

Assessment of the patulin contamination level in selected apple-based products available in retail in Poland

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The aim of this study was to assess the level of patulin (PAT) contamination in selected apple-based products, including food intended for infants and young children, available in retail in Poland in 2016-2020. The products examined were selected due to their availability on the market and all of them derived from conventional production. All products examined contained only apples in their composition, with no other fruit added. The obtained results were related to the provisional maximum tolerable daily intake (PMTDI) of PAT established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) (WHO 1995). In addition, in selected age groups, exposure assessment resulting from the intake of this toxin in the diet, was estimated. Only 2 of the 47 examined apple products contained PAT. The determined levels of contamination were significantly lower than a maximum acceptable level specified in the provisions of the food law at the level of 50.0 µg kg⁻¹ for apple juice. When considering the value of the average daily consumption of apple juice in Poland, the PAT daily intake from apple juice did not exceed the tolerable daily intake in any of the examined samples and amounted to a maximum of 1.6% of PMTDI. As for one portion recommended for consumption, the result obtained was also below tolerable daily intake and constituted a maximum of 38.7% of PMTDI. These findings are a particularly valuable source of information for farmers, traders and consumers, and confirm health safety of apple juice and apple-based products intended for infants and young children.

Key words: mycotoxins, apple juice, products intended for infants and young children, risk assessment

Introduction

Mycotoxins, toxic metabolic products of certain types of moulds, are commonly found in the environment, causing pathological changes in human and animal organisms. Their presence is not related to the development of industry or transport, which are thought to be the main factors responsible for the occurrence of food contaminants, but connected with the inevitable occurrence of moulds in the environment (Postupolski et al. 2019). The level of mycotoxin contamination of food products is an important parameter of their safety. Patulin (PAT) is a mycotoxin biosynthesized by filamentous fungal species such as *Penicillium* (*P. carneum*, *P. expansum*), *Aspergillus* (*A. clavatus*, *A. giganteus*), *Byssoschlamys* (*B. fulva*, *B. nivea*) and *Paecilomyces* (*P. variotti*). It is most often found in apples and their processing products, such as juice or cider (Marin et al. 2011, Saleh and Goktepe 2019). This toxin was also detected in tomatoes, apricots, cherries, black currants, quinces and grapes (Ostry et al. 2018). According to the International Agency for Research on Cancer (IARC), PAT has been classified in the group 3, which means that there are not enough studies confirming its carcinogenicity (IARC 2020). Toxicity assessments proved that it affects negatively important organs, damaging, for example, liver and kidney (Pal et al. 2017). It is commonly believed that fruit susceptibility to microbial damage after harvesting depends to a large extent on the stage of maturity. Fruit become more susceptible to fungal invasions as the maturation process progresses. This is due to increased sugar content in the fruit, a weakening of the induced defence response and a change in the pH of the fruit (Alkan and Fortes 2015). Technological treatment in the fruit and vegetable industry (pasteurization, micro-filtration or clarification) do not completely reduce the initial PAT content in the processed raw material (Diao et al. 2018). One of the low cost and effective methods to reduce PAT in apples is the mechanical removal of the contaminated parts of the apples (Babali et al. 2017). The current legal act in the field of mycotoxin contamination of foodstuffs is the Commission Regulation (EC) No. 1881/2006 of December 19, 2006, setting maximum levels for certain contaminants in foodstuffs (EU 2016a).

The act has been changed many times in terms of the contaminant levels and types of foodstuffs; changes were introduced, as much as 9 times, to the mycotoxins levels, limited foodstuffs as well as new threats, e.g. citrinin. This provision sets maximum contamination levels for aflatoxins, ochratoxin A, PAT, deoxynivaleneol, zearalenone, fumonisin, and citrinin. The values set for PAT are: 50.0 µg kg⁻¹, for apple juice; 25 µg kg⁻¹ for apple-based products; and 10 µg kg⁻¹ for food intended for infants and young children.

Poland is the largest producer of apples in the European Union and the third largest in the world. Our country's share in the global production in 2014–2016 was about 4%, and 26% in the EU. After China, Poland is also the second largest producer and exporter of concentrated apple juice in the world (NASC 2018, FAOSTAT 2022). The domestic production development is associated with the use of high-quality raw materials and a well-developed and modern processing industry (NASC 2018).

According to the Central Statistical Office in Polish estimate, the apple harvest reached 3.1 million tonnes in 2019 (Statistic Poland 2020). There is little recent data on the presence of PAT in Polish apple juices and apple-based products intended for infants and young children. Therefore, the aim of this study was to assess the level of PAT contamination in selected apple-based products, including apple juice and food intended for infants and young children. The second objective was to assess consumer exposure risk resulting from the intake of PAT from apple juices and apple-based products intended for infants and young children.

Material and methods

Material

The experimental material was apple juice and apple-based products: roasted apples and products intended for infants and young children, available in retail in Poland (in the Małopolska Voivodship) in 2016–2020. A total of 47 various samples were examined and all analyses were performed in triplicate. The samples were collected by inspectors of sanitary and epidemiological stations in the Małopolskie Voivodship, according to the Commission Regulation (EC) 401/2006 (EU 2006b). Descriptions of the examined products are given in Table 1.

Table 1. Characteristics of the examined products

Product	Product description
Apple juice	Apple juices made only from apple (no other fruit added), all juices from conventional domestic production.
Apple-based products	The examined products were roasted apples, a product made from apples only, which underwent the roasting process. The product is on the market in glass packaging. All products examined contained only apples in their composition, with no other fruit added and were produced conventionally.
Apple-based products intended for infants and young children	Apple juice intended for infants and young children, produced only from apple juice (with no other fruit added). The juices derived from domestic and EU conventional production.

PAT content in the examined samples was determined by high-performance liquid chromatography (HPLC-DAD) according to the Polish Standard (PN-EN 14177 2005). Table 2 presents the parameters of the analysis for the examined groups of food products.

Table 2. Performance parameters to patulin and food products

Toxin	Food products	Method	LOD ($\mu\text{g kg}^{-1}$)	LOQ ($\mu\text{g kg}^{-1}$)	Recovery (%)
Patulin	Apple juice				
	Apple-based products intended for infants and young children	HPLC-FL	2	5	88
	Apple-based products				

LOD=limit of detection; LOQ=limit of quantitation

Sample preparation procedure

Briefly: 10 ml of clear apple juice or 10 ml of cloudy apple juice (prepared according to methodology set out in point 6.1.2 of standards [17]) or 10 ml of another product (prepared by: weighing 10 g of the sample to a centrifuge tube, adding 150 μl of endogalacturonase solution and 10 ml of water, shaking, then leaving the tube for 2 h at 40 °C and finally centrifuging at 4500 g for 5 min), were pipetted into a 100 ml separating funnel. Afterwards, 20 ml of ethyl acetate was added and the entire content was shaken for 1 min. The layers were allowed to separate and each one was transferred into two conical flasks. The aqueous layer was taken from the flask to the same separating funnel and the extraction was repeated with a new 20 ml portion of ethyl acetate. After separating the

layers, the lower aqueous layer was transferred to the conical flask used the first time, while the upper layer to the conical flask containing the ethyl acetate fraction from the first extraction. Extraction with ethyl acetate was performed a third time, discarding (after layer's separation) the aqueous layer. The combined three acetate ethyl extracts, collected in the conical flask, were transferred to a separating funnel, while the flask was washed with 5 ml ethyl acetate, which was then added to ethyl acetate fraction in the funnel.

Afterwards, 4 ml of sodium carbonate solution was added to the acetate ethyl extract collected in the separating funnel and shaking was performed for 0.5 minutes. After partition of layers, the bottom (water) layer was taken to a conical flask. The top layer was filtered through a filter paper containing about 15 g of anhydrous sodium sulphate into a round bottom flask. The aqueous layer was transferred from the conical flask to the previous separating funnel, rinsing the flask with 10 ml of ethyl acetate and shaking the content for 0.5 minutes. After the separation, the bottom layer was rejected, while the top layer was filtered through a filter paper with anhydrous sodium sulphate, into a round bottom flask. The separating funnel was then rinsed with 15 ml of ethyl acetate and the solvent was filtered as before into the round bottom flask with ethyl acetate extract. The resulting solution was evaporated to a volume less than 1ml under reduced pressure at 40 °C in a water bath. The concentrated extract was transferred to a glass vial. The round bottom flask was rinsed with ethyl acetate. The solution in the vial was evaporated to dryness under a stream of nitrogen at 40 °C in a water bath. The residue in the vial was dissolved by adding 1 ml of water. Before transferring the solution to the HPLC injection vessel, the solution was thoroughly mixed using a mini vortex shaker. Finally, the solution was transferred to the sample vessel used in HPLC analysis.

Analytical equipment

PAT concentration was determined using a Varian liquid chromatograph equipped with a diode-array detector. Separations were carried out on a NUCLEODUR C18, Gravity 3 µm, 4.6 × 250 mm reverse phase column. The mobile phase consisted of water and acetonitrile with perchloric acid (0.0956 parts by volume; 95:5, v/v). The column operating temperature was 35 °C. Separations were performed at a flow rate of 1 ml min⁻¹. Injection volume was 50 µl. Detection of PAT was conducted at 276 nm. The retention time for PAT was 11.9. The samples were analyzed in an accredited laboratory of the Voivodship Sanitary and Epidemiological Station in Krakow.

Chemicals

Ethyl acetate, sodium carbonate, anhydrous sodium sulphate, acetonitrile, perchloric acid were purchased from Sigma-Aldrich (Poznań, Poland). SPE column were derived from Phenomenex LTD (Deutschland). The PAT standard was purchased from Merck Life Science Sp. zoo (Poznan, Poland).

Dietary exposure assessment

The results obtained allowed estimation of the risk assessment for apple juice consumers in relation to the PMTDI, set at 0.4 µg kg BW⁻¹ day⁻¹. These calculations were performed on the basis of the highest obtained PAT content (14.7 µg kg⁻¹). This analysis did not include infants and young children due to the fact that there was no PAT in the products intended for this group of consumers. When determining the average consumption of apple juice, the data of the National Union of Juice Producers (2019) were taken into account, according to which the average Polish consumer drinks approx. 31.1 l of juices, nectars and fruit drinks annually. In this structure of consumption, juices constitute 38%. Apple juice and following it orange juice are the most popular among Polish consumers due to their flavour; in 2018, the share of the former one was 27.2%. An average consumer in Poland drinks approx. 11.8 litres of juice per person per year (0.98 litres per month), which gives, on average, approximately 33 ml of juice per day, including 8 ml of apple juice. In the second variant, 200 ml of juice was considered as a daily portion recommended for consumption.

The exposure dose was calculated according to the following formula:

$$(\mu\text{g kg}^{-1} \text{ body weight per day}) = \text{mycotoxin level } (\mu\text{g kg}^{-1}) \times \text{average food intake (kg per person per day)} \times \text{average body weight}^{-1} (\text{kg}^{-1} \text{ body weight per person})$$

The maximum contamination values determined for PAT as well as lower bound (LB) and upper bound (UB) values were used for calculations. These values are applied when the percentage of results below the Limit of Detection (LOD) or Limit of Quantitation (LOQ) is higher than 50%. According to the European Food Safety Authority (EFSA 2010)

guidance: at the lower-bound (LB), results below the LOD were replaced by zero and those below the LOQ by the LOD; at the upper-bound (UB) the results below the LOD were replaced by the value of the LOD and those below the LOQ were replaced by the value reported as LOQ.

The middle bound (MB) value was calculated as the difference between UB and LB.

Calculations were based on the average body weight values given for seven age groups (coded A, B, C, D, E, F, G), according to the division set by the Nutritional Standards for the Polish population (Jarosz et al. 2020). These groups included: children aged 4–6, body weight 19 kg (A); children aged 7–9, body weight 27 kg (B); boys and girls aged 10–12, body weight 38 kg (C); boys and girls aged 13–15, body weight 52.5 kg (D); boys and girls aged 16–18, body weight 61.5 kg (E); men from 19 years, body weight 70 kg (F); and women from 19 years, body weight 60 kg (G). Consumer exposure assessment resulting from the consumption of apple juice, was estimated with regard to the PMTDI. The results were expressed as %PMTDI.

Results

Of all the samples analysed, PAT was found in 2 samples of apple juice, representing 5% of the examined material. The results obtained are presented in Table 3. The determined PAT contamination was much lower than a maximum acceptable level specified in the Regulation No. 1881/2006 (EU 2006a) at the level of $50.0 \mu\text{g kg}^{-1}$ for apple juice. Over a four-year research period, PAT was detected only in two samples of apple juice from 2017, in which amounted to $5.1 \pm 0.23 \mu\text{g kg}^{-1}$ and $14.7 \pm 0.63 \mu\text{g kg}^{-1}$. There were no PAT in any of the examined roasted apple samples. No PAT was also detected in any of the 12 samples of examined products intended for infants and young children, in which the LOD limit ($2 \mu\text{g kg}^{-1}$) was not exceeded.

Table 3. Patulin concentration in examined food samples

Product	No. positive samples/ examined samples (%)	Range (min- max, $\mu\text{g kg}^{-1}$)	Mean of positive samples ($\mu\text{g kg}^{-1}$)	Mean MB (UB-LB, $\mu\text{g kg}^{-1}$)	EU ML ($\mu\text{g kg}^{-1}$)	The number of samples above ML
Apple juice	2/28 (7.1)	5.1–14.7	9.9	4.46 (5.30–0.83)	50	
Apple-based products	0/14	nd	nd	5.0 (5.0–0)	25	0
Apple-based products intended for infants and young children	0/5	nd	nd	5.0 (5.0–0)	10	0

nd=not detected; ML=maximum level; MB middle bound value was calculated as the difference between UB and LB. LB - results below the LOD were replaced by zero and those below the LOQ by the LOD; UB the results below the LOD were replaced by the value of the LOD and those below the LOQ were replaced by the value reported as LOQ.

In Poland, the average daily intake of PAT with this apple juice did not exceed the TDI in any of the analysed cases and ranged from 0.42 to 1.55% of PMTDI. The highest value was noted in group A, i.e. children aged 4–6. In the case of one portion recommended for consumption, the PMTDI was not also exceeded and the highest result was 38.68% of this value (Table 4).

Table 4. PMTDI values for patulin with regard to individual age groups

Sample type	Consumption	%PMTDI with regard to average BW						
		A	B	C	D	E	F	G
Apple juice	8 ml ^a	1.55	1.09	0.77	0.56	0.48	0.42	0.49
	200 ml ^b	38.68	27.22	19.34	14.0	11.95	10.50	12.25

a=mean annual ingestion; b=daily portion; A, B, C, D, E, F, G age groups

Discussion

The results obtained are consistent with the findings of other authors (Table 5), according to whom PAT concentration in apple juice ranged from 1.50 to $7.35 \mu\text{g l}^{-1}$ (Polak-Śliwińska et al. 2013).

In turn, PAT content in apple juices determined by Biernaciak and Rychcik (2019) in all examined samples of conventionally produced juices, ranged from 5.65 to 29.24 $\mu\text{g l}^{-1}$. The discrepancy observed between the number of samples in which the authors determined this toxin and our own results, may be a consequence of the sample selection method used for research (samples collected in one city in one year).

Table 5. The concentration of patulin in various food products

Food sample	Contamination level ($\mu\text{g kg}^{-1}$)	Average contamination level \pm SD ($\mu\text{g kg}^{-1}$)	Year	Location	Percentages of samples with contamination level above limit (50 $\mu\text{g kg}^{-1}$ or 10 $\mu\text{g kg}^{-1}$ for baby food) (%)	References
Apple juice	1.5 do 7.4	4.4 \pm 4.1	2013	Poland	0	(Polak-Śliwińska et al. 2013)
Apple juice	5.7 do 29.2	13.3 \pm 7.5	2019	Poland	0	(Biernaciak and Rychcik 2019)
Apple juice	nd–190.7	52.8 \pm 15.6	2015	Iran	Not calculated	(Rahimi and Jeiran 2015)
Apple juice	5.8–82.2	35.4 \pm 1.7	2017	Qatar	25	(Hammami et al. 2017)
Baby apple juice	7.7–61.3	30.7 \pm 6.7	2017	Qatar	50	(Iqbal et al. 2018)
Apple juice	20.4–45.6	38.3 \pm 18.1	2018	Pakistan	20	(Iqbal et al. 2018)
Apple juice	4–122.4	45.7 \pm 6	2015	Tunisia	Not calculated	(Zouaoui et al. 2015)
Pear juice	5–231	62.5 \pm 12.4	2015	Tunisia	Not calculated	(Zouaoui et al. 2015)
Apple jam	4.7–554	302 \pm 9.6	2015	Tunisia	Not calculated	(Zouaoui et al. 2015)
Pear jam	17–325	123.7 \pm 4.1	2015	Tunisia	Not calculated	(Zouaoui et al. 2015)
Apple juice	26.9	26.9 \pm 0.3	2014	Malaysia	0	(Lee et al. 2014)
Apple juice	0.7–118.7	19.4	2009	Spain	11	(Saleh and Goktepe 2019)
Apple juice	0.67–38.8	9	2003	Belgium	0	(Saleh and Goktepe 2019)
Apple juice	2.2	2.2	2001	Sweden	0	(Saleh and Goktepe 2019)
Apple juice	0.1	0.1	2013	France	0	(Sirot et al. 2013)
Apple juice	2.5–6.0	4.3 \pm 2.5	2011	Spain	0	(Marin et al. 2011)
Apple baby food	nd	-	2011	Spain	0	(Marin et al. 2011)
Apple baby food	\leq 3	-	2015	Turkish	0	(Karakose et al. 2015)
Apple juice	nd–13.2	6.1 \pm 3.6	2013	Spain	0	(Piqué et al. 2013)

nd=not detected; SD=standard deviation

According to a review of the global literature on the PAT contamination level in apple juices, the highest average PAT level was detected in Iran (52.8 $\mu\text{g kg}^{-1}$) (Rahimi and Jeiran 2015); slightly lower values, at the level of 35.37 $\mu\text{g kg}^{-1}$, were reported in Qatar. In the products intended for children, the average level of this toxin was 30.67 $\mu\text{g kg}^{-1}$ (Hammami et al. 2017). Iqbal et al. (2018), who analysed selected fruits, juices and cocktails available in Pakistan, noted the presence of PAT in 136 (57.4%) out of 237 samples examined. The average PAT content in the examined apple juices ranged from 20.4 to 45.6 $\mu\text{g kg}^{-1}$. The highest (921.1 $\mu\text{g kg}^{-1}$) was in red grapes, while in cocktail samples fluctuated between 20.3 and 110.4 $\mu\text{g kg}^{-1}$. In 33.8% of the examined samples, PAT concentration was higher than the recommended limit in the EU, but it concerned only 2 samples of apple juices. Similar contents were reported by Zouaoui et al. (2015) in selected fruit juices available in retail in Tunisia. Of the analysed samples of fruit and fruit-based products (apple juice concentrate, apple juice, pear juice, apple and pear jam, compote), 50% contained this toxin and its concentration ranged from 2 to 889 $\mu\text{g kg}^{-1}$. In apple juice, however, the percentage of positive samples was 64.3%, and the average contamination level was 45.7 $\mu\text{g kg}^{-1}$. In turn, Karakose et al. (2015) found that PAT contents in all examined samples of baby food from Turkish markets were below the level applicable in the European Union. According to Lee et al. (2014), PAT contamination of juices obtained from selected fruits (apple, mango, pineapple, guava, lychee and tamarind) available in retail trade in Malaysia, was lower. Among 56 analysed samples, only three (5.4%) contained PAT and its level fluctuated between 13.1 and 33.7 $\mu\text{g kg}^{-1}$; in apple juice it was 26.9 $\mu\text{g kg}^{-1}$. In the European Union, the lowest mean content of PAT in apple juice was reported in France and it was 0.115 $\mu\text{g kg}^{-1}$ (Sirot et al. 2013). In other European countries, such as Spain, Belgium and Sweden, the level of PAT contamination in apple juices was higher but still acceptable (from 1.15 to 19.4 $\mu\text{g kg}^{-1}$) (Saleh and Goktepe 2019). Marin et al. (2011), who investigated PAT contamination of Spanish apple-based products such as apple juice, nectar, jam and baby food, found presence of this toxin in two samples of apple juice, amounting to 2.5 and 6 $\mu\text{g kg}^{-1}$. Studies conducted by Piqué et al. (2013) on Spanish products available in retail, confirmed that 7 out of 25 examined juice samples contained PAT and its maximum content was 13.2 $\mu\text{g l}^{-1}$.

According to Torović et al. (2017), who investigated PAT exposure resulting from dietary intake of apple-based products, the estimated PAT intake was lower and amounted to 6.6% PMTDI for children's juice. The mean total daily patulin intake, noted by Oroian et al, (2014) in Romania, was 133 ng kg⁻¹ bw day⁻¹ and accounted for 33.3% PMTDI. In turn, Zhou et al. (2015), estimated the average exposure to this toxin in China at the level of 55.4% PMTDI for children and 19.8% PMTDI for adults. The estimated exposure for adults, which was observed by Zafar et al. (2018) in Pakistan, was 40% PMTDI.

Conclusions

Only 2 of the 47 examined apple products contained PAT. Its content in one sample was 14,7 µg kg⁻¹, which constituted 29.4% of the maximum level (ML) and in the other, 5.1 µg kg⁻¹, which accounted for 10.2% ML. In the examined products, PAT was detected rarely and in low concentrations, the levels found were much more lower than the acceptable level set at 50 µg kg⁻¹. In any sample of the products intended for infants and young children, this toxin has not been determined above the LOD (2 µg kg⁻¹). The daily PAT intake with apple juice did not exceed the PMTDI in any of the analysed cases and was at a maximum of 38.68% the PMTDI. The results obtained are a particularly valuable source of information for farmers, traders and consumers, and confirm health safety of apple juice and apple-based products intended for infants and young children.

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