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Nutritional Traits of Bean (*Phaseolus vulgaris*) Seeds from Plants Chronically Exposed to Ozone Pollution

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The effect of chronic exposure to ozone pollution on nutritional traits of bean (*Phaseolus vulgaris* L. cv. Borlotto Nano Lingua di Fuoco) seeds from plants grown in filtered and nonfiltered open-top chambers (OTCs) has been investigated. Results showed that, among seed macronutrients, ozone significantly raised total lipids, crude proteins, and dietary fiber and slightly decreased total free amino acid content, although with a significant reduction of asparagine, lysine, valine, methionine, and glycine, compensated by a conspicuous augmentation of ornithine and tryptophan. Phytosterol analysis showed a marked increase of β-sitosterol, stigmasterol, and campesterol in seeds collected from nonfiltered OTCs. With regard to secondary metabolites, ozone exposure induced a slight increase of total polyphenol content, although causing a significant reduction of some flavonols (aglycone kaempferol and its 3-glucoside derivative) and hydroxycinnamates (caffeic, *p*-coumaric, and sinapic acids). Total anthocyanins decreased significantly, too. Nevertheless, ozone-exposed seeds showed higher antioxidant activity, with higher Trolox equivalent antioxidant capacity (TEAC) values than those measured in seeds collected from filtered air.

KEYWORDS: Bean seeds; ozone pollution; free amino acids; sterols; polyphenols; antioxidant activity

INTRODUCTION

Tropospheric ozone (O_3), a constituent of photochemical smog, is a secondary pollutant produced through sunlight-catalyzed reactions involving primary pollutants, such as nitrogen oxides, sulfur oxides, carbon monoxide, and hydrocarbons (*1*). Therefore, ozone is produced on bright sunny days over areas with intense primary pollution, due mainly to vehicle exhausts, fossil fuel burning, and industrial processes, in the so-called photochemical cycle (*2*). Furthermore, meteorological conditions can move the pollutant, or its precursors, from these areas toward less polluted ones, such as rural zones, with detrimental effects on natural and cultivated plant species (*2*). Because of its strong oxidizing potential (+2.07 eV), ozone is a powerful oxidizing agent capable of reacting with virtually any biomacromolecule, namely, lipids, proteins, nucleic acids, and carbohydrates, and producing reactive oxygen species (ROS) (*3*). This, in turn, causes an array of biological effects at molecular, biochemical, and physiological levels, the last affecting biomass formation and crop yield (*4*). Ozone can also activate the plant immune system (*5*), and it influences the biosynthesis of plant secondary metabolites by changing either the transcription or the activity of key enzymes of secondary metabolic pathways (*6*). These compounds, to a large extent deriving from three biosynthetic routes, the phenylpropanoid, isoprenoid, and alkaloid pathways, include a plethora of

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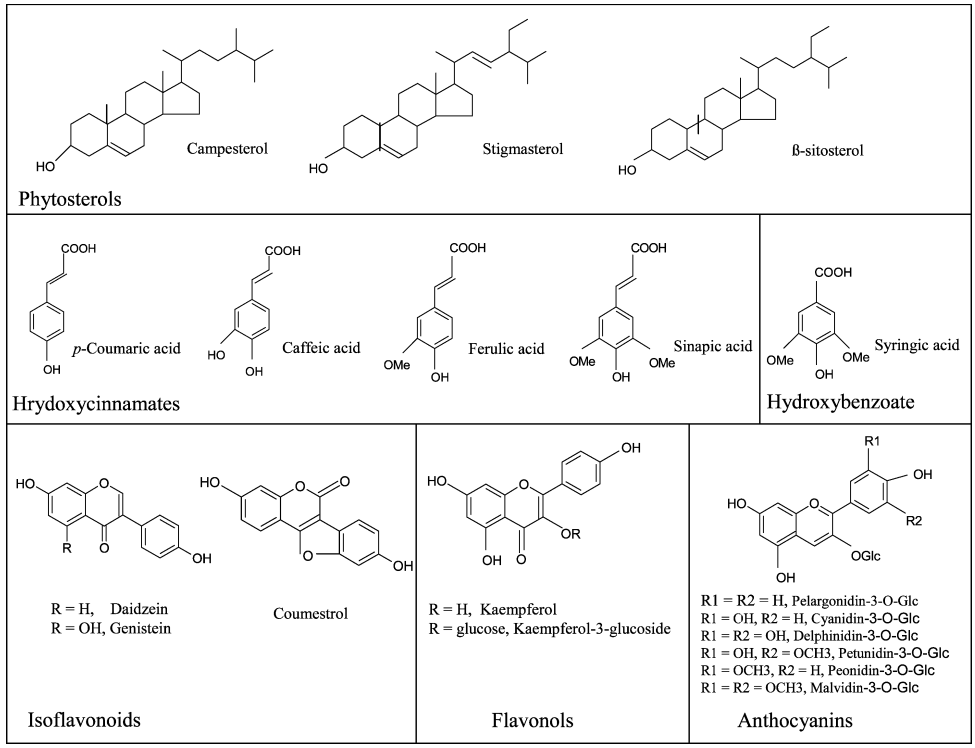

Figure 1. Phytosterol and phenylpropanoid secondary metabolites analyzed in bean seeds chronically exposed to atmospheric ozone pollution. Among phenylpropanoids, both phenolic acid (hydroxycinnamates and hydroxybenzoates) and flavonoid (isoflavonoids, flavonols, and anthocyanins) contents were determined.

protectant molecules able to improve the plant's tolerance to environmental pollutants, as well as the plant's resistance to pathogens and phytophagy. Phytochemicals arising from these pathways include not only compounds with broad-spectrum antibiotic activity but also powerful antioxidants able to efficiently scavenge ozone-induced ROS (*7*−*9*). As the precursors of secondary metabolic routes are products of the primary metabolism, a severe or long-lasting stress factor, such as ozone exposure, may induce an excessive shift between primary and secondary metabolism and, consequently, a diversion of essential available resources from growth to stress tolerance could occur.

Plants are exposed to either acute and chronic ozone doses according to the gas concentration and exposure time. An acute exposure consists of relatively high ozone concentration (>80 ppb, or 80 nL/L, or 160 μg/m^3) for a few consecutive hours, whereas a chronic exposure involves a relatively low gas concentration (<40 ppb, or 40 nL/L, or 80 μg/m^3) for the entire life of a plant, with intermittent episodes of high concentration, either periodically or accidentally (*10*). Symptoms of injury appear typically in the leaves, and pathophysiological conditions related to the ozone-induced damages include the impairment of stomatal function, the decrease of photosynthetic activity, and the induction of senescence, resulting in dysfunction of transpiration and water use efficiency, reduction of dry matter production, detrimental effects on flowering and pollen tube extension, and yield losses (reviewed in ref *4*).

Previously, we carried out experiments in open-top chamber (OTC) facilities to assess the crop yield parameters of bean (*Phaseolus vulgaris*) plants, growing in charcoal-filtered OTCs (OTCs-F) and in nonfiltered OTCs (OTCs-NF), meanwhile assessing the real O_3 uptake by leaves (*11*). Those results showed a significant reduction of crop yield in OTCs-NF, mainly due to the detrimental effect of ozone on photosynthetic activity. Particularly, plants grown in OTCs-F showed a higher number of pods per plant and of seeds per pod as well as an increment of both the weight of pods per plant and seeds per pod (*11*). In this work, we investigated the quality traits of bean seeds harvested from plants grown in filtered and nonfiltered OTCs in order to assess the impact of chronic ozone exposure on their nutritional value. For this purpose, we evaluated the difference in the content of macronutrients (carbohydrates, proteins, and lipids), dietary fiber, free amino acid, phytosterols and phenylpropanoids (hydroxycinnamates, hydroxybenzoates, collectively named simple phenols or phenolic acids and flavonoids) (**Figure 1**), as well as their antioxidant activity. So far, with the exception of a few published works on wheat and potatoes (*12*−*14*), scarce attention has been paid to possible modification of plant foodstuff quality induced by one of the most widespread pollutants, the background concentration of which has doubled since preindustrial times and has continued to rise over the past three decades with a 0.5−2% yearly rate at the mid-latitudes of the northern hemisphere (*15*). Moreover, the above-mentioned works mostly deal with starch and crude protein content, whereas this is the first study reporting a detailed analysis on a set of quality parameters influenced by O_3 pollution in a crop species.

MATERIALS AND METHODS

Experimental Facilities, Plant Material, Ozone Exposure, and Seed Harvest. Open-top chamber (OTC) facilities were localized within the Regional Forest Nursery of Curno (Bergamo), on the northern edge of the Po Valley. In this region, ozone levels are among the highest in Europe, and the critical levels for crops are frequently exceeded (*11*). Seeds of common bean (*P. vulgaris* L. cv. Borlotto Nano Lingua di Fuoco) were sown in July 2006 in two filtered OTCs (OTCs-F) and in two nonfiltered OTCs (OTCs-NF). O_3 concentrations in the chambers were continuously monitored by an ozone automatic analyzer model 1108 RS (Dasibi Italia, Milano, Italy). During the growing season (July 11−October 5), the cumulative exposures of plants over an O_3 concentration of 40 ppb (AOT40, accumulated exposure over a threshold of 40 ppb) were 535 ppb h in OTCs-F and 4675 ppb h in

OTCs-NF. Similarly, ozone stomatal fluxes referred to the projected leaf area ($AF_{ST}0$, a measurement of the amount of ozone effectively absorbed by plants) were 9055 mmol of O_3 m^{-2} in OTC-F and 20548 mmol of O_3 m^{-2} in OTC-NF (for details, see ref *11*). After harvesting, in October, part of the seeds were frozen in liquid N_2, powdered in a precooled mortar, and stored at −80 °C until analysis. Another part was left to dry and stored in desiccators at room temperature.

Starch Determination. The total starch content was determined according to the American Association of Cereal Chemists (AACC) (1983) Official Standard Method 76-13, using the Total Starch Assay Kit by Megazyme International, Ireland Ltd. (Bray Business Park, Wicklow, Ireland).

Crude Protein Content. Crude protein content was measured by nitrogen determination, using the Kjeldahl procedure (*16*), by a Mineral Six digestor and an Autodisteam semiautomatic steam-distilling unit (International PBI, Milan, Italy). Crude protein content was then calculated as % N × 6.25. All chemicals and solvents for the Kjeldahl method were provided by Carlo Erba Reagents (Milan, Italy).

Free Amino Acid Analysis. Free amino acids were analyzed with a Biochrom 20 amino acid analyzer, as previously reported (*17, 18*). All chemicals and solvents for the amino acid analysis were obtained from Biochrom.

Total Lipid Content and Phytosterol Analysis. Seeds powdered in liquid N_2 were lyophilized by an FTS-System Flex-Dry instrument (Stone Ridge, NY). The dried powders (~2.5 g each) were extracted by the Soxhlet apparatus for 4 h with $CHCl_3$, and the obtained solutions were dried in an evaporator to obtain the crude extracts.

Acid Hydrolysis and Saponification for Free Phytosterol Determination. Seeds powdered in liquid N_2 (2 g) were transferred into a flask with 25 mL of ethanol (25 mL) and dried under vacuum. Cholesterol was added to each sample (1 g), as internal standard (2 mL of a solution 1:1 w/v in ethanol), together with 2 mL of ethanol and 10 mL of 6 N HCl. The suspension was stirred at 80 °C for 60 min. After cooling, 10 mL of ethanol was added, and the mixture was stirred for a further 5 min. Finally, 50 mL of hexane/diethyl ether (1:1 v/v) was added and, after 10 min of stirring, 35 mL of the organic layer was removed and evaporated under vacuum by a Büchi Rotavapor R-114 (temperature not greater than 40 °C). The residue was treated with ethanolic pyrogallol (16 mL of a solution 3% w/v) and KOH (8 mL of a solution 4.3% w/v). After 15 min at 80 °C, the solution was cooled, and 24 mL of water and 40 mL of cyclohexane were added therein. The mixture was centrifuged at 6500 rpm for 5 min at 4 °C. Part of the organic layer (30 mL) was removed, and the solvent was evaporated in a vacuum by a Büchi Rotavapor R-114 (temperature not greater than 40 °C). Dry samples were stored at −20 °C (*19*).

Gas Chromatographic Analysis of Phytosterols. Dry samples obtained after acid hydrolysis and saponification were analyzed by GC and GC-MS to determine the content of sterols and their structure. Standards of the principal phytosterols (campesterol, stigmasterol, and β-sitosterol) and cholesterol were transformed into the corresponding trimethylsilyl ethers, according to the following general procedure: 1 mg of a single sterol and 0.5 mL of a mixture of bis(trimethylsilyl) trifluoroacetamide and dry pyridine (1:1 v/v) were left at room temperature for 1 h. The extracts were treated and analyzed with the same procedure previously described for the standards. Trimethylsilyl ethers were directly analyzed by GC with a Dani 86.10 instrument, equipped with a fused silica capillary column WCOT-CP-Sil-5 CB (Chrompack, 25 m × 0.32 mm i.d., film = 0.11 μm), carrier helium (0.45 kg/cm^3), injector, 300 °C, detector, 320 °C, oven, 180 °C (4 min) and then raised to 300 at 5 °C/min. The sterol structures were confirmed by injection of the same samples and of the standards in a Dani 3800 gas chromatograph, equipped with the same capillary column and connected to a VG 7070 EQ mass spectrometer. Retention times of sterols were 18.97 min for cholesterol, 19.11 min for campesterol, 20.20 min for stigmasterol, and 21.03 min for β-sitosterol (*19*).

Moisture and Total Dietary Fiber. Moisture was evaluated after a further drying of seeds for 24 h at 80 °C. Total dietary fiber was measured according to the Prosky method (*20*). Samples were previously delipidized with chloroform/methanol (3:1, v/v); afterward, they were gelatinized with a heat-stable α-amylase (pH 6.0, 100 °C, 30 min) and then sequentially digested with protease (pH 7.5, 60 °C, 30 min) and amyloglucosidase (pH 4.5, 60 °C, 30 min) to remove proteins and starch. Total dietary fiber was precipitated with ethanol and, after washing and drying, the residue was weighed. Results were corrected for protein and ash contents.

Phenylpropanoid Determination. For anthocyanin extraction, bean seeds (5 g) were previously dehulled as described in reff *21*. After separation from cotyledons, hulls were homogenized with 100 mL of methanol and maintained overnight at 4 °C. Samples were then centrifuged and pellets extracted again with 50 mL of methanol, for 2 h. After desiccation in a rotary evaporator at 40 °C, residues were dissolved again in 10 mL of methanol/0.3% perchloric acid (23:73), and the final extracts were stored in foil-wrapped glass vials at −20 °C. HPLC analysis of anthocyanins was performed as previously described (*17*).

Flavonoids and phenolic acids were extracted from 5 g of whole bean seeds, as described in ref *22*. HPLC analysis was performed with a Shimadzu instrument, constituted by an LC-10 AD vp pump, an FCV-10 AL vp mixer, an SIL-10 AD vp auto sampler, and an SPD-10 A vp UV detector set up on a wavelength of 320 nm. A Nova-Pak C18 column (300 mm × 3,9 mm, particle size = 4 μm) at room temperature was used. The mobile phase was constituted by (A) a solution of water/acetic acid (98:2, v/v) and (B) a solution of 0.5% of acetic acid in water/acetonitrile (1:1, v/v), maintained at a flow rate of 1 mL/min. Class VP 3,4 software was employed for chromatographic analysis.

Spectrophotometric detection of total polyphenols and total anthocyanins was performed according to the Folin−Ciocalteu assay on whole seeds, as previously described (*14*), and results were expressed as gallic acid (GAE) and malvidin (ME) equivalents, respectively. Folin−Ciocalteu reagents were obtained from Sigma-Aldrich (St. Louis, MO).

$ABTS^{\bullet+}$ Radical Cation Scavenging Activity. The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical cation decolorization test assesses the ability of hydrogen- or electron-donating antioxidants to scavenge this radical (*23*). The $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) and maintaining the mixture in the dark at room temperature for 12−16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C.

A weighed amount (ca. 200 mg) of dried seed sample was added to 5 mL of methanol/acetone/water (40:40:20, v/v/v) containing 0.1% acetic acid, and the mixture was stirred at room temperature for 12 h and centrifuged at 2000*g* for 10 min. Dilutions (10 μL) of sample surnatant, or ethanol (negative control), or standard solution of the synthetic antioxidant 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox, positive control) were mixed for 30 s with 1 mL of diluted $ABTS^{\bullet+}$ solution, and the absorbance at 734 nm was taken at 30 °C 1 and 10 min after the initial mixing. The percentage of inhibition was calculated and reported as Trolox activity (Trolox equivalent antioxidant capacity, TEAC) at time points of the reaction.

Presentation of Results. Extraction of metabolites, spectrophotometric, HPLC, GC, GS-MS, and amino acid analyses were repeated at least three times for each replicate; mean and standard deviation (SD) of values are reported, and the evaluation of the statistical significance of results was based on Student's *t* test for paired data analysis, with a probability value of <0.05 ($p < 0.05$) considered to be statistically significant.

RESULTS

Macronutrient Composition and Dietary Fiber. On the whole, the amount of macronutrients was higher in beans exposed to ozone pollution (**Table 1**). In particular, total lipid content rose by 28.5%, crude protein content by 7.88%, and dietary fiber by 14.54%. In contrast, starch content and moisture did not change significantly.

Free Amino Acid Profile. Total free amino acid content decreased (−5%) from 490 ± 9.83 to 465 ± 25.44 mg/100 g in seeds collected from filtered and nonfiltered facilities, respectively (**Table 2**). However, ozone exposure only partially

Table 1. Macronutrient Composition, Dietary Fiber, and Moisture of Seed Extracts from Beans Grown in Charcoal-Filtered (F) and Nonfiltered (NF) Open-Top Chambers[a]

	F (g/100 g)	NF (g/100 g)	% change
starch	35.69 ± 0.93	35.99 ± 1.31	+0.84
crude proteins	15.98 ± 0.75	17.24 ± 0.30	+7.88
total lipids	1.96 ± 0.24	2.52 ± 0.14	+28.57
dietary fiber	27.5 ± 0.4	31.50 ± 0.8	+14.54
moisture	8.66 ± 0.04	8.64 ± 0.08	−0.23

[a] Data (mean ± SD) derive from three separate seed extracts. Results are expressed as grams of constituent per 100 g of seeds.

modified the whole free amino acid profile of the samples, with significant decreases of the following five amino acids: asparagine (−13.5%), lysine (−10%), valine (−6%), methionine (−7%), and glycine (−22%). Contrary to the general trend, the concentrations of two amino acids significantly rose as a consequence of the ozone exposure, with increases of 55.5% in ornithine and 33.5% in tryptophan (**Table 2**).

Phytosterol Content. All three main phytosterols markedly increased in seeds from plants grown in OTCs-NF compared to those from OTCs-F (**Table 3**). In particular, β-sitosterol, the most abundant, rose 76.83%, stigmasterol, 65.69%, and campesterol, 52.17%.

Phenylpropanoids. Ozone induced a slight increase (+3.18%) of the total polyphenols in whole seeds, their contents being 3450 ± 21.1 and 3563 ± 33.4 μg/g in samples from OTCs-F and OTC-NF, respectively. However, a pronounced decrease (−43.15%) of total anthocyanins, from 95 ± 4.1 μg/g in OTCs-F to 54 ± 7.3 μg/g in OTCs-NF, was observed. Among the different anthocyanins, detected by HPLC only in the seed coat fraction, the concentrations of the 3-glucoside derivatives of delphinidin, cyanidin, and peonidin were significantly lower in seeds grown in polluted environment (OTCs-NF), whereas ozone exposure significantly raised the content of petunidin-3-glucoside and pelargonidin-3-glucoside (**Figure 2A**; **Table 4**).

With regard to the polyphenolic compounds, no significant difference was reported in the isoflavonoid (daidzein, genistein, and coumestrol) content, whereas an increase of both flavonol aglycone kaempferol and its 3-glucoside derivative concentrations was significantly detected in samples collected from OTCs-F (**Figure 2B**; **Table 4**). Finally, among hydroxycinnamates, ozone exposure significantly lowered the content of caffeic, *p*-coumaric, and sinapic acids, in whole seeds from OTCs-NF, whereas the levels of ferulic and syringic acids, the latter a hydroxybenzoic acid, did not change (**Figure 2C**; **Table 4**).

Antioxidant Activity. Extracts from ozone-exposed seeds showed higher TEAC values than those measured in seeds collected from filtered chambers, at both time points considered (**Figure 3**). After 1 min of reaction, TEAC values were 0.0323 ± 0.0014 and 0.0439 ± 0.0030 mmol equiv of Trolox/g in seeds from OTCs-F and OTCs-NF, respectively, with the higher TEAC in the latter indicative of a stronger antioxidant activity (**Figure 3**). When extracts had completed the reaction, after 10 min, TEAC values rose in both samples, reaching 0.0518 ± 0.0028 and 0.0693 ± 0.0028 mmol equiv of Trolox/g in seeds from OTCs-F and OTCs-NF, respectively (**Figure 3**).

DISCUSSION

The main aim of this work was to evaluate the effect of exposure of bean plants to atmospheric ozone pollution in terms of quality and nutritional traits of seeds. In general, the exposure to a stress can affect the nutritional properties of plant foodstuffs, particularly the plant secondary metabolites occurring in foods and the food chain and responsible for the health benefits arising from a high consumption of fruits and vegetables. In fact, these bioactive phytochemicals are compounds involved in plant resistance/tolerance against biotic/abiotic stresses (*4, 24*).

Legumes are an important component of the Mediterranean diet, and beans, in particular, are good sources of macronutrients (mainly proteins and carbohydrates). Furthermore, beyond their basic nutritional value, they provide health benefits by virtue of their minor components. Our data on macronutrients showed that starch was not influenced by ozone pollution, unlike crude proteins and total lipids, the concentrations of which increased significantly, as already reported by other authors (*25*). The functional significance of these changes may be due to the necessity of the plant, to improve the fitness of the next generation, to ensure higher contents of proteins and lipids, besides synthesizing defense metabolites. Nevertheless, in a study on seed nutritional quality of 34 bean accessions with different ozone sensitivity no correlation was found between ozone sensitivity and protein, lipid, and carbohydrate contents of the accession (*26*).

Although ozone exposure did not markedly modify the whole amino acid profile of bean seeds, we previously reported a deep change of free amino acid composition, in grapevine treated with benzothiadiazole, an inducer of systemic acquired resistance (SAR), and in transgenic *Arabidopsis* transformed with a Myb transcription factor. In both cases, the variation of free amino acid content was related to an enhanced resistance to pathogens and tolerance to abiotic stresses, including ozone (*17, 18*). Here we showed significant decreases of asparagine, lysine, valine, methionine, and glycine concentrations after ozone exposure. In leguminous plants, asparagine is the major assimilation product of nitrogen fixation in nodules and an important compound for the transport and storage of nitrogen resources, because of its high nitrogen to carbon ratio (*27*). Consequently, the reduction of asparagine in ozone-exposed seeds may be due to an impairment of nitrogen uptake from soil or its fixation in radical nodules, besides transport and storage in plant. The essential amino acid lysine belongs to the aspartate family of amino acids, arising from aspartate after seven consecutive enzymatic reactions started by dihydrodipicolinate synthase (DHDPS) (**Figure 4**) (*28*). Therefore, the low content of lysine following ozone exposure may be due to a down-regulation of the key enzyme DHDPS or an up-regulation of enzymes involved in lysine catabolism and degradation, such as lysine 2-oxoglutarate reductase and saccharopine dehydrogenase, at the level of mRNA transcription, protein translation, or enzymatic activity (*29*). In this view, the decrease of the sulfur-containing essential amino acid methionine, arising from the aspartate family as well (**Figure 4**), could be also explained (*30*). However, methionine decrement may be the consequence of direct ozone oxidation of methionine to methionine sulfoxide (*31*). The branched essential amino acid valine is the precursor of an array of plant defense secondary metabolites named phytoanticipins, including glucosinolates, isothiocyanates, and cyanogenic glucosides, mainly involved in defense against pathogens and phytophagy (*32*), whereas glycine is an osmolyte precursor of betains, other osmolytes protecting plant against water stress caused by drought and salinity (*33*). Hence, the low levels of both these free amino acids may represent an unfavorable trait for the ozone-exposed beans.

Ornithine and tryptophan were the only amino acids of which contents improved because of ozone exposure. Ornithine is a nonprotein amino acid precursor of polyamines, polycationic

Table 2. Free Amino Acid Profile of Seed Extracts from Beans Grown in Charcoal-Filtered (F) and Nonfiltered (NF) Open-Top Chambers[a]

amino acid	F (mg/100 g)	NF (mg/100 g)	*P*	functions
Glu (glutamic acid)	21.10 ± 0.60	21.54 ± 0.32	NS	nitrogen assimilation, stress induced
Gln (glutamine)	0.93 ± 0.10	0.96 ± 0.07	NS	
Asp (aspartic acid)	11.15 ± 0.26	11.35 ± 0.13	NS	
Asn (asparagine)	119.48 ± 1.62	103.15 ± 1.80	**	
Ser (serine)	0.97 ± 0.01	1.06 ± 0.10	NS	phospholipid precursors, stress induced
Ethan (ethanolamine)	0.51 ± 0.04	0.53 ± 0.03	NS	
Arg (arginine)	17.74 ± 0.19	18.13 ± 1.30	NS	arginine metabolism, polyamine precursors, stress induced
Orn (ornithine)	0.18 ± 0.02	0.28 ± 0.05	**	
Lys (lysine)	0.60 ± 0.02	0.54 ± 0.01	**	aspartate metabolism
Thr (threonin)	1.93 ± 0.03	1.87 ± 0.07	NS	
Ile (isoleucine)	1.73 ± 0.05	1.54 ± 0.16	NS	branched amino acids, secondary metabolite precursors
Leu (leucine)	2.02 ± 0.06	1.90 ± 0.14	NS	
Val (valine)	12.05 ± 0.36	11.35 ± 0.35	*	
Tyr (tyrosine)	1.17 ± 0.04	1.13 ± 0.12	NS	aromatic amino acids, secondary metabolite precursors
Phe (phenylalanine)	2.36 ± 0.05	2.16 ± 0.22	NS	
Trp (tryptophan)	6.25 ± 0.16	8.34 ± 0.23	**	
Cys (cysteine)	0.26 ± 0.02	0.28 ± 0.03	NS	sulfured amino acids
Met (methionine)	15.02 ± 0.40	13.98 ± 0.59	*	
Sarc (sarcosine)	113.66 ± 3.26	110.43 ± 9.12	NS	osmolytes, osmoprotectants
Gly (glycine)	2.63 ± 0.13	2.05 ± 0.16	**	
Ala (alanine)	9.26 ± 0.18	8.82 ± 0.38	NS	
β-Ala (β-alanine)	0.65 ± 0.04	0.60 ± 0.07	NS	
Pro (proline)	4.79 ± 0.50	4.19 ± 0.33	NS	
Hypro (hydroxyproline)	136.39 ± 3.63	131.75 ± 9.35	NS	
AABA (α-aminobutyric acid)	0.28 ± 0.02	0.29 ± 0.01	NS	
GABA (γ-aminobutyric acid)	0.34 ± 0.04	0.37 ± 0.03	NS	
His (histidine)	2.43 ± 0.10	2.56 ± 0.37	NS	metal chelator
AAAA (α-aminoadipoic acid)	5.39 ± 0.15	5.30 ± 0.27	NS	
urea	6.46 ± 0.29	7.26 ± 0.78	NS	

[a] Data (mean ± SD) derive from three separate seed extracts. Results are expressed as milligrams of amino acid per 100 g of seeds [*, $P \leq 0.05$; **, $P \leq 0.01$; NS, not significant (Student's *t* test)].

Table 3. Phytosterol Content in Seed Extracts from Beans Grown in Charcoal-Filtered (F) and Nonfiltered (NF) Open-Top Chambers[a]

phytosterol	F (mg/100 g)	NF (mg/100 g)	% increment
β-sitosterol	27.2 ± 2.8	48.1 ± 3.5	76.83
stigmasterol	17.2 ± 1.0	28.5 ± 1.7	65.69
campesterol	2.3 ± 0.2	3.5 ± 0.4	52.17
cholesterol	ND	ND	

[a] Data (mean ± SD) derive from three separate seed extracts. Results are expressed as milligrams of phytosterol per 100 g of seeds. ND, not detected.

nitrogenous compounds of low molecular weight ubiquitous in all living organisms. In plants, both free and conjugated polyamines improve ozone tolerance either by inhibiting the biosynthesis of ethylene, the phytohormone partly responsible for ozone damages, or by direct ROS scavenging, respectively (*34, 35*). Additionally, apoplastic free polyamines can form conjugates with hydroxycinnamates and phenolic acid derivatives, effective in ROS detoxification (*36*). In some model plants, tobacco and *Arabidopsis*, ozone-induced oxidative stress stimulates both prechorismate and anthranilate biosynthetic pathways (*9*), raising the level of tryptophan, but not of phenylalanine and tyrosine, the other aromatic amino acids arising from chorismate via the branch point of prephenate/arogenate (*37*). Tryptophan, besides being the precursor of the plant growth hormone auxin, is the precursor of melatonin, a powerful antioxidant significantly present in seed tissues (*38*). Furthermore, it has been suggested that free tryptophan in legume seeds can be easily absorbable at the stomach level and more apt to cross the blood−brain barrier than protein tryptophan, thus influencing the synthesis of serotonin (*39*).

Our results on phytosterols (plant sterols) showed that ozone pollution greatly improved β-sitosterol, stigmasterol, and campesterol, the main plant sterols present in seed tissues (*40*). Sterols determine the functional and structural properties of cell membrane, such as fluidity and permeability, by modulating the physical state of the lipid bilayer (reviewed in ref *41*). At the cellular level, the membrane represents the first structure directly exposed to ozone injury, its polyunsaturated fatty acids being the primary target. Ozone-induced lipid peroxidation, known as the Criegee ozonation pathway, involves the ozonolysis of alkenes of polyunsaturated chains, that is, the electrophilic O_3 addition across the carbon−carbon double bonds, impairing membrane structure and function (*4*). Thus, it seems to be conceivable that the enhancement of the phytosterol pools, in ozone-exposed plants, could assume a functional role to counteract cell membrane damage. Certainly, from a nutritional point of view, this alteration may be of interest, because of the well-known cholesterol-lowering properties of plant sterols (*42*).

About phenylpropanoid content, ozone exposure reduced the concentration of caffeic, *p*-coumaric, and sinapic acids, the three main hydroxycinnamates in bean seeds (*43*), as well as the content of both flavonol aglycone kaempferol and its 3-gluco-

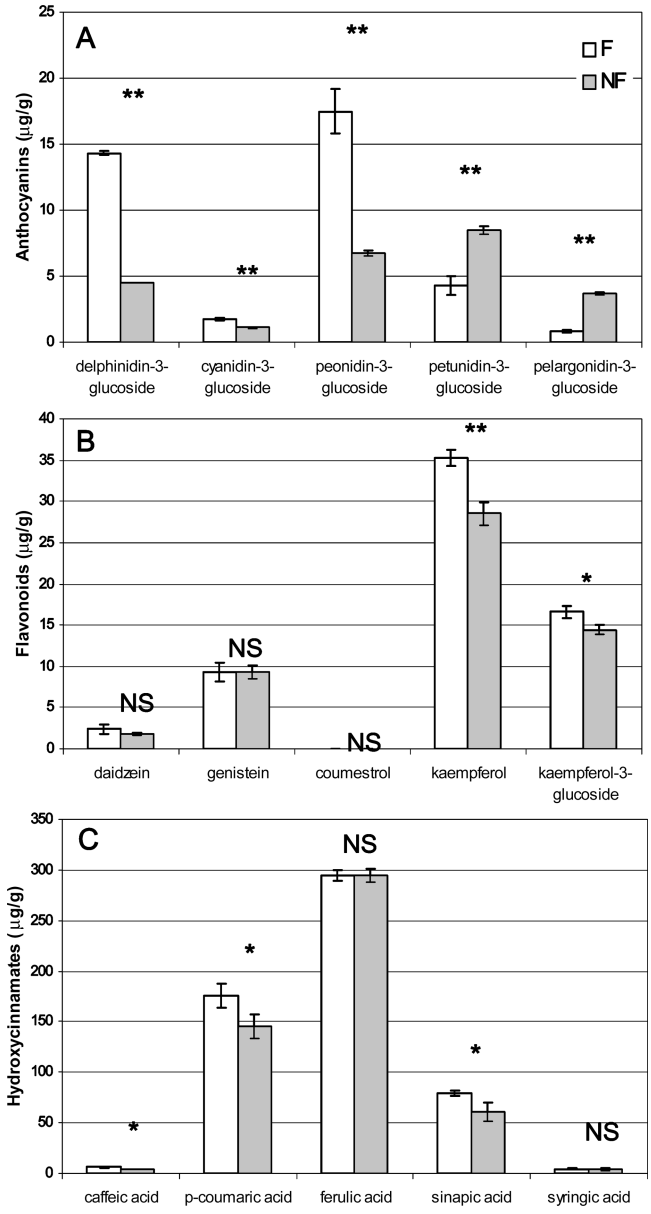

Figure 2. Anthocyanin (**A**), flavonoid (**B**), and hydroxycinnamate (**C**) contents in seed extracts from beans grown in charcoal-filtered (F) and nonfiltered (NF) open-top chambers (OTCs). Data (mean ± SD) are expressed as micrograms per gram of seeds; coumestrol values in **B** are 0.012 μg (F) and 0.011 μg (NF) [∗, $P \leq 0.05$; ∗∗, $P \leq 0.01$; NS, not significant (Student's *t* test)].

Table 4. Percentage Variation of Phenylpropanoidic Compounds in Seed Extracts from Beans Grown in Nonfiltered Open-Top Chambers (OTCs) in Comparison with Seeds Grown in Filtered OTCs

phenylpropanoid	% variation	phenylpropanoid	% variation
delphinidin-3-glucoside	−68.41	kaempferol	−19.15
cyanidin-3-glucoside	−37.57	kaempferol-3-glucoside	−13.12
peonidin-3-glucoside	−61.23	caffeic acid	−32.90
petunidin-3-glucoside	+98.36	*p*-coumaric acid	−17.26
pelargonidin-3-glucoside	+343.37	ferulic acid	−0.02
daidzein	−28.51	sinapic acid	−23.54
genistein	−0.32	syringic acid	−6.12
coumestrol	−8.33		

side derivative, abundant in different Italian ecotypes (*44*). Total anthocyanins were also reduced, although two of them, petunidin-3-glucoside and pelargonidin-3-glucoside, increased in ozone-exposed seeds. Actually, peonidin-3-glucoside, only

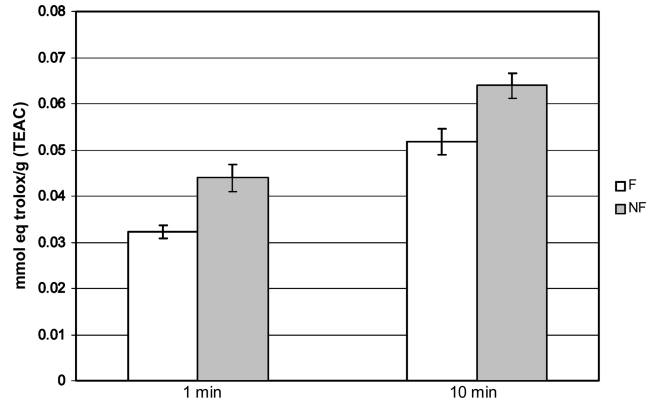

Figure 3. Antioxidant power of seed extracts from beans grown in charcoal-filtered (F) and nonfiltered (NF) open-top chambers. Trolox equivalent antioxidant capacity (TEAC) is based on the $ABTS^{\bullet+}$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid] radical cation decolorization assay at specific time points. A higher TEAC is indicative of a stronger antioxidant activity.

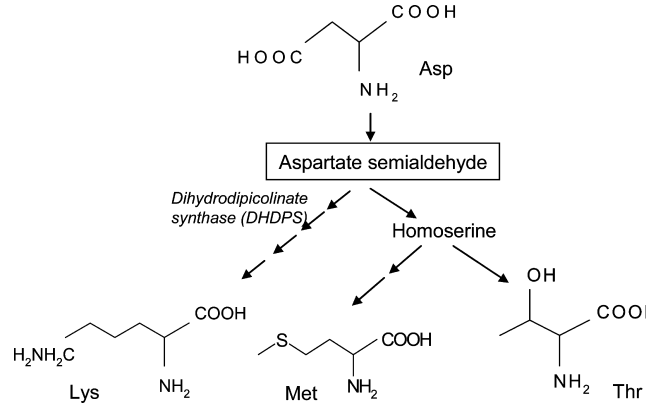

Figure 4. Aspartate pathway: lysine, threonine, and methionine belong to the aspartate family of amino acids via the common intermediate aspartate semialdehyde. Dihydrodipicolinate synthase (DHDPS) is the key enzyme of the branch leading to lysine. See the text for more details.

recently reported in *P. vulgaris*, was the most abundant anthocyanin we found, instead of delphinidin-3-glucoside, as indicated by previous studies (*45*). Flavonoid content was reduced, too, after ozone exposure, despite the observed increment of total polyphenols. This apparent discrepancy could be due to the spectrophotometric detection of total polyphenols that include proanthocyanidins (condensed tannins), polyphenolic compounds arising from the polymerization of catechin units. Interestingly, in a recent paper on the influence of preharvest ozone exposure on the quality of strawberries, no difference was reported in total anthocyanins and phenolic compounds (*46*). The increase of total polyphenols could be somehow in agreement with the higher content of the total dietary fiber found in beans grown in an ozone-polluted environment, which could be partly due to a higher content of insoluble lignin, a phenol-derived polymer itself.

Finally, with regard to the antioxidant power, the above-reported changes in phenylpropanoid composition do not contribute to an explanation of the higher TEAC of ozone-exposed seeds that can be possibly attributed to other compounds, such as tocopherols or other lipid-soluble metabolites, abundant in seed tissues and elicited by ozone (*47*).

In conclusion, although we did not take into account the effects of ozone on some unfavorable traits of bean seeds, including antinutritional factors (phytates, lectins, α-amylase and

protease inhibitors, tannins), flatulence promoters (the oligosaccharides raffinose, stachyose, and verbascose), and structural modifications detrimental for cooking (seed coat hardness) (*48*), it seems that their nutritional value is not particularly affected, if not possibly ameliorated, by the pollutant. In fact, some important traits, such as dietary fiber, phytosterol, and polyphenol contents appear to be enhanced, and the overall antioxidant power is higher. The levels of two important free amino acids, ornithine and tryptophan, are also higher, as is the total protein content. However, with regard to the latter, it must be take into account that seed storage proteins (globulins in legumes), enzyme and protease inhibitors, and pathogenesis-related proteins synthesized in response to abiotic and environmental stresses can be potentially allergenic (*49*), thus exacerbating an immunoglobulin (Ig) E-mediated hypersensitive response in atopic patients. A set of experiments are now ongoing to evaluate this important aspect.

ABBREVIATIONS USED

AACC, American Association of Cereal Chemists; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AOAC, Association of Official Analytical Chemists; GAE, gallic acid equivalent; GC, gas chromatography; MAE, malvidin equivalent; OTC-F, filtered open-top chamber; MS, mass spectrometry; OTC-NF, nonfiltered open-top chamber; ROS, reactive oxygen species; TEAC, Trolox equivalent antioxidant capacity.

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LITERATURE CITED

(1) Crutzen, P. J.; Lelieveld, J. Human impacts on atmospheric chemistry. *Annu. Rev. Earth Planet. Sci.* **2001**, *29*, 17–45.

(2) Fowler, D.; Cape, J. N.; Coyle, M.; Smith, R. I.; Hjellbrekke, A.-G.; Simpson, D.; Derwent, R. G.; Johnson, C. E. Modelling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystems. *Environ. Pollut.* **1999**, *100*, 43–55.

(3) Mustafa, M. G. Biochemical basis of ozone toxicity. *Free Radical Biol. Med.* **1990**, *9*, 245–265.

(4) Iriti, M.; Faoro, F. Oxidative stress, the paradigm of ozone toxicity in plants and animals. *Water, Air Soil Pollut.* **2008**, *187*, 285–301.

(5) Iriti, M.; Faoro, F. Review on innate and specific immunity in plants and animals. *Mycopathologia* **2007**, *164*, 57–64.

(6) Iriti, M.; Faoro, F. Ozone-induced changes in plant secondary metabolism. In *Climate Change and Crops*; Singh, S. N., Ed.; Springer: Heiderberg, Germany, in press.

(7) Facchini, P. J. Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation and metabolic engineering. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2001**, *52*, 29–66.

(8) Holstein, S. A.; Hohl, R. J. Isoprenoids: remarkable diversity of form and function. *Lipids* **2004**, *34*, 293–309.

(9) Iriti, M.; Faoro, F. Plant defense and human nutrition: the phenylpropanoids on the menu. *Curr. Top. Nutraceutical Res.* **2004**, *2*, 47–95.

(10) Krupa, S.; McGrath, M. T.; Andersen, C. P.; Booker, F.; Burkey, K. O.; Chappelka, A. H.; Chevone, B. I.; Pell, E. J.; Zilinskas, B. A. Ambient ozone and plant health. *Plant Dis.* **2000**, *85*, 4–12.

(11) Gerosa, G.; Marzuoli, R.; Rossini, M.; Panigada, C.; Meroni, M.; Colombo, R.; Faoro, F.; Iriti, M. A flux-based assessment of the effects of ozone on foliar injury, photosynthesis and yield of bean (*Phaseolus vulgaris* L. cv. Borlotto Nano Lingua di Fuoco) in open-top chambers. *Environ. Pollut.* **2008**, 10.1016/j.envpol.2008.06.028.

(12) Fangmeier, A.; De Temmerman, L.; Mortensen, L.; Kemp, K.; Burke, J.; Mitchell, R.; van Oijen, M.; Weigel, H.-J. Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. *Eur. J. Agron.* **1999**, *10*, 215–229.

(13) Fangmeier, A.; De Temmerman, L.; Black, C.; Persson, K.; Vorne, V. Effects of elevated CO_2 and/or ozone on nutrient concentrations and nutrient uptake of potatoes. *Eur. J. Agron.* **2002**, *17*, 353–368.

(14) Piikki, K.; De Temmerman, L.; Ojanperä, K.; Danielsson, H.; Pleijel, H. The grain quality of spring wheat (*Triticum aestivum* L.) in relation to elevated ozone uptake and carbon dioxide exposure. *Eur. J. Agron.* **2008**, *28*, 245–254.

(15) Vingarzan, R. A review of surface O_3 background levels and trends. *Atmos. Environ.* **2004**, *38*, 3431–3442.

(16) AOAC (Association of Official Analytical Chemists). In *Official Methods of Analysis*, 17th ed.; AOAC: Arlington, VA, 2000.

(17) Iriti, M.; Rossoni, M.; Borgo, M.; Ferrara, L.; Faoro, F. Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: primary versus secondary metabolism. *J. Agric. Food Chem.* **2005**, *53*, 9133–9139.

(18) Vannini, C.; Iriti, M.; Bracale, M.; Locatelli, F.; Faoro, F.; Croce, P.; Pirona, R.; Di Maro, A.; Coraggio, I.; Genga, A. The ectopic expression of the rice *Osmyb4* gene in *Arabidopsis* increases tolerance to abiotic, environmental and biotic stresses. *Physiol. Mol. Plant Pathol.* **2006**, *69*, 26–42.

(19) Toivo, J.; Phillips, K.; Lampi, A.-M.; Piironen, V. Determination of sterols in foods: recovery of free, esterified and glycosidic sterols. *J. Food Compos. Anal.* **2001**, *14*, 631–643.

(20) AOAC (Association of Official Analytical Chemists), Method 920.87. In *Official Methods of Analysis*, 16th ed.; Gaithersburg, MD, 1995; Chapter 32, p 7.

(21) Madhujith, T.; Shahidi, F. Antioxidant potential of pea beans (*Phaseolus vulgaris* L.). *J. Food Sci* **2005**, *70*, S85−S90.

(22) Kammerer, D.; Claus, A.; Carle, R.; Schieber, A. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *J. Agric. Food Chem.* **2004**, *52*, 4360–4367.

(23) Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C. A. Screening of dietary carotenoids and carotenoids-rich fruit extracts for antioxidant activities applying the $ABTS^{\bullet+}$ radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 378–389.

(24) Jansen, M. A. K.; Hectors, K.; O'Brien, N. M.; Guisez, Y.; Potters, G. Plant stress and human health: do human consumers benefits from UV-B acclimated crops. *Plant Sci.* **2008**, 10.1016/j.plantsci.2008.04.010.

(25) Pleijel, H.; Mortensen, L.; Fuhrer, J.; Ojanperä, K.; Danielsson, H. Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. *Agric. Ecosyst. Environ.* **1999**, *72*, 265–270.

(26) Mohamed, A. I.; Bhardwaj, H. L.; Rangappa, M.; Hoggard, G. Seed characteristics and nutrient composition of selected beans (*Phaseolus vulgaris*) with different ozone tolerance. *Plant Foods Hum. Nutr.* **1995**, *47*, 29–38.

(27) Lea, P. J.; Sodek, L.; Parry, M. A. J.; Shewry, P. R.; Halford, N. G. Asparagine in plants. *Ann. Appl. Biol.* **2007**, *150*, 1–26.

(28) Ferreira, R. R.; Varisi, V. A.; Meinhardt, L. W.; Lea, P. J.; Azevedo, R. A. Are high-lysine cereal crops still a challenge. *Braz. J. Med. Biol. Res.* **2005**, *38*, 985–994.

(29) Lima, S. T. C.; Azevedo, R. A.; Santoro, L. G.; Gaziola, S. A.; Lea, P. J. Isolation of the bifunctional enzyme lysine 2-oxoglutarate reductase/saccharopine dehydrogenase (LOR/SDH) from *Phaseolus vulgaris*. *Amino Acids* **2003**, *24*, 179–186.

(30) Azevedo, R. A.; Arruda, P.; Turner, W. L.; Lea, P. J. The biosynthesis and metabolism of the aspartate derived amino acids in higher plants. *Phytochemistry* **1997**, *46*, 395–419.

(31) Mudd, J. B.; Leavitt, R.; Ongun, A.; McManus, T. T. Reaction of ozone with amino acids and proteins. *Atmos. Environ.* **1969**, *3*, 669–676.
(32) Fahey, J. W.; Zalcmann, A. T.; Talay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5–51.
(33) Chen, T. H. H.; Murata, N. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.* **2002**, *5*, 250–257.
(34) Liu, J.-H.; Kitashiba, H.; Wang, J.; Ban, Y.; Moriguchi, T. Polyamines and their ability to provide environmental stress tolerance to plants. *Plant Biotechnol.* **2007**, *24*, 117–126.
(35) Bors, W.; Langebartels, C.; Michel, C.; Sandermann, H. Polyamines as radical scavengers and protectans against ozone damages. *Phytochemistry* **1989**, *28*, 1589–1595.
(36) Bouchereau, A.; Aziz, A.; Larher, F.; Martin-Tanguy, J. Polyamines and environmental challenges: recent development. *Plant Sci.* **1999**, *140*, 103–125.
(37) Janzik, I.; Preiskowski, S.; Kneifel, H. Ozone has dramatic effects on the regulation of the prechorismate pathway in tobacco (*Nicotiana tabacum* L. cv. Bel W3). *Planta* **2005**, *223*, 20–27.
(38) Manchester, L. C.; Tan, D.-X.; Reiter, R. J.; Park, W.; Moni, K.; Qi, W. High level of melatonin in the seeds of edible plants. Possible function in germ tissue protection. *Life Sci.* **2000**, *67*, 3023–3029.
(39) Comai, S.; Bertazzo, A.; Bailoni, L.; Zancato, M.; Costa, C. V. L.; Allegri, G. Protein and non-protein (free and protein-bound) tryptophan in legume seeds. *Food Chem.* **2007**, *103*, 657–661.
(40) Ryan, E.; Galvin, K.; O'Connor, T. P.; Maguire, A. R.; O'Brien, N. M. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. *Plant Foods Hum. Nutr.* **2007**, *62*, 85–91.
(41) Iriti, M.; Faoro, F. Lipids biosynthesis in Spermatophyta. In *Floricolture, Ornamental and Plant Biotechnology: Advances and Topical Issues*; Teixeira da Silva, J. A., Ed.; Global Science Books: London, U.K., 2006; Vol. 1, pp 359−372.
(42) Berger, A.; Jones, P. J. H.; Abumweis, S. S. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. *Lipids Health Dis.* **2004**, *3*, 5–24.
(43) Luthria, D. L.; Pastor-Corrales, M. A. Phenolic acid content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *J. Food Compos. Anal.* **2006**, *19*, 205–211.
(44) Dinelli, G.; Bonetti, A.; Minelli, M.; Marotti, I.; Catizone, P.; Mazzanti, A. Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes. *Food Chem.* **2006**, *99*, 105–114.
(45) Macz-Pop, G. A.; González-Parámas, A. M.; Pérez-Alonso, J. J.; Rivas-Gonzalo, J. C. New-flavanol-anthocyanin condensed pigments and anthocyanin composition in bean (*Phaseolus* spp.). *J. Agric. Food Chem.* **2006**, *54*, 536–542.
(46) Keutgen, A. J.; Pawelzik, E. Influence of pre-harvest ozone exposure on quality of strawberry fruit under simulated retail conditions. *Postharvest Biol. Technol.* **2008**, *49*, 10–18.
(47) Della Penna, D.; Pogson, B. J. Vitamin synthesis in plants: tocopherols and carotenoids. *Annu. Rev. Plant Biol.* **2006**, *57*, 711–738.
(48) Kigel, J. Culinary and nutritional quality of *Phaseolus vulgaris* seeds as affected by environmental factors. *Biotechnol. Agron. Soc. Environ.* **1999**, *3*, 205–208.
(49) Hauser, M.; Egger, M.; Wallner, M.; Wopfner, N.; Schmidt, G.; Ferreira, F. Molecular properties of plant food allergens: a current classification into protein families. *Open Immunol. J.* **2008**, *1*, 1–12.

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