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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 μg 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 (6H)-one) (Fig. 1), is a mycotoxin produced by a number of fungal species in the genera *Aspergillus* and *Penicillum* (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 μg 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 μg L^{-1} (Gokmen and Acar, 1998). Occasionally, very high patulin concentration in fruit juices have been found, reaching 1000 μg 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 (Kadakal 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.

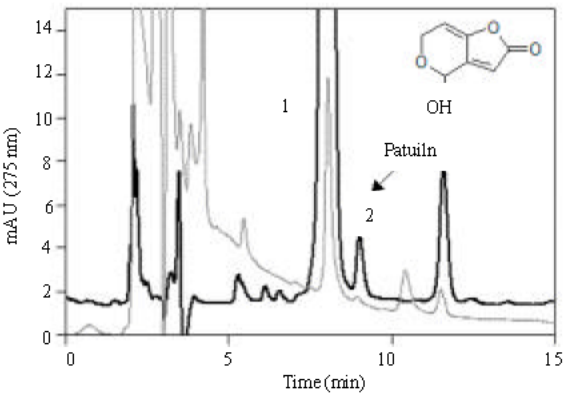

Fig. 1: HPLC chromatograms of apple juice before (grey line) and after sample clean-up (dark line). Peak legend: (1) 5-hydroxymethylfurfural; (2) patulin

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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 (ϵ = 14600). Working solution of patulin between 5500 μg 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 μg 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±3%. Attempts to improve the recovery by using SPE cartridges was unsuccessful. The limit of detection at 275 nm was 1.5 μg 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-Hydroy-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: 50120% below 20 μg kg^{-1} patulin and between 70-105% for a contamination range of 20-50 μg kg^{-1} (GURI, 2005). Recently, Piemontese *et al*. (2005) have been able to quantify patulin down to 0.5 μg 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 μg 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 μg 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.)	($\mu g\ L^{-1}$ or $\mu g\ kg^{-1}$) Range	Mean (±SD)	Median	References
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 *et al*., 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 *et al*., 2005)
	Organic	12/24	(50%)	2	0.5-69	7.1	0.48	
	Conventiona	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)
	Conventional	___	___	___	0.7-3	1.0 (±1)	___	(Beretta, 2000a,b)
	Organic	___	___	___	0.1-28	7.7 (±8)	___	
	Conventional**	___	___	2	0.7-1150	203 (±433)	___	
South Africa	___	2/13	(15%)	0	5-10	7.5	___	(Leggott and Shepard, 2001)
	for infant	2/3	(67%)	___	5	5.0	___	
Turkey	___	215/215	(100%)	99	7-376	___	___	(Gokmea and Acar, 1998)
Spain	___	82/100	(82%)	7	0.5-170	13.8	___	(Prieta *et al*., 1994)
	___	6/8	(75%)	1	3-78	27	19	(Rovira *et al*., 1993)

*Number of apple juices with patulin content exceeding the legal limit of 50 $\mu g\ L^{-1}$ or $\mu g\ kg^{-1}$; **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) hsave found the uppermost level of patulin (56 $\mu g\ L^{-1}$) to occur in organic apple juice. Ritieni (2003) has found patulin up to 74 $\mu g\ kg^{-1}$ 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 $\mu g\ kg^{-1}$) compared to the conventional products (1.0±1.1 $\mu g\ kg^{-1}$). 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 $\mu g\ kg^{-1}$ (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 $\mu g\ L^{-1}$. 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.

REFERENCES

Acar, J., V. Gökmen and E.E. Taydas, 1998. The effect of processing technology on the patulin content of juice during commercial apple juice concentrate production. Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 207: 328-331.

Baert, K., B. De Meulenaer, A. Kamala, C. Kasase and F. Devlieghere, 2006. Occurrence of patulin in organic, conventional and handcrafted apple juices marketed in Belgium. J. Food Prot., 6: 1371-1378.

Beretta, B., A. Gaiaschi, C.L. Galli and P. Restani, 2000a. Patulin in apple-based foods: Occurrence and safety evaluation. Food Additives and Contaminant, 17: 399-406.

Beretta, B., A. Gaiaschi, C.L. Galli and P. Restani, 2000b. Valutazione Della Presenza Di Patulina Inmele e Prodotti Da Esse Derivati. In: Act of the VI Congress on Chemistry of Foods. Brandolini, V. and E. Menzani (Eds.), Società Chimica Italiana, Roma, Italy, pp: 90-95.

Brause, A.R., M.W. Trucksess, F.S. Thomas and S.W. Page, 1996. Determination of patulin in apple juice by liquid chromatography: Collaborative study. J. AOAC. Int., 79: 451-455.

Canavari, M., G.M. Bazzani, R. Spadoni and D. Regazzi, 2002. Food safety and organic fruit demand in Italy: A survey. Br. Food J., 104: 220-232.

CODEX, 2001. Proposed draft code of practice for the prevention of Patulin contamination in apple juice and apple juice ingredients in other beverages. Joint FAO/WHO Food Standards Programme; Codex Alimentarius Commission, CX/FAC 01/23.

Davis, N.D. and U.L. Dinier, 1978. Mycotoxin. In: Food and Beverage Mycology. Beuchat, L.R. (Ed.), AVI, Westport, CT., pp: 397-444.

EC, 2003. European Commission Regulation 1425/2003 amending Regulation No. 466/2001 as regards Patulin. Offic. J. Eur. Union, 12.08.2003 L 203/3.

GUCE, 1991. Metodo di produzione biologico di prodotti agricoli. Gazzetta Ufficiale delle Comunità Europee, No. 2092/91, 24.06.1991, L198/1, 22.07.1991.

GURI, 2005. Recepimento della direttiva 2003/78/CE della Commissione dell'11 agosto 2003, relativa ai metodi di campionamento e di analisi per il controllo ufficiale dei tenori di patulina nei prodotti alimentari. Gazzetta Ufficiale della Repubblica Italiana.

Gökmen, V. and J. Acar, 1998. Incidence of patulin in apple juice concentrates produced in Turkey. J. Chromatography A, 815: 99-102.

Kadakal, C. and S. Nas, 2003. Effect of heat treatment and evaporation on patulin and some other properties of apple juice. J. Sci. Food Agric., 83: 987-990.

Leggott, N.L. and G.S. Shepard, 2001. Patulin in South African commercial apple products. Food Control, 12: 73-76.

Malmauret, L., D. Parent-Massin, J.L. Hardy and P. Verger, 2002. Contaminants in organic and conventional foodstuffs in France. Food Additives and Contaminants, 19: 524-532.

Martins, M.L., A. Gimeno, H.M. Martins and F. Bernardo, 2002. Co-occurrence of patulin and citrinin in Portuguese apples with rotten spots. Food Additives and Contaminants, 19: 568-574.

Mortimer, D.N., I. Parker, M.J. Shephard and J. Gilbert, 1985. A limited survey of retail apple and grape juices for the mycotoxin patulin. Food Additives and Contaminants, 2: 165-170.

Piemontese, L., M. Solfrizzo and A. Visconti, 2005. Occurrence of patulin in conventional and organic fruit products in Italy and subsequent exposure assessment. Food Additives and Contaminants, 5: 437-442.

Pietri, A., T. Bertuzzi, L. Pallaroni and G. Piva, 2001. Occurrence of ochratoxin A in Italian wines. Food Additives and Contaminants, 7: 647-654.

Plessi, M., D. Bertelli and A. Monzani, 1998. Valutazione del contenuto di patulina in prodotti per la prima infanzia a base di mela. Rivista di Scienza dell'Alimentazione, 27: 237-243.

Prieta, J., M.A. Moreno, J. Bayo, S. Díaz, G. Suárez, L. Domínguez, R. Canela and V. Sanchis, 1993. Determination of patulin by reversed-phase high-performance liquid chromatography with extraction by diphasic dialysis. Analyst, 118: 171-173.

Prieta, J., M.A. Moreno, S. Díaz, G. Suárez and L. Domínguez, 1994. Survey of patulin in apple juice and children's apple food by the diphasic dialysis membrane procedure. J. Agric. Food Chem., 42: 17011703.

Ritieni, A., 2003. Patulin in Italian commercial apple products. J. Agric. Food Chem., 51: 6086-6090.

Rovira, R., F. Ribera, V. Sanchis and R. Canela, 1993. Improvements in the quantitation of patulin in apple juice by high-performance liquid chromatography. J. Agric. Food Chem., 41: 214-216.

Southgate, D.A.T., I.T. Johnson and G.R. Fenwick, 1995. Nutritional Value and Safety of Processed Fruit Juices. In: Production and Packaging of Non-Carbonated Fruit Juices and Fruit Beverages Ashurst, P.R. (Ed.), Blackie Academic and Professional, London, UK., pp: 349.

Spotti, E. and E. Berni, 2003. Patulin determination by RP-HPLC in apple and pear purees and concentrated juices. Ind. Conserve, 78: 429439.

Tangni, E.K., R. Theys, E. Mignolet, M. Maudoux, J.Y. Michelet and Y. Larondelle, 2003. Patulin in domestic and imported apple-based drinks in Belgium: Occurrence and exposure assessment. Food Additives and Contaminants, 5: 482-489.