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Possible toxicological risk arising from contamination of grapes and derivatives by emerging mycotoxins: patulin

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Abstract. Following the acquired awareness of the presence of ochratoxin A in grape derivatives, actions were undertaken to contain this contamination, and attempts were made to evaluate the presence of any other molecule belonging to this class. Some authors have therefore reported the presence of aflatoxins, patulin and fumonisins. Patulin is classified by the IARC in group 3, which includes substances that do not have sufficient evidence of a carcinogenic effect. However, this mycotoxin may represent a real risk to the health of the consumer, since it can cause simple disorders of the gastrointestinal tract but also kidney, liver, neurological problems and compromise the immune system. The aim of this presentation was to collect and critically evaluate the available knowledge on the presence of patulin in grapes and its derivatives. The evaluation of papers found in the literature indicates that grape and derivatives have only rarely been included among the foods/drinks in which the patulin mycotoxin has been quantified. From the few data at disposal, it can be stated that patulin occurs in grapes and derivatives coming from different geographical areas with extremely variable values ranging from <LOD to 13,880 µg/kg or L. The conclusions indicate that fresh grapes, juice and must require greater attention since these products are the most contaminated and consumed by individuals of all ages, including children. Wine seems to be less frequently contaminated by patulin, perhaps due to fermentation. This knowledge, however, should be confirmed by new studies, as patuline, while not classified as a carcinogen, can exert significant toxic effects in humans. Special attention must be paid to groups at risk, as in the case of children.

1. Introduction

Exposure to mycotoxins through the food chain has been a long-standing problem. The number of identified molecules belonging to this class is progressively increasing, making it difficult to quantify and correctly evaluate the relative toxicological risk of a contaminated food. The contamination of vine products by mycotoxins was underestimated until the end of the last century when, in many geographical areas, the presence of moulds producing ochratoxin A was detected in vineyards [1]. Following the acquired awareness of the possible contamination of grapes and their derivatives, actions were undertaken to contain the problem, and attempts were made to evaluate the possible presence of other mycotoxins. Some authors have therefore searched for and reported the presence of other molecules belonging to this class, including aflatoxins, patulin and fumonisins [2]. Mycotoxins are secondary metabolites produced by moulds in particular environmental conditions that can be defined as "stressful". Among them, patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) (Figure 1) is produced by several moulds, including the genera Aspergillus, Penicillium and Byssochlamys.

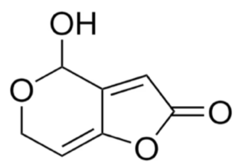

Figure 1. Chemical structure of patulin.

Among the various mycotoxins mentioned above, patulin has the peculiar characteristic of infecting mainly fruits. Tannous and coworkers [3] showed that Penicillium expansum, which is a major producer of patulin, has an optimal growth rate at 24 °C, a pH of 5.1 and an aw (water activity) level of 0.99. To get an idea of the situation at the international level, the reviews by Mahato et al. and Bacha et al. [4, 5] provided data on patulin concentrations measured in fruits grown in different countries. Although the mycotoxin concentration depends greatly on geographical areas and atmospheric conditions (temperature and humidity), the reviews cited above clearly indicate that the fruits most at risk for contamination are apples and pears. However, other food sources, including grapes and their derivatives, can contribute significantly to the dietary intake of the general population.

The growth of moulds producing patulin occurs mainly when the surface of a fruit has been damaged, for example by storms, pests (such as insects), and handling procedures. Data on patulin distribution in a rotten apple have been published by our group [6], and results are illustrated in Figure 2.

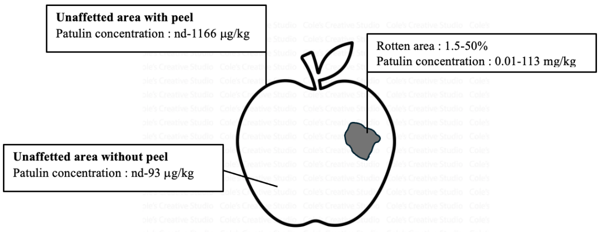

Figure 2. Patulin level in rotten and unaffected areas of an apple [7].

The most contaminated part was that affected by mould, but significant amounts of patulin were quantified both in the healthy part with peel (probably due to superficial diffusion) and in the inner part without peel (pulp). This phenomenon indicates the strong diffusion capacity of the patulin-producing moulds and/or the capacity of the mycotoxin to spread in the healthy pulp.

1.1. Toxicological aspects

IARC classified patulin in group 3, which collects substances "unclassifiable as to carcinogenicity in humans". In its conclusion dated 1986 (updated in 1998), IARC stated that there was inadequate evidence for carcinogenicity in experimental animals and no possible evaluation on carcinogenicity in humans [8]. However, toxicological studies performed in experimental animals showed that dietary exposure to patulin can produce different adverse effects, including neurotoxicity, immunotoxicity, teratogenicity and genotoxicity [9], although carcinogenic potential has not been demonstrated. More recent studies in rodents confirmed the adverse health effects due to patulin intake, in particular hepatotoxicity, gastrointestinal alterations and immunotoxicity [10].

A combined reproductive toxicity, long-term toxicity and carcinogenicity study in rats established a NOEL (No Observed Effect Level) of 0.1 mg/kg body weight (bw). Since patulin was administered only three times a week for 24 months, the NOEL was corrected to 43 µg/kg bw (0.1 mg/kg bw x 3 days/7 days). From this value, divided by a safety factor of 100, the FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) calculated a PMTDI (Provisional Maximum Tolerable Daily Intake) of 0.4 µg/kg bw/day [9].

According to the established PMTDI, many parts of the world (including the EU, USA, China, and Canada) set a maximum limit of 50 µg/kg in fruit juices and in the related concentrates. In the case of products aimed at children, the limit drops to 10 µg/kg.

To further investigate the problem, the research presented in this paper aimed to consult international scientific databases and collect as much data as possible on the presence of patulin in grapes and derivatives. The main goal was therefore to evaluate whether grapes and their derivatives (including wine) may have a significant role in relation to the intake of patulin by the general population or possible groups at risk.

2. Methods

The literature search was performed using the most important scientific databases, including PubMed, Embase, Web of Science, and CAB Abstract from 1970 to 2025. The keywords used were "wine" or “must" or "juice" or “grape" in combination with "patulin" or “mycotoxins".

3. Results

Data on the presence of patulin in foods mostly concern apples and derivatives, as they were the first products for which awareness of a possible health risk was acquired. Our group has also dealt with this topic in the past, as reported in Figure 2 [6, 7]. Abrunhosa et al. [11] collected moulds present in the vineyard and demonstrated that 33 strains of *Penicillium expansum* were able to produce patulin in grape juice.

As regards grapes and derivatives, data on patulin occurrence published in the scientific literature or by international institutions are listed in Table 1. From the data analysis, it can be stated that patulin was identified in grape and derivatives coming from different geographical areas; values were extremely variable, ranging from <LOD to 13,808 µg/kg or L.

Table 1. Patulin content in grapes and derivatives.

Grape or derivative	Origin	Method of quantification	Number of samples (positive/total)	Concentration (µg/kg or µg/L) m±SD	Concentration range (µg/kg or µg/L)	Reference
Grape	Argentina	HPLC-DAD	5/50	283±1,951	<LOD-13,808	[12]
	Pakistan	HPLC-UV	79/133	-	< LOD-505	[13]
Grape (fruit and juice)	Pakistan	HPLC-UV	112/172	53.9±98.5	< LOD-505	[13]
Grape juice	Austria	HPLC-UV	39/86	6.96±6.01	< LOD-41	[14]
	Belgium	HPLC-UV	1/9	4±12	< LOD-36	[14]
	Germany	HPLC/TLC	21/55	12 samples < 50 9 samples > 50	-	[15]
	Germany	SIDA and HRGC/HRMS	2/2	-	4.9-5.2	[16]
	Germany	HPLC-UV	2/62	2.15	< LOD-31.5	[14]
	Pakistan	HPLC-UV	33/39	16.3±11.9	< LOD-39	[13]
	South Korea	HPLC-UV/DAD	4/24	1.88±4.55	<LOD-14.5	[17]
Grape must	Austria	HPLC-UV	86/164	44.0±53.0	< LOD-750	[14]
	Czech Republic	HPLC UV-Vis	10/23	142.8±206.0	<LOD-644	[18]
Red must	Italy	LC-MS/MS	3/4^	122.8±120.8	<LOD-277	[20]
White must	Italy	LC-MS/MS	2/3^	281.0±431.6	<LOD-778	[20]
Wine	Czech Republic	HPLC UV-Vis	0/23	<LOD	-	[18]
	Germany	HPLC UV/DAD	54/96	15.6±14.6	< LOD-80	[19]
Red wine	Italy	LC-MS/MS	4/4^	406.6±283.8	82-712	[20]
White wine	Italy	LC-MS/MS	3/5^	523.8±828.3	<LOD-1,911	[20]

DAD= Diode Array Detector SIDA= Stable Isotope Dilution Assays HRGC/HRMS= Gas Chromatography/High-Resolution Mass Spectrometry

When m±SD were calculated by the authors, the negative samples were = 0 ^ all samples were from musts/wines positive to *Penicillium* spp.

Authors from the Czech Republic analysed 23 must samples, finding the presence of patulin in 10 of them with a concentration range of 143-644 µg/kg [18]. The same authors measured the mycotoxin in wines derived from the musts mentioned above: in all cases, the concentration was lower than 10 µg/kg. They therefore assumed that the mycotoxin can be significantly degraded in fermentation processes.

Data published by German researchers on wines produced from different grape varieties show some variability, as shown in Table 2 [19]. However, it remains difficult to attribute these differences to the grape variety, considering all the possible confounding factors such as growing area, climate, used oenological treatments, etc. It should be emphasized that concentrations above the established limit (50 µg/kg in fruit juices and in the related concentrates or 10 µg/kg in baby foods) were observed in a significant percentage of samples.

Table 2. Patulin content in German wines according to the grape variety [19].

Grape variety	Number of total samples	Number of positive samples	Concentration (µg/L) m±SD	Concentration range in positive samples min-max (µg/L)
Burgundy varieties	20	9	15.7±12.1	5.3-40.2
Chardon-nay	3	1	-	5.8#
Kerner	6	2	20.1^	8.3-31.8
Riesling	55	33	13.2±9.6	3.5-48.5
Scheurebe	3	3	33.5±40,.4	6.8-80
Other varieties	9	4	26.9±26.4	11.3-57.4

^ mean value (low number for SD) #only one sample

Due to the limited studies on the presence of patulin in foodstuffs and in particular in grape products, it is difficult to draw firm conclusions on the possible exposure to the mycotoxin. As an example, Table 3 lists the data on the estimated exposure to patulin coming from apple and grape juice consumption for the Austrian population [14].

Table 3. Estimation of patulin exposure from grape and apple juices in the Austrian population [14].

Group of age	Age range (years)	All population Daily intake (ng/kg bw)		Consumers only Daily intake (ng/kg bw)	
		Apple juice	Grape juice	Apple juice	Grape juice
Children	3-6	21.65	1.72	80.85	-
	7-9	20.80	2.27	61.19	45.76
	10-12	17.06	1.88	53.27	32.60
	13-14	13.52	1.74	41.04	43.41
	15-19	14.81	1.63	45.67	27.83
Adults	25	6,61	0.72	31.45	44.30
	26-35	3.83	0.42	34.61	30.37
	36-45	2.51	0.27	23,82	34.96
	46-55	2.44	0.27	34.15	45.99
	56-74	1.95	0.21	28.38	23.82
Elderly	75	2.67	0.29	-	-
	75-84	11.00	1.21	-	-

Grape juice was chosen as "marker matrix" because it is consumed across all age groups and has been the object of the most studies. In parallel, Table 3 shows the relative patulin intake due to apple juice consumption, i.e. the fruit normally considered to be the main responsible source of patulin intake. Considering the general population, grape juice provides about 10% of the amount estimated for apple juice. However, if we evaluate the intake among juice consumers only, the values between grape and apple are very similar, also suggesting the possible presence of a "risk group" represented by children. When individual juices are considered, the patulin intake is much lower than the toxicological reference established at 0.4 μg/kg bw/day (PMTDI). However, this should not be reassuring because the sum of the various sources, including fresh fruit, could bring the values much closer. The intake with wine could be less consistent, hence, greater attention should be paid to younger ages, where fruit juice consumption is higher.

4. Conclusions

Papers found in the literature indicate that the grape and derivatives sector has only rarely been included among the foods/drinks in which the patulin mycotoxin has been quantified. The conclusions that can be drawn indicate that the products to which greater attention should be paid are fresh grapes and must. Based on the few available studies, wine could be less frequently contaminated by patulin, perhaps due to fermentation, which may eliminate it. This knowledge should, however, be confirmed by new studies and analytical data since, although not classified as a carcinogen, patulin can have significant toxic effects in humans. In parallel, experimental studies should consider both new agricultural approaches to contain the spread of patulin-producing fungi in the vineyard and processes that can reduce its concentration in grape derivatives (juice, must and wine). Some positive results have been obtained in apple juice using SO_2 [21], a treatment which today, however, is recommended to be reduced as much as possible in oenology. Several other strategies used in reducing patulin levels in apple derivatives have been reported by Ziarati and co-workers [22], and they could be applied to grape derivatives to verify their efficiency.

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