Journal of Food Composition and Analysis 56 (2017) 11–17

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original research article

Soybean spermidine concentration: Genetic and environmental variation of a potential 'anti-aging' constituent

Tatsuya Sagara[a], Gregor Fiechter[b], Martin Pachner[a], Helmut K. Mayer[b], Johann Vollmann[a,*]

[a] University of Natural Resources and Life Sciences Vienna (BOKU), Dept. Crop Sciences, Konrad Lorenz Str. 24, 3430 Tulln an der Donau, Austria
[b] University of Natural Resources and Life Sciences Vienna (BOKU), Dept. Food Sci. & Technol., Muthgasse 11, 1190 Vienna, Austria

ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form 15 November 2016
Accepted 20 November 2016
Available online 21 November 2016

Keywords:
Soybean (*Glycine max*)
Spermidine
Polyamines
Free amino acids
Seed protein content
Food analysis
Food composition
Functional food
Ultra-high performance liquid chromatography

ABSTRACT

Soybean seed is one of the richest food sources of spermidine and other polyamines. Recent findings from human and animal models have confirmed spermidine as a potential anti-aging substance acting through the initiation of autophagy pathways as well as through antioxidant and anti-inflammatory properties. As this might be of relevance for selecting soybeans for soy food production, the present research addresses the natural variation of spermidine concentration of soybean to determine the influences of genotype and environmental factors on spermidine and other polyamines, and to study possible relationships between spermidine and major seed quality traits. Sixteen early maturity soybean genotypes were grown near Vienna, Austria for three seasons, and harvest samples were subject to ultra-high performance liquid chromatography (UHPLC) for determining concentrations of polyamines and free amino acids. Based on individual samples, spermidine concentration ranged between 167 and 291 mg kg^{-1} dry seed, and both genotype and growing season significantly affected spermidine level. Spermidine concentration was closely correlated to putrescine but was not related to seed protein content or other major seed constituents determined by NIRS analysis. These results demonstrate the feasibility of plant breeding approaches to modify the spermidine level of soybean which might support the future development of functional soy foods.

1. Introduction

Soybean (*Glycine max* [L.] Merr.) is the major protein and oilseed crop grown on over 117 million ha worldwide in 2014 (FAOSTAT, 2016). While most of the soybean harvest is processed for preparing protein-rich meal for livestock feeding and vegetable oil, an increasing rate of soybean is utilized for human food production which is due to the superior soybean seed composition made up of 42.1% protein and 19.5% oil on average (Wilson, 2004). Apart from its macronutritional composition, soybean contains a number of bioactive compounds such as isoflavones adding health-protecting functional properties to soy-based foods. Thus, associations between soy food consumption and a reduced risk of cardiometabolic diseases (Anderson et al., 1995; Messina, 2010) as well as chemoprevention lowering the risk of carcinogenesis (Sánchez-Chino et al., 2015) are well established from epidemiologic and cellular studies. As a consequence, health claims acknowledging the nutritional and health benefits of soybean protein and isoflavones have been granted in different countries (Xiao, 2008).

Soybean seed is also known to contain polyamines such as spermidine, spermine, putrescine and cadaverine. In particular, dry soybean is a rich source of spermidine ranging from 88 to 389 mg kg^{-1}according to different investigations (Gloria et al., 2005; Kalač et al., 2005; Nishibori et al., 2007). Thus, the spermidine level of soybean is considerably higher than that of common cereals, vegetables, root crops, fruits or animal food products (Kalač, 2014; Nishibori et al., 2007). This might be due to the superior seed protein content of soybean and in particular due to the high level of arginine which is a major precursor of polyamine biosynthesis (Miller-Fleming et al., 2015). The presence of spermidine has also been confirmed for different soy foods, although spermidine concentrations appear to be rather variable in different types of soy food: Generally, natto and tempeh have been identified as high,

* Corresponding author.
E-mail address: johann.vollmann@boku.ac.at (J. Vollmann).

http://dx.doi.org/10.1016/j.jfca.2016.11.008
0889-1575/

and tofu, soy sauce, sprouts or soy milk as low in spermidine concentration, respectively (Kim et al., 2012; Nishibori et al., 2007; Toro-Funes et al., 2015).

On a cellular level, spermidine has various important functional and regulatory properties related to the physiology of cell aging. In animal models, spermidine application reversed age-induced memory impairment and neurodegeneration through induction of autophagy (Gupta et al., 2013) which also affected lipid metabolism inducing changes in neuronal fatty acid composition (Minois et al., 2014). In human diabetes patients, spermidine-induced autophagy has been shown to reduce endothelial dysfunction which might decrease the risk of cardiovascular disease (Fetterman et al., 2016). Apart from autophagy, antioxidant and anti-inflammatory protective effects of spermidine have been demonstrated for neurodegenerative disease models (Jamwal and Kumar, 2016). As a consequence of the growing body of such findings, a number of molecular mechanisms of polyamine and particularly spermidine action have been summarized with respect to their significant anti-aging and lifespan extension effects (Minois, 2014; Soda, 2015). Recently, human intervention studies towards extending a healthy lifespan and reducing age-dependent diseases have been proposed (De Cabo et al., 2014; Longo et al., 2015). As spermidine is a nontoxic natural substance, it is considered a safe and promising candidate for clinical testing besides a very few other substances. Moreover, an elevated blood polyamine concentration in humans could rather easily be achieved by administering a high polyamine test diet through daily food supplementation with natto or other soy products, as demonstrated by Soda et al. (2009) in a preliminary study.

The growing evidence of anti-aging and other positive effects associated with spermidine suggests that the consumption of soy foods rich in spermidine and other polyamines might bear significant health effects in addition to the well-studied isoflavone components. As these findings have not been considered so far in agricultural soybean research, the objective of the present study is to investigate genetic and environmental variation in the concentration of spermidine and other polyamines in a range of soybean genotypes differing in seed protein content, and to elaborate possible relationships between spermidine and other seed quality traits such as macro-nutritional components and free amino acids. This would be of particular relevance, as information on natural variation in spermidine concentration of soybean seed is a prerequisite for developing soy food products high in polyamines. As conjugated polyamines are present at a much lower magnitude in soybean than free polyamines (Righetti et al., 2008), the present research is dealing with free polyamines only (*i.e.* cadaverine, putrescine, spermidine and spermine) without further consideration of conjugated forms.

2. Materials and methods

2.1. Plant materials and chemicals

A total of 16 soybean (*Glycine max* [L.] Merr.) genotypes of early maturity (soybean maturity groups 000 to 0 suitable for cultivation in Central European latitudes) comprising both soybean cultivars and advanced breeding lines from different crosses were used in field experiments. The selection of genotypes was based on covering a wide range of different seed protein contents, as it was hypothesized that differences in seed protein content might indicate variation in nitrogen metabolism, thus affecting spermidine and other polyamines as well.

Reagents and standards of both amino acids and polyamines were obtained either from Fluka (Buchs, Switzerland) or Sigma (St. Louis, MO, USA). Full details of all analytical chemicals used have been described elsewhere (Fiechter et al., 2013).

2.2. Field experiments

Field experiments were carried out at the Gross Enzersdorf experimental station located 25 km east of Vienna (Austria) over the three growing seasons (environments) 2012, 2013, and 2014. Each individual experiment was planted using a randomized complete block (RCB) design with two replications; soybeans were grown in single row plots of 2.5 m plot length and 50 cm row spacing. Before sowing, seed was inoculated with rhizobial bacteria (*Bradyrhizobium japonicum* [Kirchner] Jordan) using Nodular G (Serbios, Badia Polesine, Italy) for promoting nodule formation and symbiotic di-nitrogen fixation, and no additional nitrogen fertilizer was applied. At full maturity, soybean was harvested and seed was air-dried under ambient temperature conditions to a moisture level of 9–10%.

2.3. Seed protein, oil, sucrose and thousand seed weight

Ten grams of finely ground soybean seed (Cyclotec 1093 mill, Foss Tecator, Höganäs, Sweden, 2.0 mm mesh sieve) were subject to near-infrared reflectance spectroscopy (NIRS) analysis. A Bruker Matrix-I Fourier transform instrument (Bruker, Ettlingen, Germany) equipped with a RT-PbS detector unit was used for collecting near-infrared spectra. For spectroscopic prediction of seed oil, protein and sucrose content (given in g kg^{-1} based on dry matter), soybean specific calibration models were utilized as described elsewhere (Sato et al., 2012; Vollmann et al., 2011). As a measure of seed mass and seed size, thousand seed weight was calculated after counting and weighing 2×100 seeds from each sample.

2.4. Polyamines and free amino acids

Dry soybean samples (5 g per sample) were finely ground in a steel ball mill (Retsch Mixer Mill MM 400 with 50 mL stainless steel grinding jars, Retsch GmbH., Haan, Germany), and triplicate extraction of polyamines and free amino acids was carried out with 0.6 M perchloric acid. Ultra-high performance liquid chromatography (UHPLC) was carried out on a Waters Acquity™ Ultra Performance LC (UPLC™) unit (Waters, Milford, MA, USA). Sample preparation, extraction of analytes and UHPLC conditions have been described in full detail by Fiechter et al. (2013). Pre-column derivatization of polyamines and free amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) was performed using Waters AccQ.Fluor™ reagent kit (Waters, 1993). Briefly, neutralized soybean extracts or standard solutions (5 μL) were mixed with 35 μL AccQ.Fluor™ borate buffer and 10 μL AQC reagent. Mixtures were immediately vortexed for 10 s and left to incubate for 1 min at room temperature before being heated at 55 °C for 10 min to complete the derivatization reaction. Derivatized sample solutions were then filtered through a 0.20 μm syringe filter (Sartorius, Goettingen, Germany) and subject to chromatographic analysis. The protocol applied in the present study had previously been developed as a multi-analyte procedure for facilitating a simultaneous separation of major free amino acids and polyamines in food products. External standards were used for quantification of the 4 polyamines and 21 free amino acids (summarized in Table 1 and Fig. 1.), and calibration lines were established for each individual amino acid and polyamine analyzed in this study. After derivatization (equal to an additional 1:10 dilution), *on-column* amounts of AQC derivatized amino acids and polyamines ranged from 2 to 100 pmol per injection and 25 pmol for norvaline. Linear regressions were calculated and were found to be appropriate ($R^2 > 0.99$) within a linear working range of 2–50 pmol per injection for both amino acids and polyamines, respectively. An internal standard (e.g., norvaline) was additionally