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Pesticide residue levels in strawberry processing by-products that are rich in ellagitannins and an assessment of their dietary risk to consumers

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ABSTRACT

Background: According to many scientific studies, ellagitannins are beneficial for human health. One of the sources of these compounds is strawberry press-cake. This material can be used for the production of dietary fiber or ellagitannin preparations. The health safety of press cake and the preparations that are rich in ellagitannins obtained from it depend on pesticide residue levels, mainly fungicides, which are used for strawberry protection.
Aim: The aim of the work was a dietary risk assessment measured by the %ADI (acceptable daily intake) and MOE (margin of exposure) associated with the presence of pesticide residues for the consumption of strawberry processing by-products containing an amount of ellagitannins equivalent to amount present in 100 g of fresh strawberries. In our study, we investigated the contents of pesticides that are approved for use against strawberry diseases and pests.
Results: The total contents of pesticides in strawberry press-cake seeds (SPCS), exhausted strawberry flesh (ESF) and strawberry ellagitannin preparation (SEP) were 2143, 13,464 and 20,225 μg/kg, respectively. The analyzed products were dominated by fungicides as 96% of the total content of the tested pesticides. In the tested samples, we detected 11 fungicides and 3 insecticides. The dietary risk to consumer health, which depends on the presence of pesticide residues, in %ADI of daily consumption of ellagitannins (as dried extract (SEP), seeds (SPCS) or flesh (ESF)) ranged from 0.2% to 4.1% in a dose that was equivalent to 100 g of strawberries.
Conclusion: Although the pesticide residue contents in strawberry by-products are higher than in fresh fruits, the suggested doses of the by-products are lower. Therefore, the dietary risk to consumers from strawberry by-products is comparable to that from fresh fruits.

1. Introduction

The strawberry is a perennial plant of considerable economic importance. It is cultivated on commodity plantations both for the purposes of industrial processing and for direct consumption. In 2010, the world's production reached 4,400,000 t. With an annual output of approximately 200,000 t, Poland is a leading producer of strawberries in Europe [8], with 60% of the production used for frozen foods and concentrated juices [20]. The profitability of strawberry cultivation is largely influenced by biological, agricultural, and organizational factors, with the most important successful criteria being the amount of crops and fruit marketability. The fruits may be attractive not only in terms of their taste and flavor but also in terms of their nutritional and health properties [10].

Strawberries are strongly affected by diseases and pests, especially in years with unfavorable weather conditions, throughout the vegetation season and harvest. Therefore, efficient protection is crucial for commodity crops. Fruit producers around the world use plant protection agents [9]. The most dangerous strawberry diseases are *Botrytis cinerea*, *Oidium fragariae*, and *Mycosphaerella fragariae*, and the major pests are *Steneotarsonemus fragariae*, *Anthonomus rubi*, and *Tetranychus urticae* [18]. In countries belonging to the European Union, provisions concerning the use of plant protection agents and the monitoring of other pesticides in food are outlined in regulations EC 1107/2009 and 396/2005. The list of pesticides approved for use against strawberry diseases and pests in 2013, maximum residue levels (MRL) and acceptable daily intakes (ADIs) for all of the substances is given in Table 1.

The industrial processing of fruits is accompanied by the generation of considerable quantities of by-products, which may be a valuable source of substances that may impart added value to many products [4]. Industrial strawberry press cake, which is a by-product of juice production, amounts to 4%–10% of the weight of the processed fruits and is an important source of healthy substances, such as dietary fiber and

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Table 1
Synthetic pesticides approved for use in strawberry protection in Poland in 2013.

Pesticide	MRL* (mg/kg)	ADI** (mg/kg bw/day)
Fungicides		
Boscalid	10	0.04
Carbendazim	0.1	0.02
Cyprodinil	5	0.03
Fludioxonil	3	0.37
Folpet	3	0.1
Pyraclostrobin	1.5	0.03
Pyrimethanil	5	0.17
Tetraconazole	0.2	0.004
Thiophanate-methyl	0.1	0.08
Trifloxystrobin	0.5	0.1
Fenhexamid	5	0.2
Insecticides		
Acetamiprid	0.5	0.07
Chlorpyrifos	0.2	0.01
Pyridaben	1.0	0.01

*MRL — maximum residue limit; **ADI — acceptable daily intake.

polyphenols, including anthocyanins, ellagitannins, flavonols, and proanthocyanidins [1,10,24]. In several studies, ellagitannins, which are the main phenolic group in strawberries, were recognized as anti-inflammatory [11] and anti-carcinogenic agents [22]. Strawberry press cake, as a source of polyphenols that are beneficial for health, may be a valuable by-product to obtain products (ellagitannin preparations, dietary fiber preparations) with health beneficial properties ([15,24]; [13]). Ellagitannin or dietary fiber preparations may be consumed directly in the form of dietary supplements or in products enhanced by them, i.e., dairy products, bakery products, and cereals.

To date, there is no data concerning the distribution of pesticide residues in the process of producing strawberry juice taking into consideration the seeds and the seedless fraction.

In the context of the above overview of the literature, the goal of this study was to enrich the knowledge of the distribution of pesticide residues in the by-products of strawberry processing and to assess the dietary risk to consumers resulting from the presence of these residues in strawberry press cake fractions and ellagitannin preparations. We wanted to examine the safety of obtained strawberry by-products rich in ellagitannins and dietary fibers for the health of the consumer.

2. Materials and methods

2.1. Plant material

2.1.1. Fruit

The material for the study included 121 samples of fresh and frozen strawberries from the 2012 harvest season, which came from growers and traders from the Mazovia and Lublin voivodeships. It was declared that the samples were taken representatively from fields or cold rooms.

2.1.2. Press cake fractions

The material for the study was fresh industrial strawberry press cake from a modern fruit processing plant in Poland (Alpex Company, Łęczeszyce, Poland). The press cake was obtained from typical concentrated strawberry juice production, using fruits of cultivars that are common in large-scale farming in Poland. Four samples of the material (4 × 100 kg collected from the production line at 4 hours intervals) were from the 2012 harvest season, and three samples (3 × 100 kg collected from the production line at 4 hours intervals) were from the 2013 harvest season. Representative (10 kg) samples were next freeze-dried in a TG 5 freeze dryer (VEB Hochvakuum Dresden, Germany), and these samples were used for the separation of the seeds (SPCS, strawberry press-cake seeds) from the seedless fraction. For this purpose, freeze-dried press cake was sieved through a 0.8-mm screen. Large seeds were retained on the screen, while the flesh and small seeds passed through the screen. Flesh and small seeds were next sieved using a 0.6-mm screen to obtain press cake deprived of seeds. The share of SPCS in dried industrial strawberry press cake was 38 ± 2%. As a result of industrial strawberry pulp pressing and water extraction in the press, the seedless fraction was rather deprived of water-soluble substances, and therefore, that part was described as exhausted strawberry flesh (ESF).

2.1.3. Strawberry ellagitannin preparation (SEP)

Because strawberries from open commodity plantations are heavily contaminated with sand severely limits the possibility of the direct use of press cake (especially ESF) for consumption purposes [24]. Thus, one of the ways of using ESF is the production of products that are rich in ellagitannins. Part of the freeze-dried ESF was subjected to water-ethanol extraction using 5 L of an aqueous solution of 60% ethanol and 1 kg of plant material. Extraction (maceration) was carried out without mixing in two stages, each for a period of 10 h at 20 °C. The resulting extracts were combined and filtered on a Hobrafilt S40 N cellulose sheet with a 5 μm nominal retention and a 3.6-mm thickness (Hobra-Školnik S.R.O., Broumov, Czech Republic). Next, after removing the extraction solvent (ethanol), the resulting solutions were concentrated to approximately 15% dry matter. The process of ethanol removal was carried out under a reduced pressure of 135 mbar at 60 °C, and the concentration of the extract was performed at a pressure of 72 mbar at 60 °C. Both processes were carried out using a Basis Hei-VAP HL rotary evaporator (Heidolph, Schwabach, Germany). The concentrated extract was then freeze-dried (−32 °C, 48 h; Christ, Alpha 1-2 LD Plus, Osterode am Harz, Germany). This resulted in a dry polyphenol preparation referred to as the strawberry ellagitannin preparation (SEP).

2.2. Determination of pesticide residues

To ensure good recovery and high specificity of the analyzed pesticides, the QuEChERS extraction method and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) or gas chromatography with a single mass detector (GC/MS) were used. Multiple pesticide residues were extracted from plant material by liquid–liquid extraction followed by dispersive solid phase extraction (dSPE) purification. The detailed method was first published in 2003 [2] and was standardized as the European Standard EN 15662:2008 [8]. After homogenization, a 2–10 g portion of a sample was weighed into a 50 mL Teflon centrifuge tube, and 100 μL of (surrogate standard) TDCPP (tris(1,3-dichloroisopropyl)phosphate) at a concentration of 50 μg/mL was added. Then, 10 mL of deionized water was added to the dried samples, and the tube was shaken for 2 min. After that, 10 mL of acetonitrile (MeCN) was added, and the tube was shaken for 1 min. Afterwards, a mixture of 4 g of anhydrous magnesium sulfate ($MgSO_4$), 1 g of sodium chloride, 1 g of sodium citrate tribasic and 0.5 g of sodium hydrogencitrate sesquihydrate was added. The tube was shaken immediately by hand and then vigorously in a vortex for 1 min to prevent the agglomeration of magnesium sulfate salts. The extract was then centrifuged at 900 rad/s for 5 min. One milliliter of the MeCN supernatant was transferred to the dSPE tube containing 150 mg of anhydrous $MgSO_4$ and 25 mg of PSA (primary secondary amine sorbent) per mL of extract. The extract was shaken in a vortex for 30 s and centrifuged again at 900 rad/s for 1 min. For LC/MS/MS analysis, 250 μL of final extract was transferred into a 2-mL Eppendorf tube. Then, 750 μL of water, 50 μL of MeCN and 50 μL of a TPP (triphenyl phosphate) internal standard at a concentration 0.5 μg/mL in 1% formic acid in MeCN were added, and the tube was vortexed. For GC/MS analysis, 1 mL of the final extract was directly transferred to a vial, and 100 μL of MeCN along with 50 μL of a TPP (triphenyl phosphate) internal standard at a concentration 20 μg/mL was added. Before injection into the chromatographic system, the final solutions were filtered through a 0.45-μm PTFE membrane syringe filter directly into the vial. To quantify results, matrix-matched standard calibration was used.

For the LC analysis, an Agilent 1200 HPLC system with a binary pump was used. The chromatographic separation was performed using a 100-mm C18 column with a 2.1-mm internal diameter and 1.8-μm particle size (Agilent Zorbax Eclipse Plus). The column temperature was maintained at 45 °C. The injected sample volume was 10 μL. The mobile phase consisted of the following: A was water containing 0.01% formic acid and 5 mM ammonium formate and B was a mixture of acetonitrile/water (95:5, v/v) containing 0.01% formic acid and 5 mM ammonium formate. The gradient program was set as follows: 20% B was kept constant for 1 min followed by a linear gradient up to 100% B in 18 min, and then, 100% B was held constant for 2 min. The re-equilibration time was 7 min. For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was applied. The ion source parameters were as follows: gas temperatures, 325 °C; gas flow, 9 L/min; nebulizer gas, 40 psi; and capillary voltage, 4000 V.

GC–MS analysis was performed on an Agilent GC6850 gas chromatograph equipped with a split/splitless injector and coupled with a 5973 MSD mass spectrometer. A DB-35MS 30-m capillary column with a 0.25-mm i.d. and a 0.25-μm phase film diameter was used for the separation of the analytes. The carrier gas was helium at a flow of 1.0 mL/min. The temperature program was applied as follows: 95 °C (1.5 min), 20 °C min^{-1} to 190 °C, 5 °C min^{-1} to 230 °C, 25 °C min^{-1} to 290 °C (3 min) and finally 18 °C min^{-1} to 330 °C (5 min). The run time was 30 min, and 2 μL of sample was injected in the splitless mode. The injector, ion source and quadrupole were set at 250, 230, and 150 °C, respectively. The ionization was performed by electronic impact at 70 eV. In Table 2, the acquisition parameters of GC/MS and LC/MS/MS for pesticide detection in strawberry samples are given.

For the test matrices, good performance validation parameters were obtained (tested in the laboratory of the Department of Food Safety Research Institute of Horticulture, Skierniewice, Poland). All of the analyzed compounds have a LOQ at 10 μg/kg, except that of folpet, which was at 50 μg/kg. The LODs of all of the analyzed samples were below or equal to 5 μg/kg. The recoveries of all of the compounds were between 89 and 106% for fruit, 84 and 108% for SPCS, 82 and 106% for ESF and 80 and 110% for SEP. An additional verification of the method performance is successfully confirmed every year by participation in EUPT-FV, as organized by the Community Reference Laboratory (CRL) for fruit and vegetables.

2.3. Determination of the ellagitannins

Mean laboratory samples of fresh or frozen strawberry fruit, strawberry press cake seeds (SPCS), and exhausted strawberry flesh (ESF) were ground with liquid nitrogen in a IKA A11 (IKA-Analytical Mill, Staufen, Germany) laboratory mill. The total ellagitannins were determined by the HPLC method described by Sójka et al. [24] or Klimczak and Król [14], and the results were calculated as galloyl-bis-HHDP-glucose.

2.4. Risk assessment estimation

For calculating the risk assessment coming from the consumption of strawberry fruit and its processing products, a daily dose of 100 g was used. This value was used based on research conducted by Ashfield-Watt et al. [3], in which the mean daily serving of strawberries was 103 g. One-hundred grams of strawberries containing 62.5 mg of ellagitannins was found to be a common serving size of fresh strawberries consumed in the season per person. The products recovered from industrial strawberry press cakes (i.e., SPCS, ESF, and SEP) rich in ellagitannins may be considered to be an equivalent source of these compounds in the form of an appropriate dietary supplement.

To estimate the daily dose of SPCS, ESF and SEP for calculations, the mean content of ellagitannins (62.5 mg) coming from 100 g of fresh fruit and the mean contents of these compounds in the above products of 500, 1000 and 20,000 mg/100 g, respectively, were used. Therefore, to provide the body with 62.5 mg of ellagitannins, one should consume 100 g of fruit, 12.5 g of SPCS, 6.25 g of ESF or 0.31 g of SEP.

In the risk assessment of the consumption of pesticide residues from fruits, SPCS, ESF and SEP were presented in the form of %ADI, representing a chronic risk [21] for a person with a body mass of 50 kg. For the calculations, the following equation was used:

$$\%\text{ADI} = \frac{\text{Average Food Exposure (mg residue / kg body weight / day)}}{\text{ADI (mg residue / kg body weight / day)}} \times 100$$

where

$$\text{Average Food Exposure} = \frac{\text{Residue content (mg / kg)} \times \text{Daily mass of the product (g)}}{1000 \times \text{Body weight (kg)}}.$$

Chronic food risk is expressed as a percentage of ADI. If the calculated %ADI is less than 100, the risk is generally considered to be acceptable [21].

The risk assessment was also carried out using a margin of exposure (MOE), which represents the ratio of the reference point (NOAEL or ADI) for a compound to the estimated or measured level of exposure. To estimate the influence of an accumulation of pesticide residues, a combined margin of exposure (MOE_T) was applied, which was determined by taking the reciprocal of the sum of the reciprocals of the individual MOEs. When the MOE_T is greater than 1, the combined risk from

Table 2
Acquisition parameters of LC/MS/MS and GC/MS for pesticides detected in strawberry fruit, flesh, seeds and dried extract.

Pesticide	Method	Precursor ion	Quantitation ion m/z	Confirmation ion m/z	Retention time (min)
Fungicides					
Boscalid	GC/MS	–	140	342.1, 142, 344	25.1
Carbendazim	LC/MS/MS	192.1	160	132	2.9
Cyprodinil	GC/MS	–	224	225, 210	17.8
Fludioxonil	GC/MS	–	248	127, 154	19.6
Folpet	GC/MS	–	260	262, 130	19.0
Pyraclostrobin	GC/MS	–	132	164.1	23.6
Pyrimethanil	GC/MS	–	198	199	14.3
Tetraconazole	GC/MS	–	336	338, 337	16.9
Tiophanate-methyl	LC/MS/MS	343.1	151	93	9.4
Trifloxystrobin	GC/MS	–	116	131, 186, 222	20.0
Fenhexamid	GC/MS	–	97	177, 179	20.1
Insecticides					
Acetamiprid	LC/MS/MS	223.1	126	56	4.8
Chlorpyrifos	GC/MS	–	197	199, 314	16.6
Pyridaben	LC/MS/MS	365.2	147.1	309.1	19.7

exposure to the residues is considered to be acceptable [5]. The MOE and $\mathrm{MOE_T}$ were calculated for a person with a body mass of 50 kg according to following formulas:

$$\mathrm{MOE} = \frac{\mathrm{ADI\ (mg\ residue/body\ weight/day)}}{\mathrm{Average\ exposure\ (mg\ residue/kg\ body\ weight/day)}}$$

$$\mathrm{MOE_T} = \frac{1}{\frac{1}{\mathrm{MOE_1}} + \frac{1}{\mathrm{MOE_2}} + \frac{1}{\mathrm{MOE_3}} + \ldots}$$

2.5. Statistics

To illustrate the differences in the researched component contents between fruit, SPCS, ESF and SEP, the results were statistically analyzed by one-way analysis of variance and the post hoc Duncan test with a statistical significance of $p \leq 0.05$. Statistical analysis was carried by Statistica version 10 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Pesticide residue content

The examined fruits contained eight fungicides and one insecticide from the list of pesticides approved for strawberry protection. Table 3 and Fig. 1 present the qualitative and quantitative compositions as well as the frequency of detection of the active substances in plant protection products found in strawberries from the 2012 harvest. The data show that among the 121 studied samples, 43 (36%) did not contain pesticide residues, while 5 samples revealed excessive levels of individual substances. In turn, in 73 samples (60%), the content of pesticides did not exceed the MRL. A total of 177 residues were detected, but in 40 cases, the detected amounts were lower than 10 μg/kg, which was close to the limit of detection. The mean number of substances detected per fruit sample was 1.9. The typical frequency of residue occurrence in strawberries is given in Fig. 1. The figure shows that samples containing a maximum of three pesticides constituted almost 90% of the population of the studied strawberries, and the mean number of substances detected in this population was 0.95. Table 3 shows that pyrimethanil was the most frequently detected pesticide in the studied strawberry samples, and the mean content of this compound in samples in which it was present was 56 μg/kg. The other pesticides, in decreasing order of content by weight, were cyprodinil, fludioxonil and boscalid. All of the above pesticides, except for pyrimethanil, were the most often used substances for strawberry protection.

The calculated mean content of total pesticides for the population of 73 strawberry samples was 220 μg/kg (data not shown). Taking into consideration the mean contents of individual pesticides in samples in which they were present, the mean total content of pesticides may be estimated as 650 μg/kg (Table 3). According to a study by Looser et al. [17], the mean content of total pesticides in fruits from a German chain retailer was estimated at 400 μg/kg of strawberries, while the average number of pesticides detected in these strawberries was 4.7 (from 3.9 to 6.2, depending on the origin of strawberries). The most frequently detected pesticides were cyprodinil, fludioxonil, fenhexamid, and chlorpyrifos, most of which are also used for the protection of other berry-bearing plants, especially grapevines. A similar level of pesticide content (500 μg/kg of fruit) was determined for strawberries obtained from integrated cultivation [9].

All of the fungicides and insecticides (Table 3) approved for strawberry protection were detected in dried industrial strawberry press cake, which was divided into seeds (SPCS) and flesh (ESF). Thus far, in the literature, there is no available data concerning the number of pesticides or the level of pesticide concentration in strawberry press cake for the seeds and flesh separately. The ratios of the concentrations of fungicides and insecticides, except pyraclostrobin, in ESF to those in SPCS were from 2 to 9, which indicates a greater accumulation of several substances in strawberry flesh than in the seeds. The mean concentration of fungicides and insecticides in the ESF was greater than five times higher than that in the SPCS. Pesticides that were the most absorbed by the ESF were pyraclostrobin, chlorpyrifos, trifloxystrobin and fludioxonil. We observed that the contents of seven out of the eleven fungicides were significantly higher in the ESF than in the SPCS. Additionally, it should be noted that insecticides account for approximately 4% of the total determined pesticides.

Recent results for the concentrations of pesticides in grapes, must, and wine [7] indicate that some hydrophobic substances accumulate in the residue remaining after the pressing of crushed grapes and in the sediment from settled must and wine. The contents of boscalid, pyrimethanil, and procymidone were 10 to 20 times higher in the press cake than those in the must. In turn, the concentration of folpet

Table 3
Pesticide contents in samples of fruit, dried seeds (SPCS), flesh (ESF), and strawberry ellagitannin (SEP) preparation obtained from industrial strawberry press cake in μg/kg.

Pesticide	ns	Fruit[a] 2012	SPCS 2012 (n = 4)	SPCS 2013 (n = 4)	SPCS (2012 + 2013)	ESF 2012 (n = 3)	ESF 2013 (n = 3)	ESF (2012 + 2013)	SEP 2012 (n = 3)
Fungicides									
Boscalid	16	93 ± 96a	665 ± 293	843 ± 110	741 ± 237b	2308 ± 465	3367 ± 1185	2761 ± 947c	3047 ± 300c
Carbendazim	0	–	nd.	37 ± 11	16 ± 21a	nd.	107 ± 6	46 ± 57a	nd.
Cyprodinil	20	160 ± 175a	150 ± 127	283 ± 110	207 ± 131a	688 ± 471	930 ± 79	791 ± 360b	713 ± 70b
Fludioxonil	8	115 ± 97a	143 ± 95	240 ± 111	184 ± 107a	838 ± 291	1800 ± 529	1250 ± 633b	1030 ± 260b
Folpet	0	–	620 ± 520	647 ± 133	631 ± 376a	1218 ± 330	2450 ± 1429	1746 ± 1081a	9137 ± 436b
Pyraclostrobin	8	29 ± 20a	nd.	20 ± 0	9 ± 11a	nd.	3600 ± 2022	1543 ± 2251b	nd.
Pyrimethanil	39	56 ± 132a	50 ± 29	90 ± 30	67 ± 35a	145 ± 78	463 ± 49	281 ± 181b	3803 ± 65c
Tetraconazole	6	52 ± 27a	70 ± 27	117 ± 12	90 ± 32a	488 ± 158	343 ± 142	426 ± 159b	358 ± 68b
Thiophanate-methyl	4	30 ± 12	nd.	nd.	nd.	nd.	<LOQ	<LOQ	nd.
Trifloxystrobin	0	–	75 ± 19	30 ± 20	56 ± 30a	675 ± 168	143 ± 15	447 ± 308b	<LOQ
Fenhexamid	5	63 ± 43a	75 ± 54	30 ± 17	56 ± 46a	65 ± 130	130 ± 70	93 ± 106a	2113 ± 1115b
Sum of FG	–	650a	1850 ± 1135	2339 ± 319	2060 ± 864ab	6425 ± 1304	13,342 ± 5333	9389 ± 4899b	20,201 ± 6238c
Insecticides									
Acetamiprid	0	–	nd.	<LOQ	<LOQ	nd.	43 ± 12	19 ± 24	nd.
Chlorpyrifos	5	12 ± 2	75 ± 55	nd.	43 ± 56a	633 ± 772	<LOQ	364 ± 640a	<LOQ
Pyridaben	0	–	53 ± 29	23 ± 6	40 ± 26a	93 ± 185	73 ± 21	84 ± 132a	25 ± 11a
Sum of INS	–	12a	128 ± 61	23 ± 6	83 ± 70a	725 ± 843	122 ± 31	467 ± 678a	25 ± 11a
Sum of PEST	–	662a	1987 ± 1118	2364 ± 321	2143 ± 838a	7150 ± 1419	13,464 ± 5362	9856 ± 4689b	20,226 ± 6249c

Values are the means ± standard deviation; n: number of measurements; ns: number of fresh or frozen fruit samples with a given substance detected; nd.: not detected.
Mean results in the columns: fruit, SPCS (2012 + 2013), ESF (2012 + 2013), and SEP for particular pesticides marked with the same letters do not differ statistically at $p \leq 0.05$.

[a] Mean content of pesticide in the pool of fruit samples where the pesticide was detected; FG: fungicides; INS: insecticides; PEST: pesticides.

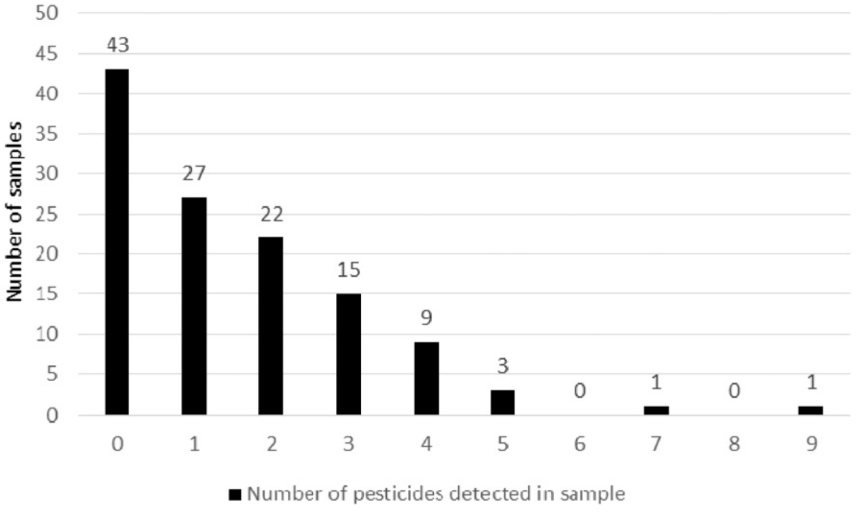

Fig. 1. Number of pesticides detected in a sample (n = 121) from the 2012 harvest season. *Footnote*: In 43 samples 0 pesticides were found, in 27 samples 1 pesticide was found etc.

in the press cake was relatively low, while it was not detected in must or wine. According to Cabras et al. [6], folpet degrades to phthalimides under the influence of light and hydrolysis, and in wine, it is decomposed by the fermentation processes. Our results confirmed some of these observations with respect to strawberry products, especially for the ESF.

The SEP preparation, rich in ellagitannins, obtained from industrial press cake contained, in decreasing order of magnitude, folpet, pyrimethanil, boscalid, and fenhexamid, with the folpet and boscalid levels characterized by considerable variability, which may be due to differences in the doses of these pesticides applied for plant protection on plantations and/or due to the greater affinity of these substances to the extractant used. The above-mentioned substances, in terms of content, represent 80% of tested pesticides. Comparing the tested products, it was shown that the highest content of pesticide residues was found in the SEP. The total content of pesticides in the SEP was almost 2-fold higher than in the ESF, ca. 10-fold higher than in the SPCS and 30-fold higher than in the fruit itself. A significant rise in the concentration of pesticide residues was observed with an increased degree of fruit processing, which in particular concerns the extract and well-pressed dried strawberry flesh.

The possibility of obtaining bioactive compounds from strawberry processing by-products is an important issue both for nutritional and health reasons as well as in terms of the recovery of valuable substances from the waste generated by processing strawberries and other fruits in the family *Rosaceae* [16]. The key question here is the pesticide residue levels in strawberries and their accumulation in the by-products of the processing of strawberries into juice as well as in the recovery products. In the industrial production of concentrated juice, the raw material becomes naturally mixed, and its composition is averaged, leading to the concentration of pesticides decreasing, while the number of substances detected in the products rises [19]. Our research confirmed this phenomenon because tested press cake and SEP contained more than 8 pesticides, and the fruits themselves only contained 2, on average. An important issue is also using pesticides that are not approved for strawberry protection. In the strawberry population that we tested (n = 121), we found a high frequency of procymidone (herbicide); however, it was found in an amount below the applied MRL. The presence of this compound may be due to its recent withdrawal.

3.2. Risk assessment

Table 4 shows the assessment of the dietary risk to consumer health for a person weighing 50 kg in terms of the %ADI, MOE and MOE_T, depending on the daily consumption of ellagitannins contained in 100 g of strawberries as well as an equivalent amount of ellagitannins present in products recovered from the press cake, that is, in 12.5 g of seeds (SPCS), 6.25 g of flesh (ESF), and 0.31 g of the ellagitannin preparation (SEP), which were characterized by variable levels of pesticide remains. The potential risk to consumers ingesting the above-mentioned amounts of strawberries varied from 0.06%ADI to 2.62%ADI and depended on the substance. The highest risks, in the case of the fruit itself, were introduced by tetraconazole and cyprodinil, for which the %ADI and MOE were 2.62 and 38 and 1.07 and 93, respectively. In summary, in the case of pesticide residues in fruits in such a quantity as in Table 4, the risk from the accumulation of a few pesticides expressed as the MOE_T is 20 and as the %ADI is 4.94. According to current knowledge, samples for which the MOE_T is higher than 1 and the %ADI is lower than 100 are considered to be safe.

In turn, the risks to consumers ingesting an equivalent dose of ellagitannins recovered from strawberry press cake in the forms of SPCS, ESF and SEP amounted to 1.63%, 4.10%, and 0.20%ADI, respectively. Meanwhile, the MOE_T values for SPCS, ESF and SEP were 61, 24, and 506, respectively. The obtained data indicated that the highest risk among the by-products was introduced by the ESF. The substances responsible for that were mainly tetraconazole and boscalid, for which the %ADI values were 1.33 and 0.86, respectively. A similar situation was found for SPCS; however, the above-mentioned substances were found in significantly lower quantities (they were not accumulated in the seeds), and the risk coming from their presence was lower. The lowest risk was calculated for the SEP, and values of 0.2%ADI and 506 for the MOE_T were found. It should be noted that under conditions of ellagitannin extraction from the ESF using an ethanol solution, some pesticide transfer to the solution occurs (including boscalid, folpet, pyrimethanil, and fenhexamid), but the concentrations of those pesticides are much lower than those of ellagitannins in the dried extract (SEP). According to the latest reports, the presence of some pesticide combinations in food products may exhibit a synergistic effect despite the low concentration of each individual pesticide [12]. In terms of the safe consumption of strawberries and strawberry products, the ADI value is the more important criteria than the substance content [23]. As a result, the risk coming from the consumption of products tested by us may be due to the higher number of pesticides rather than a higher total pesticide content. Comparing products such as the SPCS, ESF and SEP with the fruit itself indicated that these products contained 8 pesticides on average while the fruits only contained 2. In risk assessment, an important issue may be the presence of pesticides that are not allowed in strawberry protection. In our case, among illegal pesticides, mainly the presence of procymidone (data not shown) could increase the risk for the ESF by approximately 1 percentage point.

Table 4

Dietary risk to consumer health in %ADI and MOE following ingestion of equivalent amounts of ellagitannins contained in strawberries, SPCS, ESF, and SEP.

	Fruit (100 g)		SPCS (12.5 g)		ESF (6.25 g)		SEP (0.31 g)	
Pesticide	%ADI[b]	MOE[b]	%ADI	MOE	%ADI	MOE	%ADI	MOE
Fungicides								
Boscalid	0.46 ± 0.48ab	216	0.46 ± 0.15ab	216	0.86 ± 0.30b	116	0.047 ± 0.005a	2117
Carbendazim	–	–	0.02 ± 0.03a	5045	0.03 ± 0.04a	3500	–	–
Cyprodinil	1.07 ± 1.17a	93	0.17 ± 0.11a	579	0.33 ± 0.15a	303	0.015 ± 0.001a	6790
Fludioxonil	0.06 ± 0.05b	1616	0.01 ± 0.01a	8031	0.04 ± 0.02ab	2368	0.002 ± 0.000a	57,917
Folpet	–	–	0.16 ± 0.09ab	633	0.22 ± 0.14b	458	0.057 ± 0.027a	1765
Pyraclostrobin	0.19 ± 0.14ab	522	0.01 ± 0.01a	14,000	0.64 ± 0.94b	156	–	–
Pyrimethanil	0.07 ± 0,14a	1447	0.01 ± 0.01a	10,128	0.02 ± 0.01a	4832	0.014 ± 0.000a	7209
Tetraconazole	2.62 ± 1.33c	38	0.56 ± 0.20ab	178	1.33 ± 0.50b	75	0.056 ± 0.010a	1800
Thiophanate-methyl	0.08 ± 0.03	1333	–	–	–	–	–	–
Trifloxystrobin	–	–	0.01 ± 0.01a	7179	0.06 ± 0.04b	1786	–	–
Fenhexamid	0.06 ± 0.04b	1582	0.01 ± 0.01a	14,359	0.01 ± 0.01a	17,231	0.007 ± 0.003a	15,264
Sum of FG	4.71b	21[a]	1.43 ± 0.51a	70[a]	3.54 ± 1.45b	28[a]	0.196 ± 0.048a	509[a]
Insecticides								
Acetamiprid	–	–	–	–	0.00 ± 0.00	30,154	–	–
Chlorpyrifos	0.24 ± 0.03a	426	0.11 ± 0.14a	933	0.45 ± 0.80a	220	–	–
Pyridaben	–	–	0.10 ± 0.06a	1000	0.11 ± 0.16a	949	0.002 ± 0.001a	65,388
Sum of INS	0.24a	426[a]	0.21 ± 0.18a	483[a]	0.56 ± 0.86a	63[a]	0.002 ± 0.001a	65,388[a]
Sum of PEST	4.94b	20[a]	1.63 ± 0.44a	61[a]	4.10 ± 1.57b	24[a]	0.198 ± 0.048a	506[a]

Values of %ADI are the means ± standard deviation; FG: fungicides, INS: insecticides, PEST: pesticides.

Mean results of %ADI in the columns: fruit, SPCS, ESF, and SEP for particular pesticides marked with the same letters do not differ statistically at $p \le 0.05$.

[a] MOE for the sum of FG, INS and the sum of PEST were calculated as a MOE_T (as described in the Materials and methods section).

[b] Calculated based on the mean content of the pesticide in the pool of samples in which the pesticide was detected.

The results show that in fruits, tetraconazole > cyprodinil > boscalid form a descending series of pesticides presenting a dietary risk to consumer health. In the case of by-products, the descending series of pesticides presenting a dietary risk to consumers consists of tetraconazole > boscalid > cyprodinil for the SPCS and tetraconazole > boscalid > pyraclostrobin for the ESF. In the SEP preparation, which is rich in ellagitannins, the risk to consumer health decreases in the following order: folpet > tetraconazole > boscalid. In light of the above, the levels of tetraconazole and boscalid should be subjected to particularly close scrutiny if press cake or its fractions are to be used for the recovery of polyphenol substances from the waste generated in strawberry processing. An obvious alternative is the recovery of ellagitannins and related substances from raw materials free from pesticides.

4. Conclusions

By-products obtained in fruit processing to form juice are characterized by variable contents of pesticide residues. The highest content of pesticide residues was found in decreasing order in preparations rich in ellagitannins (SEP), seedless part of press cake (ESF) and seeds (SPCS). The total content of pesticides in the SEP was almost 2-fold higher than in the ESF and approximately 10-fold higher than in the SPCS.

The dietary risk to consumer health, depending on the pesticide residue presence ranged from 0.2% to 4.1% in %ADI of the daily consumption of ellagitannins as dried extract (SEP), seeds (SPCS) or flesh (ESF) in a dose equivalent to 100 g of strawberries, and thus, the risk can be compared with that presented by fresh strawberry fruits. In the case of the ESF, the risk is relatively higher due to an increased accumulation of tetraconazole and boscalid in that material.

In the industrial processing of fruits, the potential risk may be increased by the fact that in a mixed material (fruits, pulp, press cake), a high number of pesticides that are allowed to be used for strawberry protection may be found. Thus, further research investigating the synergistic action of pesticide residues on human health is needed. Alternatively, material coming from organic farming could be used.

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