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Processing factors of pesticide residues in biscuits and their relation to the physicochemical properties of pesticides

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ABSTRACT

Agricultural commodities are generally consumed as processed food. Therefore, it is indispensable to assess pesticide residues in processed products rather than only in the raw agricultural commodity, in order to approach a more realistic scenario of dietary exposure. Processing factors are important tools for dietary exposure risk assessments. In this study, processing factors for the baking process were derived for 41 pesticides in cereal bran-based biscuits. The raw materials used consisted of wheat, rye, oat, and barley grains with incurred pesticides, which originally was produced for test material for European Union Proficiency Tests. Information on physicochemical properties of pesticides was collected for understanding the fate of pesticides during the baking process. Average processing factors varied between 0.67 and 1.6. Most pesticide residues exhibited a reduction of pesticide residues of less than 24%, which correspond to a processing factor (PF) range between 1 and 0.76, showing resistance to the baking process. However, for polar compounds such as carbendazim and volatile compounds (chlorpyrifos-methyl, malathion, and pirimiphos-methyl) larger reduction rates were observed, up to 33% (PF: 0.67). In general, a prolonged baking time did not significantly affect the PF, because the main degradation process takes place within the first 6 min. However, this was not the case for the highly volatile compounds, highly polar compounds, and compounds of low degradation temperature. These latter were significantly reduced with prolonged baking time, resulting in a reduction rate of up to 95%, which means an almost complete elimination.

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Introduction


The increasing awareness of health and food quality in general has also increased the public awareness on the occurrence of pesticide residues in our food and the resulting dietary intake and risk assessment. The pesticide residue control is mainly focused on analysing raw agricultural products, because the maximum residue levels in force are set for these. Thus the vast majority of the pesticide residue data available for the estimation of the dietary intake and the related risk is for the raw agricultural products. However, a significant part of our diet is based on processed foods. Pesticide residue levels may be affected by the processing (Bajwa and Sandhu 2014).

From monitoring studies done worldwide, a variety of pesticides were detected in cereal grains (as many as in other crops) (Bakore et al. 2004). After harvesting, agricultural commodities such as cereals are subjected to different processing techniques that

could increase or decrease the levels of pesticide residues depending on the processing technique (baking, roasting, milling, steaming, boiling, etc.), the food commodity (chemical composition), and the physicochemical properties of the pesticide. The main processing techniques applied to cereal grains are milling and baking. Cereal grains, especially wheat, are typically consumed as bread, pasta, breakfast cereals, biscuits, cakes, and pastries (Stevenson et al. 2012).

In the Danish monitoring programme for pesticide residues from the period 2004–2011, samples of raw fruits, vegetables, and cereals were included as well as some processed food such as wine and samples of processed organically grown crops (Poulsen et al. 2017). Cumulative risk assessment after chronic dietary exposure was carried out (Jensen et al. 2015). Processing factors for relevant commodities were also applied to give a more realistic estimate of the dietary intake. However, human health risk assessment from exposure to

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pesticide residues in processed foods derived from wheat or other cereal grains (oat, barley, and rye) is still deficient. The studies available in the literature mainly focus on the bread-making process (Herrera-Herrera et al. 2019) because bread consumption tends to be the greatest of all cereals-based products. The pesticide residues occurring in cereals are primarily associated with the bran (Udeaan and Bindra 1973; Dors et al. 2011). Several studies on incurred pesticides in proficiency tests (Hajeb et al. 2017; Herrmann et al. 2017; Blanco Canalis et al. 2018) demonstrate that extraction efficiency increases as particle size decreases, especially for oat and barley because pesticides are more concentrated in the bran. For wheat bread production the content of bran is often low and thereby also the resulting content of pesticides. Thus by diluting the pesticide residue content originating from bran with a large fraction of flour there is a possibility of diluting the pesticide level resulting in concentrations below the detection limit, even before the baking process. We therefore chose to study the effect of baking in a high bran content food product (biscuits). Moreover, the biscuit and whole-grain market in general is increasing, with new brands emerging as the rise in demand for healthy snacks continues. The nutritional value of bran is high not only due to a high content of dietary fibre (Xhabiri et al. 2014) but also occurrence of vitamins and various minerals.

Consequently, knowledge on the effect of processing on the residue levels is vital for a refined estimation of the dietary intake of pesticide residues and the resulting potential health risk. Cereals-based products account for a large part of our diet. Therefore, the establishment of processing factors for pesticides in cereals-based products is important. When applying for the placement of plant protection products on the market under Directive 91/414, data on processing factors may be required. However there is still a deficiency in the availability of information and synchronisation among processing factors within Europe and worldwide (Besil et al. 2015).

The objective of this project is to provide information on the variation of pesticide residues levels in the bran of wheat, rye, barley, and oat to biscuits, during the baking process. By using grain produced as test material for European Union proficiency

tests that contain incurred residues of multiple pesticides, we had a unique possibility to study the effect of processing for 41 pesticides in the same setup. The data will supplement the existing available data in the BfR compilation of processing factors. In the present study, processing factors are determined by conducting a follow-up study, wherein addition to the initial product, pesticide residues are studied in the end-product (the biscuit) (European Commission 1997). Possible correlations between the physical/chemical properties of a pesticide and the observed changes in the levels after baking are studied. Therefore, data for Log P, vapour pressure, degradation temperature, and pKa were collected and the relation between the physicochemical properties of the pesticides and the baking technique was tested.

Material and methods

Chemicals

Pesticide standards (purity > 96%) were purchased from Sigma-Aldrich or LGC Standards. Pesticide standard stock solutions of 1000 mg.mL⁻¹ were prepared in toluene and stored at -18°C in ampoules under an argon atmosphere. A standard mixture of 10 mg.mL⁻¹ was prepared from these stock solutions. Working standard solutions were prepared with standard-matched calibrations with cereal blank extract. Acetonitrile (HPLC Grade 5) was purchased from Rathburn Chemicals (Walkerburn, UK). The buffer salt mixture was purchased from Thermo Scientific, and the clean-up sorbent SupelTMQuE (EN) tubes were purchased from Supelco.

Selected samples and studied pesticides

Cereal kernels, originally produced as European Union proficiency test material for cereals (EUPT-C) of wheat (EUPT-C1, -C2, and -C8), oat (EUPT-C3), barley (EUPT-C6), and rye (EUPT-C4 and -C10), also served as the test material in the present study (Poulsen et al., 2007, 2008, 2009, 2010, 2014, 2016; Poulsen & Christensen, 2012). The cereals were treated with pesticides in experimental fields for the preparation of EUPTs (EUPT-C1, -C2, -C4, -C6, -C8, and -C10) in 2007, 2008, 2009, 2010, 2012, 2014, and 2016. The Faculty of Agricultural Sciences, University of

Aarhus in Denmark performed the field spraying. The material was also used for establishing processing factors for milling, in an unpublished study. The kernels were stored in a freezer at -80°C to ensure the chemical integrity of the cereal as well as the pesticide compounds. Incurred pesticides are of higher value than spiked pesticides when assessing processing factors in processed foods, which explains why we chose to use the proficiency test (PT) material. Moreover, according to some guidelines, pesticides derived from spiked residues are considered unacceptable (OECD 2008).

Accordingly, in this study, bran fractions obtained from each of the PT materials identified above were used in this project, except for that of PT-C4 (rye), for which a mixture of bran and feed flour had to be used due to availability constraints.

Dough and biscuit preparation

The procedure for biscuit preparation that was adopted for this study mimics the representative domestic standard of biscuit (OECD 2008). Dealing with a composite food (the biscuit), it was essential to use as few ingredients as possible, to avoid dilution of the pesticides through the addition of more ingredients. However, because one key aspect of the study is its focus on the baking process, it was necessary for our procedure to be representative of the domestic standard for biscuits. Accordingly, all biscuits were prepared by the same procedure, using untreated (blank) and treated (test item) material of each of the PT materials. One batch of blank biscuits and two batches of biscuits using the test item of bran was prepared with each PT material.

The ingredients used for biscuit preparation were: cereal bran (27 g; 58.4%), baking powder (3 g; 6.5%), sugar (4 g; 8.7%), salt (0.2 g; 0.4%), butter (5 g; 10.8%), and milk (7 mL; 15.2%). The biscuits were prepared as follows:

- In a large bowl, the cereal bran, sugar, baking powder, and salt were combined and mixed thoroughly.
- Butter, that had been chilled in the freezer for 10 min, was added and the mixture was stirred.
- Slowly, milk was added, and the dough was kneaded.

- Once the dough was cohesive, the dough was transferred onto baking papers.
- Using a round biscuit cutter, 4 small biscuit pieces measuring 5 cm \times 1 cm were made, except for oat, for which only 3 pieces of 5 cm \times 1 cm of biscuits were made, due to the higher density of the oat bran. Using a spoon, the layers were gently flattened. The biscuits were prepared using all of the dough and avoiding any material loss.
- The biscuits were baked in a preheated oven at 200°C for 6 min in a temperature-controlled oven.

When conducting processing studies and deriving processing factors for baking, the main parameters that must be considered are the duration of the heating process and the applied temperature. In addition to their influence on the structure and texture of the biscuits, these parameters are also critical with regard to their potential influence on the concentration of pesticide in the end product. Therefore, to report and study the role of temperature and time on the PFs reliably, the biscuits made of rye bran (test item PT-C4) were prepared by applying three different baking conditions: baking at 200°C for 6 min, baking at 180°C for 25 min, and baking at 200°C for 20 min. The reason behind performing this last experiment with only rye bran was due strictly to the available quantity of raw PT materials.

To be able to correct the measured levels of pesticides in the biscuits and to avoid overestimating the pesticide concentration due to the dilution of the bran with other ingredients and the vaporisation of water during the baking, the yield factor was calculated for each batch of biscuits. The yield factor is defined as a percentage calculated by dividing the mass of the processed commodity by the mass of the corresponding raw agricultural commodity (RAC), then multiplying by 100 (Bempelou et al., 2018).

The value of RAC is in this case the sum of the bran and the other biscuit ingredients, which in this study amounted to 46.2 g. Analysing the ingredients based on the RAC allows for the pesticide dilution to be corrected. Each batch of biscuits was weighed before and after baking. The yield factor obtained ranged from 67% to 86%,

depending on the batch. Yield factors less than 100% were due mainly to the loss of water during baking, with only minor losses due to the adhesion of dough to the bowl. The correction using the yield factor can be made either to the final quantitative results or by adjusting the sample size of the biscuit extracted. In this study, the latter approach was chosen. Therefore, to analyse exactly the same amount of bran (2 g) in the raw material and in the final biscuit product, we extracted 2.6 ± 0.1 g of biscuits, corresponding to 2 g of bran when taking into consideration both the recipe dilution and water loss during baking.

Extraction method

After baking, the biscuits were allowed to cool for 1 h at room temperature before beginning the extraction procedure. Each batch consisting of four biscuits (or three in the case of oat) were put together, milled, and extracted in duplicate.

The bran raw material and the processed commodity (biscuit) were extracted using identical methods, specifically, a modified citrate-buffered QuEChERS (EN 15662) (CEN 2008). For the bran, 2 g of bran was weighed into a 50 mL polystyrene centrifuge tube. For the biscuit, 2.6 ± 0.1 g of biscuit was weighed. Procedural standards were added to all samples before extraction. Then 10 mL of cold water was added, followed by 10 mL of acetonitrile. To aid the extraction, a ceramic homogeniser was used. The tubes were shaken for 1 min by hand. Next, 4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of sodium citrate dihydrate, and 0.5 g of sodium citrate sesquihydrate were added. After 1 min of shaking by hand followed by centrifugation for 10 min at 4500 rpm, 8 mL of the supernatant was transferred to a clean tube and stored at -80°C for 1 h. The extracts were then thawed, and while they were still very cold, they were centrifuged at 4500 rpm for 5 min. Thereafter, 6 mL of the cold supernatant was transferred to a tube containing 150 mg of primary secondary amine (PSA) and 900 mg of magnesium sulphate. After shaking for 30 s and centrifuging for 5 min at 4500 rpm, 40 μL of 5% formic acid solution was added to the extracts. For GC-MS/MS analysis, 100 μL of acetonitrile and 20 μL of the internal standard were added to 100 μL of extract. For LC-

MS/MS analysis, 1 mL of extract was removed before clean-up with PSA. Then, 200 μL of this crude extract was diluted 1:1 with acetonitrile and 40 μL of internal standard was added before filtration using vials with built-in polypropylene filters. The internal standard added to the vials before injection allows checking the performance of the analytical system.

Eight sets of matrix-matched calibration curves were prepared with each matrix (rye, oat, barley, wheat) of raw material (bran) and with each matrix of biscuit at concentration levels of 0.333, 0.1, 0.0333, 0.01, 0.0033, and 0.001 $\mu\text{g}/\text{mL}$.

Instrumentation

For gas chromatographic separation, a Thermo ScientificTM TraceTM 1310 Gas Chromatograph coupled to a Thermo ScientificTM TriPlusTM RSH autosampler was used. The samples were injected in a programmable temperature vaporiser (PTV) through a PTV baffle liner $2 \times 2.75 \times 120$ mm for Thermo GCs (Siltek). The injection volume was 1 μL and the injection temperature was set to 70°C . Helium (99.999%) was used as carrier gas at a flow of $1.2 \text{ mL}\cdot\text{min}^{-1}$. The analytes were separated on a DB5-MS capillary column 30 m \times 0.25 mm inner diameter and a film thickness of 0.25 μm . The oven temperature program was as follows: 60°C for 1.5 min, up to 90°C at $25^{\circ}\text{C}/\text{min}$ for 1.5 min, up to 180°C at $25^{\circ}\text{C}/\text{min}$, then up to 280°C at $5^{\circ}\text{C}/\text{min}$ and finally up to 300°C at $10^{\circ}\text{C}/\text{min}$ and for 12 min. The total runtime was 42 min. For the mass spectrometric analysis, a Thermo ScientificTM TSQTM 8000 Evo was used. The electron ionisation (EI) source was used with an electron energy of 70 eV. The analyses were performed by a triple quadrupole operating in the multiple reaction-monitoring mode (MRM). The source temperature was set at 300°C , and the transfer line, at 280°C .

The pesticide residues analysis was also performed by LC-(ESI)MS/MS. The LC system employed was a Thermo Ultimate 3000 and the mass spectrometer was a Bruker EVOQ. The analytes were separated on an Accuity UPLC BEH C18 1.7 μm , 2.1×100 mm reversed-phase column. The injection volume was 1 μL . The eluents consisted of Milli-Q water with 0.1% formic acid and 5 mM ammonia solution (A eluent) and methanol (B

eluent) and a flow rate of 0.4 ml/min was applied. The analytes were separated using a gradient elution programme. Before injection, the column was equilibrated with 2% of B eluent. At the time of injection, the proportion of B eluent was increased to 35% within 0.1 min and then increased further reaching 98% at a run time of 7 min. The 98% of B eluent is then maintained for 3 minutes before the proportion is lowered again to 2% within 0.1 min and maintained until a total run time of 13 min in order to prepare the column for the next injection. The mass spectrometer was operated in multiple reaction-monitoring mode and using both positive and negative electrospray ionisation.

The list of compounds analysed by GC-MS/MS and LC-MS/MS are shown in Supplementary Table 1, along with the transitions, retention times, and collision energies.

Calculation of processing factors

The processing factor (PF) is calculated from the residue level in the processed commodity, i.e. the biscuits, divided by the residue level in the RAC, i.e. the bran:

$$Pf = \frac{\text{Residue in biscuit} \left(\frac{\mu\text{g}}{\text{kg}} \right)}{\text{Residue in raw cereal bran} \left(\frac{\mu\text{g}}{\text{kg}} \right)}$$

In cases when the residue in the raw material was less than the LOQ, no PF was calculated. A PF value greater than 1 indicates an increase in the residue during processing, whereas a PF value less than 1 indicates a decrease in the residue (due to either dilution, evaporation, or thermal degradation) (Bempelou et al. 2018a). In this study, processing factors were determined for 41 compounds. Table 1 shows the compounds incurred or spiked in the raw material for the preparation of the mentioned EUPs and the current pesticide residue concentration in $\mu\text{g/g}$.

Method performance

The repeatability was calculated as the relative standard deviation for the replications of the bran and the dough, for all of the compounds within the same PT-material, demonstrating the precision of

the method, including the dough making (two replicates), baking, extraction (two replicates), and determination steps.

Some of the PT materials (wheat, oat, barley, and rye) were incurred with the same pesticides. To determine whether one PF value could be generalised and assigned for all of those compounds, the relative deviation among different matrices was calculated as follows:

$$\frac{Pf(\text{high value}) - Pf(\text{low value})}{Pf(\text{high value})} \times 100$$

Results and discussions

The effect of baking on the residue levels of 41 incurred pesticides in high bran content biscuits, representing cereals-based products in general, was studied in order to establish PFs necessary for refined dietary intake estimations and compliance check of pesticide residue found in baked cereals-based products. Overall the results show that PFs for the baking of cereals-based products are 1 or slightly below 1 and the median PFs were found to be acceptable.

Processing factors

The processing factors obtained for each PT sample and the average PF value are presented in Table 2. Figure 1 shows the percentage of compounds that showed a decrease, an increase, or no change in residue concentration after baking with each PT-test item. The baking process reduced the concentration of most of the pesticide residues in the biscuits prepared with PT-C1, C2, C3, C4, C5, C6, and C8 ($PF < 1$). Most of the compounds exhibited a $\pm 20\%$ variation, with most results decreasing, corresponding to an average PF range of 0.80–1.20. This indicates a higher persistence of these pesticides through the baking process than other pesticides and included azoxystrobin, bixafen, boscalid, carbendazim, chlorpyrifos, cypermethrin, cyproconazole, cyprodinil, deltamethrin, difenoconazole, epoxiconazole, fenpropidin, fenpropimorph, fludioxonil, fluopyram, fluquinconazole, flusilazole, fluxapyroxad, iprodione, kresoxym-methyl, metconazole, metrafenone, prochloraz, propiconazole, prothioconazole, pyraclostrobin, spiroxamine, tebuconazole,

Table 1. Concentration of incurred pesticides ($\mu\text{g/g}$) in the bran of test items of EUPT-C1, C2, C3, C4, C8, and C10.

	Compounds	C1	C2	C3	C4	C6	C8	C10
		Wheat	Wheat	Oat	Rye	Barley	Wheat	Rye
1	Azoxystrobin	0.616		0.244	0.427	0.416	0.412	0.223
2	Bifenthrin		0.148					
3	Bixafen						0.226	0.217
4	Boscalid					1.74	0.616	0.945
5	Carbendazim	0.0274	1.25	0.713	1.04			0.132
6	Chlorpyrifos			0.571				
7	Chlorpyrifos-methyl				0.105			
8	Cypermethrin		0.256				0.0455	0.0648
9	Cyproconazole			0.571				
10	Cyprodinil			0.102				
11	Deltamethrin	0.0306			0.072		0.0432	
12	Diazinon	0.172						
13	Difenoconazole		0.127					
14	Endosulfan	0.0859						
15	Epoxiconazole		0.485			1.08	0.492	0.450
16	Fenbuconazole			0.616				
17	Fenpropidin					0.676		0.464
18	Fenpropimorph			0.164	4.50			
19	Fenvalerate			0.120				
20	Fludioxinil			0.115				
21	Fluopyram							0.735
22	Fluquinconazole				0.803			
23	Flusilazole			0.958				
24	Flutriafol				2.32			
25	Fluxapyroxad						0.655	
26	Iprodione		0.593					
27	Kresoxim-methyl				0.533			
28	Lambda-cyhalothrin			0.145	0.0770			
29	Malathion		0.0188		0.0939			
30	Metconazole			0.673				
31	Metrafenone							0.0874
32	Pirimicarb		0.171					
33	Pirimiphos-methyl	0.0119			0.0853			
34	Prochloraz		0.0302					
35	Propiconazole					0.425		
36	Prothioconazole					0.137	0.599	0.320
37	Pyraclostrobin			1.04		1.10	0.220	0.294
38	Spiroxamine						0.0785	
39	Tebuconazole			1.65		0.752		0.205
40	Triadimenol				2.51			
41	Trifloxystrobin		1.15					

triadimenol, and trifloxystrobin. Some compounds (bifenthrin and pirimicarb) were unaltered by the baking process.

The repeatability, calculated as the relative standard deviation for the replications of the bran and the dough, was less than 20% for all of the compounds within the same PT-material. Within different PT-materials (wheat, rye, oat, and barley) the lowest, highest, median PF, and the standard deviation are shown in Table 3. The compounds showed a deviation of less than 50% (Table 3). These results allow calculation of the average processing factor by combining the results obtained with different cereal matrices. It also allows the extrapolation of results to other cereal matrices. Table 4 shows the concentrations of pesticide residues in wheat raw material and biscuit with different wheat PT materials (C1,

C2, and C8) the percentage of decrease or increase of pesticide residues and the relative standard deviation of common compounds studied. Cypermethrin (30%) and deltamethrin (36%) showed the highest relative deviations (Table 4). However, these deviations were less than 50%; therefore, the median PFs were found to be acceptable, according to the EU requirements (Bempelou et al. 2018b). Only two pesticides were included in both of the rye PT materials (C4 and C10), azoxystrobin and carbendazim. The calculated PFs showed standard deviations of 8% and 35% respectively; therefore, the derived median PF was acceptable. A divergence of results was observed with the two pyrethroid lipophilic compounds, cypermethrin studied in PT-C10 (bran) and lambda-cyhalothrin, studied in rye PT-C4 (a mix of bran

Table 2. Individual and median values of processing factors obtained for 41 compounds after baking.

	Compounds	C1	C2	C3	C4	C6	C8	C10	Average PF
		Wheat	Wheat	Oat	Rye	Barley	Wheat	Rye	
1	Azoxystrobin	1.0		1.0	1.1	0.89	0.94	1.0	0.99
2	Bifenthrin		1.0						1.0
3	Bixafen						0.97	0.84	0.90
4	Boscalid					0.84	0.85	0.95	0.88
5	Carbendazim	0.84	0.69	0.73	0.68			1.0	0.80
6	Chlorpyrifos			0.97					0.97
7	Chlorpyrifos-methyl		0.67		0.69				0.68
8	Cypermethrin		0.92				0.65	0.92	0.83
9	Cyproconazole			0.86					0.86
10	Cyprodinil			1.1					1.1
11	Deltamethrin	1.1			1.0		1.2		1.1
12	Diazinon	0.67							0.67
13	Difenoconazole	1.1							1.1
14	Endosulfan	0.76							0.76
15	Epoxiconazole		1.0			0.88	1.0	0.88	0.95
16	Fenbuconazole			0.99					0.99
17	Fenpropidin			0.94		0.69		0.92	0.85
18	Fenpropimorph				0.84				0.84
19	Fenvalerate			1.3					1.3
20	Fludioxonil			0.91					0.91
21	Fluopyram							0.86	0.86
22	Fluquinconazole				0.87				0.87
23	Flusilazole			0.90					0.90
24	Flutriafol				0.94				0.94
25	Fluxapyroxad						0.74		0.74
26	Iprodione		0.88						0.88
27	Kresoxim-methyl				0.92				0.92
28	Lambda-cyhalothrin			1.4	1.8				1.6
29	Malathion		0.67		0.73				0.70
30	Metconazole			0.93			0.73		0.83
31	Metrafenone						0.84	0.83	0.84
32	Pirimicarb		1.0						1.0
33	Pirimiphos-methyl	0.61			0.84				0.72
34	Prochloraz		0.86						0.86
35	Propiconazole					0.92			0.92
36	Prothioconazole					0.81	0.76	0.87	0.81
37	Pyraclostrobin			0.93		0.76	0.75	0.89	0.83
38	Spiroxamine						1.1		1.1
39	Tebuconazole			0.95		0.87		1.1	0.97
40	Triadimenol				0.98				0.98
41	Trifloxystrobin		0.96						0.96

and feed flour). In the former, a decrease was observed, and in the second, an increase (Table 2). This variation could be due to differences in the structures of these compounds, the physicochemical composition of each matrix, and/or the particle size distribution.

Correlation between the PFs and the physicochemical properties of the pesticides

PFs obtained were related to the pesticides polarity (octanol/water partitioning coefficient), vapour pressure, degradation temperature, acid dissociation constant (pKa), and to the possible role of ingredients. The aim is to study whether the PF values can be predicted with the demonstrated

correlation to the physicochemical properties of the pesticides.

For oat, a decrease in pesticide concentration, between 1% and 14%, was observed for medium polarity compounds (Log P between 2 and 4). In barley, pesticides with medium polarity also showed a decrease in concentration during baking, on the order of 7% to 19%, which is consistent with the results obtained for oat. In wheat and rye, a decrease of a maximum of 16% and 17% was observed, respectively, for medium polar compounds. Concentrations of pesticide residues in oat, barley, and rye raw material and biscuit, the percentage of decrease or increase of pesticide residues and the physicochemical properties of studied pesticides are shown in Supplementary Tables 2, 3, and 4, respectively.

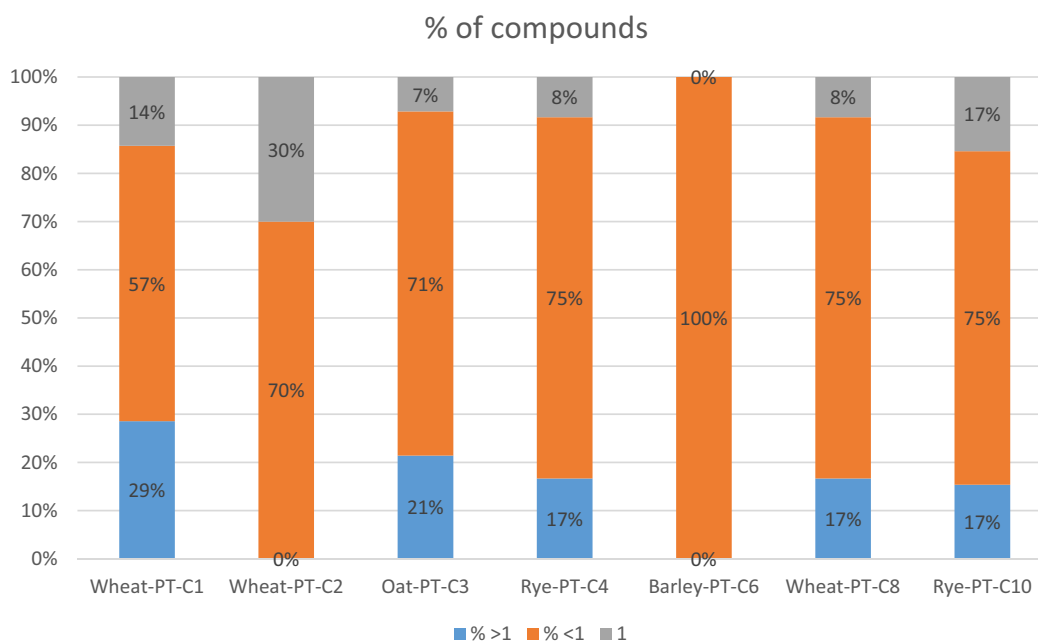


Figure 1. Percentage of compounds that showed an increase in the pesticide residues (PF>1), a decrease in the pesticide residues, (PF<1) or no change (PF = 1) with each PT-test item, for a total of 7, 10, 14, 12, 8, 12, and 12 compounds, respectively, in PT-C1, C2, C6, C8, and C10.

Table 3. Lowest, highest, and median PF, and the standard deviation obtained with the different cereal matrices.

Compounds	C1	C2	C3	C4	C6	C8	C10	PF			
	Wheat	Wheat	Oat	Rye	Barley	Wheat	Rye	Highest	Lowest	Median	RSD %
Azoxystrobin	1.0		1.0	1.1	0.89	0.94	1.0	1.1	0.89	0.99	18
Bixafen						0.97	0.84	0.97	0.84	0.90	13
Boscalid					0.84	0.85	0.95	0.95	0.84	0.88	12
Carbendazim	0.84	0.69	0.73	0.68			1.0	1.05	0.68	0.80	35
Chlorpyrifos-methyl		0.67		0.69				0.69	0.67	0.68	4
Cypermethrin		0.92				0.65	0.92	0.92	0.65	0.83	30
Deltamethrin	1.1			1.0			1.2	1.2	1.03	1.12	17
Epoxiconazole		1.0			0.88	1.0	0.88	1.02	0.88	0.95	14
Fenpropidin			0.94		0.69		0.92	0.94	0.69	0.85	26
Lambda-cyhalothrin			1.4	1.8				1.8	1.4	1.6	23
Malathion		0.67		0.73				0.73	0.67	0.70	8
Metconazole			0.93			0.73		0.93	0.73	0.83	22
Metrafenone						0.84	0.83	0.84	0.83	0.84	2
Pirimiphos-methyl	0.61			0.84				0.84	0.61	0.72	28
Prothioconazole					0.81	0.76	0.87	0.87	0.76	0.81	13
Pyraclostrobin			0.93		0.76	0.75	0.89	0.93	0.75	0.83	19
Tebuconazole			0.95		0.87		1.1	1.1	0.87	0.97	20

In barley, the highest percentage of reduction was observed for fenpropidin (31%). Fenpropidin has the highest vapour pressure (17 mPa) among the pesticides studied in barley, so this compound is volatilised more rapidly than the others, which is likely why fenpropidin decreased most significantly. Pyraclostrobin in barley also exhibited a relatively high percentage of decrease (24%), due to its low degradation temperature (200°C). Hence, thermal breakdown was more important for pyraclostrobin, because the temperature applied during the baking process was 200°C (Supplementary Table 2). In

wheat, the highest decrease (up to 39%) in pesticide concentration occurred for chlorpyrifos-methyl, diazinon, malathion, and pirimiphos-methyl. These compounds have comparatively low degradation temperatures of 175°C, 140°C, 174°C, and 162°C, respectively, which indicates that they were likely to decrease due to thermal degradation (Table 4). Moreover, the high vapour pressure of chlorpyrifos-methyl (3 mPa), malathion (3.1 mPa), and diazinon (11.97 mPa) may have contributed to these compounds' relatively high degradation rates due to volatilisation. Therefore the most-reduced residues after baking revealed average

Table 4. Concentrations of pesticide residues in wheat raw material and biscuit with different wheat PT materials, the percentage of decrease or increase of pesticide residues and the relative standard deviation of common compounds studied.

Compounds	C ($\mu\text{g}/\text{kg}$)						PF			% Decrease/Increase			Median PF	RSD %
	C1		C2		C8		C1	C2	C8	C1	C2	C8		
	Bran	Biscuit	Bran	Biscuit	Bran	Biscuit								
Azoxystrobin	0.616	0.628			0.412	0.389	1.0	0.94		2		-6	1.0	7
Bifenthrin			0.148	0.148				1.0			0		1.0	
Bixafen					0.226	0.218				0.97		-3	1.0	
Boscalid					0.616	0.525				0.85		-15	0.9	
Carbendazim	0.0274	0.0231	1.25	0.865			0.84	0.69			-16	-31	0.8	18
Chlorpyrifos methyl			0.0805	0.0537				0.67				-33	0.7	
Cypermethrin			0.256	0.235	0.0455	0.0295		0.92	0.65		-8	-35	0.8	30
Deltamethrin	0.0306	0.0336			0.0432	0.0304	1.1		0.70		10	-30	0.9	36
Diazinon	0.172	0.116					0.67				-33		0.7	
Difenconazole			0.127	0.136				1.1			7		1.1	
Endosulfan	0.0859	0.0654					0.76				-20		0.8	
Epoxiconazole			0.485	0.497	0.492	0.492		1.0	1.00		2	0	1.0	2
Fluxapyroxad					0.655	0.483			0.74			-26	0.7	
Iprodione			0.593	0.524					0.88			-12	0.9	
Malathion			0.0188	0.0125				0.67				-33	0.7	
Metconazole					0.348	0.253			0.73			-27	0.7	
Metrafenone					0.106	0.0889			0.84			-16	0.8	
Pirimicarb			0.171	0.173				1.0			1		1.0	
Pirimiphos-methyl	0.0119	0.00718					0.61				-39		0.6	
Prochloraz			0.0302	0.0261				0.86				-14	0.9	
Prothioconazole					0.599	0.454			0.76			-24	0.8	
Pyraclostrobin					0.220	0.166			0.75			-25	0.8	
Spiroxamine					0.079	0.087			1.1			10	1.1	
Trifloxystrobin			1.15	1.11				0.96				-4	1.0	

PF values between 0.67 and 0.76, which corresponded to a reduction in the pesticide content between 24% and 33%. This group of pesticides included chlorpyrifos-methyl, diazinon, endosulfan, flutriafol, malathion, and pirimiphos-methyl. A significant decrease in concentration for organophosphorus compounds during baking processes has also been reported by others (Uygun et al. 2009).

For carbendazim, which is a highly polar compound with $\text{Log } P \leq 2$, a more significant decrease was observed relative to the compounds with a moderate solubility in water ($2 < \text{Log } P < 5$). Pesticides that have lower $\text{Log } P$ values and that are more likely to dissolve in water may be lost and entrained during the evaporation of water contained in the tissue (Sharma et al. 2005). The high degradation rate of carbendazim in oat and wheat was therefore associated with the loss of water during evaporation.

In contrast, increases in concentration were observed for lambda-cyhalothrin and fenvalerate in oat (Supplementary Figure 2). These unexpected residue level increases of fenvalerate and lambda-cyhalothrin in oat during baking were 33% and 41%, respectively. The increase may be related to the compounds' lipophilic character. Both compounds are

lipophilic, with $\text{Log } P$ values of 5.01 and 5.5, respectively. Because there is no pesticide incorporation and the results were already corrected for water loss, the only explanation for the increased levels is with regard to the alteration or distortion of the fat associated with the bran. Lipid oxidation or fat conjugation to sugar during the dough-making process and fat melting during the baking process may result in the release of pesticides, that were primarily bound to the lipid in the bran, into the trapped air cells created when the fat is beaten. As a result, an increase of pesticide extractability would be observed during extraction, which explains the elevated concentrations obtained in this study. Therefore, the PF reported for fenvalerate and lambda-cyhalothrin in oat bran may not directly reflect the fate of pesticides during baking, because the concentration observed could be a matter of extractability. However, we decided to include these PF values, even though they are not completely acceptable results, because they are indicative of important qualitative effects. It was expected that the cypermethrin in rye, consistent with the results reported for wheat, would concentrate after baking due to its lipophilic character ($\text{Log } P: 5.6$), also similar to the results for lipophilic compounds in oat. However, this was not observed. Likely, because the fat contents of wheat bran (3.33%)

and rye bran (3%) are lower than the fat content of oat bran (7.03%) (USDA 2018), during baking, less fat is melted in the wheat and rye, resulting in lower amounts of liberated pesticides. This result would also be dependent on the lower intensity of the pesticides initially bonded in the matrix, which explains the resulting disparity observed regarding the decreases in wheat and rye and increase in oat.

Based on the results obtained, it was not possible to establish a link between the dissociation constant (pKa) and the fate of pesticides after baking. Theoretically, the addition of baking powder can increase the pH level of the dough, thereby increasing the rate of the associated Maillard reaction (Claire 2014). The addition of milk (lactic acid) may have neutralised the pH. In our case, the dough was baked directly after mixing, leaving no room for the fermentation process to occur and the baking time was short. Therefore, the dough may have preserved the protonated form of the acidic and alkaline pesticides. Although pH may affect the fate of pesticides during baking, nonetheless the results obtained in this study are, in general, better explained in terms of the polarity and volatility of the compounds studied.

Effect of baking parameters on processing factors

During baking, the water vapour generated inside the food continuously migrates to the surface. A prolonged baking time ensures that both the centre and surface of the processed food are heated, because a process of continuous water evaporation can be sustained. Only slight differences were observed

among the three different sets of processing parameters (temperature/time) for the moderate polarity compounds (azoxystrobin, deltamethrin, fenpropimorph, fluquinconazole, flutriafol, kresoxim-methyl, triadimenol) and for the lipophilic compound, lambda-cyhalothrin. Figure 2 shows the percentage of decrease or increase observed in pesticide residues when different baking parameters were applied. A significant decrease in pesticide residue levels was observed for chlorpyrifos-methyl (vapour pressure: 3 mPa), malathion (vapour pressure: 3.1 mPa), and pirimiphos-methyl when the biscuits were baked at either 200°C for 20 min or 180°C for 25 min. Of the pesticides included in this test item, these three have the highest vapour pressures, thus their residue levels decreased with prolonged baking time due to volatilisation. Moreover, these latter compounds have the lowest degradation points of the pesticides studied, at 175°C, 174°C, and 162°C, respectively (IUPAC 2007). A significant decrease was also observed for carbendazim residues (~60%) when baking at 200°C for 20 min and at 180°C for 25 min. Carbendazim is a highly polar compound (Log P: 1.48), such that its significant carryover and reduction percentages are due to carbendazim being highly associated with the evaporated water during the prolonged baking times.

Therefore, for compounds of moderate polarity and for lipophilic compounds, the temperature/time processing parameters are likely insignificant, unless the vapour pressure is high. In contrast, for highly polar compounds (carbendazim), the effect of time/temperature parameters is significant.

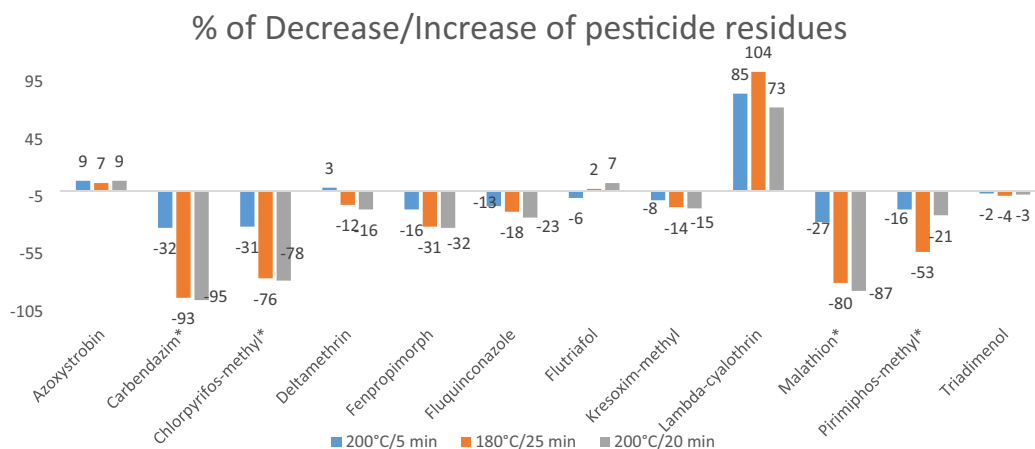


Figure 2. The decrease/increase in the pesticide residue levels in per cent following the defined baking conditions. (*) Compounds with the most significant variation.

Conclusions

It is clear that the extent of reduction or concentration of a particular active ingredient varies with the nature of pesticides, type of commodity and processing techniques. From this study, the results of the PF obtained (~1) indicate that processed baked cereals are not safer for consumption than their corresponding raw cereals. It also implies that the baking parameters might influence the pesticide residues level in processed food, depending on the pesticide properties. In addition, it was shown that the volatility, polarity, and thermal stability of a particular active ingredient could be used to understand the underlying basis of processing factors. This understanding will allow the extrapolation of processing factors to a large number of pesticides within different cereal-based processed food, thus minimising the work and time required to develop processing factors and to reach an international harmonised guidance of processing factors.

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Disclosure statement

The authors declare that they have no interests that could have appeared to influence the work reported in this paper.

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