

## Occurrence of patulin in conventional and organic fruit products in Italy and subsequent exposure assessment

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### Abstract

The occurrence of patulin was investigated in 100 conventional and 69 organic fruity foodstuffs samples commercially available in Italy by using an HPLC method with a limit of quantification of  $0.5 \mu\text{g kg}^{-1}$ . Patulin was detected in 26 (26%) conventional and 31 (45%) organic products with a significantly higher ( $p < 0.01$ ) mean concentration in the organic products ( $4.78$  vs.  $1.15 \mu\text{g kg}^{-1}$ ). Mean patulin concentrations in conventional apple juices, pear juices, other juices and fruits purees were  $3.14$ ,  $0.22$ ,  $0.19$ ,  $0.11 \mu\text{g kg}^{-1}$ , respectively, and  $7.11$ ,  $11.46$ ,  $2.10$ ,  $0.18 \mu\text{g kg}^{-1}$  in the relevant organic products. Four samples of juices (one conventional and two organic apple, and one organic pear) contained patulin at concentrations above the limit of  $50 \mu\text{g kg}^{-1}$ , four at concentrations between  $10$  and  $25 \mu\text{g kg}^{-1}$ , and the remaining ones below  $10 \mu\text{g kg}^{-1}$ . Patulin was detected ( $< 1 \mu\text{g kg}^{-1}$ ) in only three of the 23 fruity baby food samples tested (homogenized fruits, 11 conventional and 12 organic). Based on the available data on Italian intakes of fruit juices, the estimated daily intakes of patulin, were  $0.38$  and  $1.57 \text{ ng kg}^{-1}$  body weight (bw) from conventional and organic products, respectively. Estimated daily intakes of patulin for children were higher,  $3.41 \text{ ng kg}^{-1}$  bw from conventional and  $14.17 \text{ ng kg}^{-1}$  bw from organic products, but largely below the provisional maximum tolerable daily intake (PMTDI) of  $400 \text{ ng kg}^{-1}$  bw. Patulin was also found in two samples of organic apple vinegar ( $< 5 \mu\text{g kg}^{-1}$ ) and in fresh apples with rotten spots (12 out of 24 samples) with maximum levels at  $16\,402$  and  $44\,572 \mu\text{g kg}^{-1}$  for conventional and organic apples, respectively.

**Keywords:** *Patulin, Penicillium, mycotoxin, fruit juices, organic fruits, risk assessment*

### Introduction

Patulin is a mycotoxin produced by fungi belonging to *Penicillium*, *Aspergillus* and *Byssoschlamys* species. Its toxicity was evaluated in 1990 and reviewed in 1996 by the Joint Food and Agricultural Organization and World Health Organization Expert Committee on Food Additives (JECFA). Based on available experimental results it was concluded that patulin is genotoxic but no adequate evidence exists for carcinogenicity in experimental animals. Based on a no-observed-effect level (NOEL) of  $43 \mu\text{g kg}^{-1}$  body weight (bw)  $\text{day}^{-1}$  and a safety factor of 100, a provisional maximum tolerable daily intake (PMTDI) of  $400 \text{ ng kg}^{-1}$  bw was established by the JECFA and recently endorsed by the Scientific Committee for Food (SCF) (Wouters and Speijers 1996; SCF 2000). Recent studies have demonstrated that micromolar concentrations of patulin were found to induce a rapid and dramatic decrease of transepithelial resistance of

human epithelial intestinal cell lines without major signs of toxicity as assessed by the LDH release assay (Mahfoud et al. 2002).

The maximum permitted levels of patulin in fruity foodstuffs in Europe is  $10 \mu\text{g kg}^{-1}$  for fruity baby food,  $50 \mu\text{g kg}^{-1}$  in fruit juices and  $25 \mu\text{g kg}^{-1}$  in solid apple products (European Commission 2003). The new Regulation introduced a separate limit for baby food in order to protect infants and young children who have a higher fruity foodstuffs consumption compared to adults. Patulin can be reliably analysed in fruits and derived products by HPLC with UV detection. An accurate HPLC method for determination of patulin in clear apple juice, cloudy apple juice and apple puree has been validated by a collaborative study of patulin levels of  $25 \mu\text{g kg}^{-1}$  or higher and adopted by the European Committee for Standardization as European Standard (MacDonald et al. 2000; CEN 2003). This method has been recently modified to permit the determination of patulin at level of  $10 \mu\text{g kg}^{-1}$  or lower and,

successively, it was validated by a collaborative study (Arranz et al. 2004). Most available data on the occurrence of patulin refer to apples and derived products although this mycotoxin has also been found in pears, peaches, strawberries, blueberries, cherries, apricots and grapes (Majerus and Kapp 2002). Assessment of dietary intake of patulin by the population of EU Member States has been recently performed although most of the data on patulin occurrence originated from Northern Europe, and the occurrence data were not fully representative for the national situation in Italy (Majerus and Kapp 2002).

In this paper we report the results of a survey on the occurrence of patulin in conventional and organic fruity foodstuffs including apple juice, pear juice, other juices, fruits puree, fresh apple fruits and apple vinegar commercially available in Italy by using a method with a limit of quantification of  $0.5 \mu\text{g kg}^{-1}$ . The subsequent exposure in different age groups has also been assessed.

## Materials and methods

### Sample collection

Commercial fruity foodstuffs (169 samples) were purchased from Italian markets, supermarkets, hypermarkets or organic food shops and classified on the base of matrix typology (57 apple juices, 15 pear juices, 57 other juices and 40 fruits purees), origin (100 conventional and 69 organic), brand and production batch; for 17 of these products (13 apple juices, two other juices and two fruits purees) two separate samples from different batches of the same brand were tested (the second sample was collected after one–two months from the first sampling). The 40 fruits puree samples included 23 baby foods (11 conventional and 12 organic) designed for infants in their first year of life. In addition to these fruity foodstuffs, 24 fresh apples (14 conventional and ten organic) and two fresh pears (conventional) with rot symptoms were purchased from local shops in Bari province and analysed for patulin. Eight commercial apple vinegar samples (two conventional and six organic) were also analysed. The samples were collected between November 2003 and February 2004.

### Determination of patulin in fruity foodstuffs

The commercial fruity foodstuffs and fresh fruit samples were analysed by using the method of MacDonald et al. (2000) appropriately modified by Arranz et al. (2004) in order to quantify patulin at levels of  $10 \mu\text{g kg}^{-1}$  or lower. In particular, 20 g of sample instead of 10 g were used, and liquid-liquid

extraction was performed with 30 ml of ethyl acetate instead of 20 ml. The final purified extract was reconstituted with 2 ml of pH 4 water instead of 0.5–1 ml and 100  $\mu\text{l}$  instead of 50  $\mu\text{l}$  was injected onto the HPLC column.

The HPLC apparatus was an Agilent 1100 series equipped with a G 1313A autosampler, G1316A column thermostat set at 30°C, G 1315B UV diode array detector set at 276 nm, G1312A binary pump and Agilent Chemstation G2170AA Windows 2000 operating system (Agilent, Waldbronn, Germany). A stainless steel 250  $\times$  4.6 mm i.d., 4  $\mu\text{m}$ , Synergi Hydro-RP (Phenomenex) preceded by a guard column (4  $\times$  3 mm i.d.) with the same stationary phase was used. The mobile phase, eluting at a flow rate of 1 ml/min, consisted of an isocratic mixture of water-acetonitrile-perchloric acid (96:4:0.1) for 18 min followed by a 5-min washing step with an isocratic mixture of water-acetonitrile (35:65). With these conditions patulin eluted with a retention time of 13.71 min.

### Method validation

The performances of the method and of our laboratory were tested by participating in a collaborative study designed to validate a method for the determination of patulin content in spiked and naturally contaminated apple juice and fruits puree at levels of 5, 10, 15 and 25  $\mu\text{g kg}^{-1}$  (Arranz et al. 2004). The results obtained in our laboratory showed mean recoveries from spiked apple juice and fruits puree of 89.1% and 98.6%, respectively. The results of the analyses of the test samples were quite good and the data of the 24 samples analysed (12 apple juice and 12 fruits purees, 20 positive and four negative for patulin content) matched very well to the target values (Lab ID 1602003, Arranz et al. 2004). The repeatability of results for duplicate analyses ranged from 1–11% for apple juice to 1–9% for fruits puree. The limit of quantification (LOQ), calculated as signal to noise ratio of 6, was  $0.5 \mu\text{g kg}^{-1}$  for juices and purees. The method resulted to be applicable also to apple vinegar but with a higher LOQ,  $1.2 \mu\text{g kg}^{-1}$ . The identity of patulin in contaminated samples was confirmed by comparing the UV spectrum of the peak eluting at retention time of patulin with that of authentic standard of patulin (SIGMA-Aldrich Srl, Milan, Italy). Additional confirmation was performed in selected positive samples that were reanalysed by HPLC after spiking the extracts with an equivalent amount of patulin by checking the symmetry of the peak eluting at retention time of patulin. Samples with a concentration of patulin higher than the LOQ were considered positive whereas samples with concentration between the limit of detection and

the LOQ were considered negative since the identity of patulin could not be confirmed by overlapping the UV spectrum with standard due to the high noise. Mean patulin concentrations were calculated by using LOQ/6 for negative samples according to Majerus and Kapp (2002).

## Results and discussion

The occurrence of patulin in conventional and organic fruity foodstuffs is reported in Table I which also includes the results of eight apple vinegar samples, 24 fresh apples and two pears bearing rot symptoms. All results are reported as uncorrected for recovery. The incidence of positive samples was higher in organic products (45%) than in conventional fruity foodstuffs (26%).

Mean patulin concentrations in apple juices, pear juices, other juices and fruits purees were also higher in organic products as compared to conventional products. Statistical analysis of total commercial samples showed a significantly higher mean concentration in organic product, as compared to conventional products ( $p=0.0073$ , Man-Whitney test). Moreover, the maximum patulin concentrations were always found in organic products (see Table I). On the other hand, a similar incidence of positive samples was found in conventional and organic apple juices although the mean patulin concentration was higher in organic juices but not statistically significant ( $p=0.42$ , Man-Whitney test). Of the 57 positive commercial samples 86% were found contaminated with patulin at concentrations below  $10 \mu\text{g kg}^{-1}$ , 7% between 10 and  $25 \mu\text{g kg}^{-1}$ , and 7% (four samples) were above the maximum permitted level of  $50 \mu\text{g kg}^{-1}$ . Three of the samples exceeding the limit of  $50 \mu\text{g kg}^{-1}$  derived from apples (two organic and one conventional) and the fourth one from organic pears. Samples with patulin concentration between 10 and  $25 \mu\text{g kg}^{-1}$  included one apple juice ( $16.3 \mu\text{g kg}^{-1}$ , conventional), one pear juice ( $23.3 \mu\text{g kg}^{-1}$ , organic), one blueberry juice ( $13.5 \mu\text{g kg}^{-1}$ , organic) and one mixed fruits and cereals puree ( $13.0 \mu\text{g kg}^{-1}$ , organic). Of the 23 fruity baby food products (homogenized fruits, 11 conventional and 12 organic) only three samples contained patulin ( $0.5 \mu\text{g kg}^{-1}$ ), one conventional (mixture of apple and blueberry puree) and two organic (mixed fruits and apple puree).

Results of the analyses of the 17 commercial fruity products of the same brand deriving from two different batches are reported in Table II. The absence of patulin in three fruity products was confirmed in the products deriving from the second batch. Among the 14 products found contaminated in the first survey 12 of the second batch were found again contaminated with patulin. In particular, the

only product containing patulin at concentration  $>50 \mu\text{g kg}^{-1}$  in the first survey (organic apple juice) was found again contaminated at about the same level in a sample collected two months later from a different batch.

Recent surveys on patulin occurrence were focused on apples and derived products (MAFF 1999; Trucksess and Tang 1999; Beretta et al. 2000; Gökmen and Acar 2000; Lai et al. 2000; FDA/CFSAN 2001; Yurdun et al. 2001; Tangni et al. 2003), whereas few reports are available on the occurrence of patulin in other fruits juices (de Sylós and Rodríguez-Amaya 1999; Majerus and Kapp 2002). The results of this survey for apple juices are comparable with those obtained in UK reporting 28–35% of samples were contaminated with patulin at concentrations between  $5\text{--}15 \mu\text{g kg}^{-1}$ , 3–25% between  $15\text{--}50 \mu\text{g kg}^{-1}$  and 4% above  $50 \mu\text{g kg}^{-1}$  (MAFF 1999). Our results of conventional pear juices but not of other juices are comparable with those reported by Majerus and Kapp (2002) who found 17% of pear juices and 2.9% of other juices positive for patulin. The lower percentage of positive samples reported by Majerus and Kapp (2002) for other juices could be explained by the higher limit of detection of the analytical methods used which ranged from 2.0 to  $32.4 \mu\text{g kg}^{-1}$ .

The estimated intake of patulin in different age groups, calculated by using the data on the occurrence of this mycotoxin in the 169 fruity products analysed in this study, is reported in Table III. The data on the occurrence of patulin in samples of apple vinegar, fresh apple and pear fruit with rot symptoms were not considered in the calculation of patulin intake. Intakes were calculated for children, adolescent, adults, elderly and all ages by using the Italian dietary intake of fruit juices reported by Turrini et al. (2001). The daily intake of patulin in the different age groups ranged from 0.22 to  $3.41 \text{ ng kg}^{-1} \text{ bw}$  for conventional and from 0.90 to  $14.17 \text{ ng kg}^{-1} \text{ bw}$  for organic fruity foodstuffs.

The highest patulin intake was estimated for children followed by adolescent, adults and elderly. All the estimated intakes of patulin calculated from the data of the present study are far below the PMTDI of  $400 \text{ ng kg}^{-1} \text{ bw}$  proposed by JECFA and endorsed by the SCF. The patulin daily intake estimated in this study for children is comparable with that reported by Majerus and Kapp (2002) ( $28 \text{ ng kg}^{-1} \text{ bw}$ ) when the organic products are considered, whereas for conventional products it is about ten fold lower. A similar situation was observed for adults. A much greater mean patulin exposure was estimated by the US Food and Drug Administration, Center for Food Safety and Applied Nutrition. In particular, the mean daily intake of patulin was  $140 \text{ ng kg}^{-1} \text{ bw}$  for all ages and

Table I. Occurrence of patulin in organic and conventional apples, apple vinegar, apple juice and other fruits-based foods consumed in Italy.

Commodity	Origin	Positive/total	% Positives	$n > \text{LOQ}^a$ $< 10 \mu\text{g kg}^{-1}$	$n > 10$ $< 25 \mu\text{g kg}^{-1}$	$n > 25$ $< 50 \mu\text{g kg}^{-1}$	$n > 50 \mu\text{g kg}^{-1}$	Max ( $\mu\text{g kg}^{-1}$ )	Mean <sup>b</sup> ( $\mu\text{g kg}^{-1}$ )	Median ( $\mu\text{g kg}^{-1}$ )
Apple juice	Conventional <sup>c</sup>	16/33	48	14	1	–	1	53.4	3.14	0.07
	Organic	12/24	50	10	–	–	2	69.3	7.11	0.48
Pear juice	Conventional	1/7	14	1	–	–	–	1.1	0.22	0.07
	Organic	4/8	50	2	1	–	1	61.0	11.46	0.60
Other juices	Conventional <sup>c</sup>	8/45	18	8	–	–	–	1.7	0.19	0.07
	Organic	6/12	50	5	1	–	–	13.5	2.10	0.41
Fruits purees <sup>d</sup>	Conventional	1/15	7	1	–	–	–	0.7	0.11	0.07
	Organic	9/25	36	8	1	–	–	13.0	0.18	0.07
Total <sup>e</sup>	Conventional	26/100	26	24	1	–	1	53.4	1.15	0.07
	Organic	31/69	45	25	3	–	3	69.3	4.78 <sup>f</sup>	0.07
Apple <sup>g</sup>	Conventional	7/13	54	3	2	1	1	81.7	11.14	0.45
	Organic	3/9	33	1	–	–	2	50.8	11.27	0.07
Apple vinegar	Conventional	0/2	0	–	–	–	–	–	0.21	0.21
	Organic	2/6	33	2	–	–	–	4.2	1.52	0.21

<sup>a</sup>limit of quantification (signal/noise = 6), LOQ =  $0.5 \mu\text{g kg}^{-1}$ ; for apple vinegar LOQ was  $1.25 \mu\text{g kg}^{-1}$ ; <sup>b</sup>mean level was calculated using LOQ/6 for negative samples; <sup>c</sup>enclosed is 1 sample of mixture with milk; <sup>d</sup> enclosed are 23 baby food samples (homogenized fruits, 11 conventional and 12 organic); <sup>e</sup>these data are used to estimate intake of patulin; <sup>f</sup>significantly higher as compared to conventional products ( $p = 0.0073$ ); <sup>g</sup>target samples with rot symptoms. A conventional apple containing  $16\,402 \mu\text{g kg}^{-1}$  of patulin and an organic apple containing  $44\,572 \mu\text{g kg}^{-1}$  are not included in the table together with two pears (conventional), one negative and one containing  $23.7 \mu\text{g kg}^{-1}$  of patulin.

800 ng kg<sup>-1</sup> bw for 1–2-year-olds based on mean apple juice intakes of 200 and 216 g day<sup>-1</sup> (FDA/CFSAN 2001).

From the results of previous surveys it was not clear if patulin contamination was more severe in organic fruity products than in conventional ones. Moreover, a limited number of organic samples were analysed and all deriving from apple (Tangni et al. 2003; Ritieni 2003; Malmauret et al. 2002; Beretta et al. 2000). This is the first investigation performed on a significant number of both organic and conventional fruity foodstuff samples which also comprise a significant number of products deriving from fruits other than apples. The results of this investigation showed higher incidence of positive samples and mean patulin concentration in organic products as compared to conventional ones.

The results of the analysis of apple vinegar and fresh fruit samples with rot symptoms are also included in Table I. Patulin was found in two out of six organic apple vinegar samples with a maximum concentration of 4.2 µg l<sup>-1</sup>. As far as we are

concerned this is the first report on the occurrence of patulin in apple vinegar. Patulin was not detected in 12 samples of fruit vinegar analysed in Germany in 1998, with a limit of detection of 5 µg l<sup>-1</sup> (Majerus and Kapp 2002), and a previous report indicated a complete destruction of patulin during alcoholic fermentation of apple juice to cider (Stinson et al. 1978). The finding of patulin in apple vinegar suggests that patulin degradation during fermentation might not be complete and residual levels can be found in the final product. The occurrence of patulin in apple cider has been reported in samples commercially available in Norway, France, Belgium and South Africa (Tangni et al. 2003; Majerus and Kapp 2002; Leggott and Shephard 2001). Tangni et al. (2003) suggested that the source of patulin in cider might be provided by the additional juice introduced after fermentation for softening purposes.

The analysis of fresh apples with rot symptoms showed a higher incidence of positive samples in conventional fruits (58%) as compared to organic (42%). However, very high levels of patulin were measured in two individual samples of both organic (44 572 µg kg<sup>-1</sup>) and conventional apples (16 402 µg kg<sup>-1</sup>), and similar patulin levels were found in the two groups of the remaining positive samples ranging from 2.0 to 81.6 µg kg<sup>-1</sup> for conventional and from 0.5 to 50.8 µg kg<sup>-1</sup> for organic apples, with no difference between means (11.14 vs. 11.27 µg kg<sup>-1</sup>, *p* = 0.51, Mann-Whitney test) (Table I). The incidence of positive samples found in our apple samples is comparable to that one reported by Martins et al. (2002) in a survey of 351 apples with rotten spots that were found contaminated with mean patulin levels ranging from 3.05 to 80.5 mg kg<sup>-1</sup>. One of the two pears with rot spots contained 23.7 µg kg<sup>-1</sup> of patulin.

## Conclusions

All categories of processed fruits available on the market that have been considered in this study showed for organic products higher incidence and levels of contamination with patulin as compared to

Table II. Occurrence of patulin in samples of conventional and organic fruity foodstuffs deriving from two different batches.

Commodity	Origin	Patulin (µg kg <sup>-1</sup> )	
		Batch 1	Batch 2
Apple juice	Conventional	nd <sup>a</sup>	nd
	Conventional	16.3	4.3
	Conventional	0.8	53.4
	Conventional	9.1	8.3
	Conventional	0.5	0.6
	Conventional	4.8	nd
	Organic	0.9	8.4
	Organic	1.2	nd
	Organic	69.3	63.2
	Organic	1.8	5.1
	Organic	1.9	6.8
	Organic	4.9	3.5
	Organic	7.4	2.8
	Other juice	Conventional	nd
Organic		0.9	45.5
Mixed fruit puree	Conventional	nd	nd
Apple puree	Organic	2.5	0.6

<sup>a</sup>nd = not detected (limit of detection, 0.2 µg kg<sup>-1</sup>).

Table III. Estimated intake of patulin in age groups calculated for conventional and organic fruit based products.

Age group	Age	Mean consumption (g day <sup>-1</sup> ) <sup>a</sup>	Body weight (kg)	Intake of patulin (ng kg <sup>-1</sup> bw <sup>b</sup> day <sup>-1</sup> ) <sup>c</sup>	
				From conventional products	From organic products
Children	1–10	59.3	20	3.41	14.17
Adolescent	10–18	40.7	52	0.90	3.74
Adults	18–64	16.4	70	0.27	1.12
Elderly	>64	13.2	70	0.22	0.90
All ages		21.0	64	0.38	1.57

<sup>a</sup>from Turrini et al. 2001; <sup>b</sup>bw = body weight; <sup>c</sup>Mean patulin concentrations used in the calculations were 1.15 µg kg<sup>-1</sup> and 4.78 µg kg<sup>-1</sup> for conventional and organic products, respectively (see Table I).

conventional products. These findings suggest a more strict control of fruits produced under organic conditions before they are processed to juices and derived products. Based on the present study and previous reports, the overall situation of patulin contamination in fruity foodstuffs commercially available in Italy could be considered acceptable since only few samples exceeded the maximum permitted levels. The safety of fruity baby food seems to be quite acceptable since only trace levels of patulin were detected in a limited number of samples. Based on the contamination levels found in this study and the available data on Italian intakes of fruit juices, the level of exposure to patulin through the consumption of fruity food products in Italy is far below the PMTDI established by the EU Scientific Committee for Food.

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