EVALUATION OF FUNGICIDE RESIDUES IN APPLES AND THEIR IMPACT ON FOOD SAFETY

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Summary

The apple is a fruit that has beneficial health effects due to the presence of biologically active components and is considered as a functional food. But today's production of apples cannot be imagined without chemical treatment. In addition to its favorable health effects, it may also have adverse effects on human health due to the presence of pesticides that are considered as contaminants. The purpose of this research is to evaluate the fungicide residues in fruit/apples. The fungicide residues were extracted from apple cultivar Idared with QuEChERS method and analyzed by ultra-performance liquid chromatography with a triple quadrupole mass spectrometer (UPLC-TQ/MS). The analyses have shown that the apples contain pesticides penconazole (10 μg/kg), boscalide (60 μg/kg), tebuconazole (11-40 μg/kg), myclobutanil (20-70 μg/kg), fenbuconazole (70 μg/kg), pyrimethanil (12-60 μg/kg) and carbendazime (azole) with range of 100-200 μg/kg. After comparing the concentration of detected pesticides with MRLs, it can be concluded that apples are safe for consumption.

Keywords: apples, food safety, pesticides, residues

Introduction

The apple *(Malus domestica)* is one of the three basic fruits produced in Republic of North Macedonia. Apple picking starts in late summer with the early varieties and continues in the autumnal months. Apples are highly represented in the national market, and a certain percentage are also exported (Picha, 2009). The total domestic consumption of apples is estimated at about 24.748 tons, which is about 20% of domestic production, while about 21.500 tons are purchased for industrial processing, 25% of the total apple production are sold on the national market, while 75% are processed or sold on foreign markets (Ministry of Agriculture, Forestry and Water management, 2010).
Various types of apples have different chemical composition. In accordance with the research conducted by Aziz et al. (2013) there is a difference in the chemical composition between *Malus sylvestris* and *Malus domestica*. The percentage of moisture, potassium and ascorbic acid is high in *Malus Sylvestris*. The ash, fat, fiber, protein and total sugar percentage are high in *Malus Domestica*. The factors which are affecting chemical composition of apples, as well as the content of some components are: cultivar, climate, harvesting period, soil, storage and others (Mujkanović et al., 2019). The apple is a source of vitamins and minerals, soluble and insoluble dietary fiber (Chen et al., 2014). Nearly half of the amount of vitamin C is found below the skin of the apple, and the skin contains insoluble fiber. Pectin, which plays a major role in the reduction of cholesterol and prevents atherosclerosis and cardiac diseases, is present in the apple. Because of these components that play an important role in the health of people, apples and their products are considered as functional foods. The apple jam from traditional apple cultivars can be used as functional food because it contains carbohydrates, potassium, zinc, iron and can be recommended to the sport players, little children, vegans and elderly (Mujkanović et al., 2019). The consumption of apples and their health benefits with regard to cancer, cardiovascular diseases, asthma and diabetes have been researched by Boyer and Liu (2004).
Apart from health benefits, the apples can also have adverse effects on health if they contain pesticides over the MRLs and are unsafe for consumption. Today's practice shows that apples in conventional production are treated with pesticides 10 to 15 times (Jankuloska et al., 2018). Many types of fruits are consumed raw, without any thermal processing and that is a potential health risk for the consumer. Thermal processing and treating the apples with steam (110 °C, 20-25 min) and removing the apples peel from the apples (65-70 °C, 3 min) was identified as the most effective step in reducing fosalone residues and the complete elimination of fenitrothion and tolifluanide (Štěpán et al., 2005). The presence of pesticide residues and their metabolites can be a potential hazard to human health that is

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manifested by acute or chronic toxicity. The priority is to detect them in the food in order to avoid the possible risks to human health.

Pesticides are used to protect the plants during cultivation in order to decrease the damage which can be caused by harmful organisms (fungi, insects, weeds, birds, snails, spiders, nematodes, bacteria and viruses). Pesticides permitted for use as means of protection in the Republic of North Macedonia are prescribed in the list of approved active substances (Official Gazette of Republic North Macedonia, 2013). It is mandatory to manage the appearance of pesticide resistance in the pest, to uphold the waiting period, to follow the recommendations of the advisory service, as well as the instructions of the pesticide manufacturer. The European Commission has adopted a list of maximum residue levels (MRLs) for pesticide used in the production of food and feed, regulated by Regulation EC 396/2005 (Official Journal of the European Union, 2005). In the Republic of North Macedonia, the maximum residue levels (MRLs) for pesticides are regulated with the Regulation on general food safety requirements regarding maximum tolerated levels of pesticide residues in or on food (Official Gazette of Republic of North Macedonia, 2013).

Difenoconazole, fenbuconazole and trifloxystrobin in apples (Golden Delicious and Idared) were extracted with QuEChERS method and analyzed with LC-MS/MS. The concentration content was: 0.01-0.41 mg/kg difenoconazole (Jankuloska et al., 2018), 0.01 to 0.07 mg/kg fenbuconazole (in Golden Delicious apples) (Jankuloska et al., 2017b) and 0.04-0.14 mg/kg trifloxystrobin (Jankuloska et al., 2017). According to the results obtained the authors concluded that the apple is safe for consumption. The chlorpyrifos content in apple from the Resen region is smaller than the MRLs and the use of chlorpyrifos doesn't depend on the variety of apple but rather of the locations where the apple is grown (Jankuloska et al., 2017a).

Fungicide residues (quintozene, pyrimethanil, captan, folpet, tebuconazole, carboxin, flutolanil, fludioxonil) were analyzed with GC and matrix solid phase dispersion (MSPD) in fruit and vegetables (apples, carrots, orange, artichoke, tomatoes and zucchini). The concentration content was smaller than the MRLs. LOQ and LOD were under 0.1 mg/kg (Navarro et al., 2002). Azoxystrobin and trifloxystrobin were analyzed in apple with GC, with electron capture detector (ECD) and nitrogen phosphor detector (NDP) and limit of detection (LOD) were found to be 0.02 mg/kg for azoxystrobin and 0.01 mg/kg for trifloxystrobin (Giza and Sztwiertnia, 2003).

From the above stated we can conclude that pesticides play an important role in the food safety and the purpose of this research is to evaluate the fungicide residues level in fruits/apples.

Materials and methods

The experimental part of the research was carried out in Resen at two different locations (Evla and Kriveni). Field research and trials took place in stages and lasted throughout 2016. The evaluation of fungicides is done during apple vegetation until harvest time. At the end of May, the first samples were taken for analysis (hazelnut-sized apples) and transported for the analysis of residues. The second sampling stage was when apples reached the size of a walnut. The third sampling stage took place in the ripening period. The fourth sampling stage for residue analysis took place at the time of harvest (September-October). To determine the exact maturity for apple harvesting, several objective measures and indicators were used, such as the color of the skin, the hardness, content of soluble hard materials, the development of ethylene and the content of starch. Apples were randomly sampled. The mass of samples for analysis was 1 kg (for sampling). Fresh apples with peel and mesocarp without treatment were analyzed.

Extraction of pesticide residues

Following the latest trends in the extraction of pesticide residues, the QuEChERS method was applied in this study according to the MKS EN 15662:2011 standard (Standardization Institute of Republic of North Macedonia, 2011). The homogenized sample of apple fruits with weight of 10 ± 0.1 g is transferred at 50 ml centrifuge tube. 10 ml of acetonitrile and a buffered mix of salts for extraction and separation (4 g ± 0.2 g $MgSO_4$, 1 g ± 0.05 g NaCl, 1 g ± 0.05 g trisodium citrate dihydrate, 0.5 g ± 0.03 g disodium hydrogen citrate sesquihydrate) are added. The tube was closed with a cap and the extract was vigorously mixed by hand (for 1 min). After the centrifugation (5 minutes at 3000 rpm), 1 ml of the acetonitrile extract was taken and transferred into a clean-up tubes containing 150 mg primary secondary amine (PSA) and 900 mg $MgSO_4$. The tubes were closed and the mixture was shaken 30 seconds, and then were centrifuged for 5 minutes at 4500 rpm. The cleaned and acidified extracts are transferred into auto sampler vials to be used for the multi-residue determination with chromatographic method (Anastassiades et al., 2003; Jankuloska et al., 2018).

Analysis with UPLC/TQ-MS

The analysis of pesticide residues in apples was conducted with the most sophisticated separation technique: ultra-performance liquid chromatography-triple quadruple mass spectrometry (UPLC-TQ/MS), Agilent UPLC 1290, detector DAD VL Agilent 1260 G1315D (Waldbronn, Germany), Agilent triple quadruple LC/MS detector 6420 (Agilent Technologies, Santa Clara, California, USA). UPLC BEH C-18 column with dimensions of 2.1 x 100 mm and pore size of 1.8 µm was used for separation (Table 1). The temperature of the column was 35 °C and the flow was 0.4 ml/min. The volume of injection is 0.7 µl. The solvents used in extraction and analysis were distilled and checked for any unwanted impurity prior to use. The mobile phase used consisted of solvent A: 0.1% (v/v) HCOOH and 5 mmol ammonium formate; solvent B: methanol with 0.1% (v/v) HCOOH and 5 mmol ammonium formate.
The accuracy of the method was checked with a standard addition method, showing good accuracy, repeatability, and reproducibility (RSD < 10%). The limit of detection (LOD) is in the range of 0.01 to 0.07 mg/kg and the limit of quantification (LOQ) is 0.01 mg/kg for all pesticides.

Table 1. Separation parameters on UPLC for pesticide residues

Time (min.)	*Flow rate (ml)*	*Solvent A[%]*	*Solvent B [%]*
0	0.4	95	5
0.5	0.4	95	5
3.50	0.4	50	50
17.00	0.4	0	100
20.00	0.4	0	100
20.10	0.4	95	5

Results and discussion

In this research we analyzed the fungicide residues in Idared apples in four development stages from cultivation.
Table 2 shows the present fungicides in apples from two locations (Evla and Kriveni).

Table 2. Concentration of fungicide residues in Idared from locations Evla and Kriveni in the first and second stage

Pesticides	**Concentration (µg/kg)/Evla, first stage**	**Concentration (µg/kg)/Kriveni, first stage**	**Concentration (µg/kg)/Evla, second stage**	**Concentration (µg/kg)/Kriveni, second stage**
Myclobutanil	20	70	n.d	20
Fenbuconazole	70	70	n.d	n.d
Penconazole	10	n.d	n.d	n.d
Tebuconazole	n.d*	11	n.d	40
Boscalide	60	n.d	n.d	n.d
Pyrimethanil	60	20	12	n.d

*n.d- not detected

At first stage, six fungicides were detected in apples (Table 2). From obtained results we can see that detected pesticides in apples from both locations are different. Respectively, in the Evla apples, penconazole, boscalide and tebuconazole were detected, while the same were not detected in the Kriveni apples.

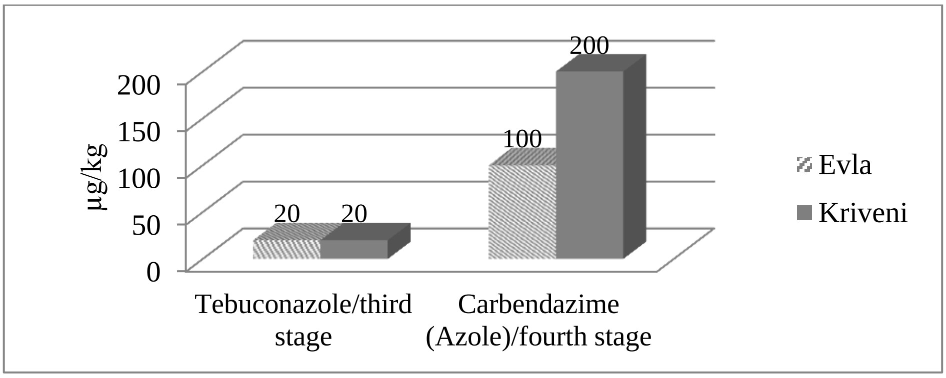

Fig. 1. Concentration of fungicide residues in Idared from locations Evla and Kriveni in the third and fourth stage of development of apples cultivation

As shown in Fig. 1 the tebuconazole fungicide is present in the apples from both locations in the same concentration of 20 μg/kg, while the remaining pesticide detected in the second stage is not present in the third stage apples. Navarro et al. (2002) used GC to analyze tebuconazole in fruits and vegetables and received a low pesticide concentration which is similar to our results. In this stage, 100 μg/kg carbendazime (azole) is detected in the Evla apples and 200 μg/kg is detected in the Kriveni apples. Similar results to these were obtained by Singh et al. (2009), during the analysis of apples grown under different conditions in which they detected carbendazime and chlorpyrifos but in low concentration.

In this study, there is a distinct presence of pesticides in all four stages, but it is also noticeable that the concentration of detected pesticides is different. The persistence of pesticides depends upon the dose of pesticides, mode of application, temperature and weather conditions at the time of application and on crop growth conditions (Tsochatzis et al., 2013; Trapp S. 2015), translocation of pesticides in nature, chemical properties, location of cultivation and type of formulations (Patel et al., 2016).

When assessing the importance of pesticides, what needs to be taken into consideration is whether their level is higher than the allowed maximum, and for that reason in this study we made a comparison of the concentration of pesticides represented in the fourth stage (time of harvest) in the apples from both locations.

The obtained results for the concentrations of the carbendazime (azole) in the Idared apples from the location of Evla and Kriveni and their maximum residue levels (MRLs) are presented in Fig. 2.

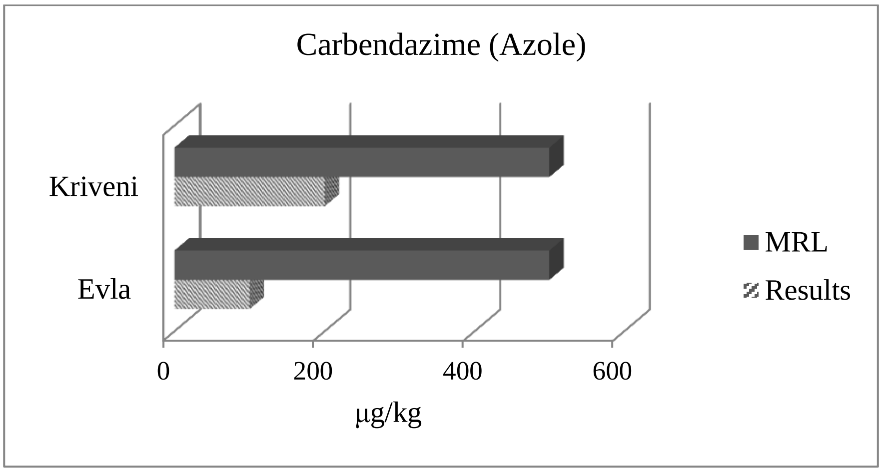

Fig. 2. Concentration of carbendazime (azole) in apples from different locations and maximum residue levels (MRLs)

Based on the results (Fig. 2) it can be seen that the carbendazime (azole) content (100 μg/kg) is at a legally tolerated level (500 μg/kg). Our results are similar to the ones obtained by Radišić et al. (2009), which analyzed the carbendazime level in fruit juices from apple, peach, orange and raspberry with LC-MS/MS. The carbendazime in apples from Kriveni is present at 200 μg/kg and this concentration is smaller than the MRLs. This result is similar to the result obtained by Morales et al. (2011), when they analyzed pesticides in peppers with LC-ESI-MS and extraction with QuEChERS. Carbendazime is systemic fungicide with protective and curative action used to protect plants against *Venturia* and *Podosphaera* in pome fruit and *Monilia* and *Sclerotinia* in stone fruit.

Conclusion

The aim of this research was to evaluate the represented fungicide residues in apples Idared from two different locations with QuEChERS and UPLC/TQ-MS. Different fungicides in different concentration content were detected in the four stages of apple cultivation. The different representation of pesticides depends on and is related to various factors and in our research, from the location of apple cultivation. But despite the different presence of fungicides in apples, they are safe for consumption.

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