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Polyamines content in plant derived food: A comparison between soybean and Jerusalem artichoke

Laura Righetti, Annalisa Tassoni, Nello Bagni *

Department of Experimental Evolutionary Biology, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

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ABSTRACT

This paper aims to compare the dietary polyamine intake coming from foods derived from two different plants: soybean (*Glycine max* L.) well known and universally utilised both fresh and processed, and Jerusalem artichoke (*Helianthus tuberosus* L.) tuber, not yet well known and scarcely utilised in the everyday diet. Free, soluble and insoluble conjugated polyamines were determined in different soy-derived foodstuffs such as milk, tofu and fermented soy sauce, and in soybeans coming from two different organic experimental fields (Imola and Altedo, Bologna, Italy). Results show that free polyamines (in particular putrescine and spermidine) were present in relevant amounts especially in tofu and soy sauce. Conversely, the *Helianthus* parenchymatous medulla tissue, which is the only edible part of the tuber, contains very low levels of polyamines, which are instead preferentially accumulated in the buds. These data could suggest a preferential utilisation of *Helianthus* tuber in the diet of people with special needs, such as patients treated by chemotherapy and patients with diabetes.

1. Introduction

Amines are basic nitrogenous compounds in which 1, 2, and 3 atoms of hydrogen in alkyl groups are replaced by ammonia. They can be classified either by the number of hydrogen atoms substituted in ammonia as primary, secondary and tertiary amines, or by the number of basic groups (NH or NH_2) as mono-, di- and polyamines. The term "biogenic amines" defines decarboxylation products such as histamine, serotonine, tyramine, phenylethylamine, tryptamine, and also aliphatic polyamines. Aliphatic polyamines such as spermidine and spermine, but also the diamines putrescine, cadaverine and agmatine, are essential for normal and neoplastic cell growth and proliferation, and can be ubiquitously synthesised from their aminoacidic precursors (Bagni & Tassoni, 2001).

Food contains a significant amount of polyamines and related compounds that are present either naturally or as a result of processing, storage and spoilage (Larqué, Sabater-Molina, & Zamora, 2007; Zoumas-Morse et al., 2007). Dietary free polyamines can be almost completely absorbed in the small intestine (Noack, Kleessen, & Blaut, 1999). However, the proportion of polyamines which may affect the large intestinal mucosa tissue is also influenced by microbial production. In fact, polyamines found in the gut lumen can be derived from the diet, synthesised by bacteria or originated from endogenous sources such as epithelial cells shed into the gut lumen (Pegg, 1986), and are involved in intestinal growth and differentiation (Peulen & Dendrifosse, 2002). After intestinal absorption, uptaken polyamines are successively metabolised in different tissues and can be eliminated from the organism by means of oxidation reactions, appearing in the urine in all their metabolic forms. The dietary intake of exogenous polyamines can be enhanced by growth factors and hormones, as well as by inhibition of intracellular biosynthesis (Morgan, Brooks, Rajanayagam, O'Sullivan, & Golding, 2000).

Polyamine levels have been determined in foods and drinks coming from animal and plant sources (see for example Bardócz, 1993; Larqué et al., 2007; Nishimura, Shiina, Kashiwagi, & Igarashi, 2006; Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997), even though for plant derived food these analyses have not generally taken into consideration the possibility of utilising specific plant derived foods for patients affected by diseases that need special feeding. Finally, very few studies have focused their attention on polyamines conjugated to hydroxycinnamic acids that are particularly abundant in some plant foods such those derived from Solanaceae (Bagni et al., 2000).

This paper aims to compare the dietary polyamine intake coming from foods derived from two different plants: soybean (*Glycine max* L.) well known and universally utilised both fresh and processed, and the tuber of *Helianthus tuberosus* (Jerusalem artichoke), not yet well known and scarcely utilised in everyday diet, at least in Italy. For its characteristics *Helianthus* tuber could become very

Abbreviations: Dap, diaminopropane; Put, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine; PCA, perchloric acid.

* Corresponding author. Tel.: +39 051 2091280; fax: +39 051 242576.
E-mail address: nello.bagni@unibo.it (N. Bagni).

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important in the diet of people that need special feeding, such as patients treated by chemotherapy and patients with diabetes.

2. Materials and methods

2.1. Materials

Organic soybean (*Glycine max* L.) seeds were obtained from experimental fields in Imola (BL3 Thesis 2) and Altedo (BL1 Thesis 1), Bologna, Italy. Soybean foodstuffs were purchased from local markets and in particular soy germs by SAB ortofrutta (Telgate, Bergamo, Italy), soy milk by Alce Nero (Monterenzio, Bologna, Italy), tofu by Compagnia Italiana Alimenti Biologici e Salutistici Srl (Bagnacavallo, Ravenna, Italy). Soy sauce by Kikkoman (Kikkoman International Inc., Brownsdale, MN, USA) was naturally fermented by *Aspergillus oryzae*.

H. tuberosus L. var. OB1 was grown in the Botanical Garden of the University of Bologna. For many years this plant was exclusively propagated by vegetative reproduction from one single original plant due to the sterility of the inflorescence, and, therefore, providing a genetically homogenous material. The dormant tubers were harvested at the end of November.

2.2. Polyamines and hydroxycinnamic acids determination

Polyamines determination by HPLC were performed as described by Tassoni, van Buuren, Franceschetti, Fornalè, and Bagni (2000).

Hydroxycinnamic acids were extracted from about 0.5 g FW of soybean seeds homogenised with 5 ml of 95% (v/v) methanol, incubated overnight in the dark at room temperature in a rotatory shaker and filtered through Whatman GF/B filters. The extracted volume was concentrated in a speed vacuum at 45 °C (Speed Vac PD1, Savant Instruments, Holbrook, NY, USA) and then diluted with water to reduced methanol concentration below 5% (v/v). The aqueous phase was loaded onto a Strata-X column (33 μm polymeric sorbent 60 mg/3 ml, Phenomenex, Torrence CA, USA) and hydroxycinnamic acids were eluted by 100% (v/v) methanol. The methanolic samples were completely dried and resuspended up to 200 μl of 1/10 acetonitrile/0.2% (v/v) acetic acid before being directly injected into the HPLC. Hydroxycinnamic acids were analysed by reverse-phase HPLC separation (Jasco Instruments, Großumstad, Germany; column Phenomenex Luna C18(2), 5 μm particles 250 × 4.6 mm, Phenomenex, Torrence CA, USA; pre-column SecurityGuard Ea, Phenomenex) equipped with an on-line diode array detector (MD-2010, Plus, Jasco Instruments), using a column oven set at 40 °C. A multi-step gradient method was applied, using acetonitrile as solvent A and water–acetic acid (99.8/0.2; v/v) mixture as solvent B. The gradient profile was: 0–3 min 9% A; 3–8 min from 9% to 14% A, 8–10 min from 14% to 16% A, 10–13 min from 16% to 20% A, 13–17 min from 20% to 37% A, 17–24 min 37% A, 24–27 min from 37% to 100% A, 27–29 min 100% A, 29–33 min from 100% to 9% A, 33–37 min 9% A, with a flow rate of 1 ml/min. Chromatograms were analysed at three different wavelengths: 285 nm for cinnamic acid, 308 nm for *p*-coumaric acid and 323 nm for caffeic, ferulic acids.

All experiments were repeated twice with similar results. All measurements were done in triplicate and the standard error did not exceed the 15% of the value.

3. Results

3.1. Polyamine content in soy-derived foodstuffs

Free, PCA-soluble and PCA-insoluble conjugate polyamines were determined in different soy-derived foodstuffs, fermented soy sauce and soybeans coming from two different organic experimental fields (Imola and Altedo). Free diaminopropane (Dap), putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm) were detected (Fig. 1), with spermidine present in higher amount in all the samples with the exception of soy sauce and soy germs, in which putrescine was more abundant. In the case of soy germs, putrescine reached 320 μM of concentration over a total free polyamine amount of 450 μM. Lower levels of polyamines were present in soy sauce, soy milk and tofu (Fig. 1). Soybeans grown in Imola organic experimental fields displayed higher free polyamines level with respect to those grown in Altedo fields. Tofu was the only soybean food containing an appreciable amount of conjugated polyamines, in particular PCA-soluble putrescine and spermidine and PCA-insoluble cadaverine (Figs. 2 and 3).

In fact, in tofu the PCA-soluble and insoluble polyamines represented, respectively, the 19% and 6% of total polyamines. PCA-insoluble conjugated polyamines were not determined in soy sauce, because the PCA extract did not present solid residues.

3.2. Polyamine content in H. tuberosus tubers

Free, PCA-soluble and PCA-insoluble conjugate polyamines were determined in different organs and tissues of *H. tuberosus* (Jerusalem artichoke). Also in *Helianthus* tuber such as in soybean foodstuffs, free spermidine was the more abundant polyamine,

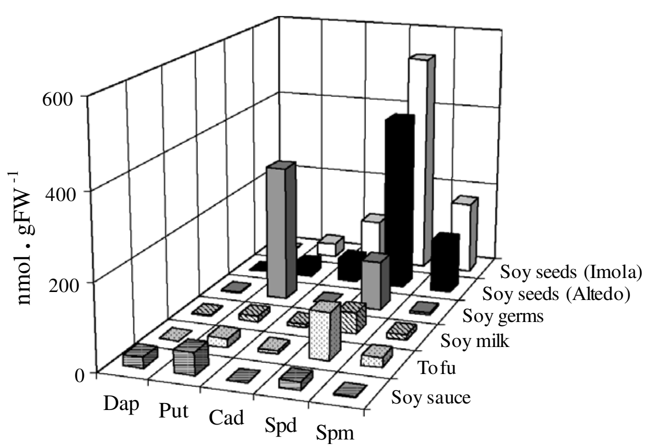

Fig. 1. Free polyamine content (nmol g FW^{-1}) in several soybean foods.

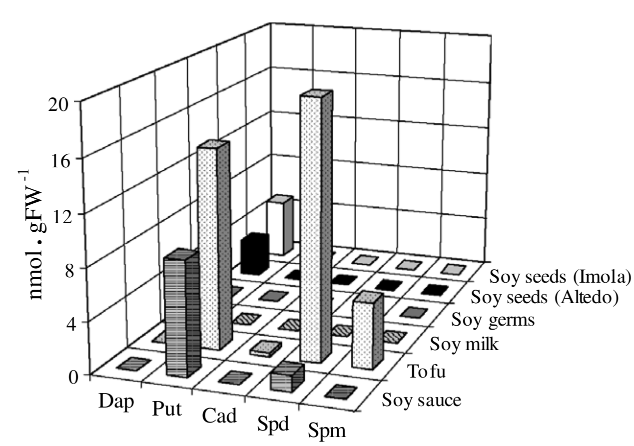

Fig. 2. PCA-soluble conjugated polyamine content (nmol g FW^{-1}) in several soybean foods.