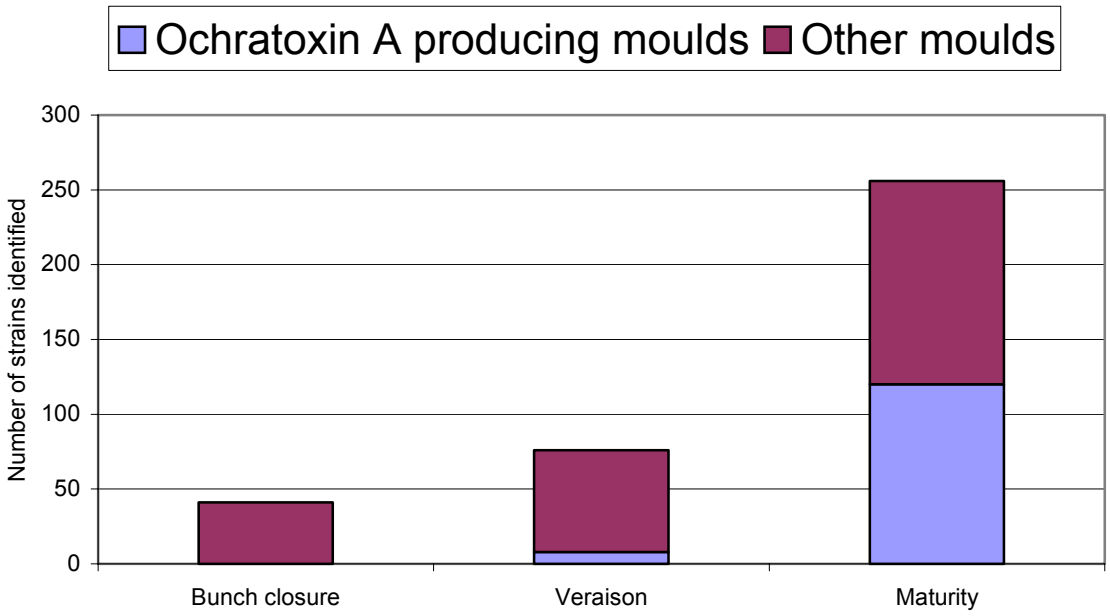

Figure 1: Evolution of the mould population during grape development (CNRS-INPT-UPS)

Aspergillus species got established very early on grapes, often before veraison. They are unable to perforate the skin and can only penetrate the berries through existing damages (bruised and burst berries, insect perforations). Once in contact with the pulp or grape juice, they can produce Ochratoxin A.

The growth of these moulds is possible at air humidity levels of 72 to 90% and temperatures of 12 to 39°C (optimum 28°C).

Figure 2: Ascospores of *Aspergillus carbonarius* (photo by INPT-ENSAT)

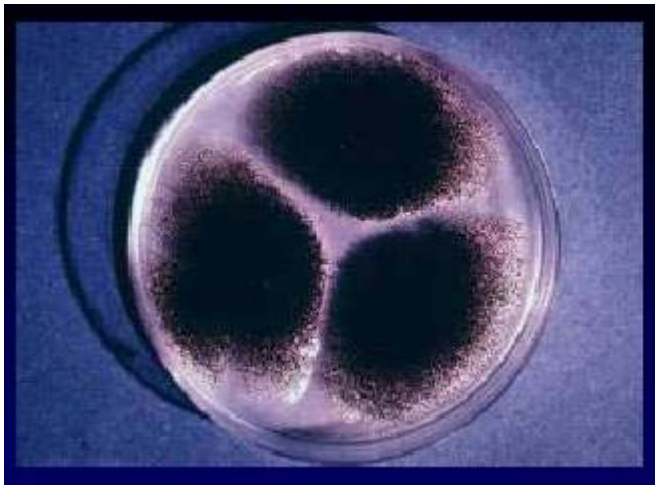

Figure 3: Growth of *Aspergillus carbonarius* on solid medium (photos INPT-ENSAT)

A stable molecule

Ochratoxin A (OTA) combines an amino acid (phenylalanine) and coumarin in its structure. Ochratoxin A is quite stable over time and against heat destruction. It can be degraded to OTB (non-chlorinated derivative), OTC (ethyl ester of OTA) or Otα.

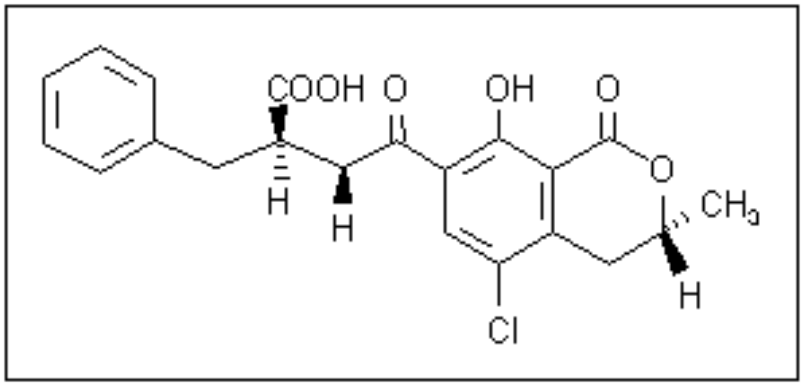

Figure 4: Molecular structure of Ochratoxin A

Toxic for humans

Ochratoxin A is a nephrotoxic substance leading to irreversible damage of the kidneys. It is considered a carcinogen (class 2B) by the International Centre for Cancer Research and may have immunosuppressive and neurotoxic effects.
The maximum tolerable daily intake is very low. According to experts, it varies between 0.3 and 0.89 µg for a person with 60 kg of weight.
The acute toxicity varies according to gender and individuals between 12 and 3000 mg for a person with 60 kg of weight.

Cereals are responsible for the main Ochratoxin A burden (45 – 50% of the average intake). Wine is responsible for the second largest contribution, with 10 – 20% of the average intake.

The Mediterranean basin is particularly affected

Contamination risk in wines
ONIVINS has conducted two surveys to assess the importance of Ochratoxin A in French wines. One was carried out in 1998 with 265 wines, and the second in 2001 with 982 wines.

The results were as follows:

- 75 % of the wines contained no Ochratoxin A, and 17% of them less than 0.5 µg/l.
- Ochratoxin A was present in all wine types: AOC (Appellation of controlled origin), regional wines, table wines, red, white and rosé wines, natural sweet wines.
- Generally, there was more Ochratoxin A in red wines than in white and rosé wines.
- Most wines with high Ochratoxin A levels originate from the Mediterranean region.

According to analyses carried out in Europe since 1996, certain wines, grape juices or natural sweet wines will contain up to 10 µg/l Ochratoxin A.

All surveys conducted since 1999 have shown that Mediterranean wines were more affected by Ochratoxin A than wines from other regions. However, not all viticultural regions are affected to the same extent as shown by analyses realized by Foulon-Sopagly with musts used for grape juice production: The risk was smaller in Provence, Venetia or the Mancha (Figure 5). Ochratoxin A has also been reported in other producing countries (Australia, South Africa…).

Viticultural regions and their OTA risk
based on analyses of musts used for grape juice production

High risk Low risk No risk

(order: counter clockwise)
Charentes
Gers/South West
Vaucluse/Rhone Valley
Penedes
Mancha
Utiel Requena
Valencia/Alicante
Languedoc/Roussillon
Sicily
Pulia
Greece
Abruzzi
Reggio Emilia
Venetia
Rhine-Palatinate

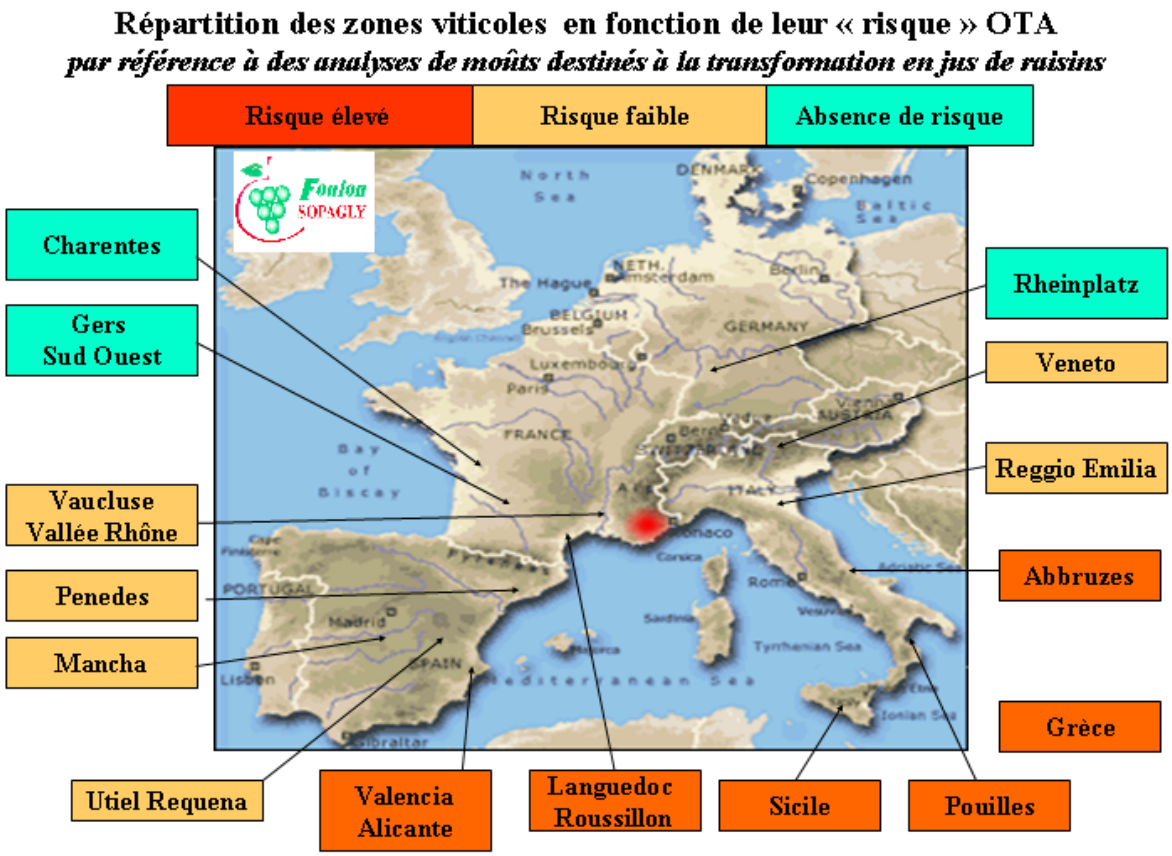

Figure 5: In spite of a certain variability, the Mediterranean vineyards are more affected by Ochratoxin A (data by Sopagly-Foulon).

Commercial constraints, and soon, legal limits.

Currently, European regulations do not impose maximum limits for Ochratoxin A levels in wines and grapes. In 2001, a European directive prescribed limits for cereals (5 µg/l). products derived thereof (3 µg/l), and raisins (10 µg/l). The maximum legal limit for wines should be defined by December 31st, 2003. In June 2002, the OIV suggested to postpone the decision to 2005 and also proposed a limit of 2 µg/l. Trade and production representatives suggested a limit of 3 µg/l for the European Union.

In spite of the temporary absence of regulations, an increasing number of countries or buyers carry out Ochratoxin A controls and apply their own limits (sometimes 0.5 µg/l).