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Evaluation of Quality and Contamination Level of Patulin in Infected Traditional and Commercial Apple Cultivars

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ABSTRACT
The aim of this study was to investigate the influence of various parameters, including water content, total soluble matter, acid content, sugar content, polyphenol content, antioxidant activity, anthocyanin content, and flavonoid content, on the quality of apples and their resistance to *Penicillium expansum* infection and subsequent patulin production. The research was conducted on four apple cultivars, namely, the traditional cultivars 'Wagener' and 'Ilzer Rosenapfel', and the commercial cultivars 'Jonagold' and 'Idared'. The results of the study provide valuable insights into the composition, quality attributes, and potential resistance to *Penicillium expansum* infection among different apple cultivars. It was shown that cultivars with lower content of polyphenols, anthocyanins, soluble matter, flavonoids, antioxidant activity and sugar had higher level of patulin. These findings have implications for the selection and cultivation of apple cultivars with desirable characteristics, such as taste, antioxidant potential, and reduced mycotoxin contamination.

KEYWORDS
apple quality; patulin; *Penicillium expansum*; food safety

KEY CONTRIBUTION
This study examined the influence of various parameters (water content, total acid content, sugar content, polyphenol content, antioxidant activity, anthocyanin content, flavonoid content) on the quality of traditional and commercial apples and their resistance to infection by *P. expansum*.

Introduction

Apples are one of the most widely consumed fruits globally and have significant economic and nutritional value (Skoko et al., 2022; Duralija et al., 2021). They are rich in essential nutrients, such as vitamins, minerals, and dietary fiber, which contribute to a healthy diet. However, apples are susceptible to fungal infections, particularly by *Penicillium expansum*, a fungus known for its detrimental effects on apple quality and the production of the mycotoxin patulin (Skoko et al., 2022). Contamination with *P*. *expansum* and patulin is a major concern in the apple industry, as it affects both the economic value of the apples and the health of consumers (Zhong et al., 2018). Patulin has been associated with various health risks, including cytotoxicity, genotoxicity, and immunotoxicity (Bacha et al., 2023). While there is an ongoing research on the resistance of apple cultivars to *P*. *expansum*, there is a lack of literature specifically focusing on traditional apple cultivars in Croatia. This gap in knowledge necessitates further investigation to assess the resistance of traditional Croatian apple cultivars to *P*. *expansum* contamination and the subsequent production of patulin. Understanding the resistance of traditional apple cultivars is essential for developing effective strategies to mitigate fungal infections and reduce patulin contamination in apples and apple-based products (Zhong et al., 2018). Additionally, gaining insight into the resistance of traditional apple cultivars in Croatia could have broader implications for sustainable agriculture and food safety. Traditional apple cultivars often carry unique genetic traits that have been naturally selected over generations, making them potentially more resilient to local pests and pathogens. The geographical and climatic conditions in Croatia provide a unique environment for apple cultivation. The diverse range of traditional apple cultivars in the region might hold untapped potential for addressing the challenges posed by *P*. *expansum* and patulin contamination (Duralija et al., 2021). By studying these cultivars, researchers can uncover valuable information about their innate resistance mechanisms, which could be harnessed for the development of disease-resistant apple cultivars through conventional breeding or genetic modification techniques. Furthermore, understanding the relationship between apple resistance and fungal contamination can lead to more targeted and sustainable approaches to pest management. Reduced reliance on chemical treatments and increased use of resistant cultivars could lead to a decrease in pesticide usage, benefiting both the environment and the health of consumers (Shen et al., 2021).

This research aims to bridge the existing knowledge gap regarding traditional Croatian apple cultivars resistance to *P*. *expansum* and patulin production by conducting thorough investigations and experiments which include determination of physicochemical parameters, bioactive profile and patulin content in infected traditional and commercial apple cultivars. The findings from this study could guide apple growers, breeders, and policymakers in making informed decisions to enhance the quality and safety of apple production.

Materials and methods

Materials

The old apple cultivars ‘Wagener’ and ‘Ilzer Rosenapfel’ were collected at Šašinovec (45°85’00.3”N, 16°17’75.2” E); and commercial apple cultivars ‘Idared’ and ‘Jonagold’ at Novaki Bistranski, Donja Bistra (45°54’57.0” N 15°52’56.0” E, Croatia). All studied apple cultivars were authenticated by a pomologist and confirm by 12 SSR markers.

Chemical Parameters

The amount of total acids was determined by the titration method with 0.1 M NaOH and expressed in percentage (as apple acid). The soluble solids were determined from the apple juice from the bottom half of the fruit in which the content of soluble solids will be determined by a digital refractometer (Atago Co., Ltd., Tokyo, Japan) and expressions in Brix (°Brix). The total dry matter (U.S.T.), i.e. the water content in apples, was determined by lyophilization to constant mass. Dehydration was conducted by

Alpha 2 - 4 LSCplus (Christ, Alpha LSCplus, Berlin, Germany) freez-dryer. Sublimation was carried out at temperatures from -35 °C to 0 °C and a pressure of 0.22 mbar, final drying and isothermal desorption was carried out at temperatures of 0 °C to 20 °C and a pressure of 0.065 mbar. (Lončarić et al., 2014). Total sugars were determined volumetrically by Luff-Schoorl, described in Lončarić et al. (2014).

Determination of Bioactive Profile

The extraction was carried out from healthy fruits. Fruits were crushed and frozen at −80 °C as soon as possible, after which the samples were lyophilized in the Alpha 2-4 LSCplus freeze-dryer (Christ, Alpha LSCplus,) and milled into a powder. The polyphenols were extracted from the obtained powder with methanol-acidified with hydrochloric acid (1%) in an ultrasonic bath, centrifuged in a centrifuge (Thermo Scientific, SL 8R, Waltham, MA, USA) and filtered through syringe filters PTFE 0.45 µm (Lončarić et al., 2014). The total polyphenol content was determined by the Folin-Ciocalteu method by measuring colour intensity at a wavelength of 765 nm on spectrophotometer (Lambda 365, Perkin Elmer, Waltham, MA, USA) (Lončarić et al., 2014). Total flavonoids, the largest group of polyphenols, were determined by a method based on the reaction of flavone and flavonol with aluminum ions and measuring absorbance at 420 nm. Concentrations of total flavonoids were converted from the previously prepared calibration curves with quercetin (Lončarić et al., 2016). Total anthocyanins, subgroup of flavonoids, was determined using the pH-differential method described by Giusti and Wrolstad (Giusti et al., 2001). Antioxidant activities were determined by a DPPH method following procedure described by Brand-Williams et al. (1995) and modified by Lončarić et al. (2016).

Patulin Determination

Before patulin determination, apple fruit (10 pcs) were sterilized by immersion for 1 min in 2% sodium hypochlorite solution, after which apples were infected with a prepared spore suspension (20 µL) of *P. expansum* with a density of 2.5 × 106 spores/mL. Apples were kept in refrigerator until maximum infection is reached. Upon reaching the maximum diameter of the colony, the infected apple sample were excluded and subjected to the determination of the produced patulin concentration. The produced concentration of patulin was determined according to the multimycotoxin “diluent and shoot” LC-MS/MS method described by Skoko et al. (2022).

Results and discussion

The present study shows a comparative analysis of the phytochemical composition of four distinct apple cultivars, namely ‘Wagener’ and ‘Ilzer Rosenapfel’ representing traditional cultivars, and ‘Jonagold’ and ‘Idared’ representing commercial cultivars. Our investigation encompasses a comprehensive exploration of various parameters including total soluble matter, water content, total sugar content, total acid content (Table 1), total polyphenol content, DPPH (2,2-diphenyl-1-picrylhydrazyl) activity, total anthocyanin content, total flavonoid content (Table 2), and patulin concentration (Table 3).

The results of total soluble matter showed that the traditional ‘Wagener’ variety exhibited a concentration of 14.4%, closely followed by ‘Idared’ with 14.5%, ‘Jonagold’ with 15.1%, and ‘Ilzer Rosenapfel’ with the highest value of 15.9%. These findings are consistent with earlier studies (Lončarić et al., 2021a; Skoko et al., 2022), which reported that traditional cultivars often have comparable soluble solid content to their commercial counterparts. The water content of investigated cultivars was between 80.46% (’Ilzer Rosenapfel’) and 83.50% (’Wagener’). The total sugar content determination showed that ‘Ilzer Rosenapfel’ (15.22 ± 0.03%) displayed the highest concentration, followed closely by ‘Jonagold’ (14.95 ± 0.03%), ‘Wagener’ (13.62 ± 0.03%), and ‘Idared’ (13.87 ± 0.00%). This alignment with previous findings (Akagić et al., 2019) suggests that traditional cultivars, despite often considered less sweet, can exhibit comparable sugar content to their commercially favoured counterparts. However, Zhong et al. (2018) stated that sugars are a key component of apple fruit that modulates the accumulation of patulin. Interestingly, the variation in total acid content, specifically in ‘Jonagold’ (0.05%) and ‘Idared’ (0.13%), indicates potential differences in fruit maturity and acidity regulation mechanisms. However, study conducted by Marín et al. (2006) showed that more acidic conditions lead to a higher production of patulin, which can mean that ‘Idared’ is more susceptible for the patulin accumulation then other three cultivars.

Table 1. Physicochemical parameters of traditional and commercial apple fruits

Apple Cultivar	Total Soluble Matter (%)	Water Content (%)	Total Sugar Content (%)	Total Acid Content (g/100 g)
’Wagener’	14.4 ± 0.00	83.5 ± 0.06	13.62 ± 0.03	0.08 ± 0.00
’Ilzer Rosenapfel’	15.9 ± 0.00	80.46 ± 0.11	15.22 ± 0.03	0.08 ± 0.00
’Jonagold’	15.1 ± 0.01	82.99 ± 0.28	14.95 ± 0.03	0.05 ± 0.00
’Idared’	14.5 ± 0.00	83.02 ± 0.60	13.87 ± 0.00	0.13 ± 0.01

Mean ± SEM based on three juice samples (n = 3).

The evaluation of bioactive profile includes determination of total polyphenol content (TPC), DPPH, total anthocyanin content and total flavonoid content (Table 2). The results of TPC unveil ‘Ilzer Rosenapfel’ as the leader with 707.63 ± 22.81 mg/kg, followed by ‘Jonagold’ (552.63 ± 29.29 mg/kg), ‘Idared’ (550.13 ± 25.23 mg/kg), and ‘Wagener’ (421.38 ± 9.44 mg/kg). These values are in agreement with earlier investigations (Lončarić et al., 2021a; Skoko et al., 2022; Lončarić et al., 2021b) that spotlight the robust polyphenolic composition of traditional cultivars. The DPPH activity, indicative of antioxidant capacity, shows a good correlation with TPC, and similar trend was noticed with ‘Ilzer Rosenapfel’ and Jonagold,’ presenting the highest value (0.29 mmol TE/kg dw) followed by ‘Wagener’ and ‘Idared’ with lower activities (0.17 and 0.27 mmol TE/kg dw, respectively). Regarding anthocyanin content, ‘Jonagold’ (5.76 mg/kg) and ‘Ilzer Rosenapfel’ (5.01 mg/kg) demonstrated a significant concentration of anthocyanin content, followed by ‘Idared’ (1.24 mg/kg), whereas in ‘Wagener’ anthocyanins was not detected. Furthermore, the analysis of flavonoid content illuminates ‘Jonagold’ as the leader (104.74 ± 3.11 g CE/kg), followed by ‘Ilzer Rosenapfel’ (187.97 ± 3.73 g CE/kg) and ‘Idared’ (139.77 ± 0.23 g CE/kg), with ‘Wagener’ exhibiting the lowest value (67.30 ± 3.11 g CE/kg). The evaluation bioactive profile was in agreement with our previous studies on traditional and commercial apple cultivars (Skoko et al., 2022; Lončarić et al., 2020; Lončarić et al., 2021a).

The importance of bioactive profile of apple fruit was underlined by several authors, indicating that polyphenols are involved in the response to patulin attack through scavenging the free radicals induced by patulin and possibility of phenolic compounds to positively contribute at the basal level to the resistance of *P. expansum* attacks in apples (Zhong et al., 2018). Furthermore, some authors point out that compounds with strong antioxidant activity have the ability to suppress the growth of *P. expansum* or the synthesis of patulin (Mahunu et al., 2018).

Table 2. Bioactive profile of traditional and commercial apple fruits

Apple Cultivar	Total Polyphenol Content (mg/kg)	DPPH (mmol TE/kg)	Total Anthocyanin Content (mg/kg)	Total Flavonoid Content (g CE/kg)
'Wagener'	421.38 ± 9.44	0.17 ± 0.00	0.00 ± 0.00	67.30 ± 3.11
'Ilzer Rosenapfel'	707.63 ± 22.81	0.29 ± 0.00	5.01 ± 0.00	187.97 ± 3.73
'Jonagold'	552.63 ± 29.29	0.29 ± 0.01	5.76 ± 0.00	104.74 ± 3.11
'Idared'	550.13 ± 25.23	0.27 ± 0.02	1.24 ± 0.01	139.77 ± 0.23

Mean ± SEM based on three juice samples (n = 3).

Lastly, the levels of patulin varied among the cultivars, with ´Wagener´ exhibiting the highest concentration (18592 ± 101.82 µg/kg), followed by ´Idared´ (4732.4 ± 57.10 µg/kg), 'Jonagold' (292.56 ± 20.93 µg/kg), and 'Ilzer Rosenapfel' (130.92 ± 0.06 µg/kg). The obtained results are extremely high. However, they are the results of intentional inoculation and growth of *P. expansum* on studied apple cultivars.

Table 3. The patulin content in infected traditional and commercial apple fruits

Apple Cultivar	Patulin µg/kg
'Wagener'	18592 ± 101.82
'Ilzer Rosenapfel'	130.92 ± 0.06
'Jonagold'	292.56 ± 20.93
'Idared'	4732.4 ± 57.10

Mean ± SEM based on three juice samples (n = 3).

Conclusions

In conclusion, our comparative analysis elucidates differences in the phytochemical composition of traditional ('Wagener' and 'Ilzer Rosenapfel') and commercial ('Jonagold' and 'Idared') apple cultivars and their resistance to *P. expansum* infection and subsequent production of patulin. The study showed that cultivars with lower total soluble matter, sugar content, total polyphenol content, antioxidant activity and anthocyanin content exhibit higher patulin levels.

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Conflicts of Interest: "The authors declare no conflict of interest."

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