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Biologically active components in seeds of three *Nicotiana* species

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ABSTRACT

Tobacco seeds, available as an untitilized byproduct of the commercial leaf production in many countries around the world, can be a valuable source of natural products with biological activity. The genus *Nicotiana* (Solanaceae) includes more than 65 species, but the most economically important and commercially cultivated is *Nicotiana tabacum* L. (common tobacco) and to a much lesser degree *N. rustica* L. (Aztec tobacco). The objective of this study was to evaluate tobacco seed oil and cakes of three tobacco species: two genotypes of *N. alata* Link & Otto (jasmine tobacco), *N. rustica*, and *N. tabacum*, with respect to their potential uses. The glyceride seed oil content was 37.6% and 40.9% for the two genotypes of *N. alata*, 37.5% for *N. rustica,* and 30.9% for *N. tabacum*. Overall, the content of phospholipids was 0.2–0.3% in the oils. Total sterol amount in the oils was 0.35–0.48%. The main component was β-sitosterol, followed by cholesterol and Δ^5-avenasterol (in *N. alata*, white petals), cholesterol and campesterol (in *N. alata*, pink petals), campesterol and Δ^5-avenasterol (in *N. rustica*), and campesterol and stigmasterol (in *N. tabacum*). In the tocopherol fraction (101, 117, 178, and 106 $mg kg^{-1}$ in *N. alata* white and pink forms, *N. rustica*, and *N. tabacum*, respectively in the oils) the most predominant (higher than 97%) was the γ-tocopherol. Overall, the main fatty acids (FA) in the three *Nicotiana* species were linoleic (61.7–67.6% range), oleic (15.5–19.0%), and palmitic (9.1–12.5%). The remaining seed cakes (after Soxhlet extraction of glyceride oil), potentially valuable nutrient by-products, were characterized with regard to their content of minerals, cellulose, proteins, and amino acids. Seed cake cellulose content varied between 32.5 and 45.2%, and protein content was 26.7–34.1%. Seed cakes were rich in mineral macro- and micronutrients, with some differences between the species. Highest content of total nitrogen and protein were found in *N. rustica* (5.5% and 34.1%, respetively), and highest content of cellulose was found in *N. alata* (white petals genotype). Potassium concentration was higher in *N. tabacum* and *N. rustica* cakes, whereas the concentrations of iron and zinc were highest in *N. alata* (pink petals genotype). Amino acid composition was dominated by aspartic acid, arginine, and threonine in *N. alata,* and by arginine, aspartic acid, and histidine in *N. rustica.* The results suggest potential alternative uses of tobacco seeds and cake as animal feed, and possibly as feedstock for new consumer human health products. The concentration of amino acids important for animal nutrition (lysine, methionine, and cysteine) in tobacco seed cakes was low, implying a need for careful combination with other animal diet ingredients.

1. Introduction

Tobacco is a perennial plant from the Solanaceae family, grown as an important cash crop for over 350 years in many countries around the world. Although the genus includes more than 65 species, *Nicotiana tabacum* L. (common tobacco) is the only commercially cultivated and economically important species. *Nicotiana rustica* L. (also known as Aztec or wild tobacco), is characterized by up to nine times more nicotine than common tobacco, and has been and continues to be used in very limited amounts in Mexico, Russia, South America, Vietnam, and other Asian countries (Kishore, 2014; Yadav et al., 2016).

Tobacco has long been a cash crop for Bulgaria, with great impact on the national economy. Three types of *N. tabacum* (common tobacco) are grown in Bulgaria: sun-cured Oriental (basma and Kaba Koulak),

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flue-cured Virginia bright, and air-cured Burley. Cured and fermented tobacco leaves are used for the production of various smoking and smokeless tobacco products, and their chemical composition and biological activity have been studied extensively (Rodgman and Perfetti, 2013). The tobacco plant, however, offers a wide range of alternative applications, such as production of biopellets from tobacco stalks as a renewable energy source, biodiesel from tobacco seeds, seed oil for the nutraceutical market, seed oil and cake for animal feeds, silage mixes, and more (Grisan et al., 2016; Rossi et al., 2013; Xie et al., 2011).

Common tobacco seeds have been considered as a source of glyceride oil, and its chemical composition has been studied previously (Ali et al., 2008; Xie et al., 2011; Zlatanov et al., 2007a). Generally, it has been shown that the yield of common tobacco seed oil and its fatty acid (FA) profile are influenced by genotype, climatic conditions, and other factors (Grisan et al., 2016; Mohammad and Tahir, 2014; Rossi et al., 2013; Zlatanov and Menkov, 2002). In previous studies, seeds from *N. tabacum* ecotypes and varieties grown in Bulgaria showed that sun-cured small-leaf Oriental tobaccos contain 30–41% glyceride oil, 1.0–1.7% phospholipids, 0.3–0.8% sterols, and 5–72 $mg\,kg^{-1}$ tocopherols. The broad-leaf tobaccos (Virginia and Burley) oil content was 38–49%, 0.3–1.5% phospholipids, 0.3–0.8% sterols, and 2–195 $mg\,kg^{-1}$ tocopherols (Zlatanov and Menkov, 2002; Zlatanov et al., 2006). In another study, the oil obtained from the seeds of broad-leaf and Oriental tobaccos contained palmitic, oleic, and linoleic FAs, with significant differences between these two genotypes of common tobacco. The dominant compounds in the sterol fraction in Oriental and broad-leaf tobacco genotypes were β-sitosterol, stigmasterol, and campesterol, again with significant differences in their content between the two genotypes. In the tocopherol fraction of Oriental and broad-leaf genotypes γ-tocopherol was dominant, and in the phospholipid fraction, phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine were the primary components, once again with significant differences in their content between the genotypes (Zlatanov and Menkov, 2002; Zlatanov et al., 2007a,b).

The chemical composition of common tobacco seeds (e.g. lipids, protein, fiber, ash) and seed oils (FAs, sterols, tocopherols, etc.) showed significant variation depending on tobacco type, variety, climatic conditions, and other factors (Ali et al., 2008; Awolola et al., 2010; Grisan et al., 2016; Majdi et al., 2012; Mohammad and Tahir, 2014; Mukhtar et al., 2006, 2007; Rossi et al., 2013; Srbinoska et al., 2005; Stanisavljevic et al., 2007b, 2009; Talaqani et al., 1986).

The seed cake remaining after extraction or cold pressing of tobacco seed oil could be considered the integrating element between biofuel and food production. Because of the presence of various biologically active substances (protein, amino acids, fatty acids, minerals, etc.) and the absence of the toxic and unpalatable nicotine, this by-product potentially could be used in animal nutrition or as a delivery system for edible vaccines to animals (Rossi et al., 2013).

As stated above, tobacco seeds contain various bioactive components with nutritional value; composition varies depending on seed origin: protein, from 18 to 41% (Ali et al., 2008; Frega et al., 1991; Mohammad and Tahir, 2014; Rossi et al., 2013; Srbinoska et al., 2005) and fiber, from 3.7 to 21.8% (Ali et al., 2008; Frega et al., 1991; Mohammad and Tahir, 2014; Srbinoska et al., 2005). In Italy, seeds of three different varieties (Bright Italia, Kentucky 104, and Bright V) had different composition of lipids, protein, fiber, and sterols (Grisan et al., 2016). Amino acid composition of tobacco seeds has also been analysed, and the results reflect the observed variations of seed chemical composition (Frega et al., 1991; Rossi et al., 2013). In a recent study, Faugno et al. (2016) established the optimal conditions (in terms of oil yield) for mechanical oil extraction from tobacco seeds, after which remains 13% oil residue in seed cakes. This component could be considered an additional asset, from a nutrition point of view, for tobacco seed cakes, if this more economic, healthy, and environmentally acceptable method for oil production gains wider popularity.

There is limited information about the chemical composition of seeds and seed oils in other tobacco species grown side by side (Koiwai et al., 1983). In 2015, the Tobacco and Tobacco Products Institute in Bulgaria started experimentally growing two (uncommon for the country) tobacco species, *N. alata* Link. & Otto and *N. rustica* L., with the intention of widening the scope of biologically active products obtained from tobacco leaves and seeds. It was hypothesized that the chemical composition of *N. alata*, *N. rustica*, and *N. tabacum* grown side by side would differ, and would depend on genotype. The objective of this study was to present a comparative analysis of biologically active compounds in seeds, seed oils, and seed cakes from two tobacco species introduced in Bulgaria (*N. alata* and *N. rustica*), and compare those with *N. tabacum* (common tobacco).

2. Materials and methods

2.1. Chemicals

All chemicals used for the analyses were of the required purity and no additional purification was done. The necessary reference samples for the identification of phospholipids were supplied from Fluka (Chemie 50 Gmbh, Switzerland), and the standard tocopherols and sterols were from Merck (Darmstadt, Germany). Thin-layer chromatography (TLC) was done on plates covered with Silica gel 60G (Merck, Darmstadt, Germany). Acetonitrile (HPLC grade) was from Sigma (Sigma-Aldrich Chemie GmbH, Germany). Aminoacid standards were purchased from Waters (Eschborn, Germany). 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQ Fluor Reagent) was used for the derivatization of amino acids.

2.2. Samples

Seeds were collected from plants of *Nicotiana alata* (represented by two genotypes, white and pink petals, flowers at full blossom); of *N. rustica* var. *rustica*, and of *N. tabacum,* variety Plovdiv 7, crop 2016. The tobacco plants were grown under identical agro-ecological and meteorological conditions on the experimental field of the Tobacco and Tobacco Products Institute (part of Bulgarian Agricultural Academy), situated in the region of Plovdiv, southern Bulgaria.

The soil type was hummus-carbonate (rendzina) with the following agrochemical characteristics: organic matter content (by Turin) 2.31%; total nitrogen (N) content (by Kjeldahl) 0.212%; mobile forms of phosphorus P_2O_5 (by Egner – Reem) 14.85 mg/100 g soil; available potassium K_2O (by Milcheva) 67.5 mg/100 g soil; soil reaction (pH in H_2O) 8.2 (Arinushkina, 1970). The meteorological conditions during the vegetation period (June–September) were characterized by an average monthly temperature of 22 °C and an average amount of rainfall of 44.5 mm. Plants were irrigated twice during the vegetation period.

Seed cakes were taken after the Soxhlet extraction of tobacco seeds with *n*-hexane and air dried.

Moisture of the fresh seeds and seed cakes was determined by oven-drying at 103 ± 2 °C (Peeva and Popova, 2007) and all values of the chemical composition of seeds were presented on absolute DW basis. The absolute weight of randomly selected 1000 seeds was determined using an electronic precision balance (Mettler-Toledo, ± 0.0001 g).

The angle of friction (θ) represents the angle (°) from the horizontal at which the seeds begin to slide on a gradually sloping surface. Two values of the angle of friction were measured $-\theta_{min}$, at which the sliding of seeds begins, and θ_{max}, at which the last seeds slide. The angle of friction was measured on two different surfaces (stainless steel and carbon steel), which are commonly used for the processing of grains (Stoyanova et al., 2008). The coefficient of static friction (μ) was calculated as $\mu = tg(\theta)$, where θ is the angle of friction from the measurements (respectively – θ_{min} and θ_{max}) for the respective surface (Stoyanova et al., 2008).