Modelling the isothermal degradation kinetics of metrafenone and mepanipyrim in a grape juice analog

Olalla López-Fernández[a], Eva Pose-Juan[a], Remedios Yáñez[b], Raquel Rial-Otero[a,*], Jesús Simal-Gándara[a,*]

[a] Nutrition and Bromatology Group, Analytical and Food Chemistry Department, University of Vigo, Ourense Campus, E-32004 Ourense, SPAIN
[b] Department of Chemical Engineering, University of Vigo, Ourense Campus, E-32004 Ourense, SPAIN

ABSTRACT

Five photodegradation products of metrafenone (MTF) and six of mepanipyrim (MEP) were identified in synthetic grape juice at 25 °C and the structures of the main reaction products established. The degradation of MTF and MEP was modelled by using three different strategies involving monitoring (a) the disappearance of the parent compound, (b) the conversion of the parent compound into its main structurally related reaction products and (c) the degradation of the parent compound to all intermediates and degradation end-products. The kinetic coefficients of degradation for these fungicides were determined and the corresponding half-lives found to be 20.8 h for MFT and 10.1 h for MEP. The proposed models afford reasonably accurate interpretation of the experimental data. Based on the results, modelling the kinetics of disappearance of the parent compound by itself does not ensure the best fit of the degradation behaviour of the fungicides.

1. Introduction

The benzophenone fungicide metrafenone, (3-bromo-6-methoxy-2-methylphenyl) (2,3,4-trimethoxy-6-methylphenyl)methanone, and the anilinopyrimidine mepanipyrim, N-(4-methyl-6-prop-1-ynylpyrimidin-2-yl)aniline, are two new generation systemic fungicides widely used to control fungal diseases of vine. Their most salient physico–chemical properties are shown in Table 1. Fungicides applied to vine have been shown to persist in grapes, and to be transferred to grape juice and wine as a result (Barba, Oliva, & Payá, 2010; Briz-Cid, Figueiredo-González, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2014; Cabras et al., 1999; Cabras & Angioni, 2000; González-Rodríguez, Cancho-Grande, & Simal-Gándara, 2009; Noguerol-Pato, González-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011). When their levels in juice or wine exceed the maximum residue limits (MRLs) established by European legislation, the quality of these products cannot be guaranteed. The European Regulation 396/2005 and its later modifications, including the Commission Regulations 2015/1040 and 2016/486, regulated MRLs for metrafenone and mepanipyrim, respectively.

Fungicides may accumulate and be metabolized through interaction with various environmental and food matrices. For example, photochemical processes play a major role in their dissipation (Anfossi, Sales, & Vanni, 2006). A sound knowledge of the mechanisms behind the degradation of fungicides 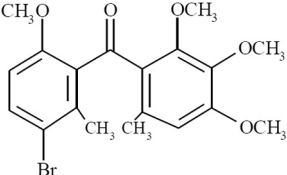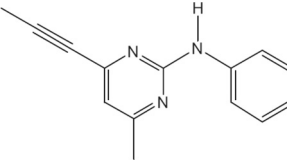is therefore essential to understand their persistence and assess the associated risks (Abbate, Borzì, Baglieri, & Gennari, 2009), and such knowledge can only be acquired with further research. The degradation of specific fungicides such as MTF and MEP has been scarcely studied. Calza, Medana, Baiocchi, Branca, and Pelizzetti (2004) examined the photocatalytic degradation of MEP in aqueous solutions containing TiO₂. More recently, Anfossi et al. (2006) studied the aqueous degradation of three anilinopyrimidine fungicides (cyprodinil, pyrimethanil and mepanipyrim) photoinduced by iron (III)–polycarboxylate complexes and identified some of the resulting photoproducts. Several studies have focused on the degradation of other anilinopyrimidine fungicides in water (Agüera, Almansa, Tejedor, & Fernández-Alba, 2000; Araña, Rodríguez, Melián, Díaz, & Peña, 2008; Vanni & Fontana, 2003). Regarding MTF, Cilla García (2013) examined its hydrolytic and photolytic degradation in different aqueous solutions and proposed various degradation pathways.

Most of the kinetic studies on pesticide degradation have modelled the disappearance of the parent compound and the formation of reaction products of toxicological concern separately (Abbate et al., 2009; Agüera et al., 2000; Anfossi et al., 2006; Araña et al., 2008; Avetta et al., 2014; Calza, Massolino, & Pelizzetti, 2008; Ruiz Suárez, Geissen, Jarquín Sánchez, Castro Chan, & Bello-Mendoza, 2013). However, other compounds formed during degradation may remain in the matrix and interact with its components. Therefore, establishing plausible degradation pathways for pesticides entails identifying all reaction

Table 1
Physico-chemical properties of the studied compounds.
Pesticide Properties DataBase, 2016.

Compound	Metrafenone	Mepanipyrim
Chemical structure		
N° CAS	220,899–03-6	110,235–47-7
Molecular weight	409.3	223.27
Solubility in water (20 °C, pH 7) mg L^{-1}	0.49	2.08
Log P (20 °C, pH = 7)	4.3	3.28
pKa (25 °C)	No dissociation	2.7
Henry's law constant (20 °C)	5.23×10^{-5}	1.02×10^{-6}

products involved. While this issue has been briefly explored (Calza et al., 2004; Calza et al., 2008; Vanni & Fontana, 2003), no kinetic coefficients for the transformations have to best of our knowledge been reported.

The main aims of this work were to examine the degradation of MTF and MEP in synthetic grape juice by using three different modelling strategies and to predict the associated kinetic coefficients of degradation. Simulating the process under controlled conditions as regards solar excitation wavelength, pH and temperature allowed the half-lives of the fungicides to be estimated and the kinetic model best fitting the degradation behaviour of each fungicide to be identified.

2. Materials and methods

2.1. Chemicals and solvents

Metrafenone (purity 99.9%) and mepanipyrim (purity 99.4%) were purchased from Fluka (Steinheim, Germany), and water and acetonitrile for liquid chromatography from Sigma–Aldrich (Steinheim, Germany).

2.2. Kinetic experiments

Kinetic experiments were performed in triplicate in Pyrex bottles containing 250 mL of synthetic grape juice. Solutions were prepared by dissolving glucose (100 g L^{-1}) and fructose (100 g L^{-1}) in water and adjusting the pH to 3.5 with tartaric acid. One bottle was spiked with MEP (4 µg mL^{-1}) and the other one with MTF (10 µg mL^{-1}). These concentrations corresponding to twice the MRLs established for those pesticides in grapes (2 and 5 µg g^{-1}, respectively) at the date of the study (May 2015). High concentrations of these two pesticides was chosen to be able to detect 100 times lower concentrations along the degradation process and to obtain high concentrations of the reaction products that can be analysed by direct injection into the HPLC system. Experiments were performed at 25 °C in presence of light from a SUNSET CPS+ machine (Atlas Material Testing Technology, Illinois, USA) equipped with a xenon arc lamp (power 1500 W, emission wavelength range 300–800 nm). An irradiance level of 250 W/m^2 was selected based on the results of previous studies (Regulska & Karpinska, 2012; Run & Schwack, 2005). Irradiation was applied over a solution surface of 362 cm^2 keeping constant the distance between the solution and the xenon lamp at 18 cm. The SUNSET CPS+ was also equipped with a system for measurement and control of Black Standard Temperature (BST) that allowed the temperature in the reaction chamber to be set and maintained. A magnetic stirrer was used to ensure good mixing of the sample in the reactor. At regular intervals, an aliquot (0.5 mL) of each solution was withdrawn to determine the pesticide

concentration at different times. Prior to chromatographic analysis, aliquots of the solution were passed through Chromafil Xtra PET-20/25 (0.20 µm) filters from Macherey–Nagel (Düren, Germany) and placed in 2 mL amber vials from Supelco (Bellefonte, PA, USA).

2.3. Chromatographic determination

Metrafenone and mepanipyrim concentrations were determined by high performance liquid chromatography with diode-array detection (HPLC-DAD). Analyses were performed on a Thermo Surveyor HPLC system equipped with an LC Plus pump, a Plus Lite autosampler, a Gecko 2000 column heater from Cil Cluzeau Info Labo (France) and a PDA Plus detector interfaced to a PC running Xcalibur v. 5.0 (TermoQuest, Madrid, Spain). A Thermo Hypersil Gold analytical column (100 × 4.6 mm, 5 µm i.d.) and a Hypersil Gold Drop guard column (10 mm × 4.6 mm, 5 µm i.d.) from Thermo Scientific (Waltham, USA) were also used. For HPLC analysis, an aliquot (25 µL) of the solution was directly injected into the column, thermostated at 35 °C, at a constant flow-rate of 1 mL min^{-1}, using the following gradient: water/acetonitrile (80:20 v/v) for 2 min; ramp to 20:80 (v/v) over 15 min and hold for 2 min. Then, the initial conditions were restored in 2 min and held for 9 min to allow the column to stabilize. The wavelengths used (and absorption coefficients) for pesticide detection and quantitation were 272 nm ($\varepsilon = 3220$ l mol^{-1} cm^{-1}) for mepanipyrim and 202 nm ($\varepsilon = 42,443$ l mol^{-1} cm^{-1}) for metrafenone (see Fig. I of the Supplementary material).

Reaction products were identified by liquid chromatography/tandem mass spectrometry (LC–MS/MS), using a TSQ Quantum Discovery triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, USA) equipped with a HESI probe operating in the positive ion mode under the following conditions: spray voltage 3500 V; capillary temperature 270 °C; vaporizer temperature 250 °C; sheath gas and auxiliary gas pressure 40 and 10 units, respectively; collision energy 15 V; and tube lens 80 V. Detection was done in the product scan and selected reaction monitoring (SRM) modes, using the precursor ions and the specific transitions (m/z) shown in Table 2. In addition, the UV spectra of the reaction products are shown in Fig. I of the Supplementary material.

2.4. Data analysis

The experimental data were fitted to the proposed kinetic model using a commercial software with a built-in optimization routine based on Newton's method (Solver, Microsoft Excel, Microsoft, USA). For this, from the integration of the corresponding differential equations that represent the variation of compounds concentration as a function of the reaction time, the corresponding integrated equations were obtained.