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Development of a comprehensive analytical platform for the detection and quantitation of food fraud using a biomarker approach. The oregano adulteration case study

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ABSTRACT

Due to increasing number of food fraud incidents, there is an inherent need for the development and implementation of analytical platforms enabling detection and quantitation of adulteration. In this study a set of unique biomarkers of commonly found oregano adulterants became the targets in the development of a LC–MS/MS method which underwent a rigorous in-house validation. The method presented very high selectivity and specificity, excellent linearity ($R^2 > 0.988$) low decision limits and detection capabilities (<2%), acceptable accuracy (intra-assay 92–113%, inter-assay 69–138%) and precision (CV < 20%). The method was compared with an established FTIR screening assay and revealed a good correlation of quali- and quantitative results ($R^2 > 0.81$). An assessment of 54 suspected adulterated oregano samples revealed that almost 90% of them contained at least one bulking agent, with a median level of adulteration of 50%. Such innovative methodologies need to be established as routine testing procedures to detect and ultimately deter food fraud.

1. Introduction

In 2002 the European Union recognised the necessity for introducing the Food Law (European Food Safety Authority, 2002) to protect consumers' health and provide improved confidence that the EU, as a major global trader in food, has the most stringent food safety laws in the world. In order to facilitate consumers' informed choices, 'the *food law should aim at the prevention of fraudulent or deceptive practices; the adulteration of food; and any other practices which may mislead the consumer*' (European Food Safety Authority, 2002). Nevertheless, to date EU legislation does not provide a definition of food fraud. In a European Parliament report 2013/2091(INI), following the horsemeat scandal, the key characteristics of food fraud were outlined and were similar to the definitions already introduced in the USA (Johnson, 2014) – i.e. non-compliance with food law and/or misleading the customer which is done intentionally and for financial gain resulting from fraudulent practices such as adulteration, substitution, tampering or counterfeiting. At the same time the 'Food Fraud Network' started to collect and exchange information in order to detect and prevent food fraud in the EU (European Commission, 2016). The largest available summary of records (over 2000) on food fraud is contained within the U.S. Pharmacopeia Food Fraud Database (U.S. Pharmacopeial Convention, 2016), which encompasses both scholarly and media reports on the subject area. According to this database, during years 2011–2012 the number of fraud records increased by 60% when compared with the period spanning 1980–2010. The first and second most affected food ingredients quoted being oils and spices respectively, with the latter accounting for 16% of all records (Johnson, 2014). Amongst these, there have been number of recent fraud incidents, potentially posing a health risk, such as addition of toxic colorants or botanic substitutes (Moore, Spink, & Lipp, 2012; U.S, 2016). In addition there have also been reports on bulking agents used for the purpose of substitution/dilution of herbs (Marieschi, Torelli, Bianchi, & Bruni, 2011; Marieschi, Torelli, Poli, Bianchi, & Bruni, 2010; U.S, 2016)

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which is in agreement with a recent study focusing on oregano adulteration (Black, Haughey, Chevallier, Galvin-King, & Elliott, 2016). Almost 25% of tested UK oregano samples (n = 78) were found to be adulterated with bulking agents, most commonly olive and myrtle leaves. The levels of adulteration in some samples was found to be over 70%. This example of adulteration is a classic case of food fraud where the aim is to mislead consumers for purely financial gain. This type of adulteration may pose health threats due to unknown status of the bulking agents, thus exposing consumers to potentially toxic compounds present in the botanical adulterants employed as well as potential microbiological and/or chemical contaminants.

Each food fraud incident has the potential to threaten consumers' well-being but also undermine confidence in the EU food market in which the herbs and spices' share is worth 1.8 billion Euro in the EU and 2.97 billion dollars world-wide (Marieschi, Torelli, Poli, Sacchetti, & Bruni, 2009). Thus, the European Parliament issued a call to develop and implement technologies and methods to detect food fraud including sensor technology and fingerprinting approach (European Parliament, 2013). Such methods, including Fourier-Transform Infrared spectroscopy (FTIR) have been already successfully implemented in food fraud detection (Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015). DNA sequencing techniques are also considered to be reliable, nevertheless, their limitations have been discussed (Parveen, Gafner, Techen, Murch, & Khan, 2016) and make them unsuitable to be employed as stand-alone tools in the field of authentication. Furthermore, according to European legislation concerning the performance of analytical methods (European Commission, 2002), those approaches would be classified as screening methods and as such they are not fit for the purpose of confirming adulteration to required legal standards, due to lack of chemical structure confirmation, if the legal action is to be pursued by industry or regulators. High Resolution Mass Spectrometry (HRMS) based fingerprinting approaches have also been employed in food authentication, however even though such methods provide the possibility of detecting unusual deviations within the sample set, instrumental analysis is time consuming, requires expensive equipment, data storage facilities and high processing power (Esslinger, Riedl, & Fauhl-Hassek, 2014). Available targeted mass spectrometry based methods designed for the purpose of adulteration confirmation usually employ food profiling approach whereby sample classification is based on analysing a selected group of matrix constituents such as flavonoids in *Ginkgo biloba* or ginsenosides in *Panax Ginseng* (Xie et al., 2006; Yuan, Wang, Chen, Ye, & Zhou, 2016). Such validated analytical methods ensure accuracy of the analytical determination (Esslinger et al., 2014), however are prone to 'targeted designed adulteration' (Sanzini, Badea, Dos Santos, Restani, & Sievers, 2011) due to increased knowledge regarding chemical composition of food commodities.

Consequently, designing an analytical approach suitable for rapid, cheap and reliable detection and confirmation of adulteration still presents a challenge. Many important issues such as selectivity and specificity of chosen markers in the presence of various botanic adulterants, methods' ruggedness as well as inherent biodiversity (De Falco et al., 2013) must all be considered during experimental design. Thus, the aim of the present study was to explore the possibility of designing a holistic system allowing for both fast and reliable FTIR screening as well as cost effective, simplified, liquid chromatography tandem–mass spectrometry (LC–MS/MS) based confirmation for the purpose of oregano adulteration detection and quantitation. The successful outcome of this study could lead to many other future applications of such a system for the analysis of other types of food fraud.

2. Materials and methods

2.1. Reference plant material and identity confirmation

A set of 38 samples were employed in the current LC–MS/MS method development and validation. Those included either certified reference materials (*Origanum vulgare* leaf and *Olea europaea* leaf referenced against vouchered (AH0024) and reagent grade biomass reference material respectively, both from LGC standards, Teddington, UK) or samples supplied with full provenance and traceability including leaves of culinary *Origanum* species such as *Origanum vulgare* and *Origanum onites* (n = 11) as well as samples of potential bulking agents including olive leaves – *Olea europaea subsp. europaea* (n = 6), *Myrtaceae* leaves including *Myrtus communis* and *Myrtus communis var. communis* (n = 6), phlomis leaves – *Phlomis x cytherea* (n = 2), sumac leaves – *Rhus coriaria* (n = 3), hazelnut leaves – *Corylus avellana* (n = 3), sage leaves – *Salvia officinalis* (n = 3) and cistus leaves – *Cistus creticus* (n = 2). In order to experimentally confirm the identification of the samples, specimens were compared morphologically against herbarium specimens and were also assessed using DNA barcoding. Material has been deposited into the DBN herbarium tissue bank in the National Botanic Gardens of Ireland (registration number DBN2017:04).

2.1.1. DNA extraction and PCR conditions

Genomic DNA was extracted from approximately 20 mg of lyophilized leaf tissue using Macherey-Nagel NucleoSpin® Plant II kits according to the manufacturer's instructions, with the exception that the cell lysis incubation step at 65 °C was extended to 45 min. Species identity was confirmed using DNA barcoding. Four regions were used, three plastid and one nuclear region. The plastid regions were *trnH-psbA* (Kress, Wurdack, Zimmer, Weigt, & Janzen, 2005), *trnL-trnF* (Taberlet et al., 2007) and *matK* (Yu, Xue, & Zhou, 2011). The nuclear region was the Internal Transcribed Spacer region (ITS) (Kress et al., 2005). Polymerase chain reactions (PCR) contained 1 μL (~50 ng) of DNA template, 1 x Bioline MyTaq™ Red Mix (Bioline, USA) and 0.5 μM of the forward and reverse primers (Eurofins, Germany) in a total volume of 20 μL. PCR cycle conditions were: 94 °C for 2 min followed by 40 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, with a final 10 min extension at 72 °C. PCR products were purified using the ExoSAP method (New England Biolabs) and sent to Macrogen Europe for direct sequencing in one direction.

2.1.2. DNA sequence analysis

DNA sequences were aligned and cleaned using Sequencher v. 4.10.1 (Gene Codes Corporation). Cleaned sequences were BLASTed against sequences available in GenBank and the top hits were checked for matches with the expected taxon. Results are presented in Table S1 (Supplementary material).

2.2. Sample preparation

The protocol described was based on the extraction published in the previous study (Black et al., 2016) and simplified herein by decreasing the volume of the extraction solvent from 2 mL to 0.5 mL, sonication time was shortened from 15 min to 10 min while final solvent exchange and filtration steps were abandoned to shorten the analysis time. Shortly, the samples were milled to a homogenous powder on a PM-100 Retsch Planetary Ball Mill (Retsch, Haan, Germany). 50 mg (±1%) was weighed out on a Discovery DV215CD Analytical Balance (Ohaus Europe GmbH, Nanikon, Switzerland) into a 1.5 mL Eppendorf tube and 500 μL of ultra-pure water (18.2 MΩ/cm)/LC–MS Chromasolv methanol