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Analytical Methods

Development of a novel gold nanoparticle-based method to determine antioxidant capacity of Brassica oilseeds, white flakes and meal

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ABSTRACT

Antioxidant capacity (AC) of Brassica oilseeds, white flakes and meal was determined by a new spectrophotometric method. The proposed assay (AuNP) based on the formation of gold nanoparticles (AuNPs) in an acetic buffer medium (pH = 4.6) was compared with the previously described silver nanoparticle-based (AgNP), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocalteu (FC) procedures.

The novel AuNP method was validated using standard antioxidants such as phenolic acids and quercetin. The AC of rapeseed, white flakes and meal varied from 10.0 to 86.7 μmol sinapic acid (SA)/g, 26.5–160.3 μmol SA/g, 6.8–103.0 μmol SA/g, 23.0–259.3 μmol SA/g and 6.9–92.1 μmol SA/g for AuNP, AgNP, FRAP, DPPH and FC methods, respectively. The proposed AuNP method is simple, precise (intra-day RSD = 0.27–2.11% and inter-day RSD = 2.05–4.87%) and accurate (recovery = 96.2–104.3%) and can be useful in the routine analysis of the AC.

1. Introduction

Brassica oilseeds commonly known as rapeseed and its by-products (white flakes and meal) are rich sources of natural antioxidants such as: polyphenols (mainly sinapic acid, sinapine, canolol and condensed tannins), tocopherols, plastochromanol-8, carotenoids, ubiquinols, phospholipids, phytosterols and antioxidative enzymes, which exhibit antiradical activity (Wanasundara, Shahidi, & Shukla, 1997). Therefore, double zero rapeseed varieties are used for the production of the healthy vegetable oil containing high amounts of omega-3 fatty acids and antioxidants without glucosinolates and erucic acid. Moreover, during the technological operations the de-oiled rapeseed from the solvent extraction is treated in two ways: (1) by the desolventizing, toasting, drying and cooling processes to produce protein rich meal as ingredient in animal feed and (2) by the flash- or downdraft-desolventizing and cooling processes to obtain white flakes, which are an intermediate material for production of protein concentrates, isolates and flour added to human food and animal feed (Maenz, 2007). On the contrary to the toasted rapeseed meal, white flakes contain undenaturated proteins due to the very mild heat treatment, therefore the extruded compound feed possesses high protein dispersability index and antioxidants level. It is noteworthy that rapeseed, white flakes and meal are valuable sources of protein and bioactive compounds (Alashi et al., 2014).

In the last years it became a known fact, that reactive oxygen (ROS) and nitrogen (RNS) species can react with DNA, lipids, protein and are implicated in the etiology of many civilization diseases such as: cancer, stroke, autism, Alzheimer's dementia, arthritis, aging, cardiovascular, Parkinson's disease and others (Szydłowska-Czerniak, 2013). However, antioxidant compounds in rapeseed and its products exhibit an antioxidant potential, hence are important in the prevention and treatment of the mentioned diseases (Alashi et al., 2014; Hassas-Roudsari, Chang, Pegg, & Tyler, 2009; Szydłowska-Czerniak, 2013). Antioxidants present in rapeseed samples have gained much attention due to their antioxidant capacity (AC) and free radical scavenging abilities, which potentially have beneficial implications in human health. Therefore, development of the novel assay for the AC determination of rapeseed and its intermediate- and by-products would assist in modernization of technological processes.

Recently, different analytical methods including ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picryl-hydrazyl), FRAP (ferric reducing antioxidant power), reducing power, ORAC (oxygen radical absorbance capacity), inhibition of linoleic acid oxidation, SRSA (superoxide radical scavenging activity), ESR (electron spin resonance) and AgNP assay based on formation of silver nanoparticles were proposed for the evaluation of the AC of rapeseed cultivars, cakes and meal

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(Alashi et al., 2014; Dwiecki, Siger, Czubiński, Nogala-Kałucka, & Lampart-Szczapa, 2012; Hassas-Roudsari et al., 2009; Khattab, Goldberg, Lin, & Thiyam, 2010; Matthäus, 2002; Szydłowska-Czerniak, Amarowicz, & Szłyk, 2010; Szydłowska-Czerniak, Trokowski, Karlovits, & Szłyk, 2010; Szydłowska-Czerniak & Tułodziecka, 2013; Szydłowska-Czerniak, Tułodziecka, & Szłyk, 2012).

The conventional analytical procedures applied to analyze the total AC of Brassica oilseeds and meal products have relative advantages but also disadvantages, which should be reduced by their modification. However, metal nanoparticles have rarely been proposed to determine the AC of foods and plants. Although, gold nanoparticles-based (AuNP) method was used for assessing the AC of beverages, various teas (green tea, *Mentha piperita* tea and *Cymbopogon citratus* tea), juice samples, stem barks of plant, *Dalbergia sissoo* Roxb., fruits of amla and Indian gooseberry, honey, fresh apples and pears, wines, Marigold, Perigon, Artichoke, soybean (Choleva, Kappi, Giokas, & Vlessidis, 2015; Liu, Liu, Yuan, Wei, & Ye, 2012; Roy et al., 2011; Scampicchio et al., 2006; Vilela, Castañeda, González, Mendoza, & Escarpa, 2015; Vilela, González, & Escarpa, 2012, 2014, 2015). Also, Zougagh, Salghi, Dhair, and Rios (2011) proposed the spectrophotometric method based on the formation of gold nanoparticles (AuNPs) to determine total phenolic acids in virgin argan oils, whereas Pelle et al. (2015) have recently reported the AuNP assay to assess the AC of extra virgin olive oils.

Nevertheless, the AuNP method was not notified for the AC evaluation of rapeseed and its by-products. Furthermore, to the best of our knowledge, the AC of white flakes was not analyzed by any conventional analytical procedure.

For this reason, the aim of this work was the development and validation of the novel AuNP method based on the AuNPs formation for determination of antioxidant potential of rapeseed, white flakes and meal extracts prepared in organic solvents and water. Moreover, relationships between the AC of rapeseed, white flakes and meal extracts analyzed by the novel AuNP and the described previously AgNP, FRAP DPPH and FC procedures were compared, evaluated and discussed using correlation and principal component analyses (PCA).

2. Materials and methods

2.1. Chemicals

All reagents of analytical or HPLC grade were purchased from Sigma-Aldrich (Poznań, Poland), whereas organic solvents (99.8% ethanol and 99.8% methanol) of HPLC grade were provided by POCH (Gliwice, Poland). All solutions were prepared with redistilled water. Acetic buffer (pH = 4.6) containing 25.5 mL of acetic acid solution (0.2 mol/L) and 24.5 mL of sodium acetate solution (0.2 mol/L) was diluted by redistilled water to 100 mL. The pH were measured using a pH-meter (Mettler Toledo, SevenGo™).

2.2. Materials

A winter open pollinated *Brassica napus* L. cultivar with a reduced content of glucosinolates and without erucic acid (double low rapeseed, R), rapeseed white flakes (WF) and rapeseed meal (M) from a technological processes were kindly donated by the vegetable oil company. White flakes were obtained from the dehulled flaked rapeseed by extracting oil with hexane and flash-desolventizing the defatted rapeseed flakes to minimize protein denaturation. Rapeseed meal was obtained from the dehulled flaked rapeseed by extracting oil with hexane and then desolventizing the defatted flakes by means of high temperature thermal processing. Rapeseed, white flakes and meal were kept in the original packing (polyethylene terephthalate) at ambient temperature prior to the AC analysis.

2.3. Procedure of antioxidants extraction

The conventional solid–liquid extraction was applied to extract total antioxidants from samples according to procedure described previously (Szydłowska-Czerniak et al., 2012). The ground rapeseed, white flakes and meal (2.00 g) and 20 mL of ethanol, methanol, 50% methanol or water in conical flasks were shaken by using an orbital shaker WL-972 (JWElectronic, Poland) for 20 min. Extractions were carried out three times at room temperature. The combined extracts were centrifuged at 4500 rpm for 15 min (MPW-54, MPW MED. INSTRUMENTS, Poland). The extracts were stored in a refrigerator until the AC analysis.

2.4. Determination of antioxidant capacity

The AC of the obtained extracts was assayed by a new AuNP method. In this procedure, 0.5 mL of each extract, 1.0 mL of acetic buffer (pH = 4.6) and 1.0 mL of 3.9 mmol/L chloroauric acid (incubated at 60 °C for 10 min) were transferred into a 10 mL volumetric flask and made up to the mark with redistilled water. After 60 min, the absorbance of a purple solutions left in the dark at room temperature were measured at 540 nm against a reagent blank (1.0 mL of acetic buffer and 1.0 mL of 3.9 mmol/L chloroauric acid made up to 10 mL with redistilled water) using a 1 cm quartz cell in a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

Calibration curves for the proposed AuNP assay were obtained using working solutions of sinapic (SA), caffeic (CA) acids between 0.015 and 0.150 μmol/mL and 0.015–0.105 μmol/mL, 0.100–0.700 μmol/mL, 0.0015–0.0225 μmol/mL for gallic acid (GA), ferulic acid (FA) and quercetin (QR), respectively.

The AC results were expressed in μmol SA (main phenolic acid in rapeseed) equivalents per 1 g of real sample.

The reaction mixture (0.12 μmol SA/mL, 0.39 mmol/L chloroauric acid) was adjusted to pH = 3.6, 4.6, 5.6, 7.0, 7.6, 8.6 and 9.6 using acetic and ammonium buffers to evaluate the influence of pH on AuNPs formation. Moreover, the effect of time on $HAuCl_4$ solution incubation (5, 10, 15 and 20 min) and reduction of gold ions by SA methanolic solution for AuNPs generation was determined as follows: 2.0 mL of 0.6 μmol SA/mL, 1.0 mL of acetic buffer (pH = 4.6) and 1.0 mL of 3.9 mmol/L chloroauric acid (was incubated at 60 °C for 10 min) were placed in a 10 mL calibrated flask, made up to volume with redistilled water and absorbance at 540 nm against a reagent blank was measured for different intervals between 0 and 60 min. Intensity of mixtures (SA, $HAuCl_4$ incubated at different temperature: 20, 40, 60, 80 and 100 °C for 10 min, pH = 4.6) color was also estimated after 60 min at 540 nm.

Furthermore, the AC of the studied rapeseed, white flakes and meal was analyzed by spectrophotometric AgNP, FRAP, DPPH and Folin–Ciocalteu (FC) methods notified in our previous work (Szydłowska-Czerniak et al., 2012).

2.5. Characterization of gold nanoparticles

The 50% methanolic extract (0.5 mL) of rapeseed meal adjusted to pH = 4.6 and 1.0 mL of 3.9 mmol/L $HAuCl_4$ solution were transferred into 10 mL volumetric flask and made up to volume with redistilled water. The mixture was kept 1 h at ambient temperature in the dark. The TEM observations were performed using a transmission electron microscope (FEI Europe, Tecnai F20 X-Twin, Czech Republic) by dropping a few droplets of AuNPs suspension on a carbon-covered microgrid and then it was allowed to evaporate of solvent under ambient conditions. The morphology of the generated AuNPs was visualized by an atomic force microscopy