Holistic evaluation of gamma-irradiation effects on green teas: New linear regression based approach applied to (+/-)ESI/MS and RPLC/UV data and comparison with PCA and CA chemometric methods

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ABSTRACT

The evaluation of the γ-irradiation effects on green teas was holistically achieved by means of a novel algorithm based on linear regression (LRA). This algorithm was compared to the discrimination power of Principal Component Analysis (PCA) and Cluster Analysis (CA). The holistic evaluation was based on positive or negative ion monitoring Electrospray Mass Spectrometry (+/−ESI/MS) data and Reversed Phase Liquid Chromatography with Ultraviolet Spectrometry detection (RPLC/UV) chromatograms, without involving any structural attribution and/or assay of the existing components. Five types of green teas (receiving irradiation doses of 0, 10 and 25 kGy) were considered. Extraction in ethanol and heated water was used. To ensure an increased definition of the profiles being compared, the LRA approach was applied on pairs of large experimental data series resulting from high frequency/high resolution acquisition rates, the resulting slopes, intercepts and correlation coefficients being considered as variables retaining the information contained in the raw data. The discrimination ability varied in the following order: LRA > CA > PCA. The information contained by the input data varied as following: (+)ESI/MS spectra > (-)ESI/MS spectra > RPLC/UV chromatograms.

1. Introduction

γ-Irradiation represents an effective way for processing and preserving the vegetal materials against microbial contamination and for increasing their storage stability (Farkas and Mohácsi-Farkas, 2011; Kumar et al., 2010; Chmielewski and Migdal, 2005; Jo et al., 2003; Thomas et al., 2008; Pereira et al., 2015). At low doses (i.e. 10 kGy), irradiation safely extends the shelf life of treated materials, without inducing formation of harmful radio nuclides (Lacroix and Quattara, 2000; FDA, 2005).

Green teas are well known for their proven uses in various therapies (Sano et al., 2004; Ide and Yamada, 2015; Fukuzawa et al., 2014; Dostal et al., 2015; Chen et al., 2015). Green teas are prepared from

unfermented leaves of *Camellia sinensis*, and are subjected to withering, pan-frying or steaming, rolling/shaping and drying before their distribution and consumption as infusions (Mishra et al., 2006).

In the literature, the study of irradiation of teas was focused in two directions: the influence of the irradiation processes on some classes of compounds (volatile fraction, amino acids, sugars, antioxidants) contained in teas (Fanaro et al., 2011, 2012, 2015; Kausar et al., 2013) and the selection of the analytical techniques being able to identify differentiations brought by the irradiation processes, including applied doses and cause-effects relationships, electron spin resonance (ESR) representing a method of choice (Polat and Korkmaz, 2008; Çam and Eng, 2010).

Other analytical techniques such as UV spectroscopy (Diniz et al.,

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2016), Near Infrared (NIR) spectroscopy (Chen et al., 2007), Direct Analysis in Real Time - Mass Spectrometry (DART-MS) (Fraser et al., 2013), High Resolution Melting Assay (HRM) (Xanthopoulou et al., 2016) and Ultra High Pressure Liquid Chromatography coupled to tandem Mass Spectrometry (UHPLC-MS/MS) (Svoboda et al., 2015) combined with various chemometric approaches were used for differentiation between different types of teas, their classification according to botanical/geographical origins, chemotypes, degree of fermentation, industrial processing methods and authentication.

The role of the chemometric approaches applied to (+/-) ESI/MS and RPLC/UV data in discriminating between standardized natural extracts was also underlined in one of our previous works (Medvedovici et al., 2014).

On our knowledge, there are no references in the literature about the evaluation of the γ-irradiation process of teas by applying chemometric approaches on (+/-) ESI/MS and RPLC/UV data. Mild ionization of a bulk mixture of compounds leads to a mass spectrum in which, at least theoretically, each ionisable compound is represented by its pseudo molecular ion. Even if the above statement is not entirely true, the number of ions generated through the ionization in mild conditions from the liquid state is significantly reduced compared to ionization from a gas phase, under electron impact conditions. Consequently, mass spectra obtained under mild ionization conditions of complex mixtures of compounds will retain information about all the ionized species, without the risk of overloading the spectral profiles. Comparison of such MS profiles, in a digitized form, may represent a valuable input for chemometric methods, for achieving discrimination against different mixtures submitted to analysis. Liquid chromatographic separations, carried out in such conditions to maximize selectivity, may also act, in digitized forms, as inputs for chemometric approaches. Such chemometric approaches are strictly holistic, without requiring structural identification/attribution and/or associated quantitative assays, being obviously based on comparison of shapes. Basic chemometric approaches, such Principal Component Analysis (PCA) or Cluster Analysis (CA) are, however, limited in their ability to compile very large input data sets, generally 1000 values series representing an upper limit. When strictly comparing shapes, their increased definition on acquisition of the profiles should be essential in achieving very sensitive differentiations. High frequency/resolution experimental profiles produce very large input data series (few thousands values for each chromatogram/spectra). In order to provide high definition profiles for comparison, we propose an alternative approach based on the Linear Regression Algorithm (LRA). Basically, the large data series were reciprocally compared by means of the linear regression approach, each individual comparison being then illustrated by the slope, the intercept and the correlation coefficient, variables retaining all the information from the initial sets of values. The principles of LRA were exposed in a previous work referring to discrimination among different types of green teas and influence of data pre-processing factors (Iorgulescu et al., 2016).

Five types of green teas were γ-irradiated at current doses of 10 and 25 kGy. Non-irradiated samples as well as the irradiated ones were then processed as aqueous infusions and ethanol extracts. Resulting solutions were separated by RPLC/UV or directly infused in an ESI source coupled to a quadrupole mass analyzer, monitoring either positive or negative ions. Chromatograms and MS spectra (in condensed or full formats) were then used as input data for PCA, CA and LRA algorithms. The final aim of our approach was to evaluate the ability of the chemometric algorithms to discriminate between the non-irradiated and irradiated materials.

2. Material and methods

2.1. Tea samples

Five commercial brands of green teas, namely Shutao, Twinnings,

Lipton, Celmar and Carrefour (further referred in text as teas 1–5) were obtained from the local market. No indication about any γ-irradiation treatments during production was found on the labels of the respective products.

2.2. Irradiation process

A GC-5000 Co-60 gamma irradiator was used for irradiation. Irradiation was carried out in the presence of air, at room temperature. An ECB dosimetric system was used for dose measurements. The absorbed dose was determined with a confidence level of 95%. All values of doses are expressed as absorbed dose in water. The green tea samples were irradiated at the doses of (10.2 ± 0.9) kGy and (25.5 ± 2.3) kGy, respectively. The irradiation time was 1 h 40 min and 4 h 10 min, respectively.

2.3. Sample preparation

Water infused samples were obtained through addition of 2 mL of boiling water to 100 mg of solid material (no mechanical processes were use to reduce the sample size or to control particle size). Vials previously tightly closed, are kept at 80 °C for 1 h. After cooling and centrifugation at $6000 \times g$, the supernatant was isolated, placed in an injection vial, sealed and used for experiments.

Ethanol extracts were obtained through addition of 2 mL of ethanol to 100 mg of solid sample (without any previous preparation of the solid material), at room temperature. The sealed vials were vortexed for 1 min at 2500 rpm and kept at room temperature and dark (an additional measure to eliminate the risks of any light influence on stability) for 7 days. After a final vortexing period of 5 min at 2500 rpm and centrifugation at $6000 \times g$, the supernatant was isolated in an injection vial, tightly sealed.

2.4. Reagents

Acetonitrile gradient grade was from Merck (Darmstadt, Germany). Ethanol of Chomasolv® grade and formic acid MS grade were from Sigma-Aldrich (Taufkirchen, Germany). HPLC grade water, characterized by a resistivity of min. 18.2 MΩ cm and a total residual organic carbon content (TOC) of max. 30 ng mL^{-1}, was produced with a TKA Lab HP 6UV/UF instrument (TKA Instruments as part of Thermo Fischer Scientific, Niederelbert, Germany).

2.5. Equipments

An Agilent 1260 series liquid chromatograph (Santa Clara, California, U.S.A.) with the following architecture, was used for RPLC separations: quaternary pump (G1311B) including a four-channel solvent degasser, automated injector (ALS - G1329B), column thermostat (TCC - G1316C), and a multi-channel UV–Vis detector (DVL - G1365D). System control and data acquisition were made with the Agilent Chemstation for LC 3D, version 04.03(16). A system built-up from the following Agilent modules: quaternary pump (G1311C) including a four-channel solvent degasser, automated injector (G1367E) with thermostat (G1316C), column thermostat (G1316C), electrospray ion source (G1948B) and a triple quadrupole mass spectrometer (G6410B) was used to acquire MS data. System control, data acquisition and processing were made with the Mass Hunter software, version B.06.00.

2.6. Chromatographic conditions

For the RPLC separations, an Eclipse XDB-C18 column, 150 mm length × 4.6 mm internal diameter × 3.5 μm particle size (Agilent Technologies) was used. The mobile phase contained acetonitrile (solvent A) and aqueous 0.1% formic acid (v/v) (solvent B) and was delivered at a flow rate of 1 mL min^{-1}. The column was thermostated at