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

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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

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

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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,

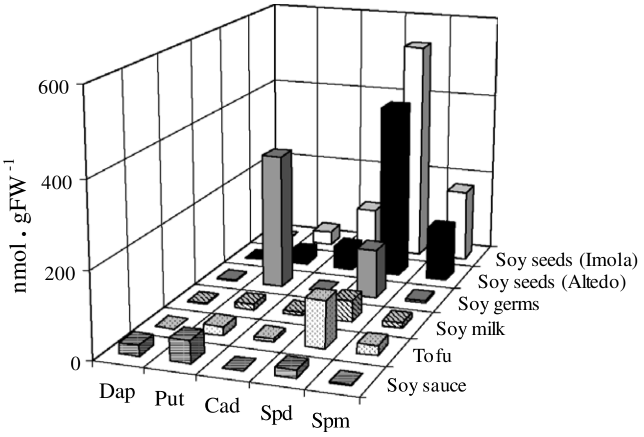

Fig. 1. Free polyamine content (nmol g FW^{-1}) in several soybean foods.

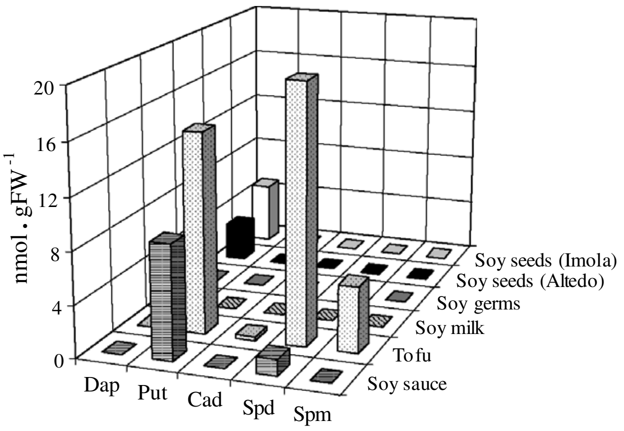

Fig. 2. PCA-soluble conjugated polyamine content (nmol g FW^{-1}) in several soybean foods.

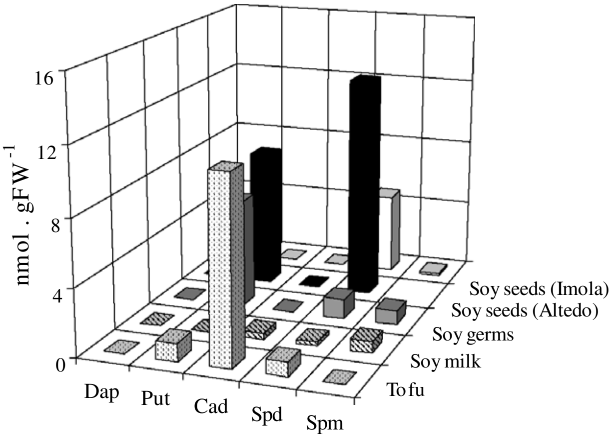

Fig. 3. PCA-insoluble conjugated polyamine content (nmol g FW^{-1}) in several soybean foods.

reaching 184 μM in buds, 80 and 49 μM, respectively, in cork and medulla tissues (Fig. 4). PCA-soluble polyamines were less abundant than the free ones, in particular spermidine was respectively 15-fold lower than the free form in the medulla tissues and 7-fold lower in buds and cork (Fig. 5). The levels of PCA-insoluble were lower than PCA-soluble polyamines with spermidine and putrescine (cork and buds) having relatively high levels (Fig. 6).

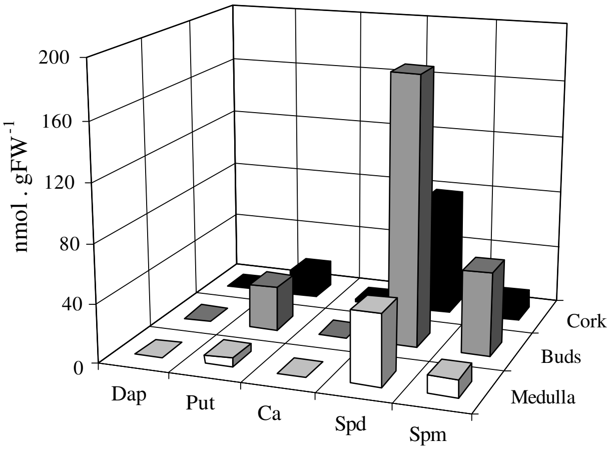

Fig. 4. Free polyamine content (nmol g FW^{-1}) in several parts of *Helianthus tuberosus* tuber.

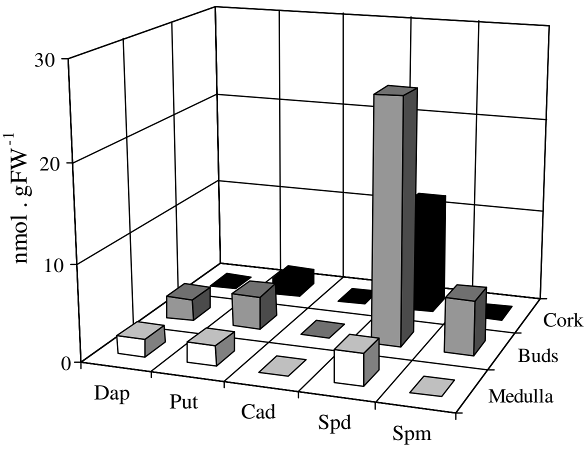

Fig. 5. PCA-soluble conjugated polyamine content (nmol g FW^{-1}) in several parts of *Helianthus tuberosus* tuber.

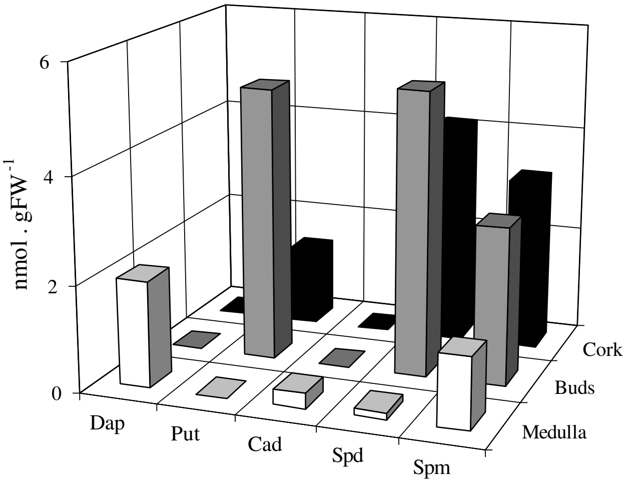

Fig. 6. PCA-insoluble conjugated polyamine content (nmol g FW^{-1}) in several parts of *Helianthus tuberosus* tuber.

In conclusion, the homogeneous parenchymatous tissue of medulla, that constitutes the majority of the tuber and is the only edible part, contains low levels of polyamines and mostly in the free form.

The levels of the different hydroxycinnamic acids (cinnamic, *p*-coumaric, caffeic and ferulic acids) utilised for the formation of hydroxycinnamic amides, present in the PCA-soluble and insoluble fractions, were determined by diode array HPLC chromatography and were present only in trace amounts in the free form.

4. Discussion

Quantifying polyamine levels in different foodstuffs and therefore assessing their everyday dietary intake, could represent an important way of reducing the concentration of polyamines in the body pool. It is in fact well ascertained that polyamines sustain cell division, growth and proliferation (Bagni & Tassoni, 2001) and therefore reducing their exogenous intake through diet could slow down cancer proliferation. Few papers have been published reporting the levels of polyamines in different plant derived foods (Larqué et al., 2007; Nishimura et al., 2006; Okamoto et al., 1997). The aim of the present research is to compare the polyamine levels of foods coming from two different plant sources: soybean, universally utilised both fresh and processed, and *H. tuberosus* tuber scarcely utilised in the everyday diet, at least in Italy.

Soybean is the raw material for manufacturing many kinds of food not only in many Asian countries, but also in Western countries. In the case of soy-derived foodstuffs, we can compare our results with those obtained by other authors (Nishimura et al., 2006; Okamoto et al., 1997), even if it has to be taken into consideration that the diverse foods come from different manufacturing sources. The comparison between our results and those of Nishimura et al. (2006) can only be done for free polyamines because the authors did not determine the amount of conjugated polyamine. Instead, Okamoto et al. (1997) measured also conjugated polyamines even if without discriminating between soluble and insoluble forms.

In particular, Alce Nero soybean milk (Fig. 1) had lower free polyamine content with cadaverine levels 16-fold lower, while tofu had 23-fold lower cadaverine and spermidine of the same order of magnitude than that of Nishimura et al. (2006). Kikkoman soybean sauce (Fig. 1) had lower polyamine content respect to fermented soybean (natto) determined by Nishimura et al. (2006) and to the four different sauces analysed by Okamoto et al. (1997). In particular, also not taking into consideration cadaverine content that in HPLC analyses performed by Okamoto et al. (1997) was eluted at the same retention time of histamine, Kikkoman soy sauce contained only 1/3 of polyamines (free plus conjugated forms) with respect to shiro soy sauce and only 1/60 with respect to tamari soy sauce that showed high levels of conjugated spermidine as well as of histamine. In addition, the polyamine levels of soybean seeds determined by Okamoto et al. (1997) were three times higher respect to those of seeds coming from Imola organic experimental fields (Fig. 1). Interestingly, free diaminopropane, which is considered to be an index of plant polyamine oxidase (PAO) activity, was absent in soy-derived foods a part from soy sauce (Fig. 1). As plant polyamine oxidases utilise in many cases different substrates compared with the enzyme present in animals and fungi through acetylation process (Seiler et al., 1981), we could assume that the absence of diaminopropane in soy-derived foods was related to the capability of fermenting microorganisms to oxidate polyamines. Okamoto et al. (1997) reported that soy sauce polyamine profile was quite different from that of soybean seeds, indicating that those polyamines were produced by decarboxylases present in the fermenting microorganisms. Conversely our results indicate that most probably the lower content of putrescine can be due to lower availability in the raw material of amino acid precursors of polyamines (such as arginine, ornithine and lysine).

The level of free cadaverine (Fig. 1) in soy-derived foods could be related to quinolizidine alkaloid biosynthesis particularly abundant in the shoot tissue of some Leguminosae plants (Lee, Pate, Harris, & Atkins, 2007; Schoofs, Teichmann, Hartmann, & Wink, 1983), even if lysine decarboxylase, enzyme responsible for cadaverine synthesis, is also present in organs which definitely do not produce alkaloids, e.g. roots and hypocotyls (Scoccianti, Torrigiani, & Bagni, 1990).

In regard to *Helianthus* results, the high levels of polyamines in buds are related to their capacity for sprouting in physiological condition. In fact the concentration of total free polyamines (about 270 μM), is able to induce cell division and growth, as demonstrated by *in vitro* experiments utilising explants of medullary parenchyma supplied with exogenous polyamines (Bagni, 1989). The very low levels of endogenous polyamines present in medulla, which is the only edible part of the *Helianthus* tuber, are not able to stimulate cell proliferation and therefore this tissue seems to be a good food candidate for people needing to lower active cancer cell proliferation, such as chemotherapic patients. *Helianthus* tubers, harvested in the dormancy period, also contain a very small amount of different plant growth regulators such as indol-3-acetic acid, cytokinins and gibberellins (Bagni, 1989) which stimulate cell division and, in particular gibberellins, seem to exert a carcinogenic effect also in animal cells (El-Mofty, Sakr, Rizk, & Moussa, 1994).

In addition to low polyamine content, *Helianthus* tuber is lacking of starch as reserve metabolite which is substituted by inuline, a D-fructofuranose polymer, in which about 35 fructose residues are linked together by β-2,1 glycosidic bonds. Inuline is widespread in the Asteraceae family and is a fructose-based carbohydrate non-digestible by humans (Flores & Flores, 1997). Thus, this tuber represents an important alternative food for the diet of patients with diabetes.

In contrast to alkaloids which are localised in few plant families, hydroxycinnamic acid amides are widespread, reaching more than 90% of total polyamines in Solanaceae, even though in many plants they represent only a small fraction of the secondary metabolites that can be formed using putrescine, cadaverine, spermidine and spermine as precursors (Bagni & Tassoni, 2001). We tried to measure the levels of the different hydroxycinnamic acids (cinnamic, *p*-coumaric, caffeic and ferulic acids) that could enter in the formation of hydroxycinnamic amides, evidencing that these compounds were present only in trace amount.

During human uptake and digestion, the oxidative breakdown of amines derived from food was extensively studied. Amino-oxidases (monoamine and diamine oxidases) in the intestine and liver degrade amines derived from food before they reach the blood (Halász & Baráth, 1998). On the contrary there is a little information about the bacterial transformation of phenolic compounds, such as anthocyanins (Aura et al., 2005), the acid forms of which were found in urine and faecal samples (Aura et al., 2002).

In the large group of phenolic compounds could also be included hydroxycinnamic amides derived from hydroxycinnamic acids. These compounds are likely to enter the colon, but no information is available about their metabolism by gut microflora. Due to the known hypotensive activity of hydroxycinnamic amides (Funayama, Yoshida, Konno, & Hikkino, 1980) it would be of great interest to study in detail their metabolism even by using an *in vitro* colon model which could offer a suitable solution to this question.

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