Food Chemistry 244 (2018) 120–127

Contents lists available at ScienceDirect

Food Chemistry

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

Untargeted metabolomics reveals specific withanolides and fatty acyl glycoside as tentative metabolites to differentiate organic and conventional *Physalis peruviana* fruits

Sandra M. Llano[1], Ana M. Muñoz-Jiménez[1], Claudio Jiménez-Cartagena, Julián Londoño-Londoño, Sonia Medina[*,2]

Faculty of Engineering, Food Engineering Program, Corporación Universitaria Lasallista, Caldas-Antioquia, Colombia

ARTICLE INFO

Keywords:
Metabolomics
Goldenberry
Organic fruit
Phytochemicals
Withanolides
Pesticides

ABSTRACT

The agronomic production systems may affect the levels of food metabolites. Metabolomics approaches have been applied as useful tool for the characterization of fruit metabolome. In this study, metabolomics techniques were used to assess the differences in phytochemical composition between goldenberry samples produced by organic and conventional systems. To verify that the organic samples were free of pesticides, individual pesticides were analyzed. Principal component analysis showed a clear separation of goldenberry samples from two different farming systems. Via targeted metabolomics assays, whereby carotenoids and ascorbic acid were analyzed, not statistical differences between both crops were found. Conversely, untargeted metabolomics allowed us to identify two withanolides and one fatty acyl glycoside as tentative metabolites to differentiate goldenberry fruits, recording organic fruits higher amounts of these compounds than conventional samples. Hence, untargeted metabolomics technology could be suitable to research differences on phytochemicals under different agricultural management practices and to authenticate organic products.

1. Introduction

Physalis peruviana (Solanaceae family), also known as goldenberry and cape gooseberry, is a yellow-orange fleshed berry that attract great interest because of its chemical composition; protein, fat, carbohydrates, minerals, vitamins, fiber and high content in bioactive compounds like carotenoids, phytosterols, physalins, withanolides and polyphenols, among others, that provide health benefits and reduce risk for certain diseases, making it a fruit of great interest for future researches (Ramadan, 2011).

Concerning fresh fruit business, international trade in organic fruits has remarkably grown over the past twenty years. There is no doubt that establishing organic agriculture is an important tool for sustainable food production with both environmental and economic benefits. Nevertheless, although in previous reports organic fruits have been considered to have a higher nutritional content than conventionally grown fruits, nowadays, scientific literature is unclear and ambiguous on the accuracy of this claim (Esch & Kariuki, 2010). That is why it is very important to prove that the organic system is better than conventional.

Due to the absence of adequate analytical methodology to differentiate organic and conventional grown crops, new analytical techniques that can distinguish between both agricultural systems are being developed. For that matter, omics approaches like metabolomics, mainly untargeted metabolomic (metabolomic fingerprinting), have allowed the detection of possible differences in the chemical composition between organic and conventional production although many results are still controversial (Vallverdu-Queralt & Lamuela-Raventos, 2016). The main advantage of this approach is its untargeted nature and it may detect unexpected changes in the food metabolome (full set

Abbreviations: 1,3-DCIP, tris-(1,3-dichloroisopropyl)phosphate; d-SPE, dispersive-solid phase extraction; EIC, extraction ion chromatogram; ESI, electrospray ionization mode; FW, fresh weight; GC–MS, gas chromatography-mass spectrometry; HPLC-DAD, high-performance liquid chromatography-diode array detection; HS-SPME, headspace-solid phase microextraction; IS, internal standard; LC, liquid-chromatography; LC–MS, liquid chromatography–mass spectrometry; MRL, maximum residue limit; MRM, multiple reaction monitoring; PCA, principal component analysis; PTFE, polytetrafluoroethylene; RT, retention time; TPP, triphenyl phosphate; UPLC-QqQ-MS/MS, ultra-performance liquid chromatography-triple quadrupole-tandem mass spectrometry; UPLC-QTof-MS/MS, ultra-performance liquid chromatography-quadrupole time of flight-tandem mass spectrometry

* Corresponding author at: Faculty of Engineering, Food Engineering Program, Corporación Universitaria Lasallista, Carrera 51, 118 Sur, 57, Caldas-Antioquia, Colombia.
E-mail address: soniamedes@gmail.com (S. Medina).
[1] These authors have equally worked on this study.
[2] ORCID: http://orcid.org/0000-0002-7231-6480.

http://dx.doi.org/10.1016/j.foodchem.2017.10.026
Received 16 June 2017; Received in revised form 23 September 2017; Accepted 6 October 2017
Available online 07 October 2017

of metabolites of low molecular weight). In this context, in recent years, the application of untargeted metabolomics to food authentication issues (geographical origin, botanical origin, adulteration cultivar origin, farming practices, among others) has gained increasing interest (Cubero-Leon, De Rudder, & Maquet, 2018). Nevertheless, there are few studies in foodstuffs that make use of untargeted metabolomics technology to analyze differences of organic and conventional farming. Related to Solanaceae family, these assays have been carried out in ketchups from tomatoes (Vallverdu-Queralt & Lamuela-Raventos, 2016) or in pepper and tomatoes (Novotná et al., 2012). These previous studies showed that the metabolome of organic and conventional crops was different; however, due to the limited number of available reports, it is still impossible to reach final and valid conclusions. For this reason, the main objective of this study was to explore the effect of organic and conventional growing conditions on the specific chemicals (carotenoids, ascorbic acid and pesticides) using targeted metabolomics and to research overall phytochemicals by untargeted metabolomics technique. As far as we know, this is the first study to investigate the influence of organic *versus* conventional crops on goldenberry metabolome, with the advantage of using two metabolomics approaches.

2. Materials and methods

2.1. Chemicals and reagents

Twenty-five LC pesticide standards (99% purity) (Abamectin, Azoxystrobin, Boscalid, Cartap hydrochloride, Cymoxanil, Cyromazine, Difenoconazole, Ethofenprox, Ethoprofos, Fenhexamid, Linuron, Malathion, Methamidophos, Methomyl, Myclobutanil, Ofurace, Picloram, Profenofos, Spinetoram, Spinosad A, Spinosad D, Spiromesifen, Tebuconazol, Triadimenol, Trifloxystrobin), internal standards (IS) (triphenyl phosphate (TPP) and tris-(1,3-dichloroisopropyl)phosphate (1, 3 DCIP)) and Q-sep® QuEChERS products were purchased from Restek Corporation (Bellefonte, PA, USA). The analyzed pesticides were mainly selected based on maximum residue limits (MRL) established for goldenberry by different regulations (Codex Alimentarius: International Food Standard 2013, European Commission 2010: Regulation EU 600/2010, Colombian Resolution 2906/07 and SANTE 11945/2015 guideline) and also for being pesticides widely used in Colombia in goldenberry conventional crops. A multi-residue stock standard solution (1000 mg L^{-1}) was prepared in acetonitrile and stored in amber glass vial at −20 °C.

Ultrapure water was used in this experiment with a resistivity of 18.2 Ω (Millipore, Bedford, MA, USA). All solvents used were LC–MS grade like acetonitrile, methanol, formic acid, acetone, hexane and dichloromethane and were purchased from Merck (Kenilworth, NJ, USA). Ammonium formate and ascorbic acid were acquired from Sigma Aldrich (St. Louis, MO, USA). Leucine-enkephalin was provided by Waters (Milford, USA).

Carotenoids (carotenes: β-carotene and xanthophylls: lutein, lycopene and zeaxanthin) were obtained from Chromadex (Irvine, CA, USA).

2.2. Plant material

Goldenberry samples (*Physalis peruviana*) from conventional crop were purchased from local markets and supermarkets in Medellin city, Colombia in 2017. Organic goldenberry samples were obtained from an online market "SiembraViva" (www.siembraviva.com), where the local farmers did not use either pesticides or agrochemicals, however it is in process to gain organic products certificate. They established organic fruit procedures, applying organic manure without the use of synthetic products. In order to verify that these organic fruits did not present any pesticides, it was necessary to check it out. The goldenberry fruits (both organic and conventionally grown) were transported in a refrigerated container of rigid construction from supermarkets to laboratory (not for more than one hour) to prevent damage and with protection against solar radiation. As soon as the fruits arrived at the laboratory, they were located in darkness at a low temperature (4 °C) for a short time (one hour) to avoid oxidation. Then, in order to obtain representative samples, 5 batches of goldenberry fruits from each cultivation system (organic and conventional) were obtained. The fruits were homogenized in a mixer using approximately 250 g of fruit and all samples were packed immediately, frozen and stored at −80 °C until the analyses period (not more than five days).

2.3. Targeted metabolomics analyses

2.3.1. Pesticides extraction and analysis by UPLC-QqQ-MS/MS

The extraction of pesticides from goldenberry samples followed the methodology described by Muñoz and colleagues (Muñoz et al., 2017) with slightly modifications. After samples extraction (in our experiment, European (EN 15662) QuEchERS extraction salt and extraction n° 26215 for the clean-up step by dispersive solid phase extraction (d-SPE) were used), the samples were centrifuged and filtered through 0.45 μm PTFE filter (Thermo Scientific, MA, USA) before UPLC-QqQ-MS/MS analysis.

A column Acquity UPLC BEH C18 1.7 μm, 2.1 × 100 mm (Waters, Milford, USA) was used. In accordance with previous study (Muñoz et al., 2017) and later we assayed different mobile phases, formic acid and ammonium formate were used as additives in mobile phases due to the fact that they produce better results for pesticides analyses than mobile phase without these additives. Hence, the mobile phases employed were as solvent A: H_2O with 5 mM ammonium formate and 0.1% formic acid and solvent B: acetonitrile. Mass spectrometer (triple quadrupole) was operated in positive electrospray ionization mode (ESI +) using multiple reaction monitoring (MRM) with two transitions (quantification and confirmation) for each pesticide (Supplementary Table 1). N_2 was used as desolvation gas at a flow rate of 650 L h^{-1} with desolvation T^a of 200 °C, capillary voltage was 3.00 kV and cone voltage 45 V. Data acquisitions were performed using MassLynx V4.1 (Waters, Milford, USA).

As internal standard (triphenyl phosphate (TPP) and tris-(1,3-dichloroisopropyl)phosphate (1,3 DCIP) were used at a final concentration of 300 μg L^{-1} and pesticides standard solution was prepared at 1 mg L^{-1} (1 ppm).

2.3.2. Carotenoids and ascorbic acid analyses

For carotenoids extraction, goldenberry samples were extracted following the methodology used in previous report (Álvarez et al., 2015). For carotenoids quantification purposes, stock solutions of β-carotene, lutein, lycopene and zeaxanthin were prepared at a concentration of 1 mg mL^{-1} in acetone and then calibration curve was prepared from 0.5 to 100 μg mL^{-1}. According to the above-mentioned report, the carotenoids content was determined by HPLC-DAD (Thermo Scientific, USA). The results were expressed as mg per 100 g of FW.

For ascorbic acid content analyses, samples (1 g) of goldenberry fruits were extracted with 1.5 mL of water, the mixture was sonicated for 10 min–25 kHz and 25 °C following the methodology described in a previous report (Briones-Labarca, Giovagnoli-Vicuña, Figueroa-Alvarez, Quispe-Fuentes, & Pérez-Won, 2013) by triplicate. Then, the extract was centrifuged and diluted with 400 μL of acetonitrile. In addition, stock solutions of ascorbic acid was prepared in water 1 mg mL^{-1} and for the quantification of this vitamin, calibration curve was freshly prepared from to 0.5 to 100 μg mL^{-1}. The results about ascorbic acid content in goldenberry samples were expressed in mg per 100 g FW. Subsequently, ascorbic acid content was determined by HPLC-CORONA Veo RS (Thermo Scientific, USA) with a specific chromatographic column C8 (5 μm, 150 × 4.6 mm) that was maintained at 50 °C (Thermo Scientific, USA).