No. 1

QUADERNS TÈCNICS DE L'INCAVI

February 2006

Guide lines in vitivinicultural practices in order to minimise levels of Ochratoxin A in vine-based products

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Note: This document takes into account the results of the research done by different Spanish (1) and European (2) research projects, and the resolution from the International Vine and Wine Organisation (3) "Code of sound vitivinicultural practices in order to minimise levels of Ochratoxin A in vine–based products" written with the contribution of experts from different countries. The present document is oriented and adapted to the conditions in Catalan and Spanish vitiviniculture to contribute to the general quality of the vine-based products.

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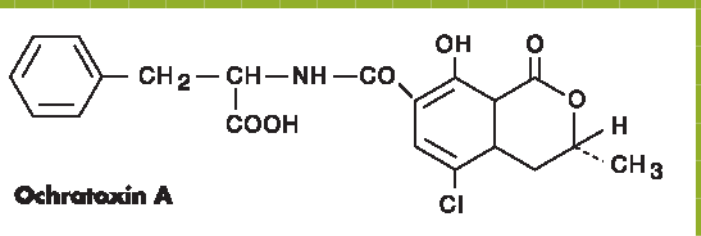
INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by fungus which grow in soils, organic matter, and in some conditions on grapes. The effects of its growth in grapes, must, wines and vinegar, addresses food safety because of negative effects on the consumer. Current legislation sets the maximum contents of Ochratoxin A (OTA) at 2,0 µg/l for wine (4).



Ochratoxin A

In Catalonia, Spain, and in general in Southern and Mediterranean countries the responsible almost in exclusivity of the presence of OTA in the grape is *Aspergillus carbonarius*, a fungus that belong to the black fungus. **The lack of grape sanity and berry integrity, mainly in the moment of maturation**, favour the growth of the fungus and the contamination by OTA in wine products.

The good winemaking practices in order to limit OTA levels in the grape and avoid to the maximum extent wine contamination have to rely in prevention measures both in the vineyard and in the cellar and are reported in the following guidelines.

GOOD CULTIVATION PRACTICES IN THE VINEYARDS

Good cultivation practices have the goal the good management of the vineyards in order to control the factors in fungus growth as well as in the environment conditions, sensivity of the varieties, vigour of the vine, canopy architecture, good conditions of the grapes, etc.

Initial growth of *Aspergillus* sp. in white grape

Viticulturists training in this aspect is a necessary factor

TRAINING OF PRODUCERS

The awareness of the viticulturist of the negative effects of the presence of OTA in the wine, is a main tool for the control of this toxin. Viticulturists training in this aspect is a necessary factor and has to address to the correct identification of the fungus most likely producer of OTA in the vineyards (*Aspergillus carbonarius*). See attached pictures and situations below in the document.

Growth of *Aspergillus* sp. in black grape

Fungus not producer of OTA. *Botrytis cinerea* (left) and *Ryzhopus* sp. (right)

Growth of *Aspergillus* sp.

Growth of *Aspergillus* sp. view inside of the cluster

Aspergillus carbonarius is a secondary infection and opportunistic fungus

The viticulturist has to take into account that *Aspergillus carbonarius* is a secondary infection and opportunistic fungus that is presented as the final step in the chain of accidents, insect attacks or other fungus that have previously affected the integrity of the berry, causing injuries in the berry.

The viticulturist has to be consciously as well of the effects and danger of a *Aspergillus carbonarius* attack is bigger for the gapes that will be macerated or in red winemaking. Consequently is necessary to know the technological processes involved in the produced grapes and follow the reccomendations below.

▶ VINEYARD ESTABLISHMENT

• Avoid the soils of too fertile areas, with high crops and tight bunches and big berries that brings the risk of breaking the skin.

• Favour vine establishment in well aerated areas while avoiding as possible humid areas.

• Draw up plots of land with adequate planting disposition, and vegetation architecture (trellising system) to facilitate planting operations, correctly position grape bunches, ensure good pest and disease control and favour the uniform ripening of the grape.

In deep and fertile soils is necessary to control the vigour

Vigour control by soil conditions

Vigour produces dense canopy in the cluster zone

Results after mechanic leaf removal

Mechanic leaf removal

PLANT MATERIAL

• Choose less vigour varieties which are less prone to develop mould and grape rot and less vigour rootstocks.

• Choose clones or biotypes within a variety which are better adapted to climatic and soil conditions in specific cultivation area and less sensitive to mould and rot development, which are oftentimes characterised by less compact grape bunches.

• Lay out homogeneous plots of land (varieties, clones) to facilitate growing operations and to ensure better crop and disease control and to obtain uniform ripening of the grapes.

GROWING TECHNIQUES

• Avoid cultivation techniques which favour vigour increase. In particular is necessary a controlled nitrogenous fertiliser application.

• Favour a correct grape bunches exposure, avoiding leaf excess in the cluster area. Clusters should be in a vertical positioning to facilitate pesticide application.

• In irrigated vineyards, irrigation should be applied to maintain quality and not as a way to increase the yields. The irrigation has to be regular and avoid the risk of berry breaking after a excessive water application.

• Carry out leaf removal in the grape cluster zone. This operation can increase the exposure of clusters, is specially necessary in warm and humid climatic conditions during grape ripening whilst recognising the need to limit the risk of sun burn.

Hand leaf removal

▶ PEST AND DISEASE CONTROL

• Avoid injuries on the berries and skin damage caused by diseases, insects, phytotoxicity and sun burn.

• Apply vine protection plans in order to control dangerous fungal diseases affecting grape quality (oïdium disease, acidic rot).

• Prevent attacks of grape berry moths and other insects which favour mould development on damaged berries; pest control need to be carried out according to biological and epidemic risk; under high risk conditions preventive treatments must be applied by using specific products and taking into account the warnings of plant protection regional services.

• Preventive control of *Botrytis* or grey mould. In conditions with risk of production of OTA it is recommended anti botrytis treatments which are actives against *Aspergillus* and have been proven scientifically (5).

• Protection strategies have to cover all ripening process respecting the safety period. This aspect is important in case of searching maximum ripening.

Spraying in the vineyard

Clusters affected by moth caterpillar *(Lobesia botrana)*

Growth of fungus after powdery mildew *(Uncinula necator)*

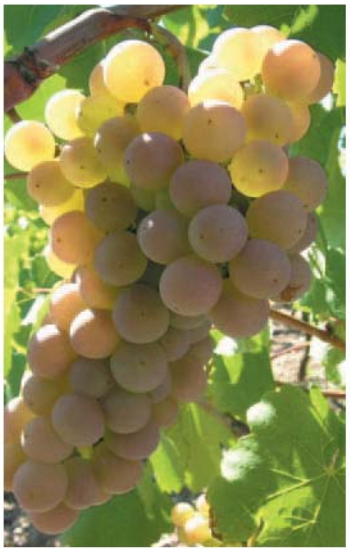
Growth of grey mould *(Botrytis cinerea)*

Collection of moth adults *(Lobesia botrana)*

PRACTICES AT HARVEST

In regions where the climatic conditions favour the growth of OTA producer fungus in wine products, in order to look after the whole quality of the grapes and wine it is recommended to apply preferably preventive measures, leaving the corrective measures only when it is absolutely necessary due to the negative effects of the latter.

Only a sound harvest can be used for human consumption



As a rule we have to remind that only a sound harvest can be used for human consumption, without the risk of lack of quality and without problems of food safety for the consumers.

The date of harvest must be decided taking into account grape ripeness, sanitary level, and forecasted climatic changes and OTA producer fungus risk. In high risk OTA areas, it is recommended to advance the harvest date.

When grapes are extensively contaminated by mould:

- The grapes can not be used for human consumption, either fresh or in raisins.
- Nor for making concentrated musts, grape juice, wine or vinegar.
- The grapes should only be used for distillation.

Mechanic harvest does not allow cluster selection

▶ PRODUCTION OF RAISINS AND RAISINED RAISINS

For production used to obtain raisins and raisined raisins (sweet wine) it is recommended that following steps:

• Ensure the hygiene of containers to be used for the harvest and/or the drying of grapes.

• Use only grapes not damaged by insects and not contaminated by mould; or select the grapes by eliminating damaged or contaminated grapes.

• Place grapes to be dried or raisined in just one layer to avoid overstacking.

• Favour progressive and uniform drying of all parts of the grape bunch.

• Take the necessary measures to avoid development of fruit fly infestation.

• For particular conditions of drying in open air, it is recommended to dry in well ventilated conditions and to cover the grapes at night to prevent condensation and humidity.

Drying raisins outdoors

Drying raisins outdoors

Selection table

Is necessary to clean well the transport trailers

▶ PRODUCTION OF WINE GRAPES

In the case that the harvest is moderately contaminated with *A. carbonarius* and destinated to wine production, it is recommended:

• Grapes damaged by mould (specially black fungus) must be eliminated before the harvest or at selection tables.

• Harvested grapes must be transported as quickly as possible to the winery in order to avoid extended waiting, especially for grapes with a high proportion of juice.

• It is important to clean containers with drinkable water under pressure after each load.

The transport to the cellar has to be as quick as possible

TREATMENT AT THE WINERY

Under conditions with a risk of OTA contamination, it is recommended to measure the contents of OTA in the musts to be used in winemaking.

In conditions of significant contamination of *Aspergillus* some steps have to be taken:

▶ PRE-FERMENTATION TREATMENTS

• In mechanical harvest add sulphites or other techniques to avoid a quick and uncontrolled fermentation.

• Evaluate in white wine making the reduction of skin maceration by crush management.

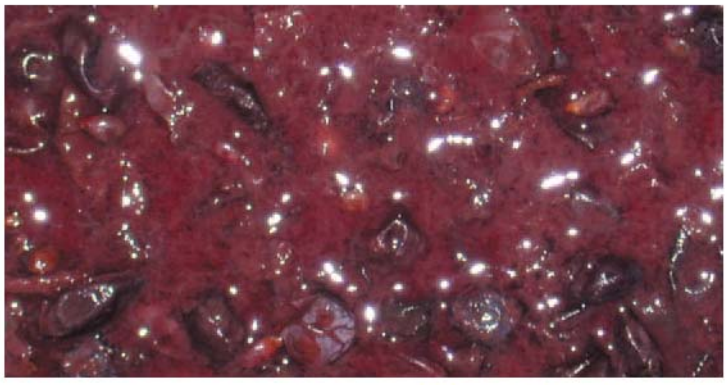
• In the case of a significant contamination of red grapes, evaluate possibility of carrying out rosé winemaking.

• Adapt pressing rate to the health status of the grape; in case of contamination, carry out small volume, low pressure quick pressings. Avoid continuous press.

• In the case of contaminated grapes, avoid using pectolytic enzymes for racking must or maceration. Quick clarifications with must filtration, centrifugation and flotation are preferable.

• Avoid harvest heating treatments and intense and prolonged maceration.

Pneumatic presses favour a soft and adequated pressing



Maceration during red wine making

Continuous presses apply agressive pressing

▶ FERMENTATION TREATMENTS

• In the case of contamination, it is preferable to treat the grapes or the musts with the lowest possible and most effective doses of oenological charcoal in order to avoid possible loss of aromatic and polyphenolic compounds when the treatment is carried out on wine according to the legislation (6).

• For alcoholic or malolactic fermentation, use yeast or bacteria which have adsorbent properties for OTA; ensure that these characteristics are guaranteed by the supplier. Note that the usage of these products only enables a partial reduction of OTA.

• It is recommended to devat as quickly as possible following fermentation.

Current clarification products (organic or inorganic fining agents) have variable levels of efficiency for reducing contents of OTA

▶ MATURING AND CLARIFICATION TREATMENTS

• Dry active yeast or inactive yeast can help reduce the OTA level.

• Maturing on lees can help in reducing the OTA level. The risks of this technique related to the organoleptic quality of wine must be evaluated.

• Current clarification products (organic or inorganic fining agents) have variable levels of efficiency for reducing contents of OTA.
- Oenological charcoal is the most efficient, it can reduced most OTA only authorised for white wines (or in experimentally according to the regulation 1622/2000 in red wines (7 & 8).
- Certain cellulose and silica gel associated with fining with gelatine only enables a partial reduction.

• Before usage:
- Become informed of efficiency of product used and application technology.
- Carry out trials with different dosages to ascertain sensorial repercussions and application rate.

REMAINDER

• Preventive measures are essentially carried out in vineyards and treatments undertaken at the wineries are solely corrective measures.

• It is recommended to check that OTA contamination is as low as possible when using wine making derivatives and by-products as food "integrators" in animal and human food.

• These recommendations are based on current knowledge and can be updated according to research to be pursued (9).

Additional information

1. *Spanish Projects: Food National Programme, Strategy Action "Improvement of quality and competitively of wines" from INIA:*
- *"Effets of quality of harvest and winemaking practices on Ochratoxin A (OTA) in wine" (OTA-Vi, VIN00-022-C3)*
- *"Study of growth of Aspergillus spp. in vine and effects of preventive control ant the post-harvest techniques on fortified wines on the reduction of Ochratoxin A levels" (OTA-Licor, VIN02-023)*

2. *European project: Contamination risk and integral control of Ochratoxin A in wine (Wine Ochra Risk, 9LK1-CT2001-01761)*

3. *Resolution OIV: VITI-OENO-1-2005. (www.oiv.int)*

4. *Maximum Ochratoxin A levels, from 2005 harvest (Regulation CE nº 123/2005; consultable at: www.gencat.net/darp/incavi.htm*
Normativa:
- *Raisins: 10.0 µg/L (0,01 mg/L)*
- *Wine: 2,0 µg/L (0,002 mg/L)*
- *Juice, Nectar: 2,0 µg/L (0,002 mg/L)*
- *Grape juice, reconstituted grape juice: 2,0 µg/L (0,002 mg/L)*

5. *According to studies in INCAVI some antibotrytis present negative effect against Aspergillus: ciprodinil, fludioxinil, and its mixture. www.gencat.net/darp/incavi.htm*

6. *Authorised wine making techniques (consultable at): www.gencat.net/darp/incavi.htm*
- *Regulation (CE) nº 1493/1999, Annex IV: point 1 letter i), (modified by Regulation CE nº 2165/2005), point 2 letter f) and point 3 letter o)*
- *Regulation (CE) nº 1622/2000, Annex IV: Limits for the use of some substances.*
- *International Code of Oenological Practices (www.oiv.int)*

7. *Use of charcoal in wine making: Regulation 1622/2000, Title III, article 41.*

8. *Report: "Effect of strategy of the disease control in vineyard on the Ochratoxin A levels in wine", in www.gencat.net/darp/incavi.htm, Documents tècnics consultables.*

9. *For further information you may contact INCAVI, incavi.vilafranca@gencat.net*

Vilafranca del Penedès, February 2006

Collaboration and support:

Generalitat de Catalunya
Agència Catalana de Seguretat Alimentària