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Short communication

Influence of storage conditions in the evolution of phytochemicals in nutraceutical products applying high resolution mass spectrometry

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ABSTRACT

An adequate knowledge about possible transformation of bioactive compounds in nutraceutical products during long storage is important in order to know potential modifications of this type of compounds. In this study, one year monitoring was performed in different types of nutraceutical products based on natural extracts (green tea, soy, royal jelly and grapes) observing the appearance of new bioactive compounds, which were not detected at the initial conditions, as well as the decrease of some of the detected compounds. To determine these transformation products, an analytical procedure based on high resolution mass spectrometry (Exactive-Orbitrap analyzer) was applied. It was noted that transformation products were detected after 3 months of storage in green tea and soy products, while 6 months were necessary to observe transformation products in royal jelly.

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1. Introduction

Nowadays, society shows a genuine concern for nutraceutical products derived from plants because their beneficial properties attributed to the content of bioactive compounds (Dillard & Bruce German, 2000). Consequently, capsules from green tea, royal jelly, soy or grapes are widely consumed. In this sense, several kinds of families of bioactive compounds are characteristic in each matrix. For instance, catechins are mainly present in green tea, whereas isoflavones are detected in soy-based products at high concentrations. Resveratrol is one of the characteristic compounds found in grapes, as well as anthocyanins, which are mostly accumulated in skins, whereas procyanidins are located in seeds (Yang, Martinson, & Liu, 2009). In relation to royal jelly, which is a secretion produced by the hypopharyngeal and mandibular glands of worker honeybees (Apis mellifera) is a complex matrix, containing water, proteins, lipids, carbohydrates, proteins (Garcia-Amoedo & De Almeida-Muradian, 2007) and polyphenols as apigenin, acacetin, biochanin A or chrysin (Ramadan & Al-Ghamdi, 2012; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008).

The concentration of these compounds can change during their storage or thermal treatment (Davidov-Pardo, Arozarena, & Marín-Arroyo, 2011), resulting in the appearance of transformation products. Thereby, the variations of the concentration of bioactive compounds in plant extracts (Correa et al., 2010; Jabri-Karoui & Marzouk, 2014; Song, Manganais, & Ferruzzi, 2015; Suárez, Romero, Ramo, & Motilva, 2011) or plant-derived products (e.g. infusions or nutraceutical products) have been evaluated in order to test several procedures that reduce their degradation (Rózek, García-Pérez, López, Güell, & Ferrando, 2010). However, this fact has been scarcely studied in nutraceutical products (Abd-Elsalam, Al-Ghobashy, Zaazaa, & Ibrahim, 2014; Friedman, Levin, Lee, & Kozukue, 2009) due to the common assumption that dry products might be inherently stable. However, some studies showed a progressive decrease in the bioactive compounds content during the storage period (Friedman et al., 2009; Wang, Sheen, & Chou, 2010). Moreover, it was observed that temperature could affect the concentration of bioactive compounds (Abd-Elsalam et al., 2014; Correa et al., 2010; Friedman et al., 2009; Rózek et al., 2010; Song et al., 2015; Suárez et al., 2011; Wang et al., 2010; Yang et al., 2009).

In order to determine possible transformation products originated during a long storage, liquid chromatography coupled to high resolution mass spectrometry (LC–HRMS) is a suitable tool, because they operate in full-scan mode and theoretically, unlimited number of compounds could be monitored. Moreover, this kind of analyzers offers the possibility of performing retrospective analysis, which allows the identification of substances not included in a target list (Gómez-Pérez, Romero-González, Vidal, & Frenich, 2015).

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In the current study, bioactive components present in four different commercial matrices (grape, tea, royal jelly and soy) stored at 5 °C have been monitored during one year to check the evolution of the detected compounds in these conditions, as well as to identify the presence of new bioactive compounds as a result of possible transformations occurred during storage conditions.

2. Experimental

2.1. Chemicals and reagents

Phytochemical standards were supplied from four different suppliers: Extrasynthese (Genay, France), Sigma–Aldrich (Madrid, Spain), ChromaDEX (Irvine, CA, USA) and Fluka (Steinheim, Germany). Purity was higher than 95% for all standards, except for some of them as chrysin, daidzin, kaempferol-7-*O*-glucoside and quercetin-3-*O*-glucoside and theaflavin, with a purity ⩾90%.

Standard solutions were prepared in methanol, ethanol, dimethyl sulfoxide or in a mixture of methanol:water (50:50 *v*/*v*), ranging from 90 to 750 mg L^{-1}. Three working solutions at 5000 μg L^{-1}, 500 μg L^{-1} and 50 μg L^{-1} were prepared in methanol and by appropriate dilution of aliquots of each individual stock standard solution. All solutions were stored in amber bottles at −18 °C in the dark and they were renewed every 6 months.

Acetonitrile (ACN) (LC–MS grade) was purchased by Fisher Scientific (Fair Lawn, NJ, USA). Dimethyl sulfoxide (HPLC grade) and methanol (MeOH) (LC–MS grade) were obtained from Sigma–Aldrich.

Ammonium acetate (purity 97%) and ethanol (HPLC grade) were supplied by Panreac (Barcelona, Spain). Formic acid (LC–MS grade) and water (LC–MS grade) were purchased by Scharlau (Barcelona, Spain). Hydrochloric acid was obtained from J.T. Baker (Deventer, The Netherlands).

The Orbitrap analyser used a mixture of acetic acid, caffeine, Met-Arg-Phe-Ala-acetate salt and Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive), and a mixture of acetic acid, sodium dodecyl sulfate, taurocholic acid sodium salt hydrate and Ultramark 1621 (fluorinated phosphazines) (ProteoMass LTQ/FT-Hybrid ESI negative) from Thermo-Fisher (Waltham, MA, USA) to accurate mass calibration.

2.2. Apparatus

A Centronic BL II centrifuge (J.P. Selecta, Barcelona, Spain), a Reax 2 rotatory agitator from Heidolph (Schwabach, Germany), and vortex mixer WX from Velp Scientifica (Usmate, Italy) as well as a coffee grinder (Orbit, Hong Kong, China) were used to process all samples.

2.3. UHPLC–Orbitrap–MS

Chromatographic apparatus consisted of an UHPLC system Transcend (Transcend 600 LC, Thermo Fisher Scientific, San Jose, CA, USA). Analyses were carried out using a Waters (Milford, MA, USA) Acquity C18 column (2.1 × 100 mm, 1.7 μm particle size).

For identification purposes, a single-stage Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific, Bremen, Germany) was used. The mass spectrometer was operated using a heated electrospray interface (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA), in positive (ESI+) and negative ionization (ESI−) modes.

Optimal ionization conditions were as follows: sheath gas (N_2, >95%), 35 (adimensional); auxiliary gas (N_2, >95%), 10 (adimensional); spray voltage, 4 kV (−4 kV in ESI−); skimmer voltage, 18 V (−18 V in ESI−); capillary voltage, 35 V (−35 V in ESI−); tube lens voltage, 95 V (−95 V in ESI−); heater temperature, 305 °C; capillary temperature, 300 °C. The automatic gain control (AGC) was set at a target value of 1×10^6.

The system was operated employing four alternating acquisition functions: (1) full MS, ESI+, without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 25,000 FWHM; scan time = 0.25 s; (2) all-ion fragmentation (AIF), ESI+, with fragmentation (HCD on, collision energy = 30 eV), mass resolving power = 10,000 FWHM; scan time = 0.10 s; (3) full MS, ESI− without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 25,000 FWHM; scan time = 0.25 s; (4) AIF, ESI−, with fragmentation (HCD on, collision energy = 30 eV), mass resolving power = 10,000 FWHM; scan time = 0.10 s. Considering the scan time of the four acquisition functions, and the polarity switching (approx. 0.27 s), an overall scan rate of 0.56 Hz was achieved. Mass range in the full scan experiments was set at *m*/*z* 100–1000, whereas for MS/MS, it was set from *m*/*z* 70–700.

All the analyses were performed without the use of lock mass, using external calibration mode. Mass accuracy was checked with multi-compound standards every day and the analyser was calibrated every two weeks with mass accuracy standards (see Section 2.1). The instrument was controlled and the data were analyzed on a computer equipped with Xcalibur™ version 2.2.1 (Thermo Fisher Scientific, Les Ulis, France) with Qual and Quan browser. ICIS peak detection was applied. For screening purposes ToxID™ 2.1.1 (automated compound screening software, Thermo Scientific) was used.

2.4. Experimental design

Four different types of commercial nutraceutical products (one royal jelly, one from grape and two from green tea (T1 and T2) and soy (S1 and S2)) were purchased in local markets located in Almería (Spain). In order to study the behavior of the samples after a long period of storage, capsules of nutraceuticals from green tea, grapes and soy from several brands were evaluated. In the case of royal jelly, samples were acquired as liquid presentations.

The identification of the compounds was performed using analytical methods developed previously in our research group (López-Gutiérrez, Aguilera-Luiz, Romero-González, Martínez, & Garrido, 2014; López-Gutiérrez, Romero-González, & Garrido, 2015; López-Gutiérrez, Romero-González, Garrido, & Martínez, 2014; López-Gutiérrez, Romero-González, Plaza-Bolaños, Martínez, & Garrido, 2015). The samples were analyzed throughout one year (acquisition of the product (t_0), 3 months (t_1), 6 months (t_2) and 12 months (t_3)). To eliminate variations between single capsules, the content of 20 capsules was homogenized using a coffee grinder. All samples were stored at 5 °C in a desiccant located in a refrigerator in a dark place.

3. Results and discussion

Taking into account that there are numerous articles that study the degradation of bioactive compounds when they are stored at room temperature or high temperatures (Abd-Elsalam et al., 2014; Correa et al., 2010; Friedman et al., 2009; Rózek et al., 2010; Song et al., 2015; Suárez et al., 2011; Wang et al., 2010), the current study was performed at low temperature in order to minimize potential degradation. Firstly, Table 1 shows the bioactive compounds detected when samples were acquired (t_0), whereas Table 2 shows the possible transformation of bioactive compounds found in four samples during storage. In order to get a reliable identification of the detected compounds, two diagnostic ions (one molecular and one fragment) with mass accuracy lower

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