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Factors governing the removal of mancozeb residues from lettuces with washing solutions

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ABSTRACT

Mancozeb was spiked in smooth and curly lettuces at two different concentrations (low and high), and lettuces were subjected to different washing treatments (with tap water, Amukine, hydrogen peroxide, acetic acid, and ammonium hydroxide) at varying time and temperature. The determination of residual levels was then carried out by using acetonitrile extraction and high performance liquid chromatography with diode array detection (HPLC-DAD). The study of analysis of variance among these experiments allowed identifying the main factors governing the removal of mancozeb residues from lettuces. In general, the oxidant character of the washing agent is the most important condition that affects removal of mancozeb from lettuces, being hydrogen peroxide more efficient than sodium hypochlorite. Moreover, other factors controlling mancozeb removal from lettuces are surface wax, concentration gradient, and also washing pH. The washing processing factor resulted to be a rest of 4% under the optima conditions (either Amukine solution for 10 min at 25 °C, or hydrogen peroxide solution for 5 min at water temperature of 15 °C).

1. Introduction

Micene WP (mancozeb 80%) from Sipcam is a broad spectrum contact fungicide used to control a wide variety of important fungal diseases on vegetable, fruit and ornamental crops. Mancozeb [manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salt] is the active ingredient in Micene WP. Although mancozeb has low acute toxicity to humans (Knio, Saad & Dagher, 2000), it is unstable in the presence of moisture or oxygen and in biological systems (Hwang, Cash & Zabik, 2003). Ethylenethiourea (ETU, imidazolidine-2-thione) is the main degradation product by hydrolysis and photolysis of mancozeb (Garcinuño, Fernández-Hernando & Cámara, 2004). ETU has been found to produce thyroid disorders and tumours in laboratory animals (WHO, 1988), and also genotoxic effects (Dearfield, 1994). However, ETU was downgraded to Group 3 ('not classifiable as to its carcinogenicity to humans') by the International Agency for Research on Cancer (IARC, 2001).

Nowadays, consumers demand food products without pesticides but with high sensorial and nutritional qualities. Although the correct use of fungicides does not cause problems of public concern in health and environmental areas, if inappropriate abusive treatments are applied without respecting safety recommendations indicated by the manufactured, undesirable residues can remain on vegetable food products after harvest. In fact, the main exposure to pesticides for humans is via food, especially by fruits and vegetables (Claeys et al., 2011). In a previous study, we observed the presence of mancozeb and other dithiocarbamates residues in fresh fruits and vegetables collected at small shops and large food chains from Galicia (North-Western Spain) (López-Fernández, Rial-Otero, González-Barreiro & Simal-Gándara, 2012). DTC residue concentrations exceeding the Maximum Residue Limit (MRL) were observed in 6% of the fruit and vegetable samples analysed (total of 150 samples), especially on lettuce for which some samples contained residues of DTCs three times higher than the MRL (5 mg kg^{-1}).

After harvest, food products are subjected to different house or industrial handling process (washing, peeling, heating, transformation processes, etc.) that usually reduce pesticide residue levels (Bajwa & Sandhu, 2011; González-Rodríguez, Rial-Otero, Cancho-Grande, Gonzalez-Barreiro & Simal-Gándara, 2011; Kaushik, Satya & Naik, 2009). This effect is also higher for contact pesticides such as mancozeb than for the systemic ones. The study of the effect of different processes for pesticides removal from vegetables is mandatory, being washing one of the main removal processes. Generally, for washing, the chlorine-releasing compounds are considered as first election by those searching for antimicrobial activity, for being inexpensive and easy to use, and for have a broad

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spectrum of activity (Pérez-Gregorio, González-Barreiro, Rial-Otero & Simal-Gándara, 2011). Hypochlorites are the most frequently used forms of chlorine, with sodium hypochlorite receiving broadest application. Chlorine can oxidize organic matter in foods or in water, and in the latter case by-products such haloforms and haloacetic acids, which are potentially carcinogenic and mutagenic, can be formed (Oelmez & Kretzschmar, 2009). Hydrogen peroxide (Beerli, Vilas Boas & Piccoli, 2004; Khadre & Yousef, 2001) or organic chlorinated products (sodium dichloroisocyanurate, potassium dichloroisocyanurate, dichloroisocyanuric acid and trichloroisocyanuric acid) are alternative sanitizing agents that gained interest in recent years (Beerli et al., 2004). In addition, acetic acid, citric acid, ascorbic acid, peroxyacetic acid and ozone were also proposed by other authors (Hwang, Cash & Zabik, 2001; Zohair, 2001). For instance, peroxyacetic acid treatments showed a reduction of 44–99% of mancozeb residues in apples depending of the treatment time and acid concentration (Hwang et al., 2001). Chlorine and ozone treatments have shown to be effective in the reduction of fungicide (captan) and insecticide (azinphos-methyl, formetanate hydrochloride and propargite) residues in apples and apples products (Ong, Cash, Zabik, Siddiq & Jones, 1996).

Processing factors (PFs), calculated by a ratio between the pesticide concentration in the processed commodity and the pesticide concentration in the raw commodity, can be useful to estimate the level of pesticide exposure at the point of consumption after processing (Ling et al., 2011). Washing PFs for several pesticides including organochlorine and organophosphorus pesticides in plant foods have been reported in the last years (Aguilera, Valverde, Camacho, Boulaid, & García-Fuentes, 2012; Samriti, Chauhan, & Kumari, 2011; Soliman, 2001). However, there is little information on the behaviour of dithiocarbamate fungicides with washing of plant foods. In fact, until now, no processing factors for ethylene-bis-dithiocarbamates (EBDCs) have been available for lettuces. The aim of this study was to evaluate the impact of regular disinfection treatments (water, amukine, hydrogen peroxide, ammonium hydroxide and acetic acid) on the reduction or elimination of mancozeb residues from lettuces treated with Micene WP (mancozeb 80%).

2. Experimental

2.1. Chemical, solvents and disposables

Mancozeb [CAS No. 8018-01-7] (purity 96.8%) was purchased from Fluka (Steinheim, Germany). Water and acetonitrile for liquid chromatography were purchased from Sigma–Aldrich (Steinheim, Germany). L-cystein [CAS No. 52-90-4] (purity ≥ 97%) and dimethyl sulfate (purity ≥ 99%) were from Sigma–Aldrich. Other reagents used were: ethylenediaminetetraacetic acid disodium salt 2-hydrate PA-ACS (EDTA, purity ≥ 99%), sodium chloride PA-ACS-ISO (purity 99.5%), magnesium sulphate anhydrous QP (purity 96%), di-sodium hydrogen phosphate anhydrous (Na_2HPO_4, USP) purissimum-CODEX (purity > 98%), sodium di-hydrogen phosphate 1-hydrate (NaH_2PO_4, USP, BP) PRS-CODEX (purity > 98%), all of them from Panreac (Barcelona, Spain). A pH 7.8 aqueous buffer was prepared with 53.5 mL of 0.2 M NaH_2PO_4 plus 947 mL of 0.2 M Na_2HPO_4.

For solid–liquid extraction (SLE) and derivatization, samples were placed in 50 mL polypropylene centrifuge tubes from Sterilin (Newport, UK). Polypropylene tubes were centrifuged in a Rotina 35 R centrifuge from Hettich Lab Technology (Tuttlingen, Germany). Strata SI-1 silica cartridges (55 μm, 70 A, 500 mg, 6 mL) from Phenomenex (Utrecht, The Netherlands) were used as solid-phase extraction (SPE) minicolumns for purification. Organic extracts were concentrated in an Eppendorf vacuum concentrator model 5301 (Hamburg, Germany). Final organic extracts were filtered through a 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) prior to chromatographic analysis.

2.2. Standard solutions

A stock standard solution (ca. 100 mg/L) of mancozeb was separately prepared as a turbid liquid in acetonitrile by weighing approximately 1 mg of the analyte to nearest 0.01 mg into a 10 mL volumetric flask and diluting to volume. Stock standard solution was stored in the dark at 4 °C and was prepared each two weeks due to the instability of the standard. Working solutions were prepared daily in distilled water or buffer solution by appropriate dilution.

2.3. Sample preparation

The extent of pesticide reduction depends upon the washing operations, nature of pesticide molecule and other preparatory steps used. Residues of several pesticides are removed with reasonable efficiency by different types of washing processes (Bajwa & Sandhu, 2011). In this work, we study the effect of different washing procedures in order to check their effectiveness on the removal of mancozeb residues from lettuces of two varieties (smooth and curly) bought in local markets of Ourense (NW Spain). No residues of mancozeb above the detection limit were observed in these samples.

The leaves of a half lettuce head were spiked with mancozeb at two levels above the detection limit by dipping them, for 1 h, in 1 L of aqueous solutions of Micene WP with concentrations of 5 and 10 mg kg^{-1} of mancozeb. Afterwards, lettuce samples were dried using a salad centrifuge. In order to confirm the initial concentration of mancozeb residues on the treated samples, a portion of them (5 g) was directly analyzed (unwashed sample).

Fortified samples were submitted to several washing procedures by dipping them in 500 mL of each washing solution according with the scheme showed below:

a) *Tap water.* This is the most common treatment for washing vegetables.
b) *Amukine.* Amukine (Angelini Farmaceutica S.A.; Barcelona; Spain) is a commercial solution (1.15 g of sodium hypochlorite solution in 100 mL) for disinfect vegetables. Samples were treated according to the manufacture instructions that recommend use 50 mL of Amukine in 2.5 L of water.
c) *Hydrogen peroxide.* Lettuces were immersed in an aqueous solution that contains 6% of H_2O_2 (purity 30%; Sigma–Aldrich).
d) *Acetic acid.* Acidic solutions were found to be in some cases more effective than neutral solutions on the removal of some compounds such as organochlorine and organophosphorous pesticides (Radwan, Abu-Elamayem, Shiboob & Abdel-Aal, 2005; Soliman, 2001; Zohair, 2001). Samples were immersed in a 50 mg L^{-1} aqueous solution of acetic acid (Sigma–Aldrich).
e) *Ammonium hydroxide.* Samples were immersed in a 50 mg L^{-1} aqueous solution of ammonia (purity 25%; Panreac).

All treatments were made at two temperatures of the washing solution (15 °C corresponding to the tap water temperature and 25 °C) except in the case of acid acetic and ammonia treatments where only the treatment at 15 °C was developed. In addition, the effect of contact time (5 and 10 min) was evaluated. Higher times were not considered due to the typical washing length in a commercial processing operation is approximately 10–15 min (Hwang et al., 2001). Three replicates by treatment were done.