Food Control 20 (2009) 469–475

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

HACCP to control microbial safety hazards during winemaking: Ochratoxin A

Adolfo J. Martínez-Rodríguez, Alfonso V. Carrascosa *

Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas (CSIC), C/Juan de la Cierva 3, 28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 13 February 2008
Received in revised form 9 July 2008
Accepted 23 July 2008

Keywords:
HACCP
Ochratoxin A
Wine
Aspergillus

ABSTRACT

In this work, an effort is made to elucidate the main hazard of microbial origin in winemaking, the presence of ochratoxin A (OTA), which has not yet been considered from an HACCP (Hazard Analysis and Critical Control Points) point of view, and for which a limit of <2 μg/L has recently been set by the Office International de la Vigne et du Vin (OIV, International Vine and Wine Organisation) and EU authorities. We describe and outline the incoming OTA safety hazard in every stage of the process, from the grape harvest to distribution of the final product. We also propose preventive measures and determine the critical factors and critical limits in the process. The study reveals that the principal CCP for wine safety is the reduction to $\leqslant$1% of grapes contaminated with OTA before the grape juice is obtained. Preventive measures and limits for the percentage of grapes contaminated with OTA are given.

1. Introduction

The application of HACCP concepts to food production initially focused on microbiological safety and quality hazards (ICMSF, 1988), but now also include physical and chemical hazards (Mortimore & Wallace, 2001). As these were generally accepted and successful, they were included in the EU regulations (Directive 93/43/ECC on food hygiene) and also accepted by the United States Federal Department of Agriculture (CPR-123). In European countries, like Spain, this has been incorporated in the legislation (Real Decreto 2207/1995 from December 28, which defines hygiene standards for food products), and, accordingly, the application of HACCP has become a mandatory regulation. It is, also, compatible with quality assurance systems like the ISO 9000 series (International Standardization Organization) (Carrascosa, 2005).

The application of HACCP to winemaking has mainly focused on quality aspects (Briones & Úbeda, 2001; Christaki & Tiza, 2002; FIAB., 1997; Hyginov, 2000; Kourtis & Arvanitoyannis, 2001; Morassut & Cecchini, 1999). Not all of these works include microbiological hazards and none of them takes into consideration the possibility of microbiological health hazards for the consumer resulting from wine drinking.

Recently, the European Union set maximum limits for ochratoxin A (OTA) in must and wine after the 2005 grape harvesting process at: 2 μg/L (EU, 2005). This limit, which had been proposed earlier by OIV (2002), has been recommended and is mandatory for all member states. As this is currently the only toxic substance of microbial origin for which there is a specific wine international law, this paper will discuss the application of HACCP during the winemaking process to control the formation and concentration of OTA in wine.

2. Methods

There are seven different activities, called the seven principles in the Guidelines of the *Codex Alimentarius* (FAO, 1997), which are necessary to establish and apply an HACCP plan (Mortimore & Wallace, 2001). A detailed analysis of the OTA hazard throughout the winemaking process, including all main and supportive stages from harvesting to bottling, which had not been done before, was carried out according to the HACCP seven principles, following the 12 tasks recommended by the FAO (1998b). No approach was followed to analyse the quality hazards during the winemaking process as this has been proposed previously (Christaki & Tzia, 2002).

3. Results and discussion

The application of seven principles and twelve tasks to the winemaking process in relation with OTA production are presented below.

3.1. Task 1: The HACCP team

The staff working in each cellar to study implementation of the HACCP method should include a microbiologist and an experienced enologist.

* Corresponding author. Tel.: +34 15622900; fax: +34 15644853.
E-mail address: acarrascosa@ifi.csic.es (A.V. Carrascosa).

0956-7135/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2008.07.015

3.2. Task 2: Product description

To start the hazard analysis, it is necessary to make a complete description of the wine to be made, including its customer specifications, by filling out a minimum generic form like the one shown in Fig. 1. This form should also include relevant information on safety of OTA depending on the limits suggested by the regulations, which are also included. This information is obtained from tests carried out before HACCP was implemented.

3.3. Task 3: Identify the intended use of the product

The intended use of the product must appear on its form; in this case, the intended use is human food. Considering the nature of wine, the form should also include recommendations for its proper consumption, as well as its incompatibility with medications, if any, the precautions to take into account by sectors of the population affected by illnesses, recommending some limitations in wine intake, etc.

3.4. Task 4: Construct the process flow diagram (PFD)

The flow diagram consists in a detailed list of all the stages needed to prepare any kind of wine and the order in which these stages should be carried out. None of the previous HACCP studies applied to wine considered the stages prior to the harvest as part of the winemaking process, assuming that the wine was prepared exclusively in the cellar (Briones & Ubeda, 2001; Christaki & Tzia, 2002; FIAB, 1997; Hyginov, 2000; Kourtis & Arvanitoyannis, 2001; Morassut & Cecchini, 1999).

It is important to point out that the flow diagram is the base for any further hazard analysis and to establish the critical control points. It is, therefore, generally recommended to attach a detailed description of the operations to be carried out to the flow diagram itself, including some additional information on raw materials, additives, containers they come in, storage characteristics, as well as the activities to be performed during the process, time/temperature profiles of the different stages, equipment and design characteristics, plan of the facilities, customer and distribution problems, etc. (Mortimore & Wallace, 2001).

The flow diagram should also include the stages after the product is obtained until it reaches the consumer, in order to establish circumstances that might potentially affect product safety and which should be taken into account.

PRODUCT DESCRIPTION

1. Name of the product(s)	WINE (type, wine of origin, etc.)
2. Description and important characteristics of the final product	pH, alcoholic content, sulphur dioxide, tasting, etc. **<2µg/L of OTA**
3. Potential use of the product	HUMAN CONSUMPTION
4. Bottling	
5. Shelf life	
6. Place of sale	
7. Labelling instructions	
8. Special control during storage and distribution	

Date: _______________ Approved by: ________________

Fig. 1. Product description form example, adapted from: FAO (1998, 2003).

Fig. 2 shows the generic flow diagram which we consider should serve as a base for any other flow diagram applying the HACCP process to risks such as the presence of OTA, adapted to each specific case. We have included stages which are normally ignored in the flow diagrams of the winemaking process published earlier, such as vineyard growing, grape harvest, and transportation to the cellar. We consider these stages to be essential in the case of hazards such as OTA. We have assembled the operations needed to prepare the must to be fermented (destemming, crushing, clearing, etc.) in the stage called "must preparation". All the stages needed to make the wine, which follow the fermentation process (devatting, racking, fining, etc.) correspond to "transformation to wine". This generic flow diagram should be considered as a guideline and each wine cellar must prepare its own flow diagrams to comply with the HACCP related legislation.

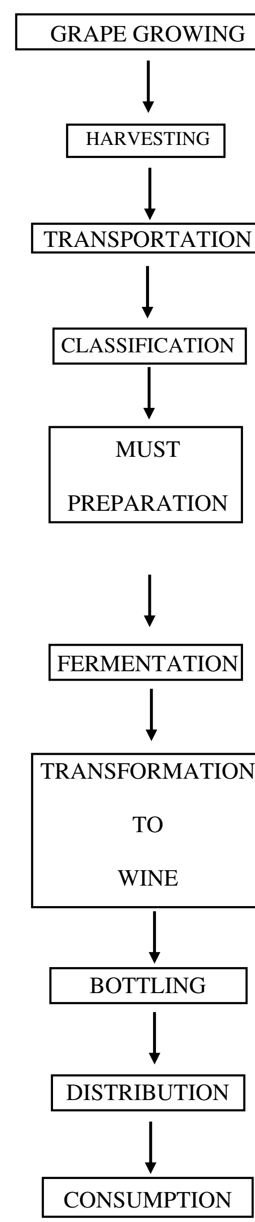

Fig. 2. Generic winemaking flow diagram, including the viticultural stages (growing and harvesting) which are crucial to control OTA by the HACCP system.

3.5. Task 5: On site confirmation of the flow diagram

This operation, known as "walking the line", consists in verifying, step by step, that all the information regarding materials, practices, controls, etc., has been taken into consideration by the team during preparation of the PFD. Where appropriate, information of interest such as the time of harvest, maximum transportation time, transportation conditions to the cellar, time and temperature of the different stages, etc., should be included. The farm should be visited as often as necessary to ensure that all the relevant information regarding the presence of OTA has been collected.

3.6. Task 6: Identify and analyse hazards (HACCP Principle 1)

Once the hazard has been identified, the risk (the probability that the specific hazard will occur) must be assessed. Like probability, risk ranges from 0 to 1, but is sometimes qualitatively evaluated as low, medium or high. Only hazards considered by the HACCP team to constitute an unacceptable risk, like in this case the presence of ⩾2 μg/L of OTA, are carried forward to Task 7 (HACCP Principle 2).

After this, appropriate control measures should be considered. These measures consist in any action or activity that may be used to control the identified hazard, in order to prevent, eliminate, or reduce it to an acceptable level.

3.6.1. Hazard Identification

3.6.1.1. Classical food pathogen microorganisms. Wine gets naturally contaminated during its production process. Microbial hazard analysis has been carried out before for wine, although incompletely, i.e., only studying microbial groups responsible for the organoleptic effects (Briones & Ubeda, 2001; FIAB, 1997; Hyginov, 2000; Kourtis & Arvanitoyannis, 2001; Morassut & Cecchini, 1999; Christaki & Tzia, 2002), but excluding microbes that could be potentially pathogenic for humans.

A single or combined effect of alcohol, polyphenols and pH cause the loss of viability in wine of food pathogens such as *Aeromonas hydrophila, Bacillus cereus, Campylobacter jejuni*, *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* (Bellido et al., 1996; ICMSF, 1996; Marimón, Bujanda, Gutiérrez-Stampa, Cosme, & Arenas, 1998; Sheth, Wisniewski, & Franson, 1988; Weisse, Eberly, & Person, 1995), and even in viruses such as the one causing Hepatitis A (Desenclos, Klontz, Wilder, & Jun, 1992). On the other hand, the effect of the increase in gastric secretion and the intestinal motility produced by wine intake is well known (Bujanda, 2000; Pfeiffer, Holgl, & Kaess, 1992), and it makes the intestinal invasion by pathogens harder. For similar reasons, it has been suggested that this intake limits the infective capacity of intestinal pathogens, including *Helicobacter pylori*, the main cause of chronic gastritis and duodenal ulcers (Brenner, Rothenbacher, Bode, & Adler, 1999).

Despite the above, in the scientific literature reviewed there are no data available on the effect of wine intake on epidemiologic outbreaks caused by microbial food-borne infections. However, there are several microbial toxic substances that might be present in wine.

3.6.1.2. OTA in wine. Some of the different types of mycotoxins that have been found in the case of grapes and its derivatives are listed in Table 1. Although the presence of OTA in wine has been considered for a long time by many researchers to be, at least, a potential hazard, there were no references to the subject in the wine literature until the late nineties (Gottardi, 1997). OTA is a mycotoxin with nephrotoxic, carcinogenic, teratogenic, immunotoxic, and possibly neurotoxic effects and has been associated in humans with Balkan endemic nephropathy (Delage, d'Harlingue, Colonna & Bompeix, 2003; Soleas, Yan, & Goldberg, 2001; Zimmerli & Dick, 1996). The daily tolerable dose of OTA is very low, and ranges from 0.3 to 0.89 μg/day for a 60 kg person. The intake of 12–3000 mg/60 kg person may cause acute toxicity. The Joint FAO/WHO [Food and Agricultural Organization of the United Nations/World Health Organization] Expert Committee on Food Additives established a provisory tolerable weekly intake of OTA at 100 ng/kg bw, which corresponds to 14 ng/day/kg bw.

Table 1
Mycotoxins identified in grapes and derivative products (adapted from Delage et al., 2003)

Mycotoxin	Substrate	Producing fungus	References
Byssoclamic acid	Grape	*Byssochlamys fulva* *Byssochlamys nivea*	Samson, Hoekstra, Frisvad, and Filtenborg (1996)
Citrinin	Must	*Penicillium citrinum* *Penicillium expansum*	Vinas, Dadon, and Sanchis (1993)
Patulin	Must	*Byssochlamys fulva* *Byssochlamys nivea* *Penicillium expansum*	Frisvad and Thrane (1996)
Ochratoxin A	Grape, must, and wine	*Aspergillus carbonarius* *Aspergillus fumigatus* *Penicillium pinophilum*	Cabañes et al. (2002)) and Bau et al. (2004)) Battilani and Pietri (2002)

Next to cereals, wine has been identified as the second largest food source of OTA (Cabañes et al., 2002), and was detected there for the first time in 1995 (Zimmerli & Dick, 1996). The *Codex Alimentarius* Committee has found that over 15% of ochratoxin A consumed in Europe comes from grapes or its derivatives (FAO, 1998a). Probably, for this reason a maximum limit has been set for ochratoxin A (OTA) in must and wines of 2 μg/L after the 2005 grape harvesting process (EU, 2005). As this is currently the only toxic substance of microbial origin for which a specific wine international law exists, this paper focuses on the control of this mycotoxin, because, unlike other mycotoxins like patulin in apple juice (FAO, 2003), an HACCP protocol to control ochratoxin A in wine is not yet available.

The average OTA content in red wine in Europe is 0.19 μg/L, and the total daily intake of red wine is estimated at 171 g (FAO, 1998a), with a range of 0.01–3.4 μg/L of mycotoxin in this kind of wine. The occurrence and concentration of OTA is higher in humid and hot years, in temperate regions and the south, in sweet wines made from over-mature grapes or raisined grapes, and increases from white wine to rosé to red wine (Battilani & Pietri, 2002; Burdaspal & Legarda, 1999).

Studies of the presence of OTA in northern Spanish wines have shown the stability of OTA in the substrate (at least for 12 months) and the enormous importance of factors such as the harvest year which, due to the changes in weather conditions from year to year, results in different percentages of the analysed wines showing the mycotoxin, for example from 86% for 1 year (range: 0.056–0.316 ng/mL) to 15% for the next year (range: 0.074–0.193 ng/mL) (López de Cerain, González-Peñas, Jiménez, & Bello, 2002). Other studies carried out on the Mediterranean coast have concluded that the concentration of OTA in the analysed wines ranges from <0.01 to 0.76 ng/mL, and the authors estimate its intake at 0.01 ng/kg bw/day (Blesa, Soriano, Moltó, & Mañes, 2004). Regarding humans, OTA has been found in the plasma of patients with chronic kidney failure in concentrations from 0 to 11.7 ng/ mL, and in healthy subjects in concentrations from 0 to 4 ng/ mL. These values are similar to those of other European countries (Pérez de Obanos, López de Cerain, Jiménez, González-Peñas, & Bello, 2001).

3.6.1.3. Microorganisms producing OTA in wine. The *Aspergillus ochraceus* and *Penicillium verrucosum* mould species produce OTA in cereals (ICMSF, 1996), but have not often been isolated in vine and grapes. The origin of OTA in grapes and its derivatives is essentially attributed to development of the *Aspergillus carbonarius* species (Bau, Bragulat, Abarca, Minguez, & Cabañes, 2004; Cabañes et al., 2002), and, to a lesser degree, to some other species such as *Aspergillus niger* corresponding to *gen. Aspergillus Secc. Nigri* (Bau et al.; 2004; Serra, Abrunhosa, Kozakiewicz, & Venancio, 2003), *Aspergillus fumigatus* and *Penicillium pinophilum* (Battilani & Pietri, 2002).

In order to simplify the application of the HACCP, we consider that OTA results from the development of *Aspergillus carbonarius*, although other species of *Aspergillus Secc. Nigri* may also contribute slightly. We understand that by considering OTA a chemical hazard, just because it is a chemical substance, may make it more difficult to identify control measures, most of which will affect the development of *Aspergillus carbonarius*.

3.7. Identification in the product flow diagram (PFD) of the phases where contamination with OTA is more likely to occur

Aspergillus carbonarius is a strict aerobe so it can only grow in wine processing before harvest (Serra et al., 2003). It must be during this period that OTA, which will later be detected in wine, is synthesized due to the development of *Aspergillus carbonarius*. If grapes have suffered extensive and earlier damage, the probability of developing *Aspergillus carbonarius* will be higher and also the final amount of OTA in wine. Thus, the probability of surpassing recommended levels of OTA increases when damaged fruits are used (Serra et al., 2003).

In other products of plant origin, the sometimes long storage after harvest and before processing is also considered to be a phase in which they may become contaminated by mycotoxins and storage times longer than 48 h at temperatures of 10 °C or more are not accepted (FAO, 2003). In the case of winemaking, grapes are normally crushed immediately after being harvested, but we also recommend these conditions to prevent the development of mould before the crushing step.

It would be interesting for future research to study the degree of damage necessary for *Aspergillus carbonarius* to grow, the time required after and the environmental conditions required for OTA to appear in the grapes, the relationship between mould growth and OTA concentration in grapes, etc.

3.8. Possible mycotoxin control measures

Although HACCP was conceived as a system to improve and ensure the quality of food safety and hygiene for the agricultural and the processing sector (ICMSF, 1991), it has been mainly implemented in the latter.

Control measures to be used during the grape growing stage would be mainly preventive and would be used to prevent the development of *Aspergillus carbonarius* and, consequently, the synthesis of OTA. It is an opportunistic pathogen, because, unlike other fungi (oidium, botrytis, mildew, etc.) it does not have any infection mechanism as such. In addition to phytopathogenic fungi, several factors affect the defense capacity of grapes and reduce the effectiveness of the skin barrier: insects (wasps, citrus mealybugs, fruit flies and Mediterranean fruit flies, grape leafrollers, etc.), birds, and the physiological-climatological conditions (hydric stress).

The main control measures to be taken, that must be included in the GAP [Good Agricultural Practices], are those applied to prevent grape skin damage, and will serve to keep plants in optimum health so that they will be able to defend themselves against possible parasites. These GAP should also include measures to prevent hydric stress, and damage caused by moulds and insects. Damage caused by birds, or other causes, must be compatible with human safety. A good preventive GAP strategy could be used against birds (elimination of natural shelters) or they could be directly frightened away (optical, acoustic effects, etc.). Fungicidal treatments, such as the addition of sulphur, cupric products, organic fungicides, etc., may be used in the case of phytopathogenic fungi and insecticides against insects. This would also hinder grape skin damage due to the attack of microbes and invasion by *Aspergillus carbonarius*.

Other preventive control measures could include the use of transgenic grapes resistant to hydric stress or to damage caused by phytopathogenic fungi (Colova-Tsolova, Perl, Krastanova, Tsvetkov, & Atanassov, 2001; Kikkert, Thomas, & Reisch, 2001; Vivier & Pretorius, 2000). However, because of the long time needed to transform the vineyards (3–8 years) and the fact that transgenic foods are more difficult to market, we expect this kind of preventive measure to take a long time to be implemented.

If, despite the preventive measures, the fruit is damaged and mould is growing, OTA reduction control measures should be used where appropriate. To establish whether or not they are appropriate, it is necessary to know if *Aspergillus carbonarius* has developed and OTA is being produced above the acceptable level. For the first case, a microbiological analysis and the taxonomic study of the isolated moulds are required; in the second case, grapes should be analysed for OTA. In certain foods of plant origin existing criteria establish that fruits damaged with toxigenic moulds should be discarded, such as in the case of patulin in apple juice or aflatoxin in the case of corn meal or copra meal, where the control measure recommends eliminating 99% of the mould damaged fruits which show a colour associated with the presence of these toxigenic mould species (FAO, 2003). We believe that, in the case of OTA in grapes, this level of damaged grape reduction would also have to be applied. Currently, there are no visual methods to identify the presence of *Aspergillus carbonarius*, which could be used to classify the fruits and separate them from those among them damaged by this fungus, despite the fact that the presence of the fungus could be visually clear. Thus, until some reliable visual *Aspergillus carbonarius* identification system is developed, it is necessary to analyse some clusters from plots attacked by moulds to establish the presence of *Aspergillus carbonarius* and/or OTA before reducing the damaged grapes. If the mould is present, contaminated grapes should be reduced to prevent pest propagation and thus reduce the hazard. If it is not possible to do this immediately, the grapes will have to be classified during harvest to reduce the contaminated ones. Finally, if the grapes can only be classified in the wine cellar and not in the vineyard during harvest, contaminated fruits will be removed here.

Based on these considerations, we may assume that traceability is a subject related to the measures to control OTA in wine. It is crucial to know the precedence of each batch of grapes to be crushed, so that those that may have been contaminated with OTA can be reduced. To establish which these are, we will have to visually inspect them for moulds and later confirm the presence of *Aspergillus carbonarius* or OTA.

Other preventive control measures include applying a detoxification process (Coker, 1997) if the level of OTA in must results unacceptable. Understandably, detoxification methods should neither compromise product safety, nor they decrease its sensory quality. However, there is not much information available on OTA detoxification and this is not very specific. Once it is available, this operation may also be considered as a hazard reduction measure.

It is possible that OTA control measures may need to be included in the GAP, GSP [Good Sanitarian Practices], GMP [Good Manufacture Practices], etc., because they have not been considered due to the lack of current regulations.

3.9. Task 7: Determine critical control points (CCPs) (HACCP Principle 2)

With the help of the decision tree of the *Codex Alimentarius* (FAO, 1993; FAO, 1997), the team will determine the CCPs in the PFD. The general objective is to attempt that no contaminated grapes arrive at the wine cellar. In order to achieve this, the preventive measures listed in Table 2 will be applied. The classification of grapes in the wine cellar, reducing the damaged ones, will correspond to the CCP. Visual examination will be the control parameter. The preventive measures should be applied during vineyard growing and the reduction measures, during the classification process.

There are no bibliographic data available that suggest the development of *Aspergillus carbonarius* or other *Aspergillus* section *nigri* species during must preparation; hence, OTA must be attributed to an earlier development of mould. Its concentration may decrease as a result of interactions between OTA and suspended solids and posterior decanting (Del Prete et al., 2007; Nunez, Pueyo, Carrascosa, & Martinez-Rodriguez, 2008), or as a consequence of the microbial metabolism (Angioni et al., 2007) Until this and other phenomena are not better understood and their influence on the elimination of OTA may be quantified and its effects on the sensorial quality of wine established, we recommend that the aforementioned guidelines be followed, applying the CCP where OTA contaminated grapes have to be eliminated. Once their efficiency has been proven, they may be included in the HACCP.

3.10. Task 8: Establish critical limits for each CCP (HACCP Principle 3)

The critical limits are usually established on immediate assessment parameters at the CCPs, such as temperature, pH, etc., which may provide information about the presence of the hazard. In the case of OTA, the parameter will be the percentage of grapes damaged by *Aspergillus carbonarius*, and the critical limit will be that the value does not exceed 1% of contaminated grapes in the crushing process, as stated for other fruit products (i.e. apple juice, copra cake and meal, and pistachios) in which mycotoxin production is the hazard (FAO, 2003). If *Aspergillus carbonarius* or OTA has been previously detected in the grapes, these critical limits are very advisable, understanding that the unit of fruit is a cluster or considering <1% of grapes to be damaged.

As new detoxification methods appear, there should be new critical limits for the corresponding control parameters. In the case of other fruits, high-pressure water jets are used to eliminate the parts damaged by mycotoxigenic moulds and thus reduce the level of mycotoxins causing the hazard, and it is necessary to set critical limits for parameters such as water pressure to guarantee that pressure is high enough, but not so high as to cause fruit damage (FAO, 2003). Currently, these detoxification methods are being perfected and should be considered for future HACCP developments, but we have not taken them into account here because we consider the data available so far to not be conclusive.

3.11. Task 9: Establish a monitoring procedure (HACCP principle 4)

Monitoring is the mechanism used to confirm that the critical limits are met at any CCP.

In the specific case of OTA in wines, visual monitoring will be used, checking the degree of damage and mouldiness of grapes while they grow. If more than 10% of grapes are damaged and mouldy in the vineyard, analysis should be carried out to establish the presence of *Aspergillus carbonarius* and OTA. In the first case, it will be necessary to work with staff specialized in taxonomy of filamentous fungi and, in the second case, to work with workers specialized in chemical analysis, preferably with experience in mycotoxins, and to use the most validated methodology available to determine OTA in grapes and wine.

Commercial kits based on immunoaffinity or similar techniques, capable of detecting 2 μg/L of toxin, and validated for must and/or wine, will be useful for a quick determination of OTA. However, the determination by other analytical methods, could also

Table 2
Preventive measures and critical factors to control OTA in wine

Stage	Hazards/reasons	Preventive measures	Critical factors/limits/controls
1. Grape growing	Development of *Aspergillus* spp. *Secc. Nigri* (mainly *Aspergillus carbonarius*) due to the damage of grape walls as a consequence of hydric stress, diseases of grapes by other fungi or by insect pests, etc., and synthesis of OTA	Application of good agricultural practices (GAP). Use of fungicides. Use of pesticides	Inspection of GAP. Timely use of pesticides and fungicides. If fungi development is detected, OTA content must be monitored (<2 μg/L). If OTA is detected, damaged grapes must be harvested to prevent propagation
2. Grape harvesting	Grape damage or injuries caused by handling	Care taken of practices during harvesting (careful grape handling). Harvesting of uninfected grapes. Application of good hygiene practices to prevent grape contamination	Inspection of the care taken of practices during harvesting. Measurement of OTA residues (<2 μg/L) if grapes damaged with fungi have been detected. If OTA is detected, damaged grapes must be removed
3. Transportation	Presence and development of *Aspergillus* spp. *Secc. Nigri* (mainly *Aspergillus carbonarius*) in grapes as a consequence of grape injury resulting from their crushing or mishandling during transportation	Smooth transportation. Fast transportation of grapes	Inspection of the grape transportation process. Transportation time of grapes as short as possible
4. Classification, CCP	Grapes or raisins infected with *Aspergillus* spp. *Secc. Nigri* (mainly *Aspergillus carbonarius*) which have OTA	Removal of infected grapes or raisins	<1% of infected grapes. Measurement of OTA residues (E.C. directives must be satisfied) if grapes damaged with fungi are detected
5. Must preparation	OTA in must	None	OTA content must be <2 μg/L
6. Fermentation	OTA in must	None	OTA content must be <2 μg/L
7. Bottling	OTA in wine	None	OTA content must be <2 μg/L
8. Distribution	OTA in wine	None	OTA content must be <2 μg/L
9. Consumption	OTA in wine	None	OTA content must be <2 μg/L

serve for this purpose. This analysis will allow acceptable and unacceptable batches of grapes to be crushed to be separated according to their OTA content.

Each farm will be responsible for setting up a visual inspection schedule for its vineyards, as well as the frequency to carry out analysis to establish the presence of *Aspergillus carbonarius* or OTA. The relevant authorities will probably help the farms to analyse the presence of the mould or its toxin.

GMP should be enough to prevent the proliferation of *Aspergillus carbonarius* and the increase of OTA during the rest of the process.

3.12. Task 10: Establish corrective actions (principle 5)

If monitoring determines that critical limits are not met, demonstrating that the process is out of control, corrective actions should be adopted. There should be two kinds of corrective actions: those which aim to recover control (e.g. if the OTA concentration in grapes is exceeded, the corrective measure should consist in reducing the affected bunches at least by 99%); and those which would consist in isolating the product for the period during which the CCP is out of control and modifying the destination of the product, eliminating it or classifying it as a lower quality product or submitting it to a new production process (e.g. when it can be mixed with other wines to reduce OTA concentration or detoxify it with some procedure should there be any).

If grapes are classified during the winemaking process, this classification process consisting in monitoring and the subsequent elimination of damaged grapes or grapes with OTA will constitute the most effective corrective action, because it will prevent the grapes with OTA from being crushed, which will lead to the production of wine with less than 2 μg/L OTA.

3.13. Task 11: Verify the HACCP plan (principle 6)

One of the most important methods to verify the plan is through audits, comprised of independent and systematic examinations carried out in order to establish if you say what you do (documentation analysis) and if you do what you say (records on the HACCP practice). For other products of plant origin affected by mycotoxin hazard, three monthly audits are recommended (FAO, 2003).

The system may be verified in the following ways:

- By taking samples to analyse them by using a method different from the one used to monitor it.
- By asking the staff, especially the person in charge of monitoring the CCPs.
- By observing the operations at the CCPs.
- By ordering official external audits from an independent auditor.

Periodical mouldy damaged grape analysis should be designed to verify that the acceptable limits have not been exceeded. If they are exceeded, it should be possible to detect where failure has occurred in order to establish in which stage the hazard has become out of control. It may be necessary to change the critical limits or to validate or introduce new control measures. If an examination of the deviations and destinations of products shows an unacceptable degree of control at a specific CCP, changes should be made.

OTA concentrations in the final product should not exceed the limits established in the legislation. If the contrary happens, the traceability system and the existing records will allow the failure and the batch that has become out of control to be located, and thus to find the CCP that has become out of control, modifying this if necessary.

3.14. Task 12: Keep records (principle 7)

Keeping records is an essential part in the correct application of the HACCP plan, as it demonstrates that the correct procedures have been followed, critical limits have been respected, monitoring systems have been appropriately used and the necessary corrective actions have been carried out. The implementation of traceability systems with batch identification all the way back to the vineyard will make it possible to accurately trace products contaminated with OTA, which is essential for their elimination. Records that should be kept include all those related to processes and GMP and GHP procedures, CCP monitoring, compliance of critical limits, deviations and corrective actions.

Acknowledgements

This work has been supported by Grants ALIBIRD-CAM S-0505/AGR/000153 (Comunidad Autónoma de Madrid), 25506 FUN C FOOD (CONSOLIDER-IMAGENIO 2010) and AGL2006-02558 (Spanish Ministry of Education and Science) and PIE-200670l188 (Consejo Superior de Investigaciones Cientificas).

References

Angioni, A., Caboni, P., Garau, A., Farris, A., Orro, D., Budroni, M., et al. (2007). *In vitro* interaction between ochratoxin A and different strains of *Saccharomyces cerevisiae* and *Kloeckera apiculata*. *Journal of Agricultural and Food Chemistry, 55*, 2043–2048.

Battilani, P., & Pietri, A. (2002). Ochratoxin A in grapes and wine. *European Journal of Plant Pathology, 108*, 639–643.

Bau, M., Bragulat, M. R., Abarca, M. L., Minguez, S., & Cabañes, F. J. (2004). Ochratoxigenic species from Spanish wine grapes. *International Journal of Food Microbiology, 98*, 125–130.

Bellido, J. B., Gozález, F., Arnedo, A., Galiano, J. V., Safont, L., Herrero, C., et al. (1996). Brote de infección alimentaria por *Salmonella enteritidis*. *Possible efecto protector de las bebidas alcohólicas. Medicina Clinica, 107*, 641–644.

Blesa, J., Soriano, J. M., Moltó, J. C., & Mañes, J. (2004). Concentration of ochratoxin A in wines from supermarkets and stores of Valencian Community (Spain). *Journal of Chromatography A, 1054*, 397–401.

Brenner, H., Rothenbacher, D., Bode, G., & Adler, G. (1999). Inverse graded relation between alcohol consumption and active infection with *Helicobacter pylori*. *American Journal of Epidemiology, 149*, 571–576.

Briones, A. I., & Ubeda, J. F. (2001). Elaboración de un plan APPCC en una bodega. *Tecnología del Vino May/June*, 89–93.

Bujanda, L. (2000). The effects of alcohol consumption upon the gastrointestinal tract. *American Journal of Gastroenterology, 95*, 3374–3382.

Burdaspal, P. A., & Legarda, T. M. (1999). Ocratoxina A en vinos, mostos y zumos de uva elaborados en España y en otros países europeos. *Alimentaria, 36*, 107–113.

Cabañes, F. J., Accensi, F., Bragulat, M. R., Abarca, M. L., Castellá, G., Minguez, S., et al. (2002). What is the source of ochratoxin A in wine? *International Journal of Food Microbiology, 79*, 213–215.

Carrascosa, A. V. (2005). APPCC para la elaboración del vino: Ocratoxina A. In *Microbiologia del vino*. Madrid: AMV.

Christaki, T., & Tzia, C. (2002). Quality and safety assurance in winemaking. *Food Control, 13*, 503–517.

Coker, R. D. (1997). *Mycotoxins and their control: Constraints and opportunities NRI Bulletin 73 Chatham*. United Kingdom: Natural Resources Institute.

Colova-Tsolova, V., Perl, A., Krastanova, S., Tsvetkov, I., & Atanassov, A. (2001). Genetically engineered grape for disease and stress tolerance. In K. A. Roubelakis-Angelakis (Ed.), *Molecular biology & biotechnology of grapevine* (pp. 411–432). Amsterdam: Kluwer Publ. Co.

Del Prete, V., Rodriguez, H., Carrascosa, A. V., De las Rivas, B., Garcia-Moruno, E., & Munoz, R. (2007). *In vitro* removal of ochratoxin a by wine lactic acid bacteria. *Journal of Food Protection, 7*, 2155–2160.

Delage, N., d'Harlingue, A., Colonna Ceccaldi, B., & Bompeix, G. (2003). Occurrence of mycotoxins in fruit juices and wine. *Food Control, 14*, 225–227.

Desenclos, J. C. A., Klontz, K. C., Wilder, M. H., & Jun, R. A. (1992). The protective effect of alcohol on the occurrence of epidemic oyster-borne hepatitis A. *Epidemiology, 3*, 371–374.

EU (European Union) (2005). Regulation (EC) N 123/2005 of January 26, 2005, amending Regulation (EC) n 466/2001 regarding Ochratoxin A. *Official Journal of the European Union*, L 25/3-5.

FAO (1993). Guidelines for the Application of the HACCP system. In *Training considerations for the application of the HACCP system to food processing and manufacturing*, FAO/WHO/FNU/FOS/93.3 11, Geneva.

FAO (1997). HACCP system and guidelines for its application. Annex to CAC/RCP 1-1969, Rev. 3. In *Codex Alimentarius food hygiene basic texts*, FAO/OMS, Rome.

FAO (1998a). Position paper on ochratoxin A, Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, Thirty-first Session, The Hague, The Netherlands, 22–26 March 1999, CX/FAC 99/14.

FAO (1998b). Food quality and safety systems – a training manual on food hygiene and the hazard analysis and critical control point (HACCP) system. Food Quality and Standards Service Food and Nutrition Division. Roma.

FAO (2003). Manual sobre la aplicación del sistema de Análisis de Peligros y de Puntos críticos de control (APPCC) en la prevención y control de las micotoxinas. Estudio FAO Alimentación y Nutrición no. 73. Roma.

FIAB (1997). Aplicación del Sistema de Análisis de Riesgos y Control de Puntos Críticos en Vinos. Valencia: Semana Vitivinícola.

Frisvad, J. C., & Thrane, U. (1996). Mycotoxin production by food-borne fungi. In R. A. Samson, E. S. Hoekstra, J. C. Frisvad, & O Filtenborg. Baarn (Eds.), *Introduction to food-borne fungi* (pp. 251–260). The Netherlands: Centraalbureau voor Schimmelcultures.

Gottardi, G. (1997). Come utilizzare in modo semplice il sistema HACCP nel settore enologico. *L'Enotecnico, 33*, 20–27.

Hyginov, C. (2000). *Elaboración de vinos Seguridad-Calidad-Métodos Introducción al HACCP y al control de los defectos*. Berlin: Springer.

ICMSF (1988). Microorganisms in Foods IV. Application of the hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality. Blackwell Scientific Oxford: Publications Limited.

ICMSF (1991). El sistema de análisis de riesgos y puntos críticos. Zaragoza: Acribia.

ICMSF (1996). Microorganisms in foods V. ICMSF. Microbiological specifications of food pathogens. London: Chapman and Hall.

Kikkert, J. R., Thomas, M. R., & Reisch, B. I. (2001). Grapevine genetic engineering. In Kalliopi A. Roubelakis-Angelakis (Ed.), *Molecular biology and biotechnology of grapevine* (pp. 387–404). The Netherlands: Kluwer Academic Publishers.

Kourtis, L. K., & Arvanitoyannis, I. S. (2001). Implementation of hazard analysis critical control point (HACCP) system to the alcoholic beverages industry. *Food Review International, 17*, 1–44.

López de Cerain, A., González-Peñas, E., Jiménez, A. M., & Bello, J. (2002). Contribution to the study of ochratoxin A in Spanish wines. *Food Additives and Contaminants, 19*, 1058–1064.

Marimón, J. M., Bujanda, L., Gutiérrez-Stampa, Mª. A., Cosme, A., & Arenas, J. (1998). Antibacterial activity of wine against *Salmonella enteritidis* pH or alcohol? *Journal of Clinical Gastroenterology, 27*, 179–180.

Morassut, M., & Cecchini, F. (1999). HACCP in oenological industry: A system of self-control not only for hygienic-sanitary hazards. *Italian Food Beverages Technology, 16*, 8–12.

Mortimore, S., & Wallace, C. (2001). *HACCP: Enfoque práctico*. Zaragoza: Acribia.

Nunez, Y. P., Pueyo, E., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2008). Effects of aging and heat treatment on whole yeast cells and yeast cell walls and on adsorption of ochratoxin A in a wine model system. *Journal of Food Protection, 71*, 1496–1499.

OIV (2002). CST 1/2002 adopted in 2002 by the OIV member states during the General Assembly of the OIV in Bratislava (Slovakia).

Pérez de Obanos, A., López de Cerain, A., Jiménez, A. M., González-Peñas, E., & Bello, J. (2001). Ocratoxina A en plasma humano: Nuevos datos de exposición en España. *Revista de Toxicología, 18*, 19–23.

Pfeiffer, A., Holgl, B., & Kaess, H. (1992). Effect of ethanol and commonly ingested alcoholic beverages on gastric emptying and gastrointestinal transit. *Clinical Investigations, 70*, 487–491.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., & Filtenborg, O. (1996). *Introduction to food-borne fungi* (5th ed.). The Netherlands: Centraalbureau voor Schimmelcultures.

Serra, R., Abrunhosa, L., Kozakiewicz, Z., & Venancio, A. (2003). Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. *International Journal of Food Microbiology, 88*, 63–68.

Sheth, N. K., Wisniewski, T. R., & Franson, T. R. (1988). Survival of enteric pathogens in common beverages: An in vitro study. *American Journal of Gastroenterology, 83*, 658–660.

Soleas, G. J., Yan, J., & Goldberg, D. M. (2001). Assay of ochratoxin A in wine and beer by high-pressure liquid chromatography photodiode array and gas chromatography mass selective detection. *Journal of Agricultural and Food Chemistry, 49*, 2733–2740.

Vinas, I., Dadon, J., & Sanchis, V. (1993). Citrinin-producing capacity of *Penicillium expansum* strains from apple packinghouses of Lerida (Spain). *International Journal of Food Microbiology, 19*, 153–156.

Vivier, M. A., & Pretorius, I. S. (2000). Genetic improvement of grapevine: Tailoring grape varieties for the third millennium. *South African Journal of Enology and Viticulture, 21*, 5–26.

Weisse, M. E., Eberly, B., & Person, D. A. (1995). Wine as digestive aid: Comparative antimicrobial effects of bismuth salicylate and red and white wine. *British Medical Journal, 311*, 1657–1660.

Zimmerli, B., & Dick, R. (1996). Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. *Food Additives and Contaminants, 13*, 655–668.