Food Control 70 (2016) 318–324

Contents lists available at ScienceDirect

Food Control

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

Free tocopherols as chemical markers for Arabica coffee adulteration with maize and coffee by-products

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ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form
10 June 2016
Accepted 11 June 2016
Available online 13 June 2016

Keywords:
Authenticity
Fraud
Coffee husks
Corn
HPLC
LDA
SIMCA
Vitamin E

ABSTRACT

Coffee adulteration with coffee by-products is increasing, due mostly to its chemical and sensorial similarity with ground roasted coffee. Based on the recognized effectiveness of tocopherols as markers for coffee adulteration with maize, we have further explored their ability to distinguish adulterations with coffee husks, the main residue of coffee dry processing. For the purpose, a ground roasted arabica coffee sample was adulterated with 5–50% (w/w) of roasted husks, cleaned roasted husks (without the inner parchment layer), and roasted maize. Extracted lipids were analysed by normal-phase HPLC, with florescence detection, and the tocopherol amounts analysed by mean tests, regression analysis, PCA, LDA and SIMCA. γ–tocopherol, detected in residual amounts in roasted coffee, was inversely proportional to coffee purity, being the compound that better contributed for adulteration detection, independently of the adulterant tested. Coffee richness in β–tocopherol, in opposition to all the adulterants tested, also contributes for this discrimination. Based on the tocopherol profiles, adulterations can be perceived at the lowest amounts tested (5%), but higher amounts are necessary for identification of the adulterant, namely ≥10% for maize and from 20% upwards for coffee by-products. For heavy adulterations is even possible to distinguish between husks and cleaned husks. This method is simple to implement in food analysis laboratories, with applicability for adulteration screening or to complement other instrumental methods.

1. Introduction

Coffee is one of the beverages with higher consumption worldwide, due mostly to its sensorial and stimulating attributes, together with an increased recognition of its potential health effects (Ludwig, Clifford, Lean, Ashihara, & Crozier, 2014). However, due to coffee's high commercial value, particularly Arabica coffee, it has been the target of adulteration for decades. This is of particular concern in Brazil, the world greatest coffee producer, with a high percentage of adulterated roasted coffee samples sold in the internal market (Domingues et al., 2014; Souto et al., 2015). Coffee adulteration, besides being against legislation and consumers rights, can also affect the sensorial attributes and chemical composition of the beverage, while reducing its beneficial health effects. In addition, the absence of studies on the health effects of roasted adulterants cannot disregard some doubts over its safety.

The most common coffee frauds include addition of roasted and ground vegetable products of lower commercial value, as maize, barley, rye, wheat, etc. (Jham, Winkler, Berhow, & Vaughn, 2007; Toci, Farah, Pezza, & Pezza, 2016). These products are distinct from coffee, enabling their distinction through physical and chemical characteristics, but the most recent frauds using coffee by-products, as coffee husks and stems, represent an increased challenge for analytical chemists (Reis, Franca, & Oliveira, 2013a; Toci et al., 2016).

Several analytical methods have been developed over the years to detect coffee adulteration, following the increased number of

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http://dx.doi.org/10.1016/j.foodcont.2016.06.011
0956-7135/

adulterants and availability of instrumental methods. The simplest and older detection methods used optical microscopy, naturally limited, semi-quantitative, and requesting specialized technicians for the purpose (Pauli et al., 2014). Presently, the techniques that grant higher efficiency include chromatography and infrared spectroscopy. Chromatographic techniques, associated with diverse detection systems, allow the identification of chemical adulteration markers, including oligosaccharides (Garcia et al., 2009; Nogueira & Lago, 2009; Pauli et al., 2014), fatty acids (Jham, Berhow, Manthey, Palmist, & Vaughn, 2008) and tocopherols (Jham et al., 2007; Winkler-Moser et al., 2015). Several works have also been published in the spectroscopic field, using Near Infrared Spectroscopy (NIRS), Raman Spectroscopy (RS) and Fourier Transform Infrared Spectroscopy (FTIR), and proving fast and non-destructive approaches (Briandet, Kemsley, & Wilson, 1996; Ebrahimi-Najafabadi et al., 2012; Tavares et al., 2012; Reis et al., 2013a,b; Winkler-Moser et al., 2015). Most of the published works associate chemometric tools, as PCA (*Principal Component Analysis)*, *LDA (linear discriminant analysis)* and the classification method SIMCA (*Soft Independent Modeling by Class Analogy*), among others (Aquino et al., 2014; Domingues et al., 2014; Ebrahimi-Najafabadi et al., 2012; Pauli et al., 2014; Reis et al., 2013a,b; Toledo, Hantao, Ho, Augusto, & Anderson, 2014; Winkler-Moser et al., 2015).

Tocopherols (α, β, γ and δ), together with tocotrienols, are vitamin E homologues naturally present in plants and their products (Saini & Keum, 2016). Tocochromanols have already been used as authenticity markers in several food matrices, including vegetable oils (Codex Stan-210, 1999), butter (Górnaś et al., 2014a), margarines (Górnaś & Siger, 2015a), fats used as ingredients in bakery products (Mignogna, Fratianni, Niro, & Panfili, 2015), or even fruit cultivars (Barreira et al., 2009). Tocopherols are also present in coffee (Folstar, Van der Plas, Pilnik, & De Heaus, 1977), protecting coffee lipids from oxidation. However, while most plant products are characterized by the presence of α-, γ-, and sometimes δ-tocopherol (Saini & Keum, 2016), coffee has a distinct pattern, being richer in β-tocopherol (González, Pablos, Martí, León-Camacho, & Valdernebro, 2001; Jham et al., 2007; Alves, Casal, & Oliveira, 2009a; Górnaś et al., 2014b), a condition that is only observed in some matrices, as wheat germ oil (Schwartz, Ollilainen, Piironen, & Lampi, 2008) or apple seeds (Górnaś et al., 2015b). Indeed, tocopherols have also been reported as authentication markers for coffee adulteration with maize, based on γ-tocopherol increase (Jham et al., 2007), being equally useful to distinguish coffee species (arabica and robusta), before and after roast (Alves, Casal, & Oliveira, 2009b; González et al., 2001; Górnaś et al., 2014b).

Based on coffee distinct tocopherol pattern, and knowing that each plant tissue might have different tocopherols distribution patterns (Horvath et al., 2006), the objective of the present work was to evaluate the ability of tocopherols to distinguish coffee subproducts, in particular husks and cleaned husks (deprived of the inner parchment fibrous layer). Maize adulteration was tested for comparative purposes due to its elevated prevalence as adulterant and known ability to be distinguished on the basis of the tocopherol profile (Jham et al., 2007).

2. Material and methods

2.1. Chemicals

Tocopherols (α, β, γ and δ) were purchased from Sigma (Saint Louis, USA). Accurate concentrations of the n-hexane standard solutions prepared were regularly confirmed by UV absorbance, based on their molar absorptivities (Eitenmiller & Landen, 1999). The internal standard tocol was obtained from Matreya Inc. (PA, USA) and diluted accurately to 0.2 mg/mL in n-hexane. HPLC grade n-hexane was obtained from Merck (Darmstad, Germany) and 1,4-dioxane from Fluka (Madrid, Spain). Butylated hydroxytoluene (BHT), used as antioxidant, was obtained from Aldrich (Madrid, Spain). All other reagents were of analytical grade from diverse suppliers.

2.2. Sample preparation

Six samples of each adulterant - maize, husks and cleaned husks - were gently given by the COCAM Company (Catanduva-SP, Brasil) and by the Coffee Department in the Federal University of Lavras (UFLA, Lavras-MG, Brazil). Each adulterant sample was roasted separately, using a cylindrical roaster with air cooling (Rototec, model RT-12).

Six coffees samples, including arabica (n = 3), robusta (n = 2), and an unknown commercial blend (n = 1) were collected. One of the Arabica samples (#C1) was used as basis for adulterations. A Probatino equipment was used for coffee roasting (Leogap, Brasil; 1 kg capacity).

A dark medium roasted degree was chosen both for coffee and adulterants, as it represents the most common roast in Brazil. All roasts started at 200 °C, with a medium roast time of 29, 23, 5:06 and 3:14 min for maize, coffee, cleaned husks and husks, respectively, and final average temperatures around 224, 261, 207, and 212 °C, again respectively.

All roasted samples were ground to 20 mesh with an electronic device (Pinhalense, ML-1, Brasil). A portion of each individual roasted adulterant and coffee samples was packed individually (n = 6 × 4) for further analysis. Adulterations were performed in triplicate, with 5, 10, 20, 30, 40 and 50% (W/W) of each roasted maize, husks, and cleaned husks samples, using the previously mentioned arabica coffee sample (C#1). A total of 54 adulterated coffee samples were prepared. All samples were individually packed in polyethylene/aluminium foil, sealed, and stored at −20 °C until analysis.

2.3. Tocopherols extraction and quantification by HPLC

The methodology used for tocopherol extraction and analysis was based on the method developed for roasted coffee by Alves et al. (2009a). Briefly, a 200 mg amount of sample was homogenized with 1 ml of absolute ethanol with BHT (50 μg/ml), internal standard solution (tocol; 50 μl) and 2 ml of n-hexane in 15 mL amber glass vials (Supelco, Bellefonte, PA). After homogenization for 30 min in a VV3 vortex with a multiple sample support (VWR, PA, USA), the vials were left overnight at 4 °C. After attaining room temperature, the samples were further homogenized for 15 min. Separation and cleaning of the organic phase was achieved by addition of NaCl solution (1%; 1 ml), and centrifugation. Extraction was repeated with a second 2 ml portion of hexane. The hexane extracts were combined, passed over anhydrous Na_2SO_4, concentrated to 500 μl under a nitrogen stream (40 °C) and transfer to amber injection vials.

Chromatographic analysis were performed in a Jasco HPLC equipment (Japan) equipped with an auto-sampler (AS-950; 20 μl), an isocratic pump (PU-980), a diode-array detector (MD-910) connected in series to a fluorescence detector (FP-920; programmed at 290 nm/300 nm). Normal phase separation was achieved with a Supelcosil TM LC-SI (3 mm) 75 × 3.0 mm (Supelco, Bellefonte, PA) at room temperature (21 °C), using an isocratic mixture of n-hexane and 1,4-dioxane (98:2), with a 0.7 mL/min flow.

The compounds were identified by comparison with authentic standards and by their UV spectra. Semi-quantification was achieved directly by the relative tocopherol areas, while quantification