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ORIGINAL CONTRIBUTION

Health-promoting substances and heavy metal content in tomatoes grown with different farming techniques

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Abstract *Background* Organic farming is a production technique that imposes major restrictions on the use of fertilizers, pesticides, feed additives and veterinary drugs and for this reason consumers perceive organic foods to be healthier. The content of health-promoting molecules such as ascorbic acid, β-carotene, lycopene and salicylic acid are important aspects of the nutritional quality of organic foods. *Aim* To evaluate health promoting substances and the heavy metal content of tomato berries grown using conventional, integrated pest management (IPM) and organic farming techniques. *Methods* Moisture was determined by drying, crude protein by the Kjeldhal method, and ashes by incineration at 550°C. Ergosterol, ascorbic acid, β-carotene, lycopene and salicylic acid were determined by HPLC. The levels of heavy metals were measured by atomic absorption spectroscopy. *Results* Compared to crops grown using conventional and IPM methods, organic tomatoes contained more salicylic acid but less vitamin C and lycopene. Organic tomatoes had higher Cd and Pb levels but a lower Cu content. Organic fruits had a slightly higher protein content than conventionally cultivated fruits, but the difference was minimal and consequently the nutritive significance was poor. *Conclusions* Farming techniques may have an impact on the quality of tomatoes. Their higher salicylate content supports the notion that organic foodstuffs are more wholesome. However, the lower lycopene and ascorbic acid levels of organic tomatoes are not to be regarded as positive. No residues of pesticides and ergosterol were detected.

Key words organic farming – lycopene – tomato – quality – salycilic acid

Introduction

Organic farming is an agricultural production technique that strictly limits the use of the fertilizers, pesticides, feed additives and veterinary drugs which are widely used by conventional farmers. Organic farmers try to preserve or increase soil fertility by adopting agronomic techniques such as crop rotation, planting nitrogen-fixating plants and using organic materials (mainly manure). Plant protection also relies on the selection of pest-resistant varieties and on the adoption of adequate agronomic practices. Therapy, if required, can only be based on non-synthetic molecules such as pyrethrins, plant extracts, conventional copper-based products and other preparations including those based on *Bacillus thuringiensis*. Molecules such as pyrethroids, i.e. pyrethrin

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semi-synthetic derivatives, are also allowed. In integrated pest management (IPM) pesticide are used only in very few cases, and molecules with a low environmental impact are preferred. There is discussion about the nutritional value of organic vegetables compared to their conventional counterparts. Woese et al. [30] found no significant difference in the protein content of organic and conventional vegetables. Their review, however, pointed out that in half of the cited studies, lower nitrate levels and higher vitamin C content were detected in organically farmed products. Furthermore, a higher dry residue was always found in organic leafy vegetables [30, 31]. These studies also show a tendency towards higher levels of ascorbic acid and lower amounts of nitrates in organic products. Further research has investigated the content in anti-oxidant molecules but there is a lack of results on IPM crops [3, 9, 21]. One of the most interesting molecules of plant origin is salicylic acid, which has been proposed as an anti-inflammatory agent [18] and in the treatment of patients with heart disease.

According to findings obtained by Baxter et al. [5], soups made with organic vegetables contain more salicylic acid than do soups containing conventionally farmed vegetables and this may have a protective effect on human health. Among the contaminants found in vegetables, heavy metals may reach different levels depending on their content in the soil and the type of fertilization used [19, 20, 26] for this reason the type of farming techniques can affect the heavy metal content of foods. Since factors such as climate, soil characteristics and type of management are major confounders in efforts to compare the effects of organic versus conventional farming on the nutritional value of foodstuffs, the aim of this study was to compare the content of some health-promoting molecules (vitamins, lycopene, salicylate) and heavy metals in tomatoes grown in a field trial using conventional, IPM or organic farming methods, under similar management, soil and climate conditions.

Methods

Samples

Tomato samples of the same cultivar (PS1296) were harvested from three experimental plots situated in the same farm and sufficiently far apart to prevent the drift of chemical treatments from conventional to organic or IPM. Organic tomatoes were fertilized with 30 t ha^{-1} of manure. In the IPM lots, plants were fertilized with 0.5 t ha^{-1} of a N–P–K fertilizer (11:22:16) plus 50 kg ha^{-1} of calcium nitrate and 50 kg ha^{-1} of a ferti-irrigation product (340 g kg^{-1} of nitrogen). In the conventional production lots, 700 kg ha^{-1} of 11:22:16 and 250 kg ha^{-1} of calcium nitrate were used. No herbicides were used on the organic plants, though copper (4 times, 2.5–7.0 kg t^{-1}) and sulfur (1 time, 5 kg t^{-1}) were used to protect against fungi. The soil acting pyrethroid Teflutrin (10 kg ha^{-1}) was used in IPM and conventional plots. The control of *Phytopora infestans* was carried out with two fungicide treatments in the IPM fields (diclofluanide and oxadixil) and for conventional tomato plots. In conventional and IPM plots the control of *Heliotihis armigera* was carried out using pymetrozine (1 treatment).

The experimental plots were divided into ten sub-plots, from each of which eight samples of products were collected, then mixed and one final sample (weighting approximately 10 kg) for each sub-plot was obtained. The sub-plots were the experimental units and ten independent samples for each farming technique were analyzed. An independent company certified that the farming techniques were in accordance with EU regulations on organic farming, and that the organic fields had been cultivated organically for at least 3 years.

Chemical analysis

Immediately after harvest the fruits were cleaned and stored at −20°C until analysis. Before analysis the samples were diced and homogenized using an Ultra-Turrax blender. As suggested by AOAC [2] the tomatoes were scalded (3 min at 70°C) and then cooled in water. Moisture was determined by drying at 70°C under vacuum (450 mmHg) for 4 h [2], crude protein using the Kjeldhal method [2], and ashes by incineration at 550°C [2].

Pesticides

Ten grams of homogenized sample and 12 g of Chemtube Hydromatrix (Varian, Palo Alto, CA, USA) was mixed and transferred to a chromatographic column where the phytochemicals were eluted with 100 ml of Cl_2CH_2. The eluate was evaporated to dryness at 35°C by rotary evaporation, quantitatively transferred with 10 ml of Cl_2CH_2, than reduced at 1 ml under N_2 flux. The sample was filtered and injected in GC equipped with an Electron-trap detector and a nitrogen–phosphorous detector. In addition to products used in this experiment and previously cited in "Samples", the multiresidue analysis considered: fungicides (acyclanine, dicarboximide, phthalimide, fenexamide, pyrimidine derivatives, strobilurin, and triazole), pesticides (acaricides, chlorinated hydrocarbon, organophosphate, oxadiazine, piretroides), herbicides (several molecules and Triazine) Analytical standards of the fungicide and pesticides utilized were purchased from Sigma (St. Louis, MO, USA). The limits of detection (LOD) and quantitation (LOQ) were determined by the signal-to-noise approach. The LOD for all the selected pesticides were defined at levels resulting in signal-to-noise ratios of 3 and 10, respectively (≤0.01 mg kg^{-1}) and LOQ (0.03 mg kg^{-1}). The recovery values were estimated at levels ranging from 0.1 to 1 mg kg^{-1}. Average recoveries for four replicates ranged from 83 to 94%.

Heavy metals

The homogenized sample was dried at 80°C for 12 h and then ground (1 mm) in a laboratory mill (Thomas Scientific, Swedesboro, NJ, USA). Five grams of the sample were weighed and placed in an HPV80 Milestone microwave mineralization unit (Milestone Inc., Shelton, CT, USA). The sample was mixed with 5 ml of nitric acid (695 g kg^{-1}) and 2 ml of perchloric acid, and then mineralized. After mineralization the solution was analyzed with atomic absorption spectroscopy (AAS) Perkin Elmer Analyst 600 (Perkin Elmer, Norwalk, CT, USA) equipped with a Zeeman effect corrected graphite furnace. For Cu, Cd and Pb, the LOD was 0.2 μg kg^{-1} and the LOQ, 1 μg kg^{-1}. Standard solutions were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). The recovery was 97% for Pb, 96% for Cu and 98% for Cd.

Ergosterol

Ergosterol analysis was performed on homogenized material using the method of Schwadorf and Müller [25] modified according to AFNOR [1]. The analysis was performed in subdued light. Briefly, 50 ml of ethanol, 150 ml of methanol, and 20 g KOH were added to 50 g of sample. The mixture was refluxed for 30 min at 60°C, cooled to 20°C, and filtered through a filter paper. Twenty millilitres of the filtrate were transferred to an Extrelut column and after 15 min ergosterol was slowly eluted with 90 ml of *n*-hexane. The eluate was evaporated to dryness at 35°C, quantitatively transferred to a 10-ml volumetric flask and brought to volume with *n*-hexane-isoamyl alcohol (98:2). An aliquot (1–2 ml) was filtered (0.45 μm) and injected (20 μl) in HPLC system equipped with UV–Vis detector.

A Superspher Si-60 column (4 μm particle size, 125 × 4 mm i.d.) was used at room temperature with a mobile phase of *n*-hexane-isoamyl alcohol (98:2) at 1.0 ml min^{-1}. The UV detector was set at 280 nm. Standard ergosterol was purchased from Sigma (St. Louis, MO, USA). The LOD and LOQ were determined by the signal-to-noise approach. The LOD (0.2 mg kg^{-1}) and LOQ (0.7 mg kg^{-1}) were defined at levels resulting in signal-to-noise ratios of 3 and 10, respectively. The recovery values were estimated at levels ranging from 2.0 to 10 mg kg^{-1}. The average recoveries for the four replicates ranged from 86 to 91%.

Vitamin C

The analysis was performed in subdued light. Fifty grams of homogenized tomatoes was added to 50 ml of 85% phosphoric acid and filtered through a Buckner filter. The filtrate was transferred to a 250-ml volumetric flask, brought to volume with 85% phosphoric acid, filtered and injected (20 μl) in HPLC equipped with a UV–Vis Diode Array detector set at 244 nm.

A Spelcosil ABZplus® column (5 μm particle size, 250 × 4.6 mm i.d.) was used at room temperature, with a mobile phase of 0.05 M NaH_2PO_4, pH 3, at 1.0 ml min^{-1}. The standard was purchased from Sigma-Aldrich (Milan, Italy). The LOQ was 0.5 mg kg^{-1}. Recovery values were estimated at levels ranging from 50 to 500 mg kg^{-1}. The average recoveries for the four replicates ranged from 93 to 102%.

Lycopene and β-carotene

The analysis was performed in subdued light, homogenized samples (20 g) were extracted with methanol and filtered with a Buckner filter. Tomato residues on the filter were extracted with 200 ml of dichloromethane for 90 min with a wrist-action shaker. The mixture was filtered through a Buckner filter and the filtrate was evaporated with a rotavapor at 30°C. The residue was redissolved with hexane-2-propanol (90:10) with sonication and transferred to a 10-ml volumetric flask.

An aliquot (1–2 ml) was filtered (0.45 μm) and injected (20 μl) in an HPLC system equipped with a UV–Vis detector set at 474 nm. An Amino column (4 μm particle size, 250 × 4 mm i.d.) was used at room temperature, with a mobile phase of hexane at 1.0 ml min^{-1}. Standard: lycopene and *β*-carotene were purchased from Sigma (St. Louis, MO, USA). The LOD and LOQ were determined by the signal-to-noise approach. The LOD (0.2 mg kg^{-1}) and LOQ (0.6 mg kg^{-1}) were defined at levels resulting in signal-to-noise ratios of 3 and 10, respectively. The recovery values were estimated at levels ranging from 2 to 10 mg kg^{-1}. The average recoveries for the four replicates ranged from 88 to 93%.

Salicylic acid

Ten grams of homogenized product were added to 10 ml of 25% NaOH, stirring with a mechanical stirrer. The pH was lowered to 1–2 with HCl 10 M and liquid/liquid extraction was performed in a boiling extractor with 50 ml of diethyl ether for 12 h. The residue was recovered with an acetonitrile solution–water–acetic acid (25:75:5 v/v/v) and subjected to HPLC analysis (20 µl) (Agilent Hewlett Packard 1100) with fluorimetric detector λ excitation = 300 nm and λ emission = 400 nm. The column was a Phenomenex Luna C18 (2) with 25 cm length, 4.6 mm in diameter and 5 µm packing. The eluting mixture was milliQ—85% H_3PO_4 CH_3CN 60:0.1:40. The LOQ was 0.01 mg kg^{-1}. The recovery values were estimated at different concentrations ranging from 0.05 to 20 mg kg^{-1}. The average recoveries, for three replicates per concentration, ranged from 86 to 102%. Standard: salicylic acid (99% purity) Aldrich (Milan, Italy).

Statistics

A completely randomized design was adopted, focusing principally on the agronomic technique. Statistical analysis was performed with the PROC GLM of the SAS statistical software (SAS 8.0) and based on one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls multiple comparison test. The level of significance was $P < 0.05$. The correlations between nutrients were performed with the PROC CORR of the SAS statistical software.

Results

Health-promoting molecules

This study found no differences in moisture between samples grown using different farming methods (Table 1). We found higher levels of ashes in the organic farming samples and also the protein content of organic tomatoes was higher than in the other tomatoes (Table 1). The ascorbic acid levels found in organic samples were lower than those found in the other farming systems under investigation while farming techniques were not observed to influence β-carotene content (Table 1). Our results do show significant differences in lycopene content depending on the farming system, with greater build-up in conventionally farmed tomatoes (Table 1). Although lycopene is a precursor of β-carotene, no significant inverse relationship can be observed between the two molecules (Table 2). Our experiment shows that farming techniques affected the concentration of salicylic acid, the levels of which are higher in organic than in conventional or IPM tomatoes (Table 1).

Table 1 Nutrient content (on a fresh basis) of tomatoes obtained with three different agricultural practices

Type of production	Moisture (g kg^{-1})	CP (g kg^{-1})	Ash (g kg^{--1})	Lycopene (mg kg^{-1})	Vitamin C (mg kg^{-1})	β-carotene (mg kg^{-1})	Salicylic acid (mg kg^{-1})	Cd (µg kg^{-1})	Pb (µg kg^{-1})	Cu (mg kg^{-1})
Organic	920.5 (2.5)	14.6 $(0.41)^c$	0.65 $(0.06)^b$	37.17 $(5.5)^a$	118.2 $(7.6)^a$	3.26 (0.35)	0.745 $(0.05)^c$	33.0 $(6.3)^b$	37.8 $(15)^b$	0.49 $(0.03)^a$
Conventional	926.3 (1.5)	10.5 $(0.24)^a$	0.49 $(0.05)^a$	48.90 $(1.8)^b$	214.0 $(16.0)^b$	2.95 (0.20)	0.462 $(0.03)^b$	2.0 $(1.7)^a$	3.4 $(1.9)^a$	0.46 $(0.05)^a$
IPM	921.9 (3.98)	11.4 $(0.18)^b$	0.38 $(0.05)^a$	35.35 $(2.0)^a$	212.07 $(29.8)^b$	2.81 (0.41)	0.213 $(0.03)^a$	23.0 $(9.2)^b$	1.6 $(1.2)^a$	0.65 $(0.05)^b$
P of the model	0.3422	0.0001	0.0044	0.0238	0.0056	0.5952	0.0001	0.0101	0.0107	0.0171

Values are mean. Standard error is in brackets
CP crude protein, *IPM* integrated pest management
[a,b,c]Student–Newman–Keuls multiple comparison test ($P < 0.05$)
$n = 10$ for each type of products, except for salicylate (organic $n = 7$) and vitamin C ($n = 6$)

Table 2 Pearson's correlation coefficients between nutrients' content

	DM	CP	Ash	β-carotene	Lycopene	Vitamin C	Salicylate	Cd	Pb	Cu
DM	.									
CP	0.247	.								
Ash	0.218	0.445*	.							
β-carotene	−0.067	0.221	0.015	.						
Lycopene	−0.336	−0.313	0.150	−0.089	.					
Vitamin C	0.019	−0.525*	−0.292	0.123	−0.015	.				
Salicylate	0.029	0.682**	0.599*	0.010	−0.408	−0.576#	.			
Cd	0.204	0.424*	−0.053	0.047	−0.153	−0.263	−0.196	.		
Pb	0.049	0.567**	0.492#	0.098	0.167	−0.343	0.595*	0.161	.	
Cu	0.268	−0178	−0.094	−0.058	−0.211	0.055	−0.476#	0.272	−0.182	.

DM dry matter, *CP* crude protein
* $P < 0.05$, ** $P < 0.01$, # $P < 0.08$
$n = 10$ for each type of products, except for salicylate (organic $n = 7$) and vitamin C ($n = 6$)

Heavy metals

Conventional tomato samples showed the lowest contamination levels for Cu and Cd. No significant differences in Cd levels were observed between the berries grown with IPM systems versus organic techniques, whereas higher levels of Cu were found in the IPM products (Table 1).

Plant protection products and ergosterol

For all the tested phytochemicals and ergosterol, a marker of fungal contamination, the analytical methods never detected samples with concentrations higher than the LOD

Discussion

Health-promoting molecules

The absence of differences in moisture between samples grown using different farming methods does not agree with the conclusions of the study by Fjelkner-Moding et al. [13], in which organic vegetables were found to have a higher dry matter content. Another study by Woese et al. [30], however, compared organic and conventional feedstuffs and found no clear-cut correlation between the farming method and dry matter content. The higher levels of ashes in the organic farming samples could be due to greater mineral absorption induced by more organic activity in the soil [16]. The higher nitrogen fertilization of organic tomatoes (150 vs. 120 and 63.5 kg ha^{-1} of N, respectively, for conventional and IPM) could be the reason for the higher protein content in organic berries. However, the slight difference observed in this study is of limited nutritional value, confirming the substantial equivalence of protein value of tomatoes obtained using different production techniques [7]. The low levels of ascorbic acid found in organic samples agree with the paper of Auclair et al. [4] but not with the findings of Asami et al. [3], Carnovale [9], Woese et al. [30], Caris-Veyrat et al. [8] and Lucarini et al. [23], who reported higher vitamin C content in organic vegetables and explained this increase as the plant's response to the higher oxidative stress to which organic vegetables are exposed. According to Dumas et al. [12], it is the nitrogen-based fertilization that reduces ascorbic acid synthesis, probably because lower levels of foliage growth require the shading of fruits, which triggers less ascorbic acid synthesis. In our study, the organic tomatoes received more N-based fertilizers, which caused an increase in their protein content. The existence of an inverse correlation between protein content and vitamin C levels ($r = -0.525$, $P < 0.05$) (Table 2) is logical since it corresponds to an inverse relationship between N availability and vitamin C synthesis, as observed by Woese et al. [30]. If we consider the vitamin C requirements established for Italy (60 mg day^{-1} for adults) and the Italy's estimated tomatoes consumption (100.6 g day^{-1} [28]) the consumption of organic tomatoes covers about 20% of requirements, this percentage rising to 35% for conventional or IPM products.

Our findings show no significant difference in the β-carotene content of berries grown using the three farming techniques, but the previously published results are also not univocal. Brandt and Mølgaard [7] reported a positive relationship Between N-based fertilization and β-carotene content, whereas Woese et al. [30] found no difference in this parameter depending on the use of conventional versus organic farming techniques. Caris-Veyrat et al. [8] did report higher content of β-carotene and lycopene in organic versus conventional tomatoes but Lucarini et al. [23] found a higher content of β-carotene in conventionally grown tomatoes and in the same work no differences were detected for lycopene, as confirmed also by Kopp et al. [21]. The absence of an inverse relationship between β-carotene and its precursor lycopene could be due to the high temperatures (>30°C) observed during the typical maturation period of the tomato berries that inhibited lycopene synthesis. There is not a clear requirement for lycopene. Kucuck et al. [22] obtained a reduction in the malignity of prostate cancer giving 30 mg day^{-1} of lycopene to men with a diagnosed prostatic cancer, while Fuhramn et al. [15] reported a reduction of LDL-cholesterol in subjects receiving a 60 mg day^{-1} of lycopene. Using lycopene content reported in Table 1 and data on tomato consumption of Turrini et al. [28], the estimated intake of lycopene ranges from 3.56 mg day^{-1} for IPM to 4.92 mg day^{-1} for organic fruits.

In both cases these are low values. Salicylates are molecules of plant origin, produced in response to attack by animal or plant pests [10], with anti-inflammatory properties, the most famous of which, acetyl-salicylic acid, is the active component of Aspirin®. Low-dose of Aspirin® is standard care in patients with a history of cardiovascular disease (CVD). The levels of salicylic acid observed in our experiment are similar to those reported by Venema et al. [29] but lower than values obtained by Swain et al. [27]. Our experiment shows that farming techniques have a clear effect on the concentration of salicylic acid, a molecular indicator of plant distress [11] the levels of which tend to be higher in organic foodstuffs [5]. The farming technique associated with the highest level of stress to crops was organic farming, most likely because of the poor efficacy of plant protection mechanisms in organic crops. The intermediate levels of salicylate found in conventional tomatoes probably arise from the oxidative stress induced by herbicide treatments, which are typical of conventional farming. It is difficult to understand the real effect of this difference of salicylate content on human health, assuming a per capita tomato consumption in Italy (salad and sauces) of 100.6 g day^{-1}, as estimated by Turrini et al. [28], we obtained an intake of this molecule ranging from 21.4 (IPM) to 74.9 μg day^{-1} (organic), a very low value if compared to the salicylic acid content of aspirin for long-term use (100 mg in Italy) or to the dose ranging 75–150 mg day^{-1} that proved to be effective for the prevention of CVD [6, 17]. Probably the higher content of salicylate in organic tomatoes is not sufficient to obtain serum levels of this nutrient comparable to those obtained with aspirin. But the high level of salicylate in organic foods, detected also by Baxter et al. [5], should be regarded as one of the most important aspects of quality in organic foods. However, we have to consider that Baxter et al. [5] compared soups sold commercially and containing different types of vegetables without considering factors affecting salicylate content such as cultivation and processing methods. Olsson et al. [24] hypothesized that the biological significance of health-promoting molecules contained in tomatoes (lycopene, vitamin C, β-carotene and salycilic acid), could be greater than expected on the basis of the concentration of each single molecule, because a synergistic action between them could occur. This could open new perspectives in the evaluation of the effect of health-promoting compounds of plant origin.

Heavy metals

The concentration of Cd, was <1 mg kg^{-1} in all three experimental plots, while Cu content ranged from 33.0 mg kg^{-1} in IPM plots to 36.5 in organic soil, the Pb content was 13.1, 13.9 and 13.4 mg kg^{-1} for IPM, organic and conventional plots, respectively, so differences in heavy metals content can not be due to soil composition. The values found are in agreement with the findings of Gundersen et al. [16]. In the present study, the detected levels of contaminants were found to be markedly lower than the maximum limits allowed by Law and were: 100 μg kg^{-1} for Pb and 50 μg kg^{-1} for Cd (EU Regulation 1881/2006). Copper is considered to be essential to human health (RDA of 1.2 mg day^{-1} for an adult in Italy and 0.9 mg day^{-1} in the USA) but may be harmful if taken in excessive amounts. At present there are no regulatory limits for Cu in foods, but a provisionable tolerable weekly intake (PTWI) 3.5 mg kg^{-1} of body weight has been established by Food Standard Agency of UK [14]. Based on data of tomato consumption in Italy [28] and assuming that an adult man (70 kg weight) eats one portion a day of tomatoes, the weekly intake of Cu ranges from the 9.3% of PTWI for conventional fruits to 13.1% for IPM fruits. The higher levels of Pb in organic samples could be explained by a higher input of this metal through manure [26], while the positive correlation of Pb with salicylic acid (Table 2), could be explained by a stressful action of Pb on the plant, which would react by increasing the synthesis of salicylic acid, a marker of distress on the plant [11].

Conclusions

Although our goal was not to draw general conclusions on the quality of organic food, our findings show that the farming technique may have an impact on the quality of tomatoes. The higher protein content observed in organic fruits has a low nutritional significance, because tomatoes are a poor protein source, but the higher salicylate content support the supposition that organic foodstuffs are more wholesome. On the other hand, the lower lycopene and ascorbic acid levels of organic tomatoes are not to be regarded as positive. No residues of pesticides and ergosterol were detected.

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