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8. Occurrence of patulin in organic and conventional apple juice. Risk assessment

Ester Piqué[1], Liliana Vargas-Murga[1,2], Jesús Gómez-Catalán[1] and Joan Maria Llobet[1]

[1]*Toxicology Unit, GRET-CERETOX, INSA-UB, Food and Nutrition Torribera Campus, University of Barcelona, Spain;* [2]*Biothani Europe. S.L: Can Lleganya, Sant Feliu de Buixalleu, Spain*

Abstract. Organic foods are promoted as environmentally friendly and its consumption has become increasingly popular. Nevertheless, organic farming practices could produce commodities more susceptible to fungal attack and as a result, food with higher levels of mycotoxins. Patulin is a mycotoxin mainly present in apples and apple-based products. In this paper, we report our recent results and an update of the patulin occurrence and their risk in conventional and organic apple juices around Europe.

Introduction

Mycotoxins are secondary metabolites produced by some filamentous fungi. The main genera related to mycotoxins production are: *Alternaria, Claviceps, Fusarium, Aspergillus* and *Penicillium*. Some of them are responsible of the presence of mycotoxins in the crops (*Alternaria, Claviceps* and, *Fusarium*) and, some of them in post-harvest processes (*Aspergillus, Penicillium* and *Fusarium)* [1,2].

The term mycotoxin was used for the first time in 1962 after a veterinary crisis in England in which approximately 100,000 turkey poults died. A toxin

Correspondence/Reprint request: Dr. Ester Piqué, Toxicology Unit, Public Health Department, University of Barcelona, Spain. E-mail: esterpique@ub.edu

produced by fungi was found responsible of this crisis. Up to now, there are around 300 different mycotoxins identified, but the most important for human health are aflatoxins, ochratoxins, tricotecenes, citrinin, patulin, zearalenone, ergot alkaloids and fumonisins [1].

In general, mycotoxicosis refers to toxic effects or poisoning due to mycotoxins. These effects can be acute and chronic effects and range from irritation to severe damage including hepatic and kidney toxicity, neurotoxicity, immunotoxicity, skin irritation, disorders of reproduction and endocrine systems, and carcinogenic effects. Nowadays, chronic toxicity of many mycotoxins, characterized by low-dose exposure over a long time period, is usually of greater concern than acute toxicity, particularly in developed countries [3].

The principal route of exposure to mycotoxins is through intake of contaminated food. Its presence in levels higher than tolerable represents a threat to food safety and a risk for human health. In fact, mycotoxins are the most important chronic food safety risk factor and of greater risk than synthetic food contaminants, plant toxins, food additives, or pesticide residues [4]. Although it would be impossible completely eliminate mycotoxins in food, it is important to ensure that their levels do not pose an excessive health threat. Consequently, regulations relating to mycotoxins in food and feed have been established. What is more, over the past years, several studies have been done in order to monitor the human exposure to mycotoxins and determine the associated risks [3].

Some of these monitoring studies include the analysis of organic food. This is not surprising considering that typical organic farming practices are characterized by very strict limits on chemical synthetic pesticide (fungicides, herbicides, insecticides) and synthetic fertilizer use. This fact could lead to produce food more susceptible to fungal attack and mycotoxin contamination. Must be taken into account that the most important factors that influence the presence of mycotoxins in food are the insect attack, damage to vegetables during harvest and the temperature and humidity during storage [3,5].

In the last years, consumption of organic foods have become increasingly popular for a number of reasons, the most significant of which is the perception of health and wellness benefits associated with naturally grown foods. Specifically, organic foods are promoted as being safer, better-tasting, environmentally and farmer friendly. In addition, consumers are choosing organic foods because several foods crisis have caused a health concern. Consequently, organic farming has been raised in Spain, especially in Catalonia, as well as in whole Europe, since the nineties. However, the health benefits of consuming organic compared to conventional foods are still unclear. Current scientific literature cannot state that organic food is healthier than conventional alternatives and future research is needed [6].

In the following sections, the occurrence of patulin in organic and conventional apple juice in Europe and the risk of their intake are analyzed and the recent findings performed by our group are reported.

1. Patulin

Patulin is a mycotoxin produced by some filamentous fungi of the genus *Aspergillus, Penicillium* and *Byssochlamys*. These fungi can growth in fruits and vegetables, but the apples and apple-based products are considered the main source of this mycotoxin and *Penicillum expansum* the main responsible [7].

Chemically, patulin [4-hydroxy-4-H-furo(3,2-c)pyran-2(6H)-one], is a water soluble unsaturated heterocyclic lactone (Fig. 1) [8].

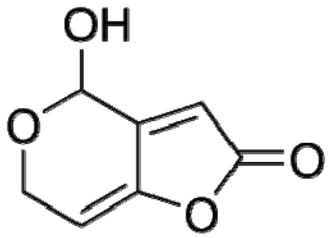

Figure 1. Chemical structure of patulin.

1.1. Toxic effects

Although patulin has antibiotic properties and it was firstly proposed for therapeutic purpose, in 1960s it was reclassified as mycotoxin due to its toxicity. Several toxicological studies have been done in order to characterize the patulin toxicity, only a few using *in vivo* models. In acute and short-term *in vivo* studies, patulin caused gastrointestinal effects as, distension, ulceration and haemorrhage. In chronic studies in rats, patulin caused neurotoxicity, immunotoxicity and genotoxicity. Reproductive and teratogenicity *in vivo* studies showed that patulin was embriotoxic. Regarding its potential as a human carcinogen, it has been classified as group 3 (Group 3- Not classifiable as to its carcinogenicity to humans) by International Agency for Research on Cancer (IARC) [8]. Recent studies have also demonstrated that patulin alters the intestinal barrier function [9].

Patulin has electrophilic properties, thus high reactivity to cellular nucleophiles. It is believed to exert its cytotoxic and chromosome-damaging effects mainly by forming covalent adducts with essential cellular thiols especially with sulfhydryl groups. These properties, led to cause cross linking with proteins, enzyme inhibition, depletion of glutathione and genetic damage including chromosomal aberrations [10-12].

1.2. Regulatory aspects

Worldwide, regulations regarding to mycotoxins have been established to protect the consumer from the harmful effects of these compounds. Since 1981 the number of countries with specific mycotoxin regulations has been increased from 33 to 100 in 2003. In Europe, the first harmonized regulations for mycotoxins in human food came into force in 1998 [13].

Nowadays, patulin is one of the most regulated mycotoxins; many countries have regulation for this mycotoxin, especially in apple products like juice or puree. The great majority of these countries have established a 50 μg/kg as a reference level. In particular European legislation have been established a maximum permitted level of 50 μg/kg for patulin in apples juices, 25 μg/kg for apple puree and 10 μg/kg if these products are intended for infants (children under the age of 12 month) and young children (children aged between one and three years) (Commission Regulation 1881/2006) [14].

2. Occurrence of patulin in apple juice

The occurrence of patulin in apple-based products like apple juice is of health concern, thus, it is important to monitor and control final products to confirm that patulin levels are below the established limits according to the European regulation, and also, check the population exposure and the derived risk.

The occurrence of patulin in apple juices, commercially available on many European countries has been reported, including Belgium [15,16], Denmark [17], Germany [18], Greece [19], Italy [20-25], Portugal [26,27], Spain [28-37], Sweden [38], and The Netherlands[39], which are summarized in Table 1.

The extent and the degree of patulin contamination in apple products, reported in these studies, are affected by a variety of factors including origin and characteristics of samples and the method of analysis. With regard to the samples, the food category (i.e. fruit apple, apple fruit from concentrate, apple nectar and mixed juices), the cloudiness (i.e. clear and cloudy), the production method (i.e. conventional, organic and integrated), the place of production (i.e. local or imported), and the production-size (industrial or handicraft-made) are usually taken into account. Referring to the method of analysis, another crucial factor, the type of extraction, purification and determination used are of great importance for the results.

The incidence of patulin is of great variability as a consequence of different criteria considering positive samples. For instance, some authors consider samples with patulin concentrations higher than limit of detection as positives samples [15,28,35] while others consider as positives, samples with patulin concentrations higher than limit of quantification [21,30].

The comparison of the results obtained by different authors about the patulin content in apple juices is complex and difficult due to the different analytical conditions, the information provided in the publications, and the number of samples analysed. Considering the mean value, none of the values

Table 1. Occurrence of patulin in apple juices reported in several European studies.

Country, region (study period)	Sample characteristics	N	% P	Max µg/kg or µg/L	Mean µg/kg or µg/L	Reference
Belgium, Flanders	Clear	67	16.4	-	14.1	[15]
	Cloudy	110	10.0	-	30.3	
	Conventional	90	13.3	-	10.2	
	Organic	65	12.3	65.7	43.1	
	Handcrafted	22	9.1	-	10.5	
	Total[a]	177	12	-	22.2	
Belgium (2001)	Clear	25	84	38.8	7.8	[16]
	Cloudy	18	77.8	26.1	10.7	
	Conventional	39	82.1	-	9.2	
	Organic	4	75	-	6.5	
	Belgian	29	79	38.8	10.3	
	Imported (France. Germany. Switzerland)	14	86	10.6	6.4	
	Industrial	32	81.3	24.2	7.0	
	Handicraft-made	11	81.8	38.8	14.6	
	Total	43	81.4	38.8	9.0	
Denmark, Copenhagen (2006)	Total	20	70	**122.5**	35.9	[17]
Germany (1996 – 1997)	Commercial	10	-	26.0	-	[18]
	Home-made	2	-	23.9	-	
	Total	12				
Greece (October 2004 – June 2006)	Total	29	100	11.8	5.6	[19]

Table 1. Continued

Country, region (study period)	Sample characteristics	N	% P	Max µg/kg or µg/L	Mean µg/kg or µg/L	Reference
Italy (2008)	Local	20	-	30.0	-	[20]
	Imported	15	-	30.0	-	
	Total[b]	35	88.6	30.0	18.1	
Italy (April – November 2005)	Clear	28	50.0	47.9	10.8	[21]
	Cloudy	25	44.0	44.9	7.6	
	Conventional	32	59.4	44.9	8.9	
	Organic	21	28.6	47.9	9.9	
	Total[b]	53	47.2	47.9	9.3	
Italy	Conventional	14	64.0	4.5	2.5	[22]
	Organic	8	38.0	22.0	9.8	
	Integrated	4	75.0	1.8	1.2	
	Total	26	58.0	22.0	-	
Italy (November 2003 – February 2004)	Conventional[c]	33	48	**53.4**	3.1	[23]
	Organic	24	50	**69.3**	7.1	
	Total	57	49	**69.3**	-	
Italy, Naples	Conventional[d]	7	42.9	**56.4**	30.4	[24]
	Organic	7	42.9	33.2	21.6	
	Integrated	1	0	0.0	0	
	Total	15	40	-	-	
Italy	Conventional	21	-	3.03	1.01	[25]
	Organic	21		28.2	7.7	
	Conventional & cloudy	12	83.33	**1150**	-	
	Total	33	-	-	-	
Portugal, Lisbon (August 2007- March 2009)	Clear[g]	32	12.5	5.5	-	[26]
	Cloudy	36	66.7	42.0	-	
	Conventional[e]	49	-	42.0	-	
	Organic	19	-	9.2	-	
	Total	68	41	42	-	
Portugal, Porto	Conventional	9	77.8	12.6	-	[27]
	Organic[f]	3	100	8.9	-	
	Total	12	83.3	12.6	-	
Spain, Valencia (2008)	Total	28	7.14	6.0	4.2	[28]
Spain, Navarra-Pamplona (2008)	Total[g]	20	70	27.09	-	[29]
		20	70	29.61	8	

Table 1. Continued

Country, region (study period)	Sample characteristics	N	% P	Max µg/kg or µg/L	Mean µg/kg or µg/L	Reference
Spain, Catalonia (June – July 2008)	Clear & conventional	71	42.3	15.0	8.1	[30]
	Conventional[g]	12	25	9.2	7.5	
	Total	83	39.7	15.0	-	
Spain, Navarra	Conventional	95	67.4	**118**	-	[31]
	Organic	5	60	17.4	-	
	Total	100	67	**118**	19.4	
Spain	Total	28	7.1	6.0	-	[32]
Spain, Navarra	Total	20	100	**107**	41.3	[33]
Spain, Valencia	Total[h]	17	29.4	**50.9**	-	[34]
Spain, Madrid (2004 – 2005)	Local	13	15.4	7.5	1.3	[35]
	France (southeast)	8	0.0	-	-	
	Portugal (north)	4	0.0	-	-	
	Total[i]	25	8.0	7.5	-	
Spain, Madrid (April – December 1992)	Local	60	-	-	-	[36]
	Local	40	-	-	-	
	Total	100	82	**170**	13.8	
Spain, Catalonia	Total	8	75	**78.0**	-	[37]
Sweden (1996 (autumn) – 1998 (summer))	Total	39	12.8	< 50	-	[38]
The Netherlands	Conventional	5	0	-	-	[39]
	Organic	5	20	-	-	
	Total	10	20	-	-	

Notes: N, number of samples; P, positive samples; numbers shown in bold are out of law limit; - data not given in the study; [a]including apple juices for babies; [b]juices with 50 %, less or more than 50 % of fruit content; [c]enclosed is 1 sample of mixture with milk; [d]one of them was claimed GMO; [e]enclosed are 10 infant drink; [f]enclosed are two apple nectar; [g]samples intended for infants; [h]enclosed apple-containing beverages; [i]samples intended for young children.

reported by these studies exceed the patulin maximum permitted level of 50 µg/kg or 10 µg/kg (labelled and sold as intended for infants and young children) established by the EU regulation. Nevertheless, taking into account the maximum levels found in samples, several surveys carried out in Spain, Italy, Belgium and Denmark have reported levels of patulin over 50 µg/kg.

Comparisons of the mean patulin contamination level in clear and cloudy apple juices were reported in Belgium [15,16], Italy [21] and Portugal [26] with disagreeing results. Most of these surveys found that the incidence of patulin were higher in clear juices than in cloudy ones [15,16,26]. Only one study [21] found more positive samples in cloudy juices. However, regarding to mean and maximum values of patulin found in samples, almost all studies reported higher values in cloudy juices. The solid parts of cloudy juices are richer in proteins compared to the liquid phase, and probably patulin could interact with these proteins. In some cases, depending on the analysis method, this patulin bound to proteins could not be detected during analysis producing an underestimation on patulin content [40], which could explain the different results obtained.

Regarding the analysis of organic and conventional apple juices, some reports performed in Belgium [15,16], Italy [21-25], Portugal [26-27], and The Netherlands [39] showed significantly differences and contradictory results. Baert [15] did not found significant differences between the incidence of patulin in conventional (13%) and organic (12%) apple juices marketed in Belgium, however, the mean concentration of patulin in contaminated samples was significantly higher in organic (43.1 µg/L) than in conventional (10.2 µg/L) apple juices. On the contrary, Tangni [16] found higher mean concentration of patulin (9.2 µg/L) in contaminated conventional apple juices than in organic ones (6.5 µg/L). According to the surveys carried out in Italy, all except one [24] found that organic apple juices were much more contaminated than conventional. The two Portuguese surveys [26,27] have found higher levels of patulin in conventional apple juices than in organic ones. Finally, one Dutch survey [39] showed higher incidence in organic apple juices than in conventional ones.

A recent study has been done by our group in order to analyse the content of patulin in apple juice from organic and conventional production systems in Catalonia (Spain). None of the studies previously done in Catalonia have compared this production systems regarding patulin contamination. In fact, only one study has been done in Spain analysing organic apple juice [31], but the number of organic samples analysed were very few comparing with those conventional and also, mean value of patulin was not reported. In our study, twenty-four samples were analysed. The results showed a higher incidence of

Table 2. Patulin exposure for consuming apple-based juices from European countries.

Country, region	Population group	Body weight (kg)	Consumption (mL/day or kg/day)	Apple juice characteristics	Patulin intake (µg/kg bw/day)	Percentage of the PMTDI[a]	Reference
Belgium. Flanders	Preschool children	-	200	Conventional	0.009	2.3	[41,42]
				Organic	0.019	4.8	
				Handcrafted	0.010	2.5	
Belgium	Children	10	200	All apple juices[b]	0.180	45.0	[16]
	Adults	60	-		0.030	7.5	
Greece	Children	20	0.1 – 0.25	Conventional[c]	0.020	5.0 – 12.5	[19]
	Adolescent	55	0.1 – 0.5		0.008-0.042	2.0 – 10.5	
	Adults	70	0.1 – 0.25		0.010-0.020	2.5 – 5.0	
Italy	Children	20	59.3	Conventional[d]	0.003	0.9	[23]
				Organic	0.014	3.5	
	Adolescents	52	40.7	Conventional[d]	0.001	0.2	
				Organic	0.004	0.9	
	Adults	70	16.4	Conventional[d]	0.000	0.1	
				Organic	0.001	0.3	
	Elderly	70	13.2	Conventional[d]	0.000	0.1	
				Organic	0.001	0.2	
Italy	Adults	60	500 – 1000	Organic	0.224[e]	60.0	[25]
	Adults	60	500 – 1000	Conventional & cloudy	9.6	2400.0	
Spain. Valencia	Infants	48.2	64	Conventional	0.002	0.5	[28]
	Adults	74.6	64	Conventional	0.03	0.8	

Table 2. Continued

Country, region	Population group	Body weight (kg)	Consumption (mL/day or kg/day)	Apple juice characteristics	Patulin intake (μg/kg bw/day)	Percentage of the PMTDI[a]	Reference
Spain. Navarra	Infants	10	130	Apple juices intended for infants	0.104	26.0	[29]
Spain. Catalonia	Babies	11.3	20.4	Conventional[f]	0.040	10.3	[30]
	Infants and teenagers	48.2	64.53		0.008	1.5	
	Adults	74.6	63.95		0.005	1.2	
Spain. Navarra	Babies	10	130	Conventional & organic	0.252	63.0	[31]
	Children	25	200		0.155	38.8	
	Adults	70	200		0.055	13.8	
Spain. Valencia	Children	20	50	Fruit juices[g]	0.012	3.0	[34]
	Adults	60	61		0.005	1.2	
	Babies	12	216		0.086	21.6	
	Adults	64	200		0.015	3.8	
Sweeden	Children	-	-	All juices[h]	0.009-0.024	2.25 – 6.0	[38]
	Adults	-	-		0.004-0.011	1.0 – 2.8	

Notes: -, data not given in the study; [a]Provisional Maximal Tolerable Daily Intake (PMTDI) 400 ng/kg bw; bw = body weight; [b]considering all apple juices (clear & cloudy; conventional & organic; local & imported; and industrial & handicrafted); [c]considering all fruit juices (apple, orange and mixed fruits); [d]considering all fruit juices (apple, pear, other) and fruit purees; [e]calculated with the maximum patulin concentration by Baretta; [f]considering all apple products (compote, multifruit compote and apple juices for babies); [g]enclosed apple-containing beverages; [h]considering all juices.

positives samples in organic juices (72.7%) when comparing with conventional (15.4%) ($p<0.05$, Chi Square Test). Moreover, the mean concentration of patulin was also higher in organic (9.3 μg/kg) than in conventional apple juices (1.4 μg/kg) ($p<0.01$, Mann Whitney Test). None of the samples were above of the EU maximum permitted level.

3. Risk assessment

Few studies, concerning risk assessment of daily patulin intake through the consumption of apple juices, have been carried out in different European countries, including Belgium [41,42], Greece [19], Italy [23,25], Spain [28-31,34] and Sweden [38]. The key data of all studies are shown in Table 2.

The exposure assessment of patulin in apple products was calculated for different population groups (i.e. infant, children, adults and elderly), taking into account the body weight, the daily consumption of apple juice and the patulin content. Due to a great variability observed of body weight and the consumption data of apple juices for the same population group, comparisons of the results obtained by different authors about the patulin intake in apple juices have been difficult.

The Joint Expert Committee on Food Additives (JEFCA), a scientific advisory body of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), allocated a provisional maximum tolerable daily intake (PMTDI) of 0.4 μg/kg body weight /day for patulin based on reproduction toxicity and carcinogenicity studies. In these studies, a NOEL of 43 μg/kg body weight/day was established and then a safety factor of 100 was applied (WHO, 1995).

Infants and young children are considered to be more susceptible to all the toxins than adults because of their lower body weight, higher metabolic rate, lower ability to detoxify, and incomplete development of some organs and tissues [43]. All studies undertaken in Europe showed that, infant and children were the main group exposed to patulin, in comparison with adults. Our results (Table 3) were also accordance to that. Consequently, they represent the highest risk group because they are the most susceptible, but also because their large dietary intake of apple-based foods.

The patulin intake derived from consumption of organic apple juices were in general higher than from conventional ones. Baert [41] reported for Belgium children a higher patulin intake for organic juice (0.019 μg/kg bw/day) than conventional one (0.009 μg/kg bw/day). Piemontese [23] compared four population groups (children, adolescents, adults and elderly), in all cases, the estimated daily intake derived from consumption of organic

Table 3. Exposure of the Catalonian population to patulin though apple juice consumption.

Population group (age)	**Body weight (kg)**	**Consumption mL/day or kg/day**	**Apple juice characteristics**	**Patulin intake (µg/kg bw/day)**	**Percentage of the PMTDI[a]**
Infants and young children (0-3)	12	20.4	Conventional	0.002	0.5
			Organic	0.016	4.0
Children (4-18)	40	64.5	Conventional	0.002	0.5
			Organic	0.015	3.8
Adults (19-66)	70	63.9	Conventional	0.001	0.3
			Organic	0.008	2.0

[a]Provisional Maximal Tolerable Daily Intake (PMTDI) 400 ng/kg bw; bw= body weight

apple juices was higher than from conventional. However, another Italian study [25] did not show this result.

Our results (Table 3) showed a highest patulin intake for organic apple juice, 0.016 µg/kg bw/day, 0.015 µg/kg bw/day, 0.008 µg/kg bw/day (infants, young children and adults respectively). Regarding to infants and young children, the daily intake of 20.4 mL of organic apple juices contributed to 4 % of the PMTDI. Considering the worse scenario for infants and young children, which implies a consumption of 200mL per day of the most contaminated brand of apple juice, the patulin intake was 0.223 ìg/kg, which represents more than a 50% of PMTDI. This suggests that in some cases, the intake of patulin could be close to PMTDI.

In summary, although the intake of patulin derived from consumption of organic apple juice were higher than that derived from conventional apple juice, all the estimated intakes of patulin calculated from the data of the studies presented in Tables 2 and 3 are below the PMTDI of 400 ng/kg bw proposed by JECFA and endorsed by the Scientific Committee of Food (SCF).

4. Conclusion

In recent years, several surveys have been conducted in Europe in order to analyse patulin levels in organic and conventional apple juices and to determine the associated risk for human health. Although the incidence and levels of patulin were, in general, higher in organic than in conventional food, patulin intake in the population groups studied does not represent a health concern. All the estimated intakes of patulin calculated from the data of the studies presented, including our study, are below the PMTDI. However, there are vulnerable groups, such as infant and children, with higher risk associated to the consumption of these apple products.

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