ABSTRACT – A mixture of pesticides, namely carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite, were spiked to different types of fruits (apple, orange, peach, pear and strawberry). Home-made jams were prepared with these samples by cooking the grinded spiked fruits with sugar and ultrapure (u.p.) water, in an open pan. The QuEChERS extraction method was applied to the blank fruits, to the spiked fruits and to the home-made jams. The extracts of home-made jams and fruits were analyzed by LC-QTOF-MS to ascertain the occurrence of the pesticides metabolites and to determine the number of co-extracted matrix components from the fruits and from the jams. All samples were also analyzed by µLC-QTRAP-MS/MS and the concentrations of the spiked pesticides in the fruits were compared to the concentrations in the home-made jams for the estimation of a processing factor for each analyte (pesticide concentration measured in the jams/pesticide concentration measured in the raw fruits).

KEYWORDS: Home-made jams; Pesticides; Stability study; Processing factors; LC-QTOF-MS.

1. INTRODUCTION

The concerns about pesticide residues in food have increased in recent years due the fact that the residues may have negative health effects. Every year many new multiresidue methods are developed for test different raw foods for pesticides. But, not so many efforts have been made to monitor pesticides and metabolites in processed foods. Cooking might alter and degrade the chemical structure of analytes. Therefore, in order to evaluate in detail the hazardous effects of pesticides on human health to analyze cooked foodstuffs appear to be a more reasonable choice of sample (Martin et al., 2013; Park et al., 2011).

Many studies have shown significant reductions in pesticide residues during household or industrial food processing (Bonnechère et al., 2012; Keikotlhaile et al., 2010; Kaushik et al., 2009). However, it should be noted that food thermal processing can concentrate residues or convert them to more toxic metabolites (Huan et al., 2015).

Primary processed foods result from the application of physical, chemical, biological processes, or a combination of them, to raw agricultural commodities (RAC). Up to now, there has
been no international agreement on setting maximum residue limits (MRL) for primary processed commodities, with the exception of some specific commodities such as spices; despite in some countries (e.g. European Union - EU member states) the common practice is to derive them from the MRL set for the RAC by applying the corresponding processing factors (Pazzirota et al., 2013).

Pesticide metabolites and breakdown products may occur in many environmental compartments, like animal feed or food for human consumption. In food analysis the most relevant metabolites and degradation products are specifically included in the definition of residue, on the basis of their toxicology and presence in significant amounts (Hernández et al., 2008). The detection and characterization of relevant pesticide metabolites in food is an important task in order to evaluate their formation, kinetics, stability and toxicity (Polgár et al., 2012).

Liquid chromatography tandem quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) can be efficient in target analysis of pesticide metabolites. In this application, the theoretical exact masses of the target analytes are extracted from the full spectrum acquisition data to reconstruct exact-mass chromatograms, which can depict the presence of the selected pesticides and metabolites in the samples. The accurate-mass capability of TOF analyzers allows reliable confirmation of the detected compounds identity, normally with mass errors below 5 mg kg\(^{-1}\) in routine analysis (Ferrer et al., 2006; Hernández et al., 2008).

The increased resolution and high mass accuracy (Gilbert-López et al., 2010; Lacina et al., 2010; Polgár et al., 2012) offer the ability to perform accurate mass measurements of the ions of interest, facilitating the elucidation of the elemental composition for confirmation/elucidation studies. In contrast, quantitation capabilities are yet a drawback of LC–high resolution (HR) MS instruments compared with triple quadrupole technology, at least in terms of linearity and linear dynamic range (Polgár et al., 2012).

Considering the information described above the goals of this study were, to investigate the effect of cooking on pesticides spiked to the fruits, to compare the pesticides concentrations found in the spiked home-made jams and in the spiked fruits and estimate processing factors.

2. MATERIALS AND METHODS

2.1 Reagents, Solvents, Standards and Instrumentation

HPLC grade acetonitrile and magnesium sulfate (98%) were supplied by Sigma Aldrich (Steinheim, Germany). Formic acid, analytical grade (≥ 96%), sodium citrate tribasic dehydrate (99%) and disodium hydrogenacetatesesquihydrate (99%) were purchased from Fluka (Steinheim, Germany). Sodium chloride (99%) was obtained from J.T. Baker (Deventer, Netherlands). C\(_{18}\) (40 µm) was from Varian (Middelburg, Netherlands) and PSA from Supelco (Bellefonte, USA).

The analytical-grade pesticide standards, purity > 98%, were acquired from Dr. Ehrenstorfer (Augsburg, Germany) and from Riedel-de Haën (Seelze, Germany). The isotopically–labeled internal standards, dichlorvos-d\(_6\), dimethoate-d\(_6\), linuron-d\(_6\) and malathion-d\(_{10}\), were purchased from CDN Isotopes (Quebec, Canada). The solid standards were used to prepare individual standard stock solutions in acetonitrile at the concentration of 1000 mg L\(^{-1}\).

The 5 pesticides individual solutions were further used to prepare a solution with a mixture of them (100 mg L\(^{-1}\)) in acetonitrile. The standard mixture solution was used to spike the fruits previously to the preparation of the home-made jams and to spike the fruits (500 µg kg\(^{-1}\)) to perform recovery studies.

The LC was equipped with an autosampler, a binary pump (Agilent 1290 Series Infinity LC, Agilent) and a Zorbax Extend-C\(_{18}\) column (Agilent, Santa Clara, USA). The injection volume was 10 µL and two mobile phases were used. The flow rate was kept at 0.5 mL min\(^{-1}\).
The QTOF-MS (Agilent 6550 Accurate Mass QTOF-MS; Santa Clara, USA) system was equipped with a Dual Spray Agilent Jet Stream ion source working in the positive electrospray (ESI) mode. The QTOF-MS/MS was used as a TOF-MS system working in the full-scan mode (Malato et al., 2011; Gómez-Ramos et al., 2011) and Agilent MassHunter Workstation Software (version B.06.00) was used for data processing.

The micro flow liquid chromatography-triple quadrupole-linear ion trap-mass spectrometry (µLC-QTRAP-MS/MS) was already described in a previous work (Reichert et al., 2015). Thereby the same methodology, equipment and conditions were used here.

2.2 Samples, Home-Made Jam Preparation and Extraction Procedure

Fruits samples from 5 different types were purchased in local markets of Almería (south of Spain). They consisted of 2 different samples (n=2) of apples, oranges, peaches, pears and strawberries. All the samples were individually ground, afterwards, a portion of each sample was used to prepare the home-made jams, a second portion was used to prepare the acetonitrile extract to ascertain if the samples were “blanks” or not, and the third portion was spiked to perform recovery studies. For the fruits spiking procedure, before the jams preparation, the grinded and homogenized samples were weighted into a container and spiked at 500 µg kg\(^{-1}\) with the standard solution of the 5 pesticides. The jam preparation and extractions procedure was done as illustrated in the Figure 1.

Figure 1 - Home-made jams preparation scheme (A) and QuEChERS extraction procedure (B).

2.3 The Pesticides Stability Study and Processing Factors

Carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite were selected for this work because they have known metabolites and were detected in the many jam samples analyzed in a previous study (Reichert et al., 2015). The standard solution with the pesticides mixture was spiked to the grinded fruits (500 µg kg\(^{-1}\)) before the cooking to prepare the home-made jams. As described in the literature, carbendazim has as metabolite benzimidazole, chlorpyrifos can be metabolized into 3,5,6-
thrichloro-2-pyridinil; imidacloprid has 2 known metabolites, imidacloprid olefin and imidacloprid-5-hidroxi, iprodione has dichloroaniline, 3,5- as metabolite and propargite can generate cyclohexanol-2-(4-tert butyl-phenoxy) (EURL, 2013). Thus, the acetonitrile extracts of the home-made jams were analyzed via LC-QTOF-MS to ascertain the presence or absence of the metabolites.

To estimate a processing factor for each pesticide, the samples were analyzed via µLC-QTRAP-MS/MS. So, the pesticide concentrations (calculated from the peak areas) measured in the home-made jams (prepared from the fruits spiked at 500 µg kg\(^{-1}\)) were compared with the pesticide concentrations in the fruits also spiked at 500 µg kg\(^{-1}\) (pesticide concentration in the jams/pesticide concentration in the raw fruits).

3. RESULTS AND DISCUSSION

By the HR LC-QTOF-MS analysis of the extracts of the home-made jams, none of the 6 known pesticides metabolites was detected. In the Figure 2 are displayed the total ion chromatograms of spiked fruits and home-made jams, where can be seen the peaks of the pesticides. The absence of the metabolites indicated that the pesticides remained stable across the conditions used for the jams preparation, e.g. cooking, presence of water and sugar, pH variations (according to the different fruit types), etc. The other small peaks that can be eventually noticed in the chromatograms correspond to co-extracted matrix components and/or to compounds that were not the focus in this study.

Figure 2 - Total ion chromatograms of apple, pear (spiked at 500 µg kg\(^{-1}\)), apple jam and pear jam (prepared from spiked fruits at 500 µg kg\(^{-1}\)), obtained by LC-QTOF-MS analysis.

Comparing the concentrations of the pesticides in the home-made jams and in the spiked fruits, variations were observed, depending on the analyte and the fruit type used to prepare the jam. But, the average percentages of carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite that were measured in the jams corresponded to, respectively, 50%, 30%, 60%, 50% and 50% of the concentration found in the spiked fruits. So, the factors of 0.5, 0.3, 0.6, 0.5, 0.5, respectively, could be estimated for the pesticides.

Two logical explanations have been found for lower concentration of the pesticides in the jams in comparison to the fruits. The first one is the dilution of the compounds, due the fact that to prepare the home-made jams the fruits (spiked at 500 µg kg\(^{-1}\)) were mixed with sugar and u.p. water in the proportion of 5:5:2 (m/m/m). So, the pesticides concentration in the mixture (fruit, sugar and water), was divided by a factor of 2.4, resulting in the concentration of 208 µg kg\(^{-1}\) (before the
cooking). But, as most of the water evaporates during the cooking process, the pesticide concentration changed again when the cooking finished.

Dilution is compatible with factors estimated for carbendazim, iprodione, propargite (0.5) and imidacloprid (0.6). As the water evaporates during cooking, the final jam products had basically the components from the fruits and sugar, which were in equal proportions, resulting in 2-fold dilution of the pesticides. Contrariwise, the dilution did not explain completely the processing factor of chlorpyrifos (0.3). In consequence, an additional possibility, that pesticide may be in part volatilized was raised. According to Bonnechère et al. (2012) and Keikothlaile et al. (2010) during cooking the pesticides can volatilize, hydrolyze and suffer thermal breakdown. It has been already demonstrated in other publications (Bonnechère et al., 2012; Keikothlaile et al., 2010; Kumar et al., 2005) that high temperatures increase the volatilization of the pesticides which were investigated here. Chlorpyrifos has the highest vapor pressure (1.34 mPa) at 20 °C among all the pesticides, that probably contributed to its volatilization besides the dilution and resulted in the processed factor of 0.3.

4. CONCLUSIONS

The effect of cooking on five pesticides which have known metabolites was investigated in this study. By the LC-QTOF-MS analysis of the home-made jams none of the pesticides metabolites was detected. Due this fact, it was plausible to conclude that the pesticides remained stable over the jam preparation process. Further, the analysis of the home-made jams and spiked fruits showed that the pesticides concentration was 40 to 70% (depending on the compound) lower in the jams than in the fruits. This was mainly attributed to the dilution caused by the addition of sugar and u.p. water to the spiked fruits to prepare the jams in the cases of carbendazim, imidacloprid, iprodione and propargite and to the dilution followed by a volatilization during cooking process, as observed for chlorpyrifos. Therefore, the processing factors of 0.5, 0.3, 0.6, 0.5 and 0.5 have been estimated for carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite, respectively, in the home-made jam making process.

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6. REFERENCES


