INFLUENCE OF ECOPHYSIOLOGICAL FACTORS ON THE PRESENCE OF OCHRATOXIN A IN DRIED VINE FRUITS: A REVIEW

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Grapes derived products, especially dried vine fruits (raisins, sultanas, currants), are very often used in human nutrition, and along with wine, are of significant economic importance in Mediterranean countries, especially in Spain, Greece, Turkey and Italy. The diversity of climate in the areas where grape is grown, indicate that moulds, potential producers of ochratoxin A (OTA), are ubiquitously distributed. Considering this fact, OTA itself is commonly isolated from these products. Great efforts are taken to eliminate ochratoxigenic moulds and preform detoxification of the products, but efficient methods have not been found so far. Because of that, the inhibition of mould growth is of great importance.

It is well known that the ecophysiological factors highly influence the presence of toxigenic moulds in different food products, including production of OTA. The aim of this work was therefore to summarize results obtained so far on the presence of OTA in dried vine fruit, and to outline the influence of ecophysiological factors on mould and OTA presence in this commodity.

KEY WORDS: dried vine fruits, ochratoxin A occurrence, ecophysiological factors,

INTRODUCTION

Grapes and dried vine fruit are one of the most important agricultural products and, consequently, are of major commercial interest. Grape is served as fresh, dried vine fruit, preserved and processed in jellies or jams, crushed for juice or wine making (1).

In view of the importance of the production scale, dried vine fruit presents second product that is made from grapes, after wine. Today, dried vine fruits are usually commercially classified in three groups: raisins (dried white grapes), sultanas (dried white grapes from seedless varieties) and currants (dried black seedless grapes) (2).

With grape drying, as one of methods of preservation, great profit is made in several countries that are famous for vine growing. Countries that are recording significant dried vine fruit production are Greece, Turkey, Mexico, Chile, Australia, South Africa, Iran and India (1). In the Mediterranean region, vine is one of the most important agricultural crops. In support of that, two of the biggest dried vine fruits producers in world, are in

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this region, Turkey and Greece (3). Taking in consideration the fact that Serbia is not produ-
ducing dried vine fruit, great share of imported dried vine fruit originate from these
countries.

In order to obtain dried vine fruit of good quality, both physical (berry size, berry
colour, the nature of waxy cuticles) and chemical (moisture content, sugar content and
acidity) fruit properties at harvest affect dried vine fruit quality. These properties are in-
fluenced by several factors, and some of them cannot be manipulated by the grower (vari-
ety, the age of vine, soil and climate conditions), while some others, such as soil im-
provement, irrigation management, nitrogen and potassium nutrition, etc., which can be im-
proved by the grower. All these parameters together are of essential significance in fine
quality vine production (1).

Grape ripening is accompanied by increase in the sugar content, decrease in acidity
and formation of specific colour, texture, aroma and taste, and creation of favourable
conditions for growth of specific microorganisms, such as moulds.

Grape harvest can be done manually for the purposes of table grapes production or
mechanically for dried vine fruit, grape juice or wine production.

Drying is probably one of the oldest and the most cost effective methods for fruit pre-
servation. Even though, new preservation methods and ability to supply market with fresh
fruits during the whole year are developed, drying stayed one of the most used technol-
ological operations worldwide, especially in the countries of extensive growth. Drying
also keeps diversity of the product on the market and responds to consumers demands.
Grape drying can be performed in several ways: a) „dry-on-vine” method, where grapes
is dried on vine directly, b) drying in open sun (traditional method), where grape bunches
are spread over either the ground or on a platform in thin layer directly exposed to the sun
(this method can be done also in shadow), c) drying under controlled conditions in drying
chambers. In Mediterranean countries, grapes are often dried by exposing to the solar
radiation, or in drying chambers performing cold or hot procedure (4).

In order to improve drying process, pre-treatments are done to enhance water diffusi-
on through the berry waxy cuticle and improve generally quality of dried vine fruit. Com-
monly used pre-treatments are dipping in the hot water or application of the chemicals
(sulphur, soda, ethyl or methyl oleate emulsions). When grape is dried in open sun, rapid
quality drop might be observed, which can be expressed in colour change and presence of
foreign matter.

Dried vine fruit is a nutritionally worthy food. It is rich in potassium, sugar, dietary
fiber, iron and vitamin A. Grape and its products are rich in phenolic compounds which
demonstrate a wide range of biochemical, and pharmacological effects, including antican-
cerogenic, antiatherogenic, anti-inflammatory, antimicrobial, and antioxidant activities. It
is eaten as a snack food without further processing and is used for cooking, baking and
brewing (1, 3).

Among technological parameters of quality (size of the berries, uniformity, condi-
tions of the berry surface, moisture content, and chemical composition) for creating heal-
thy safe product, microbiological quality (absence of the decay, moulds, yeasts and fo-
 reign matter, insect infestation) is of essential importance (1).

Because of its frequent use, dried vine fruit is extensively investigated, including
frequent contaminants such as toxigenic moulds, whereas mycotoxins are extremely ha-
zardous to human health (1, 2). In view of this, the aim of this work was to summarize results obtained so far on the presence of ochratoxin A (OTA) in dried vine fruit, and to outline the influence of ecophysiological factors on mould and OTA presence in this commodity.

OCHRATOXIN A – PROPERTIES AND OCHRATOXIN A PRODUCING MOULDS

Mycotoxins are extracellular toxic secondary metabolites from a large number of filamentous fungi. They are not essential products of metabolism, so their production is takes place only in certain circumstances. Alimentary intake of mould toxins provokes intoxications, so called mycotoxicoses (5).

Because they cause rot and can have pathogenic effects, moulds play an important role in fruit and vegetable spoilage. They can be found in nature in every region where suitable conditions for their development are present.

In the largest group of toxigenic moulds that are infesting fruits, species form the genera *Alternaria*, *Penicillium* and *Aspergillus* are included, and the toxins that can be found in fruit tissue are aflatoxins, ochratoxin A, patulin and *Alternaria* toxins. Some of these mycotoxins are cancerous and most of them have stable chemical structure during the processing and because of that they are present in final product, and can easily reach the consumer. Consumers will notice evident fruit spoilage, and skip intake, but with processed products situation is different - mycotoxins remain in the food due to their stability and present significant source of these toxins. A common characteristic of all mycotoxins is that their production and amount depend on the substrate on which they are growing, water activity of the substrate (a_w), temperature, and interaction with other mycopopulation on/at the substrate (6).

OTA (Figure 1) was first discovered as a metabolite of *Aspergillus ochraceus* in 1965, during a large investigation made on fungal metabolites, which was performed in order to find new mycotoxins. Soon after that, OTA was found in corn sample in the USA. International Agency for Research on Cancer (IARC) defined OTA as potential cancerous agent for people from group B2 (7). OTA exhibits nephrotoxic, carcinogenic, immunotoxic, teratogenic and mutagenic effects (2), and also disturbs physiological state of cell in many ways, primarily by inhibiting the enzymes responsible for synthesis of phenylalanine tRNA complex. Besides, it inhibits mitochondrial ATP production and promotes lipid peroxidation (6).

There is an opinion that OTA is involved in the human disease called Balkan Endemic Nephropathy (BEN) and can promote cancer of the urinary tract. BEN is a chronic nephritis that often occurs in the populations living in areas bordering with the Danube and Sava River in parts of Romania, Bulgaria, Serbia, and Croatia (5). Because of its chemical structure, phenylalanine-dihydroxycoumarin derivatove, OTA is very resistant on heating and hydrolysis (6).
Figure 1. Structural formula of ochratoxin A

Ochratoxin A is an extracellular metabolite produced by some species from the genera *Aspergillus* and *Penicillium*. Ochratoxigenic species from these genera are presented in Table 1 (2).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Section</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em></td>
<td><em>Circumndati</em></td>
<td><em>ochraceus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>melleus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ostianus</em></td>
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<td></td>
<td></td>
<td><em>sclerotiorum</em></td>
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<td></td>
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<td><em>elegans</em></td>
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<td></td>
<td></td>
<td><em>stenynii</em></td>
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<td></td>
<td></td>
<td><em>westerdijakiae</em></td>
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<tr>
<td><em>Flavi</em></td>
<td></td>
<td><em>alliaceus</em></td>
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<td></td>
<td></td>
<td><em>glaucis</em></td>
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<tr>
<td><em>Nigri</em></td>
<td></td>
<td><em>carbonarius</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>niger „aggregate“</em></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td><em>Viridicata</em></td>
<td><em>nordicum</em></td>
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<tr>
<td></td>
<td></td>
<td><em>verucosum</em></td>
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</tbody>
</table>

*A. niger* “aggregate” presents the most complicated taxonomic subgroup, which includes eight morphologically very similar taxons: *A. niger*, *A. tubingensis*, *A. acidus*, *A. Brasiliensis*, *A. costaricaensis*, *A. lacticoffeatus*, *A. piperis* and *A. vadensis* (8). With respect to *A. niger* usage for enzyme and citric acid production intended for human consumption, it is very important that industrial isolates do not produce OTA (9, 10). Apart from the mentioned ochratoxigenic moulds, some species from *Aspergillus* genus (*A. Albertensis*, *A. auricomus* and *A. wentii*) show the ability for OTA biosynthesis only in special conditions (11). Further investigations revealed that, regardless from the substrate, *A. Carbonarius* (section *Nigri*) is the main ochratoxigenic fungus, where its isolates are in 75 to 100% cases ochratoxigenic (investigation was done on 48 isolates from dried grapes in Argentina) (12).
The *Aspergillus* section *Circumdati* has some of the most significant OTA producers (Table 1). Recent investigations have shown that *A. steynii* has a different ochratoxigenic ability depending on the origin of isolation (13).

The genus *Penicillium* has two ochratoxigenic species – *P. nordicum* and *P. verrucosum* (2, 14). According to the literature data, some other *Penicillium* species (*P. aurantiogriseum, P. chrysogenum* etc.) are also having ability to produce OTA (15).

According to the data (16), at least 77 countries have definite regulations for mycotoxins, while 13 countries are known to have no regulation; whereas there are no available data for about 50 countries, most of which are in Africa (17, 18). On the basis of the opinion adopted by the European Food Safety Authority (EFSA) it was considered necessary and appropriate for the protection of public health to establish maximum levels for ochratoxin A in those foodstuffs that are significant contributors to the exposure of OTA or for those foodstuffs that are not necessarily a significant contributor to the exposure of OTA, but there is evidence that there can be found a very high level of OTA in these commodities. Those commodities are: cereals, dried vine fruit (currants, raisins and sultanas), coffee, wine, grape juice, foods for infants, green coffee, spices and liquorice (19, 20). The European Union legislation authorities have set up a maximum level for OTA in dried vine fruit 10 μg/kg.

**MYCOPOPULATIONS AND OTA IN DRIED VINE FRUIT**

A large number of investigations have been carried out in order to determine mycopopulations that are forming on grapes during ripening process (21, 22), but in spite of that, there is not enough data regarding ochratoxigenic mycopopulations during the drying process. After discovering OTA in dried vine fruit in the late 90’s, focus was directed on the representation and importance of contaminants from genus *Aspergillus* section *Nigri* on this commodity (23, 24). In the MAFF (Ministry of Agriculture, Fisheries and Food, U.K.) investigations, 301 dried vine sample from the market was tested on OTA presence and 286 were contaminated with this mycotoxin (9% had concentration of OTA higher than the European limit). MacDonald et al. (23) examined 60 samples of dried vine fruit from UK market, and in 88% cases OTA was found. These samples were imported from Greece and Turkey. Average contamination was around 9.0 μg/kg in currants, 4.6 μg/kg in sultanas and 7.5 μg/kg in raisins from Greece, and around 5.7 μg/kg for sultanas from Turkey. After these investigations the European Committee set a maximal concentration of OTA in dried vine fruit at 10 μg/kg. Studies carried out after the adoption of regulations have revealed that contamination of dried vine fruit with OTA is worldwide, although mean concentration level is low and under the European limit (3, 25-28). Table 2 shows the results of numerous investigations on OTA in dried vine fruit (29).

*A. carbonarius* is contributing mostly to dried vine contamination with OTA (30-36). *A. niger* also participate in OTA production, because it is often isolated, but its strains do not always have ochratoxigenic properties (30, 32-37). Iamanaka et al. (37) isolated ochratoxigenic species *A. ochraceus* from Brazilian dried vine fruit. OTA contamination in dried vine fruit can be prevented by controlling black *Aspergillus* species right before harvest, with fast drying after the harvest, and eliminating mouldy berries during process (32-34).
Table 2. OTA concentration in dried vine fruit from different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Dried vine fruit type</th>
<th>Incidence of contamination with OTA</th>
<th>OTA level (mean) μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>raisins and currants</td>
<td>96/118(^a)</td>
<td>&lt;0.1-34.6</td>
</tr>
<tr>
<td>Germany</td>
<td>raisins and currants</td>
<td>101/106</td>
<td>≤ 21.4</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>raisins</td>
<td>17/20</td>
<td>0.3 – 19.5</td>
</tr>
<tr>
<td></td>
<td>sultanas</td>
<td>17/20</td>
<td>0.5-20</td>
</tr>
<tr>
<td>Morocco</td>
<td>raisins</td>
<td>6/20</td>
<td>0.05-4.95</td>
</tr>
<tr>
<td>Turkey</td>
<td>sultanas</td>
<td>179/264</td>
<td>0.026-54</td>
</tr>
<tr>
<td>Greece</td>
<td>sultanas</td>
<td>17/27</td>
<td>≤ 13.2</td>
</tr>
<tr>
<td>Brasil</td>
<td>sultanas</td>
<td>29/43</td>
<td>0.1-33.9</td>
</tr>
<tr>
<td>Canada</td>
<td>raisins</td>
<td>67/85</td>
<td>max. 26.6 (2.29)</td>
</tr>
<tr>
<td></td>
<td>sultanas</td>
<td>36/66</td>
<td>max. 26.0 (3.11)</td>
</tr>
<tr>
<td></td>
<td>currants</td>
<td>2/2</td>
<td>max. 4.85 (2.81)</td>
</tr>
</tbody>
</table>

\(^a\) – number of OTA contaminated samples / total number of samples

Australian dried vine fruit, dried in open sun, that is not preserved with SO\(_2\) was in 100% contaminated with \(A.\ niger\) and allied species, which grew during the drying process and showed a high degree of resistance against strong solar radiation in continental parts of Australia (32). In addition to the above species, much rarely isolated are species from the genera \(Penicillium,\ Alternaria,\ Epicoccum,\ Trichoderma, Rhizopus\) and \(Cladosporium\) (32). From currants, species \(Xeromyces\ bisporus\) was isolated (38).

In comparison with other grape products, dried vine fruits belong to the group of products that are usually the most contaminated with OTA, where concentration of this toxin reaches the level of 50-70 μg/kg (39).

The investigations showed that OTA level in dried vine fruit of the Mediterranean area is much higher than of non-Mediterranean areas (Chile, USA and Australia). In favour of these results, researchers from Greece, Stefanaki et al. (40), published a study on OTA in Greece’s dried vine fruit - sultanas, raisins and currants. Sampling was done during storage and production. Sultanas appeared to be less contaminated (mean 2.1 μg/kg) than currants (mean 2.8 μg/kg). However, 7.5% of tested samples had OTA level higher than 10 μg/kg, exceeding the limit prescribed by the European regulatory EC No.1881/2006.

The altitude of vine growing and drying have a significant influence on the OTA concentration. Dried vine fruit from vineyards at the sea level of cc 500 m was less contaminated than from vineyards from 1000 m a.s.l. (41-43).

In Turkey, Meyvaci et al. (3) have investigated sultanas in the period from 1998 to 2000, and tested 264 samples on OTA presence (Table 2). They showed that 9.8% of samples had OTA level higher than the European limit (10 μg/kg); the average OTA concentration was 3.4 μg/kg, and maximum 54 μg/kg.

Another author (44), investigated 53 samples of Turkish sultanas intended for European market and found that 4% of them had a higher level of OTA than it is prescribed by
the European regulations, while contamination of all samples were in the range from 0.5 to 58 μg/kg.

In Morocco, of 20 samples of raisins tested on OTA presence 30% were contaminated, but the level of OTA did not exceed the limit prescribed by the European regulations (10 μg/kg) (45).

In Californian dried vine fruit OTA level was lower than in the Mediterranean, while in the Argentinian one the level of contamination was similar (31).

On Serbian markets, commonly dried vine fruit is imported from Turkey, Greece and Iran. Investigations have revealed that all tested samples were contaminated with moulds. The isolated species belonged to 4 genera: Aspergillus (60%), Eurotium (40%), Penicillium (40%) and Mucor (20%), and the the most ubiquitously isolated species was A. niger (in 33% of samples). Ochratoxin A was isolated from dried vine fruit, and ranged between 40-56 μg/kg. These values exceed the limit prescribed by the European regulatory (10 μg/kg) (46, 47).

INFLUENCE OF ECOPHYSIOLOGICAL FACTORS ON OTA BIOSYNTHESIS

Fungal growth and OTA production are determined by a wide range of different factors. They can be roughly defined as physical, biological and chemical, and they are interacting between themselves and influence further fungal growth and mycotoxin production (48).

The OTA synthesis can be conditioned due to the effects of intrinsic (water activity, pH, nature of substrate) and extrinsic (climatic conditions, temperature and humidity) factors. Species from the genera Aspergillus and others have the ability to grow on various substrates, also in different climatic regions, and because of that they are distributed worldwide. Growth of species from the genus Aspergillus is often related with regions of warm climate (48).

It is very hard to predict the level of contamination of agricultural products and food with mycotoxins, because it depends on numerous factors, such as: moisture content, temperature, type of product, presence of endogenous fungal species, storage conditions, time of storage, type and time of transportation (49,50). Control of certain parameters could reduce production of mycotoxins (11).

Water activity (a_w)

Fungal growth and mycotoxin production are determined by numerous abiotic and biotic parameters and their interaction. Water activity is perhaps the most critical factor that influence the germination, growth and establishment of moulds on nutrient worthy substrates (51). Esteban et al. (52) were investigated the effect of different water activity values on OTA production using twelve A.niger isolates, cultured on Czapek Yeast Agar (CYA) and on Yeast Extract Sucrose agar (YES) where a_w ranged from 0.82 to 0.99. For A. niger, it is known that minimal a_w needed for fungal growth is 0.77 (53), but for OTA production it is found that optimal a_w is 0.90-0.99, depending on the strain and culture medium.
Romero et al. (54) investigated a mixture of four *A. carbonarius* strains (isolated from dried vine fruits) on OTA production at 30°C on CYA using different a_w values (0.83, 0.85, 0.87, 0.89, 0.90, 0.93 and 0.95). They find out that limiting water activity of mixed inoculum for OTA production is 0.87 a_w, indicating xerotolerant behaviour of *A. carbonarius* isolates. Pitt and Hocking (38) reported that the lower a_w limit for ochratoxin A production is near 0.92 a_w. *A. ochraceus* grows optimally at 0.95 - 0.99 a_w, but minimal a_w needed for fungal growth is 0.77.

Regarding discussions of the mentioned authors, it can be suggested that lowering on 0.85 a_w can inhibit OTA production. This fact can be used to control and prevent OTA production in dried vine fruit (11).

**Temperature**

After water activity, second limiting factor for OTA production is temperature. Optimal temperature for OTA production is between 25-30°C for *A. ochraceus* (55), 10-20°C for *A. carbonarius* (56) and for *A. niger “aggregate”* 20-25°C (57). Because of their ability to grow in a wide range of temperatures, OTA can be constantly formed in field. This fact is very important for some products, such as grapes and dried vine fruit, because main contaminants are the species of *A. carbonarius* and a few species from *A. niger “aggregate”*, also main producers of OTA.

Ouselati et al. (58) examined the temperature effect and light exposure on the fungal growth and mycotoxin production. They simulated average Tunisia’s temperatures (night temp./daily temp. - 20/30, 30/37 and 25/42°C), where night temperature lasted 11h and daily 13h. The tested isolates were six strains of *A. carbonarius* isolated from Tunisian vineyards. The influence of these temperatures was examined on the OTA production of *A. carbonarius* using synthetic nutrient medium (SNM) similar to grape composition between the onset of ripening and ripeness (0.99 a_w). The highest concentration of OTA was observed in the temperature regime of 20/30°C. Also, the sunlight influence was tested using the same strains, at constant temperature of 25°C with 11h of darkness and 13h of light. Control sample was inoculated - medium incubated only in darkness and in light for 24h. The highest concentration of OTA was found in the samples that were incubated for 24h in light. Between the samples incubated in the regime 11/13h - darkness/light and 24h - darkness, the differences were not significant. Concentrations of OTA produced under different conditions of temperature and day/night regime, did not appear to be significant, but, on the other hand, light enforced fungal growth, directly implying higher OTA level in the samples (59).

*A. carbonarius* produces OTA optimally at cooler temperatures: 15°C and 0.95 - 0.97 a_w or 20°C and 0.98 - 0.99 a_w. Little or no OTA was formed at temperatures above 35°C. *A. ochraceus* grows strongly at 37°C, indicating a maximum for growth of at least 40°C. The growth was reported down to 0.79 a_w on glucose/fructose media and down to 0.81 a_w on media based on NaCl (38). *A. ochraceus* grows at moderate temperatures, and on commodities with a_w higher than 0.80, so it can be frequently isolated in storage. Because of the ability to grow at high temperatures, *A. carbonarius* is often related with fruit ripening, especially with grapes (11).
Pardo et al. (60) indicated that the germination occurred down to 0.80 \text{ a}_w \text{ at 20 or 30}^\circ \text{C and 0.85 a}_w \text{ at 10}^\circ \text{C.} A. \text{westerdijkiae} \text{ and } A. \text{steynii} \text{ are closely related to } A. \text{ochraceus} \text{ with perhaps slightly faster growth rates on CYA at 25}^\circ \text{C, but the growth does not occur at 37}^\circ \text{C.} \text{ The growth temperatures for } A. \text{niger} \text{ are: minimum 6-8}^\circ \text{C, maximum 45-47}^\circ \text{C, and optimum 35-37}^\circ \text{C (33, 38).} 

Investigations of ochratoxigenic moulds showed that \textit{P. verrucosum} can grow on some commodities only under temperatures of 30^\circ\text{C} \text{ and at 0.80 a}_w \text{ (11).} 

In any case, storage conditions are of great importance, so keeping the temperature and a_w away from optimums, can reduce mould growth and OTA production.

**Effect of the substrate**

Among the many different parameters that can determine expression of the ochratoxigenic character of moulds, substrate has an important role (66). Same isolates of one species grown in the same conditions (temperature, humidity) produced different amounts of OTA, depending of the medium (67). Medina et al. (66) tested ochratoxigenic capability of \textit{A. ochraceus} in the same liquid media that had different natural supplements, such as corn extract, peptone, coconut extract. They also optimized and designed semi-synthetic media that could stimulate OTA production by ochratoxigenic strains of \textit{A. ochraceus}.

The amount of free carbon (C), pH value, presence of some metals and chemical composition of the medium, are directly implicated in the biosynthesis of OTA by some species from the \textit{Aspergillus} and \textit{Penicillium} genera (66, 67). According to Muhlercoert et al. (60), \textit{A. ochraceus} produces OTA at a pH in the range from 5.5 to 8.5. The presence of some metals directly influences the pH value and thereby the biosynthesis of OTA. For example, addition of 0.12 mg/l of Zn, at the pH 6.5 increased by 50% colony growth of \textit{A. ochraceus} and also OTA production. On the other hand, addition of Fe in the same amount, decreased the colony growth by 40% and as well as the OTA production. In same investigations, the concentration of OTA in the substrate was not in correlation with biomass, and increased with the decrease of glucose in the substrate. Lactose does not stimulate fungal growth and along with that biomass, but has a positive effect on OTA production.

Pitt and Hocking (38) reported that \textit{A. ochraceus} grew well between the pH 3 and 10, and slowly at the pH 2.2, \textit{A.niger} on pH 4.0-6.5 and \textit{A. carbonarius} grew over a wide pH range (2–10).

**Effects of climate conditions**

The influence of different climate conditions and regions on the growth of toxigenic moulds and occurrence ochratoxigenic capability, and OTA concentration in the final product are known in the agricultural industry (61). In order to determine closely the influence of climate and regions on grapevine contamination, many researchers have investigated small-scale field plots. However, the obtained results were not always in accordance with the expectations. A few investigations have shown a certain correlation between toxin concentration and the area of vine growth in the Mediterranean region, i.e. Greece and Italy. Results have revealed that in southern regions grape is contaminated
with moulds that are having higher predilection for OTA production (40, 62). For example, OTA is found more frequently in wines originating from warmer regions (South Europe) than in the ones from colder regions (63). In Greece, higher concentrations of OTA are found in wines from islands with wet than from islands with more continental climate (64). Also, Battilani et al. (65) reported strong effect of rainfall on OTA concentrations in grape bunches, but on the other hand Belli et al. (56) did not find correlation between these factors.

Leong et al. (32) have reported that *A. carbonarius* is main OTA producer in Australia, which characterized only a few isolates of *A. niger* species. From tested strains, ochratoxigenic ability of *A. carbonarius* isolated from grapes was the highest and outreached 37.5 μg/kg.

**Interaction of the main ecophysiological factors**

Estimation of the interaction of the main ecophysiological factors and different fungus species is very important in designing a model in order to minimise the risk of OTA in foodstuffs.

Valero et al. (68) have investigated in vitro the effect of biotic factors on *A. carbonarius* growth and OTA production, using SNM. The tested strains were isolated from dried vine fruit and grapes. They were cultured on the same SNM agar plate with one positive OTA *A. carbonarius* strain, at two different temperatures (20 and 30°C) and two aw values (0.92 and 0.97).

The differences in the OTA biosynthesis at both temperatures and at 0.92 aw were not significant. At 0.97 aw and temperature of 30°C, the production of OTA was reduced when *A. carbonarius* was grown in paired cultures (comparing that grown alone). At 0.97 aw and 20°C, there was no clear interaction between moulds, and the level of synthesis of OTA remained the same.

The reduction in the OTA production by toxigenic moulds that are cultured together on the same medium at 30°C and at 0.97 aw can be explained on various ways: a) limited space for *A. carbonarius* growth because of the growth of the other cultured species, which normally leads to lower OTA production; b) antagonistic fungi consume specific nutrients needed for OTA production; c) OTA decomposition by other moulds, and d) interaction between moulds can provoke excretion of the substances that diffuse towards *A. carbonarius* colonies and blocking the OTA production. It was suggested (68) to keep the drying temperatures above 30°C, to prevent potential OTA biosynthesis.

In Australia, Leong et al. (33) revealed that 25°C is an optimal temperature to prevent the synergistic effect between *A. carbonarius* (5 strains) and *A. niger* (2 strains) for OTA production. They found that the optimal mould growth was at 15°C, at an optimal aw in the range of 0.95-0.98.

**CONTROL MEASURES TO REDUCE THE OTA LEVELS IN DRIED VINE FRUIT**

Contamination with OTA can be significantly reduced by using appropriate agrotechnical measures and fungicides in the vineyards, in order to prevent growth of some
species from the *Aspergillus* genera (2). At harvested fruit, SO$_2$ can be used as efficient reagent to control mould growth (69) and the presence of residual SO$_2$ (that is usually added to prevent browning) can help to control the development of moulds. Besides, the rapid drying of grapes at a temperature above 30°C reduces $a_w$ to safe levels that are not favourable for mould growth and OTA production. Elimination of the discoloured berries and the ones that are dark coloured after drying, can reduce the final OTA concentration in the products, because OTA contamination is related with this changes (32-34). Occasionally, frequent turning over of grapes during the drying process is important, because moisture accumulation and increased sugar level in grapes can stimulate growth of *Aspergillus* section *Nigri* and biosynthesis of OTA (42).

Drying methods can influence on final concentration of OTA in dried vine fruit (70). Drying in controlled conditions (temperature and humidity) is favourable comparing drying in open air under solar radiation. Drying in chambers at 50°C prevents OTA production because that temperature inhibits the growth of *A. carbonarius*, and partly degrades already formed toxin, due to the elevated temperature. Dipping of dried vine fruit in olive oil or ethyl-oleate can enhance water permeability thought the berry membrane and also eliminate some OTA. However, these procedures are very expensive and they are performed only in cases when severe mould contaminations are observed. Investigations (71, 72) have revealed that in the case of drying under controlled conditions, in climate chambers with monitored temperature and humidity, 24% less contamination occurred than in the cases when grapes were dried in open sun.

**CONCLUSION**

On analysing the investigations that have been done worldwide, it clearly comes out that great effort should be put in the optimisation of fruit drying process, as well as in the application of adequate agricultural measures in vineyards, because the only way to prevent OTA production is to avoid microbiological contamination. Microbiological contamination, especially with moulds can incur not only big economic losses, but also favourable conditions for the production of OTA, which is very toxic and hazardous to human health. OTA has been frequently isolated from dried vine fruit, and in many cases, its entration was above the pean limit, which implicates that this commodity can be a significant source of this mycotoxin. It has been shon that the drying process has an important influence on the OTA level. Drying in open sun is frequently applied, but it brings the risk of secondary contamination with foreign matter, insects and microorganisms. The mycotoxicological quality of dried vine fruit is influenced by the region of origin and climatic conditions of area where grapes are harvested and dried.

**REFERENCES**


УТИЦАЈ ЕКОФИЗИОЛОШКИХ ФАКТОРА НА ПРИСУСТВО ОХРАТОКСИНА А У СУВОМ ГРОЖЂУ

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Производи од грожђа, пре свега сушени производи, веома често се користе у људској исхрани и, заједно са вином, имају веома велик економски значај у земљама Медитерана, посебно у Шпанији, Грчкој, Турској и Италији. Разноликост у клими и регионима на којима се узгаја грожђе, указује на то да плесни, потенцијални произвођачи охратоксина A (OTA), имају велико распрострањење. Самим тим, овај токсин се често изолује из ових производа. Велики напори се улагају у циљу сушбивања раста охратоксигених плесни и детоксификације већ контаминираних производа. Међутим, значајно ефикасних метода за детоксификацију и елиминацију ОТА из намирница још увек нема. Из тог разлога спречавање раста токсигених плесни је од непобитног значаја.

Данаас је веома добро познато да екофизиолошки фактори имају јак утицај на присуство охратоксигених плесни на различitim прехрамбеним производима, као и на производњу ОТА. Циљ овог рада је, стога, да сумира резултате који су до сада добијени на пољу испитивања присуства ОТА у суvim производима од грожђа и да истакне утицај екофизиолошких фактора на присутност плесни и ОТА у овој врсти производа.

Кључне речи: суво грожђе, појава охратоксина А, екофизиолошки фактори

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