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Abstract

One hundred and twenty five pear, peach, apricot and mixed juices were bought in Italian supermarkets and organic food shops and analysed for the patulin content. An overall incidence of 34.4% was observed in the fruit juices, with a mean contamination level of 3.6 μg kg^{-1}. No one sample trespassed 50 μg kg^{-1}, the maximum permitted threshold according to the European legislation. Anyway, 19 positive samples contained more than 10 μg kg^{-1} of patulin, the maximum level admitted in baby foods. The incidence of patulin contamination was significantly higher in pear juices (64.1%) than in apricot, peach or mixed juices. Statistical analysis (p = 0.002, Kruskal-Wallis test) showed a significantly higher level of patulin in pear (5.1 μg kg^{-1}) and mixed juices (4.9 μg kg^{-1}) than in the other ones. A slightly higher incidence of positive samples was found in conventional juices (35.7%) compared to the organic ones (29.6%). The magnitude between the mean contamination levels was similar in conventional (3.6 μg kg^{-1}) and organic (3.3 μg kg^{-1}) juices (p = 0.474, Mann-Whitney test). Samples were further divided based on the fruit content in fruit juices, fruit nectars and fruit drinks. In fruit nectars the incidence of positive samples (37.8%) was slightly higher than in fruit drinks (27.2%), but the mean patulin contamination was higher in fruit drinks (5.2 μg kg^{-1}) than in fruit nectars (3.4 μg kg^{-1}). Anyway, the magnitude between the two means was not statistically different (p = 0.734; Mann-Whitney test). Italian juices had a higher incidence of patulin (35.3%), with a lower mean content (3.5 μg kg^{-1}), compared to the incidence (22.2%) and level (4.1 μg kg^{-1}) of foreign juices commercialized in Italy. The magnitude of the means between Italian and foreign juices was not statistically different, according to Mann-Whitney test (p = 0.616). The estimated intake of patulin ranged from 0.1 to 1.5 ng kg^{-1} bw for the whole population and from 0.3 to 5.1 ng kg^{-1} bw for the consumers only. The highest patulin intake was estimated for consumers of pear juices, followed by consumers of mixed juices. The daily intake of patulin deriving by the consumption of pear juices suggests that also pear juices, though a minor patulin source, could be monitored for their patulin content in order to control the mycotoxin contamination, especially in countries, such as Italy, where pear nectars are preferred as fruit drinks.

Key words: fruit juices, HPLC, mycotoxin, *Penicillium expansum*, risk assessment

Introduction

Patulin (4-hydroxy-4H-furo [3,2c] pyran-2[6H]-one) is a mycotoxin produced by a large group of fungal species, belonging to over 30 genera, including *Penicillium* spp., *Aspergillus* spp. and *Byssochlamys* spp. (Weidenbörner 2001). The most important producer of patulin is *Penicillium expansum* that is able to attack most fruit both in orchard and in warehouse, causing a disease known as blue mould. The pathogen is usually associated with other microorganisms that colonize the fruit first causing lesions used by *P.expansum* to begin the growth (Snowdon 2007). *P.expansum* is an ubiquitous organism, that can survive to many adverse conditions and contaminate different foodstuffs (Moake et al. 2005). Almost all *P.expansum* strains are patulin producers (Andersen et al. 2004).

In animal studies, patulin has been found to damage the gastrointestinal and respiratory systems (Hayes et al. 1979; Mahfoud et al. 2002), DNA and many enzymes (Cooray et al. 1982; Wichmann et al. 2002), so that a provisional maximum tolerable daily intake (PMTDI) of 0.4 µg kg^{-1} body weight (bw) has been set (JEFCA 1995). Based on this PMTDI, patulin is regulated in the European Union (EU) at levels of 50 µg kg^{-1} in fruit juices and fruit nectars, 25 µg kg^{-1} in solid apple products and 10 µg kg^{-1} in apple-based products for infants and young children (European Commission 2006).

A study carried out by the Directorate General – Health and Consumer Protection (2002) of the EU on the assessment of the dietary intake of patulin by the population of EU member states showed that apple juice and apple nectar are the main sources of patulin intake in most countries, particularly for young children. However, typical Italian fruits such as peaches, pears and apricots lead the nectar sector in Italy (Zenith International 2004) and fruit nectars are mainly used as children and kids snacks (Muraca 2000). Although most of the researches about the contamination level of patulin focused on apple and apple products (Moake et al. 2005), other fruit can be heavily damaged by blue mould. Some studies, carried out by using LC/UV or LC/MS, reported the presence of patulin in commercial fruit juices coming from blueberry (Piemontese et al. 2005, 1 sample with 13.5 µg kg^{-1}), citrus (Majerus and Kapp 2002; 2 samples, mean 25 µg kg^{-1}), grape (Rychlik and Schieberle 1999; 2 samples with 4.9 and 5.2 µg kg^{-1}; Majerus and Kapp 2002; 324 samples, 39% positive, range 3-750 µg kg^{-1}), and pear (Majerus and Kapp 2002, 100 samples, 17% positive, mean 5.8 µg kg^{-1}, range 0.03-91 µg kg^{-1}; Piemontese et al. 2005, 15 samples, 33% positive, mean 6.2 µg kg^{-1}, max 61.0 µg kg^{-1}). Few data about the contamination of peach and apricots juices are available, although Buchanan et al. (1974) and Frank et al. (1977) showed the contamination by patulin produced by *P. expansum* on pears, peaches and apricots.

The aim of this work was to evaluate the occurrence and level of patulin in peach, apricot and pear juices. Moreover, different fruit juices containing pear, peach and/or apricot juices in different concentrations were considered (mixed juices). A second aim of the work, was the investigation of the possible influence of the agricultural production process employed (conventional or organic), of the fruit product typology (fruit juices, fruit nectars, or fruit drinks) on the occurrence and level of patulin contamination. Finally, the exposure to patulin in the Italian population and, particularly, in the consumers of fruit juices was assessed.

Materials and methods

Sampling

One hundred and twenty five fruit juices were bought in supermarkets and organic food shops of north-western Italy during the period January-September 2007. The products analyzed included most of the national and imported pear, peach, apricot and mixed fruit drink products sold in Italy. They were classified based on the agricultural production practices used (conventional/organic), the origin (Italian/foreign), and the fruit content (fruit juices/fruit nectars/fruit drinks). According to the European legislation (European Council 2001), fruit juices should have 100% fruit content, while pear, peach, and apricot nectars should have respectively at least 50%, 45%, and 40% fruit content. The other fruit based products are called fruit drinks.

Sample preparation and analysis

The samples were analyzed according to the method described by Spadaro et al. (2007) with slight variations. Cloudy products were left overnight in contact with pectinase enzyme solution (Sigma Chemical Co.®, St Louis, MO, USA; 5U/g of juice), and then centrifuged at 4,500 rpm for 5 min. Thirty grams of clarified juice were collected and liquid-liquid extracted with ethyl acetate (Sigma Chemical Co.®). The procedure was carried out three times. The organic phase was dehydrated with

15 g of sodium sulphate anhydrous (Merck®, Darmstadt, Germany) and then evaporated to dryness (Rotavapor Laborota 4000, Heidolph®, Schwalback, Germany). The clean up was performed using C_{18} SPE column (200 mg, 6 ml, J.T. Baker®, Phillipsburg, NJ, USA), previously activated with 10 ml of toluene (Sigma Chemical Co.®). The columns were cleaned up with 4 ml of toluene and, for elution, 4 ml of toluene and ethyl acetate solution (1:1) were used. The samples were collected in 4 ml vials and completely dried (Reack-Therm III, Pierce®, Rockford, IL, USA). Samples were resuspended with 1.5 ml of acetic acid solution (4.35 mM, pH 4.0) and transferred into HPLC vials.

The HPLC apparatus was an Agilent® 1100 series equipped with G 1379 degasser, G 1313A autosampler, G1316A column thermostat set at 30 °C, G 1315B UV diode array detector set at 276 nm, G1311 quaternary pump and Agilent Chemstation G2170AA Windows XP operating system (Agilent®, Waldbronn, Germany). A stainless steel analytical column (250 x 4.6mm i.d., 4 μm, Synergy Hydro-RP C18; Phenomenex®, Torrance, CA, USA) preceded by a guard column (4 x 3 mm i.d.) with the same stationary phase was used. The mobile phase, eluting at a flow rate of 1ml/min, consisted of an isocratic mixture of water – acetonitrile – perchloric acid (96:4:0.1) for 16 min, followed by a washing step with an isocratic mixture of water–acetonitrile (35:65). Hundred microliters of sample were injected onto the HPLC column and the retention time of patulin was 11.82 min. The amount of patulin in the final solution was determined by using a calibration graph of concentration versus peak area and expressed as ng/ml, achieved by injection onto the HPLC column of 100 μl of standard solutions of patulin (Sigma Chemical Co. ®) prepared according to the method described by Arranz et al. (2004). The standard solutions had concentrations of 500 ng ml^{-1}, 400 ng ml^{-1}, 250 ng ml^{-1}, 100 ng ml^{-1} and 50 ng ml^{-1} of patulin and were prepared the same day of HPLC analysis, in order to avoid patulin degradation.

The recovery was determined on a blank fruit juice spiked at three concentrations of patulin (8, 30 and 50 μg kg^{-1}). Each test was performed three times and the mean recovery values were respectively 85.2, 89.5 and 88.9%. The repeatability ranged from 4.5 to 8.3% for triplicate analyses. The limit of detection (LOD) and the limit of quantification (LOQ) based on the IUPAC definition (Thompson et al., 2002) were respectively 1.2 and 1.7 μg kg^{-1}. The high value of the regression coefficient ($R^2 \geq 0.99$) obtained indicated a good linearity of the analytical response.

Statistical analysis

Samples with a concentration of patulin higher than the LOD were considered positive, whereas samples with concentrations lower than LOD were considered negative. Mean patulin concentrations were calculated by using 0 for negative samples. Experimental results are reported as mean ± standard deviation and maximum. The Kruskal-Wallis test was used to compare the mean patulin levels in apricot / peach / pear / mixed juices, and in fruit juices / fruit nectars / fruit drinks, while the Mann-Whitney test was used to compare the mean patulin levels in conventional / organic juices, and in Italian / foreign juices, using the null hypothesis that the levels were not different. The χ^2 test was used to compare the patulin contamination frequencies of the different categories of fruit juicess. Statistical analyses were performed by using the programme SPSS Release 12.01 (2003).

Results and discussion

Out of 125 samples analyzed, 43 having a patulin concentration higher than the LOD were considered positive (Table 1), ranging from 1.2 to 33.4 µg kg^{-1}. An overall incidence of 32.0% was observed in the fruit juices, with 24 samples having less than 10 µg kg^{-1} and 19 samples having more than 10 µg kg^{-1}. Positive samples were further divided as having more or less than 10 µg kg^{-1}, the threshold limit established for baby foods for infants and young children (European Commission 2006). Anyway no one sample trespassed 50 µg kg^{-1}, the maximum permitted threshold according to the European legislation. A mean contamination level of 3.6 µg kg^{-1} was found in the samples analyzed.

The levels and incidence of patulin contamination have been largely assessed in apples and apple-derived products (Moake et al. 2005). This study aimed at assessing the contamination level of patulin in fruit juices containing apricot, peach, pear, or one or more of the previously named fruits mixed with other fruits. To our knowledge, this study represents the most extensive investigation performed on such fruit juice typology. Looking at the fruit species content, the incidence of patulin contamination was significantly higher in pear juices (64.1%) than in apricot, peach or mixed juices. Statistical analysis (p = 0.002, Kruskal-Wallis test) showed a significantly higher level of patulin in pear (5.1 µg kg^{-1}) and mixed juices (4.9 µg kg^{-1}) than in the other ones.

P. expansum can cause significant losses in pear fruits (Snowdon 2007). By considering that most of this pathogen isolates are able to produce patulin (Andersen et al. 2004), patulin can be easily found in pears and pear derived products. Moreover, patulin is stable in pH ranges of 3.3-6.3 (Jefferys 1952) and in substrates with low levels of sulfydryl compounds (Scott and Somers 1968), both features are typical of pears. Based on studies carried out by Laidou et al. (2001), removal of blue mould rot of pears cannot protect consumers from the presence of patulin, because up to 25% of the mycotoxin can be detected in the sound tissues around the diseased ones.

The mixed juices analyzed in this study represent the second source of contamination of patulin, based on the incidence and level of the mycotoxin. This could be explained either with the lower care deserved to the production of mixed juices, often containing higher quantities of sugars and other additives, or with the general presence of a certain percentage of apple juice, the major source of patulin, together with other fruit juices.

In Table 2, the fruit juices analyzed were divided based on the agricultural production process employed (conventional or organic), on the fruit content (fruit juices, fruit nectars, or fruit drinks), and on the origin (Italian or foreign juices). As already found by analyzing pure and mixed apple juices (Spadaro et al. 2007), a slightly higher incidence of positive samples was found in conventional juices (35.7%) compared to the organic ones (29.6%). The χ^2-test showed that the frequencies of patulin occurrence in conventional and organic juices were comparable ($p = 0.483$). Also the magnitude between the mean contamination levels was similar in conventional (3.6 µg kg^{-1}) and organic juices (3.3 µg kg^{-1}). The hypothesis that the mean patulin contamination levels in conventional and organic juices were not different was accepted ($p = 0.692$, Mann-Whitney test). It should be noticed anyway that organic juices contain, on an average, higher fruit content (54%) than conventional ones (45%), being more exposed to patulin contamination. Generally sound fruit, which is properly picked and stored, is less subjected to blue mould rot and, consequently, to contain patulin (Sydenham et al. 1995). The absence of significant differences, based on the agricultural production methodologies used, can be explained with the fact that the presence of patulin is a strong indicator of poor agricultural practice, either conventional or organic (Codex Alimentarius 2003), although *P. expansum* mainly develops after harvesting, where the main discriminating factor becomes the removal of decayed and damaged fruits during juice processing.

Samples were further divided based on the fruit content: fruit juices should have 100% fruit content, while pear, peach, and apricot nectars should have respectively at least 50%, 45%, and 40% fruit content. The fruit based products with lower fruit content are called fruit drinks. Statistical data (Russo et al. 2002) indicate that national per capita consumption of nectars equals approximately to 6 liters, while 100% fruit juice consumption is lower (4 liters per capita). Per capita consumption of fruit drinks – with a lower fruit content and supplemented with vitamins and other nutritious ingredients – is approximately 4 liters per person. In the 5 samples containing 100% fruit, patulin was not found. In fruit nectars the incidence of positive samples (37.8%) was slightly higher than in fruit drinks (27.2%), but the mean patulin contamination was higher in fruit drinks (5.2 µg kg^{-1}) than in fruit nectars (3.4 µg kg^{-1}). Anyway, the magnitude between the two means was not statistically different ($p = 0.734$; Mann-Whitney test). As already hypothesized for the mixed juices, and most of the fruit drinks are mixed fruit drinks, the higher patulin content could be explained by the lower attention used in the production process of such products, where the lower quality of the fruits can be easily corrected by adding sugars and additives. Since fruit drinks are often produced by using raw materials poor in quality, the probability to retrieve patulin and others similar contaminants in fruit drinks is increased (Moake et al. 2005).

Finally the samples were divided by considering their origin. Italian juices had a higher incidence of patulin (35.3%), with a lower mean content (3.5 µg kg^{-1}), compared to the incidence (22.2%) and level (4.1 µg kg^{-1}) of foreign juices commercialized in Italy. The magnitude of the means between Italian and foreign juices was not statistically different, according to Mann-Whitney test (p = 0.616). Anyway it should be noticed that it was difficult to find pear, peach and apricot foreign juices commercialized in Italy (only 9 samples out of 125), because pears, peaches and apricots are typical products of the Italian horticulture.

The estimated intake of patulin in the total population and, particularly, in the consumers was calculated based on the patulin contamination in the pear, apricot, peach and mixed fruit juices analyzed. Apple juices are the most consumed fruit juices around the world. However, in Italy apple juice is not so popular as in other Countries. Apricot, peach and pear juices are highly consumed by a large share of the Italian population, and they are used as baby-foods (Zenith International 2004). By considering (Turrini et al. 2001) that the mean daily consumption of fruit juices in Italy is 21.0 g, and that only 29.8% of the population consume fruit juices (70.5 g day^{-1}), the intake of patulin was calculated in four different scenarios, where the fruit juice consumed was only apricot, or pear, or peach, or mixed juice (Table 1). The daily intake of patulin ranged from 0.1 to 1.5 ng kg^{-1} bw for the whole population and from 0.3 to 5.1 ng kg^{-1} bw for the consumers only. The highest patulin intake was estimated for consumers of pear juices, followed by consumers of mixed juices. All of the values are from two to three magnitude orders lower than the PMTDI recommended by JEFCA (1995) which is 400 ng kg^{-1} bw day^{-1}. The intake of patulin from apricot, pear, peach and mixed juices could be considered similar to the values found by Piemontese et al. (2005) that anyway showed a higher intake of patulin by consuming organic than conventional fruit juices.

In conclusion, the average level of contamination of apricot, peach, pear and mixed juices analyzed in this work appears to be generally low, by considering that none of the samples exceeded the threshold of 50 µg kg^{-1} imposed by the European regulations. Anyway, the incidence of patulin found in pear juices showed that also pears – besides apples – can be substrates for patulin contamination. The daily intake of patulin deriving by the consumption of pear juices suggests that also pear juices, though a minor patulin source, could be monitored for their patulin content in order to control the mycotoxin contamination, especially in countries, such as Italy, where pear nectars are preferred as fruit drinks.

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Table 1 - Patulin contamination in apricot, pear, peach and mixed juices marketed in Italy.

	Mean fruit concentration (%)	Positive (%)	Number of samples			Mean[a] ± (µg k
			Positive/total	<10 µg kg^{-1}	>10 µg kg^{-1}	
Apricot juices	41	25.9	7/27	2	5	3.6 ±
Pear juices	54	64.1	25/39	17	8	5.1 ±
Peach juices	48	6.7	2/30	2	0	0.3 ±
Mixed juices	43	31.0	9/29	3	6	4.9 ±
TOTAL JUICES	47	34.4	43/125	24	19	3.6 ±

[a]Mean level was calculated using 0 for samples lower than LOD.

Table 2 – Patulin contamination in different categories of apricot, peach, pear and mixed juices marketed in Italy.

	Mean fruit concentration (%)	Positive (%)	Number of samples		
			Positive/total	<10 µg kg^{-1}	>10 µg kg^{-1}
Conventional juices	45	35.7	35/98	21	14
Organic juices	54	29.6	8/27	3	5
Fruit juices	100	0.0	0/5	0	0
Fruit nectars	49	37.8	37/98	23	14
Fruit drinks	28	27.2	6/22	1	5
Italian juices	48	35.3	41/116	24	17
Foreign juices	39	22.2	2/9	0	2

[a]Mean level was calculated using 0 for samples lower than LOD.

Table 3 – Estimated intake of patulin in the total population and in the consumers only calculated for pear, apricot, peach and mixed fruit juices.

	Mean Consumption (g day^{-1})[a]	Intake of patulin (ng kg^{-1} bw[b] day^{-1})			
		From apricot products	From pear products	From peach products	From mixed juices
Population	21.0	1.1	1.5	0.1	1.5
Consumers only	70.5	3.6	5.1	0.3	4.9

[a] from Turrini et al. 2001: consumers represent 29.8% of the population; [b] bw=body weight; c mean patulin concentration used in the calculations were 3.6 μg kg^{-1} for apricot juices, 5.1 μg kg^{-1} for pear juices, 0.3 μg kg^{-1} for peach juices and 4.9 μg kg^{-1} for mixed juices.