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Polyamines in conventional and organic vegetables exposed to exogenous ethylene

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ABSTRACT

Relationships between endogenous levels of polyamines by thin layer chromatography (TLC) and gas chromatography (GC), nitrate and response to the application of ethylene were established between organic and conventional vegetables (broccoli, collard greens, carrots and beets), both raw and cooked. Responses to ethylene showed that organic plants were less responsive to the growth regulator. The levels of free polyamines obtained by TLC were higher in organic vegetables. Organic broccoli showed higher levels of putrescine (Put), and cooking resulted in lowering the overall content of these amines. Conventional collard green showed the highest level of putrescine in the leaves compared with organic. Tubers of carrots and beets contain the highest levels of Put. These plants also contain high levels of spermine. GC analysis showed the highest polyamines contents compared with those obtained by TLC. Cooking process decreased putrescine and cadaverine content, both in conventionally and organically grown vegetables. Organic beets contain lower NO_3^- compared with its conventional counterpart.

1. Introduction

The interest in organically produced foods has increased all over the world in response to intensive agricultural practices, which can impact both human health and the environment. However, current controversies regarding the production systems exist due to a variety of factors, as well as an increase in world hunger, involving more than one billion humans. Estimates show an approximate increase in world population up to nine billion by 2050 (Godfray et al., 2010). Among factors contributing to an increased demand for food include climate adversities, especially with respect to global warming, which would directly affect the photosynthetic rate and plant productivity (Fedoroff et al., 2010). Concerns about the lack of food drive the scientific community to ask governmental agencies for higher flexibility for the agricultural production systems and to overcome the popular prejudice against the use of biotechnology, in particular the production of genetically modified foods could increase productivity and lower the amount of required pesticides (Fedoroff et al., 2010; Godfray et al., 2010).

It is believed that the most important research challenge will be to deepen our knowledge of plant metabolic pathways for the selection of organisms resistant to various injuries. The hypothesis would be that these plants, which serve as candidate sources for resistance genes, can be used as a reservoir of genetic improvements for other cultivated varieties. According to some researchers, a higher level of secondary metabolites could be a reason for plant resistance (Rossetto, Shiga, Vianello, & Lima, 2013). Other studies also show the same tendency, i.e., organic plants can be more resistant to some types of stress (Reeve et al., 2010).

Higher plants under biotic and abiotic stresses, as well as those growing in organic soils without the use of chemical controls, are stimulated to produce various substances, such as ethylene. This growth regulator was reported to influence defense gene expression in several plants (Lorenzo, Piqueras, Sánchez-Serrano, & Solano, 2003). Moreover, several classes of other substances are simultaneously produced under stress conditions in plants, such as phenylpropanoids (cyanogenic glycosides, glucosinolates, etc.), phenolic compounds (phenolic acids, flavonoids, etc.), alkaloids and terpenes (Schmelz, Leclere, Carroll, Alborn, & Teal, 2007). Among these substances, polyamines (PAs) exert a protective effect against various types of injuries as they favor the stability of cell membranes (Bae et al., 2008; Zhang et al., 2013a).

Many studies have shown the protective effects of different PAs against stress derived from excess salt, alkali, water, heavy metals, ozone, among other sources (Groppa & Benavides, 2008;

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Roychoudhury, Basu, & Sengupta, 2011; Zhang, Shen, Li, Meng, & Sheng, 2013b). Conversely, PAs can limit the growth of plants and their massive ingestion in the diet may become a risk factor for human health, even if they possess beneficial dose dependence (Moinard, Cynobera, & De Bandt, 2005; Zhang et al., 2013a; Zhang et al., 2013b). Research studies to determine the dose–response of these substances, both for food safety and for the elucidation of regulatory effects in plants, are still lacking. In addition, in plants, PAs and ethylene share a common precursor, *S*-adenosyl methionine (SAM), and this amine is capable of interfering with the ethylene biosynthesis (Bouchereau, Guénot, & Larher, 2000).

Generally, liquid chromatography (HPLC) represents the most used technique for polyamine determination, even if other methods were applied both in animal and vegetal samples. According to Flores and Galston (1982), a good correlation between polyamine determination by thin layer chromatography and high performance liquid chromatography (HPLC) exists, but no examples can be found in literature comparing PAs assessment by TLC and gas chromatography.

Therefore, the present work aims to study the levels of PAs, identified in vegetable crops cultivated under conventional and organic manure submitted to exogenous ethylene and quantified by thin layer chromatography and gas chromatography. Broccoli, collard greens, beets and carrots have been selected due to the high level of consumption in Brazil and others countries. They also contain several bioactive compounds, important for human health.

2. Materials and methods

2.1. Plant materials

Vegetables used in the present study were broccoli (*Brassica oleracea* L. cv Italica, Ramoso Piracicaba), collard greens (*B. oleracea* L. var. *acephala* D.C.), beet (*Beta vulgaris* L.) and carrot (*Daucus carota* L.). These vegetables were selected because they are among the most diffuse in Brazil as vegetable foods.

Vegetables were grown in Botucatu, São Paulo State, Brazil (22° 53′ 09″ South latitude, 48° 26′ 42″ West longitude, and at 804 meter altitude) and were harvested at the optimal period. Conventional and organic cultivations were performed in separate areas.

2.2. Processing

Broccoli crops were harvested at 90 days after sowing. Organic and conventionally grown plants were at the same physiological phase of maturation at harvest. Plants were morphologically separated into inflorescences (I), leaves (L) and stalks (S). A portion of broccoli was processed as raw, and the other portion (100 g) was cooked (boiling in an inox pan, 2 l of capacity, with 100 ml of water) for 5 min. The cooking procedure was carried out on the entire broccoli plant (I + G + S), and separate containers were used for organic and conventionally derived broccoli. Collard greens were harvested 80 days after sowing and the leaves processed as raw and cooked (5 min without added water). The roots of beet (pulp, peel, leaves, stalk) and carrot (pulp, leaves and peel) were analyzed at 70 and 120 days after sowing, and processed raw and cooked (45 min in boiling water, inox pan, 2 l of capacity, 100 g of samples with 300 ml of water). For all considered vegetables, the cooking time was selected as the period needed to reach the final cooking temperature in all tissue sections. Temperature in sample tissues was measured by metal penetration thermometer. After these treatments, the plants were frozen in liquid nitrogen and stored at −20 °C.

2.3. Methods of analysis

2.3.1. Free polyamines (PAs_{free}) by TLC

Free polyamines (PAs_{free}) were determined by thin layer chromatography (TLC) according to Flores and Galston (1982) with modifications introduced by Lima, Abdallah, Takaki, Ramos, and Ono (2008) as follows: plant materials, raw and cooked were powdered by manual grinding in liquid nitrogen, was homogenized for 1 min in 5% (v:v) cold perchloric acid (Merck, 70%) using a miniturrax (Marconi, Brazil). After centrifugation (8000×*g*, 20 min at 4 °C), 4.5 mol l^{-1} Na_2CO_3, containing 18.5 mmol l^{-1} dansyl-chloride in acetone (Sigma, 95%), was added to the supernatant. The reaction was carried out at room temperature and protected from light for 16 h. Then, 0.87 mol l^{-1} proline (Sigma, 99%) was added, and samples were maintained at room temperature for 30 min. Toluene (Sigma) was used to extract dansylated PAs. Aliquots (20 μl) were applied manually with Hamilton syringe (50 μl) onto activated (1 h at 110 °C, before use) glass plates (Adamant®Silica gel 60G, 0.25 mm, (20 × 20 cm), Macherey–Nagel) and separated in a TLC developing tank, using as mobile phase chloroform:triethylamine (7.5:1). The plate was allowed to dry at room temperature (22 ± 2 °C), then dried with a hair dryer until the excess of solvent disappeared before interpretation.

Putrescine (Put) (Sigma, 98%), spermidine (Spd) (Sigma, 99%) and spermine (Spm) (Sigma, 99%), were used as standards. The entire procedure was monitored under UV light at 254 nm. PAs_{free} were quantified by comparison against standards by fluorescence emission spectroscopy (excitation at 350 nm and emission at 495 nm), in a Video Documentation System, using the Image Master 2.0 Software (Amersham Pharmacia Biotech 1996). The calculation of quantitative analysis was done based on the area obtained in the standards and the samples. PAs_{free} content was expressed as nmol g^{-1} fresh weight (FW).

2.3.2. Free and conjugated polyamines extraction and quantification by GC

Polyamine determination by gas chromatography (GC) was optimized according to Kataoka (1996), with some modifications. The extraction of amines of both raw and cooked vegetable materials was performed in the solution of trifluoroacetic acid (TFA, Sigma–Aldrich, 99%) at 5% in water (v:v). Extract aliquots were filtered through a 0.45 μm polyvinylidenedifluoride (PVDF) membrane (Millex™-Millipore) and submitted to drying with N_2 gas. Standards as putrescine (Sigma, 98%), spermine (Sigma, 99%), agmatine (Sigma, >97%) and cadaverine (Cad, Sigma 98%) were prepared in the same manner. After drying, standards and samples were subjected to acylation by trifluoroacetic anhydride (Sigma, ⩾99%) for 24 h at room temperature in acetonitrile (JT Baker) (1:1, v:v). The acylation was carried out in a microwave oven (30 s at 360 W) and then dried under a nitrogen flow. Finally, acylated derivatives were re-suspended in acetonitrile and injected in the gas chromatograph (GC mod. 17A, Shimadzu, Japan). Standards were injected individually and as mixtures. The chromatographic method consisted in the manual injection of a 2 μl aliquot of acylated derivatives, re-suspended in acetonitrile, in a sandwich sequence: 1 μl air + 2 μl sample + 1 μl air and using a split mode (20:1 ratio). The separation was performed with a BP10 capillary column (30 m × 0.25 mm id) (14% cyanopropyl phenyl polysiloxane). PAs were detected by a thermal conductivity detector (TCD). Injector and detector temperatures were set to 280 °C, and a ramp program was run from 160 °C, at 5 °C min^{-1} up to 280 °C. The flow rate of carrier gas (He) was maintained at 0.8 ml min^{-1} according to Aflalaye, Anguel, Sternberg, Raulln, and Vidal-Madjar (1996). PA contents were expressed in nmol g^{-1} fresh weight (FW).