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Determination of trace thiophanate-methyl and its metabolite carbendazim with teratogenic risk in red bell pepper (*Capsicumannuum* L.) by surface-enhanced Raman imaging technique

Jiang-Lin Li [a,b], Da-Wen Sun [a,b,c,*], Hongbin Pu [a,b], Digvir S. Jayas [d]

[a] *School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China*
[b] *Academy of Contemporary Food Engineering, South China University of Technology, Guangzhou Higher Education Mega Center, Guangzhou 510006, China*
[c] *Food Refrigeration and Computerized Food Technology (FRCFT), Agriculture and Food Science Centre, University College Dublin, National University of Ireland, Belfield, Dublin 4, Ireland*
[d] *Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada R3T 2N2*

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A B S T R A C T

Surface-enhanced Raman scattering (SERS) imaging coupling with multivariate analysis in spectral region of 200 to 1800 cm^{-1} was developed to quantify and visualize thiophanate-methyl (TM) and its metabolite carbendazim residues in red bell pepper (*Capsicum annuum* L.). Least squares support vector machines (LS-SVM) and support vector machines (SVM) models based on seven optimized characteristic peaks that showed SERS effects of TM and its metabolite carbendazim residues were employed to establish prediction models. SERS spectra with first derivative (1st) and second derivative (2nd) method were subsequently compared and the optimized model of 1st-LS-SVM acquired showed the best performance (RPD = 6.08, $R_P^2 = 0.986$ and RMSEP = 0.473). The results demonstrated that SERS imaging with multivariate analysis had the potential for rapid determination and visualization of the trace TM and its metabolite carbendazim residues in complex food matrices.

1. Introduction

Fungicides are widely used in agricultural farming to enhance yields and quality of fruit and vegetables. Thiophanate-methyl (TM) is a benzimidazole fungicide extensively applied in pre- and post-harvest, mainly to control diseases caused by ascomycota fungal pathogens in vegetables (Ye, Zhou, & Wang, 2008). However, TM is unstable in plants and to certain extent can be converted to carbendazim (methyl benzimidazol-2-ylcarbamate) that causes endocrine disruption, embryotoxic and teratogenic effects (Makita, Hashimoto, & Noguchi, 1973; Sciarrillo et al., 2008). Therefore, TM fungicide and its metabolite carbendazim residues in agricultural products constitute a significant health risk, which is classified as toxicity class U of agricultural chemical by the World Health Organization (WHO) Classification of Pesticides by Hazzard (IPCS, 2009). The Codex Alimentarius Commission (CAC) has set the maximum residue limit (MRL) of TM fungicides and its metabolite carbendazim in the range 0.05–5 mg kg^{-1} according to the food category (CAC, 2013). Red bell pepper (*Capsicum annuum* L.) is a member of the solanaceae family occupying rich capsanthin in capsicum genus for endowing the reddish colour to product. With respect to production volume and commercial value, red bell pepper is regarded as one of the most significant fruit and vegetables, and the permitted MRL of both TM fungicide and its metabolite carbendazim are 2 mg kg^{-1} for red bell pepper (Gomes, Petito, Costa, Falcão, & de Lima Araújo, 2014).

Most applied fungicides find their ways as "residue" into the environment involving the retention/liberation from soils, the pollution of water resources and eventually the terrestrial and aquatic food chains, thus undergoing constant concentration along with the potential, long-term and insalubrity risks for humans (Winteringham, 1971). Most of the fungicide residue analytical methods in this sample mainly consist of two key preparation steps: extraction of target analytes from the matrix, and partitioning of the residues in an immiscible solvent or clean-up of analytes from matrix co-extractives (Barbini, Vanni, Girolimetti, & Dommarco, 2007). After the preparation steps, detection analytical methods, such as gas chromatography (GC) (Amer, Shehata, Lotfy, & Monir, 2007), gas chromatography-mass spectrometry (GC–MS)

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* Corresponding author at: Food Refrigeration and Computerized Food Technology (FRCFT), Agriculture and Food Science Centre, University College Dublin, National University of Ireland, Belfield, Dublin 4, Ireland.
E-mail address: dawen.sun@ucd.ie (D.-W. Sun).
URLs: http://www.ucd.ie/refrig, http://www.ucd.ie/sun (D.-W. Sun).

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(Calderon-Preciado, Jimenez-Cartagena, Penuela, & Bayona, 2009) and gas chromatography–ion trap mass spectrometry (GC-ITMS) (Tao et al., 2009), have recently made a great advance in inspecting fungicides due to the high separation power, selectivity and identification capabilities of MS. Moreover, GC coupled with various sensitive detectors, such as electron capture detector (ECD) (Amvrazi & Tsiropoulos, 2009), nitrogen phosphorus detector (NPD) (Hernandez-Borges, Cabrera, Rodriguez-Delgado, Hernandez-Suarez, & Sauco, 2009), flame ionization detector (FID) (Parveen, Khuhro, & Rafiq, 2005), pulsed flame photometric detector (GC-PFPD) (Salvador, Frenich, Gonzalez, & Vidal, 2006) and flame photometric detector (FPD) (Darko & Akoto, 2008), improved the quantification procedures of inspecting fungicides residues, which is suitable for the application in different sample matrices. On the other hand, there are also other common quantification methods like high performance liquid chromatography (HPLC) (Ye et al., 2008), liquid chromatography–mass spectrometry (LC–MS) (Economou, Botitsi, Antoniou, & Tsipi, 2009) and low-pressure gas chromatography–mass spectrometry (LP–GC/MS) (Walorczyk & Gnusowski, 2006) for the determination of fungicides. However, these current used methods require complicated pretreatments for separation, extraction, purification, detection, and sample clean-up, making the entire procedure tedious and time consuming. Therefore, it is necessary to develop sensitive and reliable methods for monitoring fungicide residues in vegetables to guarantee food safety and quality.

Rapid and non-destructive spectroscopic or imaging techniques such as NIR spectroscopy, computer vision (Jackman, Sun, Allen, 2009; Wang & Sun, 2002; Wu & Sun, 2013a) or hyperspectral imaging (Barbin, Elmasry, Sun, & Allen, 2012; Barbin, ElMasry, Sun, & Allen, 2013; ElMasry, Kamruzzaman, Sun, & Allen, 2012; Elmasry, Barbin, Sun, & Allen, 2012; Feng & Sun, 2012, 2013; Feng et al., 2013; Kamruzzaman, ElMasry, Sun, & Allen, 2012; Kamruzzaman, ElMasry, Sun, & Allen, 2013; Liu, Sun, & Zeng, 2014; Wu & Sun, 2013b, 2013c) have been developed and widely used for food quality and safety monitoring and evaluation, among them, surface-enhanced Raman scattering (SERS) technology has been an attractive spectroscopic tool in recent years that provides rich molecular fingerprints on analytes at trace levels or even at single-molecule levels (Zheng & He, 2014). Due to the superiority of great sensitivity, high selectivity, minimal sample preparation and non-destructive nature, SERS technology has been widely pursued in various fields mainly on the detection of complex biological structures (Efeoglu & Culha, 2013), intracellular bacteria and virus study (Bhardwaj, Srinivasan, & McGoron, 2015), material performance (Pisarek et al., 2014), and monitoring mycotoxins, additives, pesticide and veterinary drug residues in foods (Hernandez-Castillo et al., 2015; Lee & Herrman, 2016; Liu et al., 2013; Olavarria-Fullerton, Wells, Ortiz-Rivera, Sepaniak, & De Jesus, 2011; Rajapandiyan, Tang, & Yang, 2015). The SERS enhancement is generally due to the improvement of the electromagnetic field on the nanostructured metal surfaces, leading to the increased sensitivity in SERS measurement and achieving a high enhancement factor in a well-designed SERS substrate larger than 10^6 (Le Ru, Meyer, Blackie, & Etchegoin, 2008; Zhou et al., 2015). Unfortunately, the biggest obstacle to the expansion of the practical applications for SERS sensing is the lack of highly efficient substrates with uniform and high density hotspots (Zhang, Lee, Phang, Lee, & Ling, 2014). Recently, extensive efforts have been devoted to construct different SERS substrates from zerodimensional clusters (Au or Ag nanospheres and nanocubes) to 3D hierarchical nanoscaffolds (nanoarrays, branched nanotrees, and nanobutterfly wing), and it has been shown that the structure, such as size, shape or interparticle and molecule-metal distances of nanoparticles, has an important impact on SERS performance (Liu, Sun, Huang, Li, & Liu, 2015; Tan et al., 2012). However, among the large number of SERS applications reported, SERS could only provide spectral information without the spatial distribution in these applications. On the other hand, digital imaging is only used for showing monochrome or colour images, and thus no spectral information and chemical components can be determined. Given the above limitations, surface-enhanced Raman scattering imaging technique (SERS imaging) is another significant development for SERS measurements, which combines SERS and digital imaging into a new powerful analytical technique to obtain additional spatial information on the basis of SERS in order to identify the presence and to visualize the distribution of interesting trace targets (Mamián-López & Poppi, 2015). In this technique, a region of the sample is designated and each SERS spectrum is obtained at each pixel point on the surface of sample. Then interesting chemical information about the analytes in sample can be extracted from the mapping region. The main advantage of this approach is to provide identification and distribution of constituents with concentrations at the microgram-per-liter levels. In the total mapping area, the bulk concentration can be low in a mass of pixel but the concentration of this specific analyte can be high in some pixel (Albuquerque & Poppi, 2015).

However, very few applications of SERS imaging in complex systems or mixtures have been reported, especially for agricultural and food products, such as red bell pepper matrices, which are full of vitamin C, vitamin A, vitamin B6, folic acid and potassium elements that have great interference on a univariate research, and thus SERS studies have been mainly concentrated on how to achieve sufficient homogeneity of the distribution of nanoparticles into the medium (Varenne, Makky, Gaucher-Delmas, Violleau, & Vauthier, 2016). It is hard to gain a SERS signal only from the analyte in the complex matrices, and multivariate data analysis methods should be utilized as other hyperspectral imaging applications (Mamián-López & Poppi, 2015; Sun, 2010). Therefore, in the current study, SERS imaging technique coupling with multivariate data analysis was applied to explore the feasibility of predicting trace TM fungicide and its metabolite carbendazim residues with teratogenic risk in red bell pepper products. Such a study was not only an improvement of current limited endeavor for detection of TM fungicide residues using SERS technology, but also an application of SERS for accurate quantitative analysis of trace TM fungicide and its metabolite carbendazim residues. The results could be considered as a basis for further development of highly sensitive and rapid SERS imaging for non-destructive determination of certain pesticide, herbicide or fungicide in other food products.

2. Material and methods

2.1. Chemicals and reagents

Silver nitrate ($AgNO_3$), tri-sodium citrate ($Na_3C_6H_5O_7$), sodium chloride (NaCl) and acetonitrile were obtained from Aladdin Reagent (Shanghai, China). TM fungicides were purchased from Shandong Caoda Chemical Co., Ltd. (Shandong, China). Red bell peppers (*Capsicum annuum* L.) were organic free of fungicides and were purchased from a local supermarket. The water used was from a Milli-Q® Ultrapure Water Purification System (Millipore, Brussels, Belgium). All other reagents belonged to analytical reagent grade without further purification.

2.2. Synthesis of silver nanoparticles sol

Silver nanoparticles were synthesized by the classical Lee–Meisel method (Lee & Meisel, 1982). First, 50 ml of 10^{-3} M $AgNO_3$ aqueous solution was brought to the boil by a heating mantle for 15 min. Next, 1 ml of 1% tri-sodium citrate solution was added to