Food Chemistry 212 (2016) 528–536

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

# Multiclass pesticide analysis in fruit-based baby food: A comparative study of sample preparation techniques previous to gas chromatography–mass spectrometry

Mateus H. Petrarca [a], José O. Fernandes [b], Helena T. Godoy [a,*], Sara C. Cunha [b,*]

[a] Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862 Campinas, SP, Brazil
[b] LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

ARTICLE INFO

*Article history:*
Received 21 January 2016
Received in revised form 17 May 2016
Accepted 5 June 2016
Available online 6 June 2016

*Keywords:*
Pesticides
Baby food
QuEChERS
d-SPE
DLLME
Contaminants

ABSTRACT

With the aim to develop a new gas chromatography–mass spectrometry method to analyze 24 pesticide residues in baby foods at the level imposed by established regulation two simple, rapid and environmental-friendly sample preparation techniques based on QuEChERS (quick, easy, cheap, effective, robust and safe) were compared – QuEChERS with dispersive liquid-liquid microextraction (DLLME) and QuEChERS with dispersive solid-phase extraction (d-SPE). Both sample preparation techniques achieved suitable performance criteria, including selectivity, linearity, acceptable recovery (70–120%) and precision (⩽20%). A higher enrichment factor was observed for DLLME and consequently better limits of detection and quantification were obtained. Nevertheless, d-SPE provided a more effective removal of matrix co-extractives from extracts than DLLME, which contributed to lower matrix effects. Twenty-two commercial fruit-based baby food samples were analyzed by the developed method, being procymidone detected in one sample at a level above the legal limit established by EU.



## 1. Introduction

Currently, a wide variety of ready-to-eat fruit and cereal-based foods is available for consumption by infants (aged less than 12 months) and young children (between one and three years old). It is well known that fruits and cereals provide important nutrients, including vitamins and minerals, to humans, particularly in the early stage of growth. However, the presence of pesticide residues in baby food ingredients implies a potential risk to this vulnerable consumer group, since their metabolic pathways are still immature and food consumption rates per body weight is higher when compared to adults. Evidence suggests that early exposures to pesticides and others environmental toxicants increases the risk of developing chronic diseases, including certain cancers and neurodegenerative diseases, as well as dysfunctions in the endocrine and reproductive systems (Hulin et al., 2014; Labord et al., 2015; Landrigan, Garg, & Droller, 2003; Landrigan, Kimmel, Correa, & Eskenazi, 2004).

Considering that fruit- and cereal-based baby foods are used as part of a diversified diet for infants and young children, the European Community (EC) has fixed a standard maximum residue limit (MRL) of 0.01 mg/kg for pesticides in these products, while more restrictive MRLs were established for certain pesticides of the greatest concern, such as fipronil (0.004 mg/kg), cadusafos (0.006 mg/kg) and ethoprophos (0.008 mg/kg). The Commission Directive 2006/125/EC states also the pesticides that must not be used in agricultural production intended for the production of baby foods, such as the organophosphate insecticide omethoate (EC, 2006).

Notwithstanding the effort to control and minimize risk of using pesticides in fruit and cereal cultures, their presence has been reported in foods intended for infants and young children throughout the world. For instances procymidone, chlorpyrifos and phosalone were detected in apple-based baby foods during a monitoring program between 2001 and 2003 in the Czech Republic (Stepán, Tichá, Hajslová, Kovalczuk, & Kocourek, 2005). The fungicides carbendazim, imazalil and thiabendazole were detected by Gilbert-López, García-Reyes, Ortega-Barrales, Molina-Díaz, & Fernandéz-Alba (2007) in more than 60% of the baby food samples analyzed, including juices, purée and follow-on formulae from Spain and the United Kingdom. In another study, the same compounds were detected in 18 of 25 fruit-based baby food samples collected in Spain (Gilbert-López, García-Reyes, & Molina-Díaz, 2012). Azoxystrobin (2.31 μg/kg) and thiabendazole (3.04 μg/kg) were also detected in cereal-based baby food and powdered

* Corresponding authors.
*E-mail addresses:* helena@fea.unicamp.br (H.T. Godoy), sara.cunha@ff.up.pt (S.C. Cunha).

http://dx.doi.org/10.1016/j.foodchem.2016.06.010
0308-8146/

milk-based infant formula samples, respectively, purchased from different markets in China (Jia, Chu, Ling, Huang, & Chang, 2014). In all these studies the pesticide levels were lower than the MRL established for baby foods.

To meet the restrictive European MRLs, several analytical methods have been developed for multiclass and multiresidue analysis of pesticides in foods intended for infants and young children. Selectivity and sensitivity are considered the two main analytical parameters for pesticide monitoring in baby foods (Hercegová, Dömötörová, & Matisová, 2007). Baby foods are complex matrices, composed of a mixture of fruit purées, milk, cereal flours and/or starch, and small amounts of fat (Sandra, Tienpont, & David, 2003). Therefore, sample preparation represents one of the most critical steps when dealing with pesticide analysis in these kinds of products. Most of the studies reported for pesticide monitoring in baby foods include sample preparation based on the QuEChERS (quick, easy, cheap, effective, robust and safe) method, which involves a liquid-liquid partitioning with acetonitrile and a subsequent dispersive solid-phase extraction (d-SPE) cleanup, using a mixture of $MgSO_4$ and selected sorbents usually primary and secondary amine (PSA) and C18 (Gilbert-López et al., 2007, 2012; González-Curbelo, Hernández-Borges, Borges-Miquel, & Rodríguez-Delgado, 2012; Leandro, Fussell, & Keely, 2005; Leandro, Hancock, Fussell, & Keely, 2006, 2007; Vukovic, Shtereva, Bursic, Mladenova, & Lazic, 2012; Wang & Leung, 2009). Although QuEChERS has been demonstrated to be very effective for the multi-residue analysis of pesticides, the procedure provides a low concentration factor which can result in higher limits of detection and quantification when compared to other sample preparation techniques. Other extractive techniques also based on low consumption of solvents, such as stir bar sorptive extraction (Sandra et al., 2003), direct immersion solid-phase microextraction (Viñas, Campillo, Martínez-Castillo, & Hernández-Córdoba, 2009), multi-walled carbon nanotubes dispersive solid-phase extraction (González-Curbelo, Asensio-Ramos, Herrera-Herrera, & Hernández-Borges, 2012), and ultrasound-assisted extraction and hollow-fiber liquid-phase microextraction (González-Curbelo, Hernández-Borges, Borges-Miquel, & Rodríguez-Delgado, 2013) have likewise been applied for baby food analysis. Dispersive liquid-liquid micro-extraction (DLLME) is another recently developed extraction technique presenting unique features concerning simplicity of operation, amount of organic solvent extractor (only a few microliters) and high enrichment factor (Cunha, Pena, & Fernandes, 2015). Although more suitable for extraction of analytes from aqueous samples DLLME can be also applied to solid samples after previous extraction with acetonitrile similar to first the step of QuEChERS procedure (Cunha & Fernandes, 2011).

Considering the advantages and limitations of d-SPE and DLLME, a comparative study between these two techniques was carried out, after previous QuEChERS extraction, for the simultaneous analysis of 24 pesticides belonging to 12 different chemical classes in baby foods. Once developed the entire GC–MS method was applied in the analysis of pesticide residues in baby foods commercially available in Brazil. To the best of our knowledge, this is the first pesticide monitoring in baby food from Brazil and pioneer in comparing the selectivity and sensibility of QuEChERS-DLLME and QuEChERS-d-SPE techniques for multiclass analysis of pesticide in baby foods.

## 2. Materials and methods

### 2.1. Chemicals and solutions

Pesticide standards were purchased from Fluka (Neu-Ulm, Germany), Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de Haën (Seelze, Germany). The purity of these analytical standards ranged from 95.1 to 99.9%. Individual stock standard solutions corrected by their purity (1200–5500 mg/L) were prepared, depending on the solubility, in acetonitrile, methanol or toluene, and were stored at −18 °C. A multi-compound working solution was prepared in toluene at 100 mg/L by combining appropriate aliquots of individual stock standard solutions, and it was stored at 4 °C. The internal standard (IS) triphenylphosphate (TPP; 99% purity) was obtained from Fluka, and an isotopically labeled solution of malathion-$d_6$ (98%) was obtained from Dr. Ehrenstorfer. Working standard solutions of IS and malathion-$d_6$ were prepared in acetonitrile (100 mg/L) and acetone (2 mg/L), respectively, and were stored at 4 °C. Acetonitrile, methanol and toluene, for pesticide residue analysis, and acetone and carbon tetrachloride ($CCl_4$), high purity solvents for GC analysis, were obtained from Fluka. Analytical grade glacial acetic acid was obtained from Panreac. Anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium acetate (NaOAc) were purchased from Sigma-Aldrich Chemie, and the sorbents primary secondary amine (PSA; particle size 50 μm) from Supelco (Bellefonte, PA, USA) and C18-bonded silica (particle size 55–105 μm) from Waters (Milford, MA, USA).

### 2.2. Sampling

A total of twenty-two read-to-eat baby food samples were randomly collected from five local markets in the city of Campinas, SP, located in the South-eastern region of Brazil, between August and September 2014. All the samples were packed in plastic bags (113 g each) or glass jars (120 g each), which included fruit juice or purée (apple, banana, grape, mango, orange, papaya, pear, pineapple, plum and/or strawberry), cereal flour (rice or oat), starch, milk and/or yogurt in their composition. All the samples were kept in their original packing at room temperature until analysis.

### 2.3. Determination of the pesticides

#### 2.3.1. Sample preparation techniques

The QuEChERS procedure, based on AOAC Official Method 2007.01 (Lehotay, 2007), was employed for extraction of the pesticide residues with few modifications. Fifteen grams of homogenized baby food samples and 150 μL of TPP solution (100 mg/L) were added to a 50 mL polypropylene centrifuge tube and mixed by hand for 10 s. Then 15 mL of acetonitrile acidified with 1% acetic acid (v/v) was added and the mixture was vortexed for 1 min. After vortexing, 1.5 g of NaOAc and 6.0 g of anhydrous $MgSO_4$ were added to the tube, and this was again vortexed for 1 min, and subsequently centrifuged at 5000 rpm for 3 min at room temperature. From buffered QuEChERS extract, two procedures, dispersive solid-phase extraction (d-SPE) and dispersive liquid-liquid microextraction (DLLME), were carried out and then compared for the determination of 24 pesticides pertaining to 12 different chemical classes (Table 1).

#### 2.3.2. Dispersive solid-phase extraction (d-SPE)

One milliliter of QuEChERS extract, 50 mg of $C_{18}$, 50 mg of PSA and 150 mg of $MgSO_4$ were added to a 15 mL polypropylene centrifuge tube, vortexed for 30 s, and then the mixture was centrifuged at 5000 rpm for 2 min at room temperature. The supernatant was collected and 1 μL was injected into a GC–MS system.

#### 2.3.3. Dispersive liquid-liquid microextraction (DLLME)

One milliliter of QuEChERS extract (acetonitrile – disperser solvent) was added to a 5 mL glass vial containing 75 μL of $CCl_4$ (extraction solvent) and this was manually shaken for 5 s. The