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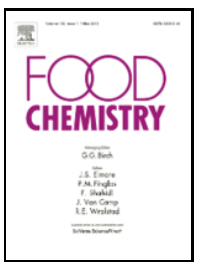

Removal residues of pesticides in apricot, peach and orange processed and dietary exposure assessment

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ABSTRACT

The effects of the industrial processing are evaluated of the removal of 16 pesticide residues in canned apricots and peaches and in orange juice. A method of multi-residual extraction that uses QuEChERS and liquid chromatography in tandem with triple quadrupole mass spectrometry was used. The method shows good linearity for the 16 pesticides studied (R2 > 0.999); it is accurate and precise (recoveries of 87–115%, relative standard deviation <8.0%). The processing factors are <0.6, indicating that all the processes significantly reduce the residue levels (spinosad, thiacloprid, pyridaben, bupirimate, flusilazole, triflumizole, flonicamid, imidacloprid, lambda-cyhalothrin, cyproconazole, fludioxinil and cyprodinil, abamectin, chlorpyrifos-methyl, hexythiazox and metalaxyl) initially present in the raw fruits and very significantly during washing/cutting, squeezing and hot pack canning (>55% loss). The risk quotient (EDI/ADI ratio) for canned foods is below 100, indicating that the potential consumer risk for the pesticides studied is practically negligent for human health.

1. Introduction

Orange, apricot and peach are popular and widely grown in Spain (>4500 t). Their nutritional properties also mean they are widely consumed (4 kg/person day) (*Mercasa, 2018*). Pesticides are necessary to ensure proper production and a wide range of insecticides and fungicides are employed so there is a need to ensure that the residues of these in the final products conform to the established Maximum Residue Levels (MRLs) in EU (EU Pesticide database, 2020).

In order to evaluate the risks of ingestion of pesticide residues in processed foods, we need to know the effects of each of the stages in the industrial process on the initial levels of residues in the raw product (*Regueiro, Lopez-Fernandez, Rial-Otero, Cancho-Grande, & Simal-Gandara, 2015; Jankowska, Kaczynski, Hrynko, & Lozowicka, 2016*). Various studies highlight the importance of the different stages in canned food production (especially, cutting, washing and heating) in the reduction of pesticide residues (*González-Rodríguez, Rial-Otero, Cancho-Grande, Gonzalez-Barreiro, & Simal-Gándara, 2011; Aguilera, Valverde, Camacho, Boulaid, & Gárcia-Fuentes, 2014*). These reductions are the result of hydrolysis, enzymatic and redox reactions or because of degradations associated with changes in temperature, the action of microorganisms, etc. (*Dordevic & Durovic-Pejcev, 2016*). Washing is the most effective procedure in residue elimination and it minimizes their ingestion by humans (*Lozowicka, Rutkowska, Jankowska, Hrynko, & Kaczynski, 2016*). It has also been shown that cutting, sealing and pasteurizing produce gradual decreases in the levels of residues (*Liu et al., 2016; Chung, 2018*). In the preparation of fruit juices, it has also been observed that the highest percentage of pesticides that are dissolvable in water is retained in the pulp (*Romeh, Mekky, Ramadan, & Hendawi, 2009*).

Processing factors (PF) represent the ratio between the levels of residues in the processed and unprocessed product. These allow us to determine if residues increase (>1) or decrease (<1) during the process. In general, these depend on the physical and chemical characteristics of the residues, especially on their solubility in water and their octanol-water partition coefficient (*Keikotlhaile, Spanoghe, & Steurbaut, 2010*).

The likelihood of toxic effects occurring from the consumption of pesticide residues depends on the concentration of residues and the amount ingested by the population. The risk is evaluated by calculating the estimated daily intake (EDI) according to the eating habits of each country and the population segment and is compared with the ADI established for each pesticide (*Pose, Fernandez-Cruz, & Simal-Gandara, 2016; Park et al., 2016; Oliva, Cermeño, Camara, Martinez, & Barba, 2017; Li et al., 2017*).

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The aims of the study are to establish the effects of each stage of the processing of the canned apricots and peaches and of the orange juice in the removal of pesticides; to evaluate the risks associated with the consumption of these foods in order to ensure greater safety for the consumers and validate the pesticide multi-residual analysis method using liquid chromatography in tandem with triple quadrupole mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Materials and reagents

The pesticides selected in this study are those commonly used in Spain to protect crops (*MAPA, 2019*): for apricot, spinosad, thiacloprid and pyridaben insecticides and the fungicides bupirimate, flusilazole and triflumizole; for peach, the insecticides flonicamid, imidacloprid, lambda-cyhalothrin and the fungicides bupirimate, cyproconazole, fludioxinil and cyprodinil; for oranges, the insecticides abamectin, chlorpyrifos-methyl, lambda-cyhalothrin, hexythiazox and the fungicide metalaxyl. The pesticides standard were provided by Dr Ehrenstorfer GmbH Trade Co. Ltd. (Ausgburg, Germany) degree of analytical standard purity >97%. To validate the analytical method 3 multi-pesticide solutions were prepared in acetonitrile for each pesticide at concentrations of 0.5, 1, 2.5, 5 and 10 mg/L: A (thiacloprid, pyridaben, spinosad, bupirimate, flusilazole and triflumizole) to study apricot; B (flonicamid, lambda-cyhalothrin, imidacloprid, cyproconazole, fludioxinil, cyprodinil and bupirimate) for peach and C (chlorpyrifos-methyl, abamectin, lambda-cyhalothrin, hexysthiazox and metalaxyl) for oranges.

Liquid chromatography quality acetonitrile was obtained from Scharlau (Barcelona. España); formic acid and ammonium formate of 95% purity; magnesium sulfate anhydrous, of 97% purity and sodium chloride, of 99.5% purity were purchased from Fluka (Buchs. Switzerland); disodium citrate sesquihydrate and dehydrate trisodium citrate of 99% purity was obtained from Sigma Aldrich (St. Louis, USA) and milli-Q water was produced by a Millipore de Purification Pak system (Billerica, USA).

An Oster Cyclotrol food crusher (Shelton, USA) and a Heraeus Cristo centrifugue (Osterode, Germany) were used for the industrial processing along with a temperature controlled refrigeration chamber and shaking bath with temperature control from Julabo (Seelbach, Germany; analytical balance (±0.1 mg) Sartorius AG (Goettingen, Germany); 2 mL amber vials for autoinjector, 32 × 11.6 mm. with capsule and septum from Ziemer GmbH (Mannheim, Germany) and single-use, screw top polypropylene centrifuge tubes of 114 × 28 mm and 50 mL from Sarstedt (Nümbrecht,Germany).

2.2. Field trials

The plots for trials are located in Murcia (SE Spain). The field containing Bulida apricot (Prunus armeniaca) was cultivated in six plots (7 × 6 m), one untreated and five treated, with four trees in each. The field containing Catherine peach (Prunus pérsica) occupied a surface of 500 m^2 in which six plots of 84 m^2 with 4 peach trees in each were marked off. For the naveline orange (Citrus sinensis) six 3.5 × 3.5 m plots, one untreated and five treated, were used with trees in each. A nearby control plot was used in all cases to guarantee identical crops and climatic conditions, although these plots were sufficiently distant to exclude any risk of cross contamination.

Phytosanitary treatments of the commercial products of each pesticide were applied (at the recommended doses for each crop) 7, 10, 14, 15 and 21 days prior to the expected harvest (recommended waiting period) (*MAPA, 2019*). Each plant-protection product was applied individually and on the appropriate date so that the day of collection they had met the PHI. The assays were performed according to Good Agricultural Practice (GAP) by applying the pesticides at the same time for each crop. Applications were performed at 75% humidity and at 26 °C. The applications of the commercial pesticide formulations were made using a Maruyama MS073D (Auburn, USA) backpack sprayer with a 2 mm nozzle. Phytosanitary treatments were carried out in the five different plots with a specific sample or analysis for each of them. The residual values shown are the average of the ones analyzed in each of the plots.

The samples of each treatment were collected at the pre harvest interval period (PHI) of each pesticide used. The main physical and chemical properties (*Turner, 2012*), formulations, dose rates and PHI for all pesticides are given in *Table 1*. The Samples were taken randomly form all the plots and 15 kg (approximately 90 units) of each fruit and for each pesticide were harvested for industrial processing. After harvesting, the fruits were packed and in opaque polyethylene bags and labeled. The bags were kept at ambient temperature and in a fixed position during their transport to the laboratory. They were protected from bumping and direct exposure to light. In the laboratory the harvested samples were crushed to obtain a small homogeneous analytical subsample and were stored at −20 °C until used. Furthermore, the samples were analyzed before completing 2 weeks in freezing and all the samples were extracted and quantified on the same day.

2.3. Processing studies

The preparation of fruits preserves simulated industrial practice at a laboratory scale (*Fig. 1*) following the same technological processes generally used in the food industry (*Paya et al., 2007a, 2007b*). The apricot canning consisted of washing with tap water at 22 °C for 2 min: splitting and stoning; canning the parts (240–250 g) with syrup at 95 °C. 14 °Brix and 0.01% citric acid; sterilization at 98 °C for 8 min and cooling at 35 °C for 10 min. For canned peaches the manufacturing process was: washing in water for 5 min. Splitting and stoning; canning the halves with syrup at 95 °C 16.5 °Brix and 0.2% citric acid; pasteurization at 100 °C for 10 min; cooling at 45 °C for 6 min. For orange juice, the procedure was washing with chlorinated water (10 mg/L of chlorine) for 3 min followed by washing with water for a further 3 min; squeezing and canning and pasteurizing at 95 °C for 5 min and cooling at 35 °C for 5 min.

2.4. Extraction and analysis of pesticide residues

Extraction was by the QuEChERS multi-residual method (*Camara, Barba, Cermeño, Martinez, & Oliva, 2017*). 10 g of the sample were put into a 50 mL polypropylene centrifuge tubes and 10 mL of acetonitrile were added (ACN). The tube was closed and manually shaken vigorously for one minute and in an ice bath. Buffer salts were added (4 g magnesium sulfate anhydrous, 0.5 g disodium citrate sesquihydrate and 1g of trisodium citrate dehydrate) and the tube was hand shaken vigorously for 1 min. This was followed by centrifugation at 3500 rpm for 5 min. The resulting extract was acidified with formic acid at 5% and was directly injected into the liquid chromatograph. In the analysis sequence, double samples were included at the beginning and at the end of the sequence as a quality control of the stability of the samples and they met the established acceptance and rejection criteria. We check the stability of stock solutions during storage regularly

Pesticide residues were determined in an Infinity Liquid Chromatograph, model 1260, coupled to an ion trap mass detector with a triple quadrupole analyzer, model Triple Quad LC/MS 6410B, with dynamic MRM scan (Agilent Technologies, Palo Alto. USA). AN Agilent Poroshell 120 EC-C18 3 mm x100 mm × 2.7 μm column was

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Table 1
Physicochemical properties. Commercial formulations. Dose rates of application, PHI, MRLs and ADIs of pesticides.

Commodity	Pesticide	Molecular weight	Water solubility (20 °C)	Log K_{0w}	Formulation	Application Dosage (g a.i./hL)	PHI (days)	MRL (mg/kg)	ADI (mg/kg bw)	Type
Apricot	Thiacloprid	252.72	1.19 g/cc	1.25	Calypso 48 SC	9.6	14	0.5	0.01	Contact insecticide
	Pyridaben	367.93	0.012 mg/L [b]	6.31	Podio WP 20	10	15	0.5	0.01	Contact insecticide-acaricide
	Spinosad	731.95	235 mg/L	2.8	Spintor 480 SC	9.6	7	0.6 [d]	0.024	Contact insecticide
	Bupirimate	361.42	22 mg/L [a]	3.8	Nimrod 25 EC	12.5	15	0.3	0.05	Systemic fungicide
	Flusilazole	315.392	45 mg/L	3.7	Olymp 10 EW	5	14	0.01	0.02	Systemic fungicide
	Triflumizole	345.75	12.5 g/L	5.10	Trifmine 30 WP EX	18	14	0.1	0.01	Systemic fungicide
Peach	Flonicamid	229.16	5.2 g/L	0.30	Teppeki 50 WG	6.5	14	0.4	0.025	Systemic insecticide
	Lambda-cyhalothrin	449.85	0.005 g/L	7.0	Karate Zeon 1.5 CS	1.5	7	0.2	0.0025	Contact insectide
	Imidacloprid	255.66	0.6 g/L	0.57	Confidor 20 LS	12.5	14	0.5	0.06	Systemic insecticide
	Cyproconazole	291.78	93 mg/L [c]	2.91	Atemi 10 WG	1.5	14	0.1	0.02	Systemic fungicide
	Fludioxinil	248.18	1.8 mg/L [a]	4.12	Switch WG	22.5	7	10.0	0.37	Systemic fungicide
	Cyprodinil	225.29	13 mg/L [a]	3.9		33.75	7	2.0	0.03	Systemic fungicide
	Bupirimate	361.42	22 mg/L [a]	3.8	Nimrod 25 EC	11.25	7	0.3	0.05	Systemic fungicide
Orange	Chlorpyrifos-methyl	322.53	1.4 g/L [a]	4.24	Dursban 48 EC	96	21	0.5	0.001	Contact insecticide
	Abamectin	1732.12	7–10 g/L	3.99	Marisol 1.8 EC	1.65	10	0.015	0.0025	Contact insecticide
	Lambda-cyhalothrin	449.85	0.005 g/L	7.0	Karate Zeon 1.5 CS	12.5	7	0.2	0.0025	Contact insecticide
	Hexythiazox	352.88	0.5 g/cc	2.53	Zeldox 10 WP	1.5	14	0.01	0.03	Contact acaricide
	Metalaxyl	279.33	8.4 g/L	1.75	Agrilaxil 25 WP	38.3	21	0.7	0.08	Systemic fungicide

[a] 25 °C.
[b] 24 °C.
[c] 22 °C.
[d] spinosad A + D; PHI = Post-Harvest Interval Period; MRL = Maximum Residue Level in UE; Log Kow = logarithm of octanol – water partition coefficient.

used thermostated at 40 °C and with a flow rate of 0.6 mL/min. The injection volume was 5 μL sample + 95 μL mobile phase. The mobile phase was ACN at 0.1% of formic acid (phase A) and H2O at 0.1% of formic acid and 2 mM of ammonium formate (phase B). The elution program was 20% A followed by a linear increase to 100% A in 10 min. This was maintained for 6 min. Before returning to the initial composition in 1 min. Analysis time was set at 14 min, with 5 min for stabilization (*Martínez et al., 2015*). Quantification and identification of the target compounds were carried out with multiple reaction monitoring (MRM) and the ESI (electrospray ionization) source was used in positive mode. The nebulizer gas was synthetic air at 40 psi and a flow rate of 9 L/min, ionization voltage was 5500 V and evaporation of solvents with synthetic air at 350 °C. *Table 1S* shows the analysis parameters for each pesticide studied.

2.5. Validation of the analytical method

The analytical method was validated following guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed (*EU, 2017*). For the validation of the analytical methodology used, the following steps were carried out: determination of possible interferences in the quantification of the compounds of interest, linearity of the detector response in matrix extract, calculating the RSD of the response factors, the back calculate concentration and R^2, recovery at two concentration levels (LOQ and 10LOQ) under repeatability and reproducibility conditions. In addition, a series of quality controls were carried out in each analysis sequence to ensure the robustness of the method. The following are entered in all the analysis sequences: calibration line, solvent, matrix extract, test sample as double sample, sample fortified at the lower level, samples, solvent, double sample and calibration line. The response linearity of the detector was determined in triplicate with spiked blank (raw, juice, canned fruits) samples of the pesticides selected at five concentrations (5, 10, 25, 50 and 100 μg/kg). To identify each pesticide (LOD), the qualifier ion must have a S/N ratio higher than 3. For the quantification (LOQ), the quantifier ion is much higher than the qualifier yielding a S/N higher than 10. The LOQ is the lowest concentration present in the extract of each matrix, whose response can be quantified accurately and precisely (signal/noise >10).

To calculate repeatability and reproducibility of the method, six fortified samples were analyzed consecutively at LOQ and 10 LOQ

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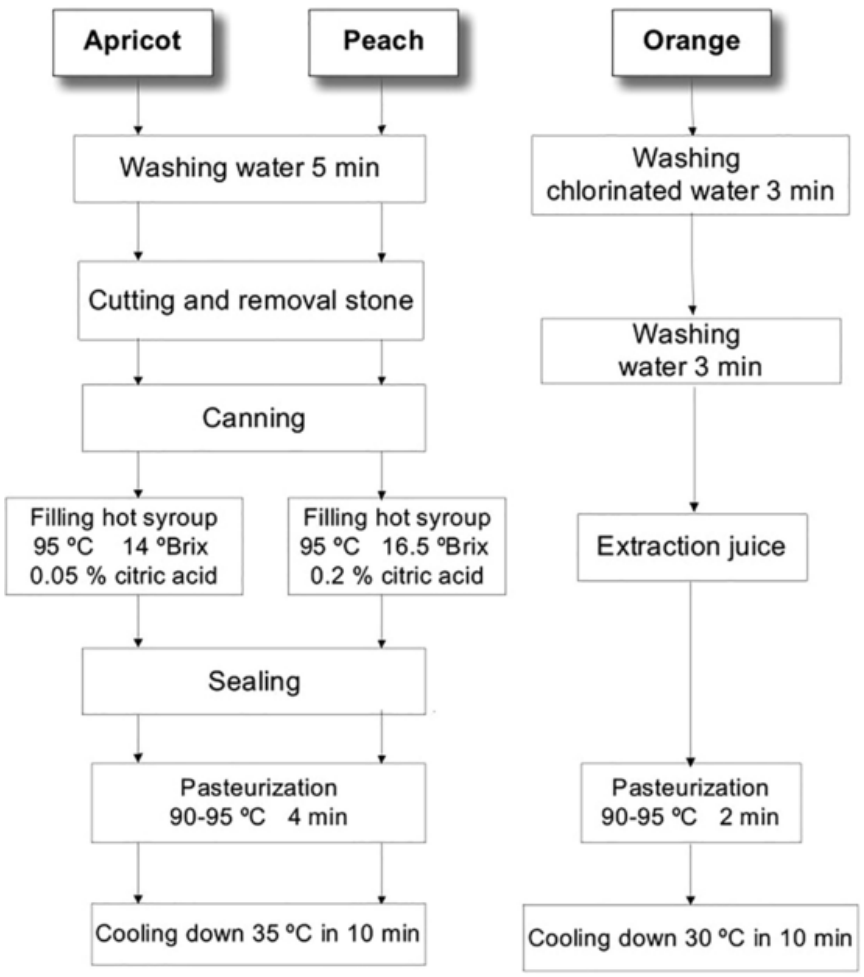

Fig. 1. Processing study of canned apricot and peach and orange juice.

levels for all the pesticides. The acceptance criterion was that the value of the variation coefficient (RSD) was ≤20%. To calculate reproducibility samples were processed on 6 different days. To evaluate the accuracy or recovery percentage 6 fortified samples were used at the two levels cited (LOQ and 10 LOQ) and the results were compared with the prepared standards, analyzing them all in the same sequence. The acceptance criterion was that the mean recovery for each set of replicates was 70–120% with an RSD ≤ 20%.

2.6. Dietary risk assessment

The mean dietary exposure values were used to predict intake of pesticides and long-term risk (*Caldas, 2017*). Risk of ingestion (RQ) was calculated as the quotient between estimated intake according to the pesticide residues present in specific food and the legally permitted daily intake (*Jardima, Britoa, van Donkersgoedb, Boonb, & Caldas, 2018*):

$$EDI(mg/kgbw) = C(kg)xFPxR(mg/kg)/kgbw$$

$$RQ = EDI/ADI \times 100$$

where C is the estimated national consumption of commodity; R is the food pesticide residues and FP is processing factors. EDI values were calculated according to the method proposed by *EFSA (2012)* considering national consumption and 60 kg as the adult body mean weight in Spain. ADI is the acceptable daily intake. This is a widely used guidance value for daily exposure to long-term intake. RQ ≤ 100 represents an acceptable risk to human health. While RQ > 100 indicates the risk of a pesticide to humans is unacceptable and higher RQ values indicate higher risks (*Zentai, Szabó, Kerekes, & Ambrus, 2016; Dong et al., 2018*).

2.7. Statistical study

In all cases, the calculation of the descriptive parameters (mean, standard deviation, variation coefficients, etc.) was carried out with IBM SPSS statistics 24.0.

3. Results and discussion

3.1. Validation

For all the pesticides used and in raw, juice and canned fruits, the LOD and the LOQ of the method took values 0.001 and 0.005 mg/kg respectively. *Table 2* shows the regression coefficients (R^2), recovery and relative standard deviation (RSD) in linearity, repeatability and reproducibility conditions for the pesticides studied in the different crops. No significant differences were found in linearity, recovery, LOD and LOQ when used raw fruits and processed commodity matrix. With the data of the calibration curve, the RSD of the response factors, the R^2 and the residuals in the relevant area (lower concentrations of the calibration curve) were calculated, obtaining for this last parameter values that ranged from 3.8% for Abamectin and 12.3% for Flonicamid, all of them less than 20%, acceptance and rejection criteria of the SANTE guidelines.

In conditions of repeatability, apricot is above 96.7% for the quantification limit and is 86.5% for the same limit times 10, and does not exceed 102.7% in the least favourable case. Again, in reproducibility, the mean values are over 94% for the level of LOQ and 86% for 10LOQ and do not exceed 102.8% for the least favourable case. All the mean values, as well as the maximum-minimum ones, are within the range accepted (70–120%). The values for peach never exceeded 20%, which is the limit established for acceptance and rejection. All mean values for repeatability and reproducibility were within the accepted range (70–120%). The values for orange were very low indeed and never above the 20% acceptance level which we established. All values were within the acceptable range of 70–120% of the validation assay.

3.2. Residues

In this supervised trials studies, the pesticide residues are quantified according to the residue definition for monitoring (EU Pesticide database, 2020): Abamectin (sum of avermectin B1a, avermectin B1b and delta-8,9 isomer of avermectin B1a, expressed as avermectin B1a); Flonicamid (sum of flonicamid, TFNA and TFNG expressed as flonicamid); Lambda-cyhalothrin ((includes gamma-cyhalothrin) (sum of R,S and S,R isomers)); Metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)); Spinosad (spinosad, sum of spinosyn A and spinosyn D). For the rest of the pesticides only monitoring parent compound.

The residual values of the pesticides found in raw foods in the main stages of the industrial processing (washing, cutting, sealing, squeezing and pasteurizing) are shown in *Tables 3 and 4* along with the corresponding processing factors. In none of the stages of commodity processing have concentrations of pesticide metabolites above LOQ been detected.

After phytosanitary treatment, thiacloprid, bupirimate and spinosad did not exceed the MRLs in raw apricots, while the rest of the pesticides all did, with flusilazole showing a value that was 50 times greater. In processed apricot, all pesticides were notably reduced with respect to the initial values during the canning process. There was a rapid decrease in the initial stages (washing, cutting and heat-sealing), followed by a slower removal during pasteurization. The transfer of residues during the various canning stages did not lead to

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Table 2
Regression coefficients (R^2), recovery and relative standard deviation (RSD) in linearity, repeatability and reproducibility conditions for the pesticides studied in apricot, peach and oranges raw (n = 5).

	Linearity		Recovery for repeatability				Recovery for reproducibility			
Spiked (mg/kg)	0.005–0.1		0.005		0.05		0.005		0.05	
	%RSD	R^2	%	%RSD	%	%RSD	%	%RSD	%	%RSD
	Apricot									
Thiacloprid	3.9	0.9999	96.7	7.0	90.3	1.1	99.0	2.3	90.7	2.4
Bupirimate	1.9	0.9999	102.7	7.2	96.2	1.0	102.8	7.6	96.8	3.3
Spinosyn A	4.8	0.9998	101.5	7.6	95.0	1.2	100.8	8.2	97.2	4.2
Flusilazole	1.9	0.9994	98.7	7.3	89.0	1.4	96.3	6.8	90.3	2.5
Spinosyn D	0.9	0.9995								
Tryflumizol	05	0.9995	101.5	7.2	94.5	1.4	98.8	9.7	92.3	3.2
Pyridaben	6.8	0.9997	97.7	6.2	86.5	3.2	94.0	5.5	86.0	4.0
	Peach									
Flonicamid	6.2	0.9998	97.3	6.4	92.7	4.6	88.7	11.3	93.8	6.1
Imidacloprid	3.2	0.9999	100.5	1.9	91.8	1.1	90.0	10.6	90.8	1.8
Cyproconazole	3.3	0.9998	107.0	2.1	95.8	1.1	97.5	6.6	96.3	4.1
Fludioxinil	2.0	0.9995	87.0	4.7	107.8	2.8	88.5	7.6	102.0	5.5
Cyprodinil	7.0	0.9998	99.0	7.8	94.2	4.1	90.5	5.6	97.2	4.6
Bupirimate	10.9	0.9997	104.5	1.5	97.2	0.7	94.3	7.3	96.7	5.2
Lambda-cyhalothrin	2.9	0.9995	99.7	6.4	88.7	6.0	99.5	8.9	92.2	6.5
	Orange									
Metalaxyl	11.1	0.9998	114.8	7.2	105.3	1.4	115.8	9.7	104.8	3.2
Chlorpyrifos-methyl	4.1	0.9999	115.2	7.0	111.2	1.1	109.8	6.3	112.3	2.4
Hexythiazox	0.8	0.9999	113.5	7.2	112.3	1.0	111.0	8.9	115.7	3.3
Lambda-cyhalothrin	14.1	0.9992	101.2	7.6	84.2	1.2	97.7	8.2	91.3	4.2
Abamectin	11.0	0.9999	111.0	7.3	100.7	1.4	105.7	6.8	101.3	2.5

Table 3
Pesticide residues (mg/kg) in the field harvested, in the processing stages and processing factors (PF) of apricot and peach canned (means ± SD, n = 5).

Pesticide	Unprocessed	Washing	%Loss	Sealing	%loss	Pasteurizated	%Loss	Mean PF
	Apricot canned							
Thiacloprid	0.223 ± 0.011	0.168 ± 0.008	24.7	0.088 ± 0.004	47.6	0.078 ± 0.004	11.4	0.35 ± 0.017
Bupirimate	0.281 ± 0.010	0.206 ± 0.007	26.7	0.087 ± 0.003	57.8	0.069 ± 0.002	20.7	0.25 ± 0.009
Spinosad A + D	0.236 ± 0.011	0.154 ± 0.005	34.7	0.072 ± 0.003	53.3	0.059 ± 0.003	18.1	0.25 ± 0.011
Flusilazole	0.509 ± 0.027	0.307 ± 0.017	39.7	0.132 ± 0.007	57.0	0.126 ± 0.007	4.5	0.25 ± 0.013
Tryflumizol	0.271 ± 0.013	0.190 ± 0.009	29.9	0.074 ± 0.003	61.1	0.034 ± 0.002	54.1	0.13 ± 0.006
Pyridaben	0.509 ± 0.029	0.410 ± 0.023	19.5	0.174 ± 0.10	57.6	0.155 ± 0.009	10.9	0.30 ± 0.017
	Peach canned							
Flonicamid	0.220 ± 0.011	0.154 ± 0.008	30.0	0.127 ± 0.006	17.5	0.122 ± 0.006	3.9	0.55 ± 0.027
Imidacloprid	0.149 ± 0.010	0.099 ± 0.006	33.6	0.065 ± 0.004	34.3	0.060 ± 0.003	7.7	0.40 ± 0.023
Cyproconazole	0.026 ± 0.002	0.016 ± 0.001	38.5	0.012 ± 0.001	25.0	0.011 ± 0.001	8.3	0.42 ± 0.025
Fludioxinil	1.159 ± 0.039	0.608 ± 0.021	47.5	0.248 ± 0.008	59.2	0.241 ± 0.008	2.8	0.21 ± 0.007
Cyprodinil	0.897 ± 0.047	0.609 ± 0.032	32.1	0.315 ± 0.16	48.3	0.284 ± 0.015	9.8	0.32 ± 0.016
Bupirimate	0.207 ± 0.010	0.102 ± 0.005	50.7	0.040 ± 0.002	60.8	< 0.005	100	< 0.01
Lambda-cyhalothrin	0.052 ± 0.002	0.042 ± 0.002	19.2	0.042 ± 0.002	0	< 0.005	100	< 0.01

Table 4
Pesticide residues (mg/kg) in the field harvested, in the processing stages and processing factors (PF) of orange juice (means ± SD. n = 5).

Pesticide	Unprocessed	Washing	%Loss	Squeezing	%Loss	Pasteurizated	%Loss	Mean PF
Metalaxyl	0.239 ± 0.014	0.206 ± 0.012	13.8	0.165 ± 0.009	19.9	<0.001	100	< 0.01
Chlorpyrifos-methyl	0.751 ± 0.044	0.571 ± 0.033	23.9	0.366 ± 0.021	35.9	0.013 ± 0.01	96.5	0.02 ± 0.001
Hexythiazox	0.020 ± 0.001	0.011 ± 0.001	45.0	< 0.005	100			< 0.01
Lambda-cyhalothrin	0.231 ± 0.014	0.177 ± 0.010	23.4	0.134 ± 0.008	24.3	0.032 ± 0.082	76.1	0.14 ± 0.008
Abamectin	0.025 ± 0.001	0.013 ± 0.001	48.0	< 0.005	100			< 0.01

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the complete removal of the pesticides, with the processing factors for all the pesticides being below 0.35, and ranging from 0.13 for triflumiziol to 0.35 for thiacloprid.

The pesticide residues in raw peaches show low concentrations and never exceed their MRLs. In canned peaches, the initial concentration decreases during the various stages of processing. Losses during washing and cutting range from 19.23% for lambda-cyhalothrin and 47.54% for fludioxinil. During sealing there is no appreciable effect for lambda-cyhalothrin and there is a slight effect ranging from 17.53% for flonicamid and 59,21% for fludioxinil. The processing factors of the pesticides in canned peach do not exceed 0.6, and range from 0.14 for bupirimate to 0.566 for flonicamid.

Following phytosanitary treatment in oranges and with a PHI of 21, 7 and 10 days, chlorpyrifos-methyl, lambda-cyhalothrin and abamectin exceeded the established MRL. In orange juices, the squeezing led to residue levels of all the pesticides decreasing by over 75% of the initial value in the whole oranges. The greater part of the residues are found in the peel and very few in the juice (<10%) (*Li et al., 2012*). In the pasteurized juice no residues of abamectin, hexysthiazox and metalaxyl were found, while the values for chlorpyrifos-methyl and lambda-cyhalothrin were 0.013 ± 0.01 and 0.032 ± 0.082 mg/kg, respectively. The processing factors of these last two pesticides were well below the unity.

The Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues are reported processing (transfer) factors for through commercial processes for metalaxil, lambda-cyhalothrin and thiacloprid in peaches and oranges. The mean PF < 0.08 for metalaxil and PF < 0.33 for lambda-cyhalothrin in orange juice and PF < 0.28 for lambda-cyhalothrin and PF < 0.66 and < 0.28 for thiacloprid in peach preserve and canned peach respectively (*JMPR, 2019*)

Washing, sealing or squeezing and pasteurization are found to produce a clear decrease in pesticide residues, coinciding with the exposed by *Lozowicka et al. (2016)*, who report that the effectiveness of washing with tap water led to a 20–68% reduction of bupirimate, lambda-cyhalothrin, fludioxonil, cyprodinil, and chlorpyrifos-methyl. On the other hand, it has been established that intensive apple washing, as the first step in apple processing during juice production, reduced the residues of chlorpyrifos-methyl and tebuconazole by 21.3 and 11.9%, respectively (*Li et al., 2016*). During boiling for 5 min, reductions of chlorpyrifos, lambda-cyhalothrin and pyraclostrobin in broccoli were 34, 43, and 34%, respectively, while the same procedure on tomato reduced, fludioxonil 69% while blanching significantly reduced pyridaben residues (*Kim et al., 2015*).

Evaluation of the linear trend between the processing factors and the octanol/water partition coefficient (log Kow) revealed moderate correlation coefficients (R^2) of 0.6846 and 0.8261, and equations y = −9.4099x + 6.1983 and y = 8.8804x + 5.6451 for canned peach and orange juice; while for apricot it was noticeably lower, 0.1647; equation y = 30.148x + 2.8964.

3.3. Risk assessment

Risk evaluation for human health seeks to estimate the nature and likelihood of adverse effects arising from exposure to pesticides both now and in the future. We evaluate the chronic risk of ingesting pesticides after the phytosanitary treatment GAP at PHI established. Through this, we aim to evaluate the potential risk of a significant presence of residues in raw commodities through comparison with the estimated daily intake (EDI) of each pesticide in each fruit and the corresponding ADI. EDI was calculated according to mean daily consumption per person of the commodities as established in the Spanish national dietary (*AECOSAN, 2016*) and risk quotient (RQ) values were calculated using the corresponding processing factors for each pesticide in canned foods (*Keikotlhaile, 2011*).

According to *OECD reports (2009)* the residue analysis for risk assessment includes the parent compound and any specified derivatives such as degradation products and metabolites considered to be of toxicological significance: abamectin (avermectin B1a and avermectin B1b); lambda-cyhalothrin (gamma-cyhalothrin and R, S and S, R isomers); flonicamid (TFNA and TFNG), spinosad (spinosyn A and spinosyn D); metalaxyl (metalaxyl-M); bupirimate (ethirimol and DE-ethyl ethirimol). Considering that ADI for bupirimate and ethirimiol are 0.05 and 0.035 mg/kg bw per day respectively and both compounds denote a number of joint toxicological actions, in our work the risk assessment was performed using the lowest ADI value derived for ethirimol. For the same reason, for dietary risk assessment of lambda-cyhalothrin, the lowest gamma-cyhalothrin has been used as the ADI value (0.0012 vs 0.0025 mg/kg bw per day). For the rest of the compounds, the ADI values are those corresponding to those established for the different pesticides in the *EU Pesticides database (2020)* following the criteria established by *EFSA (2012)*, and quantified as defined in *Section 3.2*.

Table 5 shows the mean RQa values for the general population and the specific RQb values for the population that consumes the fruits studied. The mean RQa values in raw commodities in the general population are lower than 5%, except for chlorpiryphos-methyl in orange, where it is 0.7% of the ADI, although in all cases below 100%, and hence a very low potential risk for human health in terms of residue ingestion. For the specific population that consumes these commodities, the RQb values are higher than the RQa in all cases, but never exceed the safety threshold. Above 50% ADI we find only flusilazole (87.21%) for the mean of consumers and pyridaben (55.08%) for the 95th percentile of consumers of apricot. Chlorpiryfos-methyl in orange (RQb and RQb 95 of the 209.842% and 383.636% of the ADI, respectively) and flusilazole in apricot (RQb95 of the 275.424% ADI) exceed 100%, so there is need to take precautions when using these pesticides and to control their PHI.

In the processed products, the corresponding RQPs (canned apricot and peach and orange juice) did not exceed 10% ADI, except for flusilazole in apricot, which in the mean consumer population at the 95th percentile presented RQbP and RQb95P values of 21.41 y 67.63% respectively, indicating a low risk for consumers.

4. Conclusion

The analytical method has been validated to determine residues of spinosad, thiacloprid, pyridaben, flonicamid, imidacloprid, lambda-cyhalothrin, abamectin, chlorpyrifos-methyl and hexythiazox, bupirimate, flusilazole, triflumizole, cyproconazole, fludioxinil, cyprodinil and metalaxyl in apricot, peach and oranges, by QuEChERS and LC-MS/MS extraction in tandem with triple quadrupole, with an LOQ of 0.005 mg/kg, which is well below the MRL's established in the EU and with suitable ranges of recovery and reproducibility. During the industrial processes of canned apricot and peach, washing/cutting and canning are the most influential in decreasing the levels of pesticide residues with processing factors below 0.6. In orange juice, the squeezing stage is that which most reduces residues, with >60%. In terms of risk assessment, the data indicated that the dietary intake of pesticides residues from canned fruits consumption for Spanish consumers is fairly low, with negligible health risk.

Uncited reference

Lozowicka et al., 2016.

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Table 5
ADI, EDI (mg/kg bw day) and Risk Quotients (EDI/ADI*100) of the pesticides for raw and processed foods.

	ADI	EDI_a	RQ_a	EDI_b	RQ_b	EDI_bb95	RQ_b 95	RQ_aP	RQ_bP	RQ_b 95P
	Apricot									
Thiacloprid	0.01	1.03E-05	0.103	0.000764	7.641	0.002413	24.133	0.0361	2.6728	8.4413
Bupirimate [a]	0.035	1.30E-05	0.026	0.000963	2.751	0.003041	8.689	0.0113	0.8378	2.6459
Spinosad	0.024	1.09E-05	0.046	0.000809	3.370	0.002554	10.642	0.0114	0.8424	2.6604
Flusilazole	0.002	2.36E-05	1.179	0.001744	87.209	0.005508	275.424	0.2896	21.4142	67.6308
Tryflumizol	0.05	1.26E-05	0.025	0.000929	1.857	0.002933	5.866	0.0062	0.4598	1.4520
Pyridaben	0.01	2.36E-05	0.236	0.001744	17.442	0.005508	55.085	0.0296	2.1883	6.9110
	Peach									
Flonicamid	0.025	3.86E-05	0.154	0.000692	2.770	0.001420	5.679	0.0856	1.5361	3.1492
Imidacloprid	0.06	2.61E-05	0.044	0.000469	0.782	0.000962	1.603	0.0000	0.0000	0.0000
Cyproconazole	0.02	4.56E-06	0.023	0.000082	0.409	0.000168	0.839	0.0092	0.1648	0.3378
Fludioxinil	0.37	2.03E-04	0.055	0.003648	0.986	0.007479	2.021	0.0232	0.4171	0.8552
Cyprodinil	0.03	1.57E-04	0.524	0.002823	9.412	0.005789	19.295	0.1090	1.9570	4.0123
Bupirimate [a]	0.035	3.63E-05	0.014	0.000652	1.862	0.001336	3.187	0.0328	0.5894	1.2084
Lambda-cyhalothrin [b]	0.0012	9.12E-06	0.760	0.000164	13.640	0.000336	27.964	0	0	0
	Orange									
Metalaxyl	0.08	1.30E-04	0.162	0.000668	0.835	0.001221	1.526	0	0	0
Chlorpyrifos-methyl	0.001	4.07E-04	40.742	0.002098	209.842	0.003836	383.636	0.7053	3.6324	6.6408
Hexythiazox	0.03	1.09E-05	0.036	0.000056	0.186	0.000102	0.341	0	0	0
Lambda-cyhalothrin [b]	0.0012	1.25E-04	10.443	0.000645	53.788	0.001180	98.335	1.4467	7.4511	13.6222
Abamectin	0.0025	1.36E-05	0.543	6.9854E-05	2.794	0.000128	5.108	0	0	0

EDI_a = estimated intake for all population (consumer or not); EDI_b = estimated average intake for consuming population; EDI_b 95 = estimated average intake for consumer population at the 95th percentile; RQa, RQb, RQb 95 = risk ratio raw commodities, and RQa P, RQbP and RQb 95P = risk ratio in processed foods.
[a] ADI bupimirate as ethirimol.
[b] ADI lambda-cyhalothrin as gamma-cyhalothrin.

CRediT authorship contribution statement

M.A. Cámara: Conceptualization, Methodology, Resources, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **S. Cermeño:** Methodology, Validation, Investigation. **G. Martínez:** Validation, Investigation, Resources. **J. Oliva:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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