

Survey of Patulin Contamination in Italian Apple Juices from Organic and Conventional Agriculture

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Abstract: There is an increasing concern of consumers about food safety and contaminants. In this study, 26 commercial apple juices from organic, integrated and conventional agriculture were analysed for the mycotoxin patulin by reversed-phase high performance liquid chromatography using diode array detection. The patulin content showed a skewed distribution, the average percentage of juice contamination was 58% and all samples had a patulin content below the actual safe limit of $50 \mu\text{g L}^{-1}$. Data on organic products are discussed and compared with information from the literature.

Key words: Contamination, fruit juice, HPLC, mycotoxin, statistics, organic products

INTRODUCTION

Patulin, a polyketide lactone (4-hydroxy-4*H*-furo [3, 2-*c*] pyran-2 (6*H*)-one) (Fig. 1), is a mycotoxin produced by a number of fungal species in the genera *Aspergillus* and *Penicillium* (Davis and Dinier, 1978). Patulin has been found as a contaminant in many fruits, vegetables and other foods, however the major sources of contamination for humans are apples and apple products. There is a general concern regarding the health risk associated with the consumption of patulin-contaminated foods. The EC has fixed a limit of 50, 25 and $10 \mu\text{g L}^{-1}$ patulin as the maximum allowed content of patulin in apple juice, solid apple products and fruity baby food, respectively (EC, 2003). A study conducted on 215 commercial apple juices has found that 46% of the samples exceeded the patulin level of $50 \mu\text{g L}^{-1}$ (Gokmen and Acar, 1998). Occasionally, very high patulin concentration in fruit juices have been found, reaching $1000 \mu\text{g L}^{-1}$ or more (Beretta *et al.*, 2000a, b; Malmauret *et al.*, 2002; Piemontese *et al.*, 2005).

It has been recommended that the levels of patulin in food should be reduced to the lowest that are technologically achievable by the industry to minimise the potential risk of adverse effects for humans (CODEX, 2001). About 54% patulin can be removed using high-pressure water spraying of apple before processing (Acar *et al.*, 1998), whereas the content of patulin decreases by only 25% during the heating processes of fruit juice production (Kadalkal and Nas, 2003).

The general concern about the use of additives in food processing has stimulated great attention on the marketing of foods with natural ingredients, including

foods from organic agriculture. Organic agriculture differs from the conventional type by its avoidance of synthetic chemicals for soil preparation, fertilisation and pest control (GUCE, 1991). Integrated agriculture allows the use of production methods in between the organic and the conventional types.

This study aims to determine the patulin content in three types of commercial apple juices produced with fruits obtained from organic, integrated and conventional agriculture, respectively. This research was focused on organic apple juice because of the wide consumers acceptance and the economic interest of this product. It is expected that this information will be useful to researchers, producers, consumers and regulatory agencies as well.

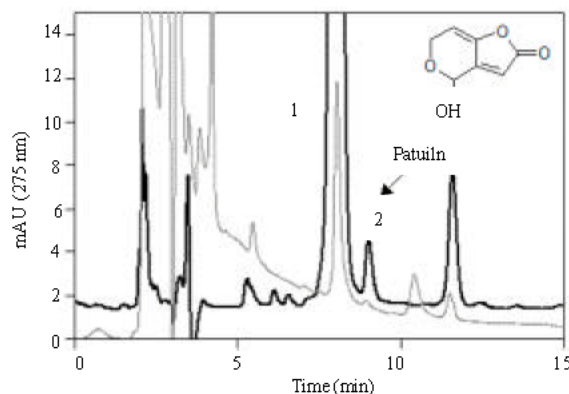


Fig. 1: HPLC chromatograms of apple juice before (grey line) and after sample clean-up (dark line). Peak legend: (1) 5-hydroxymethylfurfural; (2) patulin

MATERIALS AND METHODS

Samples: Twenty-six apple juices (8 organic, 4 integrated and 14 conventional products) were collected from producers and local markets. Cloudy apple juices were depectinised with Zimaclar (Enologica Vason, Verona, Italy) for 4 h at 40°C. Then, samples were centrifuged at 5000 g for 15 min at 4°C (Avant J25, Beckman, Milano, Italy) before liquid-liquid extraction and injection in HPLC. Each sample was analysed in duplicate and the patulin average content referred to as fruit juices with 10°Brix.

Standards and reagents: A patulin standard in crystalline form (Tecno, Trieste, Italy) was dissolved in acetonitrile (Merck, Milano, Italy) and diluted to the final concentration of 1 mg L⁻¹. This value was verified by UV spectrometer at 275 nm using the following values of Molecular Weight (MW = 154) and molar extinction ($\epsilon = 14600$). Working solution of patulin between 5500 $\mu\text{g L}^{-1}$ were prepared in acetonitrile for calibration purposes.

Sample preparation: Patulin was extracted with ethyl acetate and then cleaned-up by liquid-extraction with sodium acetate solution according to the literature (Brause *et al.*, 1996). The extracted samples were dried with anhydrous sodium sulfate and after evaporation of ethyl acetate, patulin was determined by Reversed-Phase High Performance Chromatography (RP-HPLC) with Diode Array Detection (DAD).

HPLC conditions: The HPLC system (Jasco, Tokyo, Japan) was equipped with a MD-1510 diode-array detector set at 275 nm. Data were acquired and processed using Borwin-PDA version 1.50 software (JMBS Developments, Grenoble, France). Samples were injected with a 20 μL loop using a 7125 valve (Rheodyne, Cotati, CA) onto a Adsorbosphere RP-18 column (250×4.6 mm; 5 μm i.d.) protected with a guard column of the same material (Alltech, Milano, Italy). The column operated at 30°C (Jones Chromatography, Mid Glamorgan, UK) with a flow rate of 1.0 mL min⁻¹ using isocratic elution with water as eluent A and acetonitrile as eluent B (95:5, v/v).

Method validation: Peak identification was based on retention time (t_R), spectral information and fortification technique (spiking). Peak quantification was based on the external standard method using calibration curves fitted by linear regression analysis (Statistica 5.1, StatSoft, Tulsa, OK).

The precision of the method (repeatability) was determined after five independent extractions of the same

fruit juice. To assess the accuracy of the method, a recovery study was carried out by adding patulin at four concentration levels (10, 20, 30 and 40 $\mu\text{g L}^{-1}$) to a fruit juice that was previously analysed. The limit of detection at 275 nm was evaluated as three times the signal-to-noise ratio (LOD = 3S/N).

RESULTS AND DISCUSSION

The calibration curve, relating the peak area (y) to the patulin concentration (x), yielded the equation $y = 108841x$. The linearity of response, expressed in terms of regression coefficient (r), showed a value of $r \geq 0.997$, implying a good suitability of the HPLC method. The precision in terms of area and retention time was 5% and 0.2%, respectively. The average percentage recovery of patulin was $70 \pm 3\%$. Attempts to improve the recovery by using SPE cartridges was unsuccessful. The limit of detection at 275 nm was 1.5 $\mu\text{g L}^{-1}$ patulin using an injection volume of 20 μL . The HPLC analysis of patulin in apple juice required a preliminary sample clean-up to allow the removal of interfering compounds and the concentration of patulin (Fig. 1). Patulin ($t_R = 8.8$ min) eluted after the 5-Hydroxy-Methyl-Furfural, HMF ($t_R = 7.9$ min), a compound formed during the thermal treatment of apple juices. There are a number of HPLC methods available for the determination of patulin in fruit juice and the choice should take into account several parameters, including the recovery value and the sensitivity of the method. The recovery value of patulin found in our study was within the limits set by the Italian regulation: 50-120% below 20 $\mu\text{g kg}^{-1}$ patulin and between 70-105% for a contamination range of 20-50 $\mu\text{g kg}^{-1}$ (GURI, 2005). Recently, Piemontese *et al.* (2005) have been able to quantify patulin down to 0.5 $\mu\text{g kg}^{-1}$ (based on a signal-to-noise ratio of 6) using a 100 μL injection volume. Lower detection limits can be achieved by combination of this method with more specific detection techniques such as LC-MS/MS.

Patulin is relatively stable in acid conditions and the processing of fruit juice does not ensure a product free from this mycotoxin. Patulin was detected in 15 samples and the concentration values showed a log-normal distribution skewed to the right (data not shown). The patulin content found in the apple juices is shown in Table 1. The patulin level was always below the limit of 50 $\mu\text{g L}^{-1}$, the maximum value permitted for this mycotoxin by the Italian Health Authorities. The overall percentage of juice contamination was 59% and the maximum content (22.2 $\mu\text{g L}^{-1}$) being found in organic apple juice. From a statistical point of view (Kruskal-Wallis H test, at p-level 0.05), a lack of significant

Table 1: Occurrence of Patulin in commercial apple juices found in this study and reported in the literature

Study made in	Apple juice	Positive/total (positive%)	Critical sample* (No.)	(µg L ⁻¹ or µg kg ⁻¹)			References
				Range	Mean (±SD)	Median	
Italy	Conventional	9/14 (64%)	0	1.7-4.5	2.5	2.1	This study
	Integrated	3/4 (75%)	0	1.2-1.8	1.2	1.2	
	Organic	3/8 (38%)	0	1.0-22	9.8	6.4	
Belgium	Conventional	12/90 (13%)	0	8.1-14	10.2 (±3.6)	—	(Baert <i>et al.</i> , 2006).
	Organic	8/65 (12%)	2	16-123	43.1 (±36.9)	—	
Italy	Conventional	16/33 (48%)	1	0.5-53	3.1	0.07	(Piemontese <i>et al.</i> , 2005)
	Organic	12/24 (50%)	2	0.5-69	7.1	0.48	
	Conventional	15/17 (29%)	1	6-56	24.8	—	(Ritieni, 2003)
	Organic	6/15 (40%)	1	1-74	28.3	—	
	—**	19/24 (79%)	3	0.25-115	—	—	(Spotti and Berni, 2003) (Beretta, 2000a,b)
	Conventional	—	—	0.7-3	1.0 (±1)	—	
	Organic	—	—	0.1-28	7.7 (±8)	—	
South Africa	Conventional**	—	2	0.7-1150	203 (±433)	—	(Leggott and Shepard, 2001)
	—	2/13 (15%)	0	5-10	7.5	—	
Turkey	for infant	2/3 (67%)	—	5	5.0	—	(Gokmea and Acar, 1998)
	—	215/215 (100%)	99	7-376	—	—	
Spain	—	82/100 (82%)	7	0.5-170	13.8	—	(Prieta <i>et al.</i> , 1994) (Rovira <i>et al.</i> , 1993)
	—	6/8 (75%)	1	3-78	27	19	

*Number of apple juices with patulin content exceeding the legal limit of 50 µg L⁻¹ or µg kg⁻¹; **With pulp

difference between the patulin content of juices produced with apple from conventional, integrated and organic agriculture was found. The statistical evaluation of data represents a key point for researchers. In this view, it is always important to know the distribution of the analyte in the sample in order to establish the appropriate model of data evaluation. Deviation from the normal distribution may occur for wine mycotoxins, including patulin (Prieta *et al.*, 1993) and OTA (Prieta *et al.*, 2001). The choice of the appropriate statistical analysis is critical; among the non-parametric test available, the Kruskal-Wallis H test is suitable for among-groups comparison, whereas the Mann-Whitney U test is a non-parametric alternative to the t-test for independent samples.

The percentage of apple juices contamination found in several survey studies is usually below 60% (Piemontese *et al.*, 2005; Mortimer *et al.*, 1985; Rouira *et al.*, 1993) however, highest values of juice contamination up to 100% can occur (Southgate *et al.*, 1995; Tangni *et al.*, 2003). To explain these findings two hypothesis are formulated: The apples used for fruit juice production are highly contaminated and the process of juice production only partially remove the patulin; and most of the commercial apple juices are obtained by blending stocks of low and high patulin contaminated apple juices in order to fit within the legal limit. Martins *et al.* (2000) have found a high frequency of fruit contamination, ranging between 53-89%, in several apple varieties.

Consumers usually judge the foods from organic agriculture to be more safe and healthy than conventional products (Canavari *et al.*, 2002). However, patulin content has been reported to occur at high concentration values in juice made with apples from organic agriculture (Table 1). Mortimer *et al.* (1985) have found the

uppermost level of patulin (56 µg L⁻¹) to occur in organic apple juice. Ritieni (2003) has found patulin up to 74 µg kg⁻¹ in organic apple puree. As well, Beretta *et al.* (2000 a,b) have discover more patulin in apple juices from organic agriculture (7.7±8.0 µg kg⁻¹) compared to the conventional products (1.0±1.1 µg kg⁻¹). Apple-baby-foods from organic agriculture show highest contamination rate and highest patulin content compared to conventional products (Plessi *et al.*, 1998). The latest presence in the Italian market of commercial fruit juices with a patulin content exceeding the legal limit of 50 µg kg⁻¹ (Pimontese *et al.*, 2005). Confirms the need of a continuous monitoring of patulin in fruit juices.

CONCLUSION

The early outcomes of this study show that the commercial apple juices analysed have a patulin content below the actual EU regulatory limit of 50 µg L⁻¹. Based on the present study and previous reports, due to the high frequency of contamination, the improvement of the agricultural practices and the enforcement of the analytical controls in order not to exceed the safe limit is strictly required. A further study to ascertain the traceability of patulin from the fruit to the juice will probably allows to test the hypothesis previously formulated on the origin of patulin in fruit juice.

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