

Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes

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Understanding how environment, crop management, and other factors, particularly soil fertility, influence the composition and quality of food crops is necessary for the production of high-quality nutritious foods. The flavonoid aglycones quercetin and kaempferol were measured in dried tomato samples (*Lycopersicon esculentum* L. cv. Halley 3155) that had been archived over the period from 1994 to 2004 from the Long-Term Research on Agricultural Systems project (LTRAS) at the University of California—Davis, which began in 1993. Conventional and organic processing tomato production systems are part of the set of systems compared at LTRAS. Comparisons of analyses of archived samples from conventional and organic production systems demonstrated statistically higher levels ($P < 0.05$) of quercetin and kaempferol aglycones in organic tomatoes. Ten-year mean levels of quercetin and kaempferol in organic tomatoes [115.5 and 63.3 mg g⁻¹ of dry matter (DM)] were 79 and 97% higher than those in conventional tomatoes (64.6 and 32.06 mg g⁻¹ of DM), respectively. The levels of flavonoids increased over time in samples from organic treatments, whereas the levels of flavonoids did not vary significantly in conventional treatments. This increase corresponds not only with increasing amounts of soil organic matter accumulating in organic plots but also with reduced manure application rates once soils in the organic systems had reached equilibrium levels of organic matter. Well-quantified changes in tomato nutrients over years in organic farming systems have not been reported previously.

KEYWORDS: Tomato; organic agriculture; conventional agriculture; long-term research; flavonoid; flavanol; quercetin; kaempferol

INTRODUCTION

Fruits and vegetables are rich in dietary vitamins, minerals, and fiber and are the primary source of flavonoids in the diet. Epidemiological studies suggest that flavonoids protect against cardiovascular disease (1) and, to a lesser extent, against cancer (2) and other age-related diseases such as dementia (3). Flavonoids encompass a large group of phenolic secondary plant metabolites that demonstrate potent *in vitro* antioxidant activity (4, 5) and display free radical scavenging activity (6). Most attempts to assign health-promoting activity mechanistically to the antioxidant action of individual flavonoids in foods have

been unsuccessful (7) and suggest that other mechanisms are likely involved in the health-promoting activities of these compounds.

Secondary plant metabolites such as the flavonoids function in plant defense mechanisms against herbivory, pathogen stress, and UV-B radiation (8). Many of these compounds are also the primary pigments responsible for attracting pollinators and seed dispersers. Flavonoids are produced through the phenylpropanoid pathway in plants. The key enzyme that catalyzes the biosynthesis is phenylalanine ammonia-lyase. Environmental stresses including nutrient deficiency, wounding, pathogens, and UV radiation are known to activate the biosynthesis of phenylpropanoid compounds (9, 10). Given the increasing data in support of a role for phenolic plant metabolites in the prevention of cardiovascular and other chronic diseases, efforts are underway to improve their levels in plant foods through improved cultivar selection and genetic manipulations. Alter-

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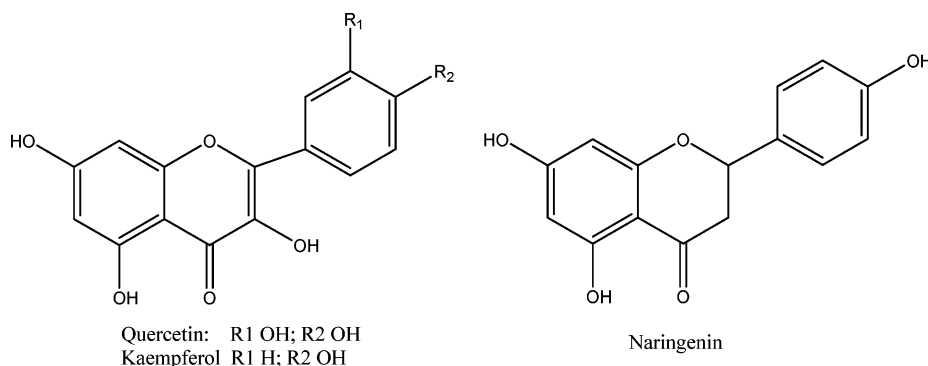


Figure 1. Structures of the flavonoid aglycones of quercetin, kaempferol, and naringenin.

natively, the phenolic content of plants may be influenced by manipulating the agronomic environment in which they are grown (11–13).

Fundamental differences between organic and conventional production systems, particularly in soil fertility management, may affect the nutritive composition of plants, including secondary plant metabolites. Organic systems emphasize the accumulation of soil organic matter and fertility over time through the use of cover crops, manures, and composts and rely on the activity of a diverse soil ecosystem to make nitrogen (N) and other nutrients available to plants. Conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids. Stamp proposed that increased crop growth and development rates and greater biomass accumulation in well-fertilized crops would also correlate with decreased allocation of resources toward the production of starch, cellulose, and non-nitrogen-containing secondary metabolites (14). Given that many secondary plant metabolites are produced for defense against herbivores and are inducible by pathogens or wounding, possible differences in pest pressure between conventional and organic systems might also influence levels in food crops.

Both conventional and organic agricultural practices include combinations of farming practices that vary greatly depending upon region, climate, soils, pests and diseases, and economic factors guiding the particular management practices used on the farm. Many of these influences change continuously, so a steady-state condition may never be achieved on most farms (15). The dynamic nature of agricultural systems also makes adequately controlled comparisons of produce quality, free from confounding influences, experimentally challenging. Comparisons of produce from different types of farms, even if they are near each other, are affected by the numerous factors mentioned. Reviews of studies comparing the nutritional quality of conventionally and organically produced vegetables demonstrate inconsistent differences with the exception of higher levels of ascorbic acid (vitamin C) and less nitrate in organic products (16, 17). However, these data are difficult to interpret because cultivar selection and agronomic conditions varied widely and different methods of sampling and analysis were used in the investigations cited. In contrast, cropping system comparisons using long-term research plots that have been managed consistently over time provide a means to overcome many of the confounding factors associated with farm-based sampling. Additionally, the effects of changes over time in cropping system behavior can be evaluated using archived soil and plant samples, and a reasonable estimate of the causes of those changes can be made.

The present study is an example of the use of long-term research to address complex processes operating in cropping systems. Its specific objective was to compare the content of the flavonoids quercetin, kaempferol, and naringenin in tomato samples (*Lycopersicon esculentum* L. cv. Halley 3155) produced in conventional and organic cropping systems that had been archived over the period from 1994 to 2004 from the Long-Term Research on Agricultural Systems project (LTRAS) at the University of California—Davis, which began in 1993 (<http://ltras.ucdavis.edu>). LTRAS was designed to detect and estimate changes in crop productivity trends and other factors correlated with sustainability, which result from differing irrigation and fertilization practices (18). It includes an organic cropping system in which maize and tomatoes are grown in rotation and compared to the same crops produced conventionally. This archive of tomato samples is unique in California and perhaps the world.

This study focused on tomatoes because the per capita consumption of tomatoes (8.1 kg per capita in 2003) and tomato products (31.1 kg per capita in 2003) in the United States is very high, second only to the potato (19). Tomatoes provide an important and significant source of vitamin C (19 mg/100 g of fresh weight), vitamin A (623 IU/100 g of fresh weight), lycopene (3.0 mg/100 g of fresh weight), and flavonoids (20, 21). The main flavonoids found in tomatoes are quercetin, kaempferol, and naringenin (Figure 1). Quercetin predominates, with levels ranging from 0.03 to 2.77 mg/100 g in fresh tomatoes and 4.77 mg/100 g in processed tomato products (22). Levels of kaempferol range from 0.01 to 0.06 mg/100 g in fresh and processed tomatoes (22).

MATERIALS AND METHODS

Chemicals and Samples. Quercetin dihydrate (3,5,7,3',4'-pentahydroxyflavone dihydrate, 98%), morin hydrate (3,5,7,2',4'-pentahydroxyflavone hydrate, 95%), and *tert*-butylhydroquinone (TBHQ, 97%) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Kaempferol (3,5,7,4'-tetrahydroxyflavone, 95%) and naringenin (4',5,7-trihydroxyflavone, 95%) were obtained from Sigma Chemical Co. (St. Louis, MO), and Indofine Chemical Co., Inc. (Hillsborough, NJ), respectively. All solvents used were of HPLC grade. Ethanol was purchased from Spectrum Chemicals (Gardena, CA).

Tomato Cultivation in the LTRAS Cropping Systems. Following uniform cropping with Sudan grass (*Sorghum vulgare* L.) in 1992 and 1993, 10 different cropping systems were established in 1993 using 0.4 ha plots (18). Each cropping system was replicated three times, and both crops or phases of the two-year rotations were present each year. Plots were large enough to allow for the use of commercial-scale farm equipment. Irrigation amounts were measured using flow meters located at each irrigated plot. Systems differ in the amount of irrigation received (rain fed or irrigated), in the amounts of nutrients applied as fertilizers, and in organic matter applied to the soil as crop residues,

winter legume cover crops, and/or composted manure. Conventional plots received herbicides and other pesticides as needed, whereas organic crops received only organically approved pesticides, such as sulfur and Bt compounds. Crop yields and total biomass were measured every year and analyzed for total N and C. Sample archiving included yearly plant and fruit samples from all cropping systems and time zero and subsequent soil samples collected every few years. Systems rather than single inputs were compared, so a valid comparison required that each system be managed carefully to achieve its potential yields. For example, both the conventional and organic maize/tomato systems had the same tomato cultivar (Halley 3155) and were irrigated similarly as needed, but the lower percent availability of organic N sources can require more total N input to meet crop N needs (24). The same cultivar was used throughout the 12-year period. The systems compared are model systems, chosen to include representative crops rather than more complex, changeable crop rotations. California farmers rarely follow fixed crop rotations as markets change, and organic farmers especially tend to have more complex rotations than the one studied at LTRAS.

Crop management practices follow best management practice guidelines in the region. Conventional tomatoes received 50 kg ha⁻¹ of an N–P–K starter fertilizer and 118 kg ha⁻¹ of ammonium nitrate as side dressing. A combination of tillage and herbicides was used for weed control. Aphids, mites, and stinkbugs occur periodically and were controlled as necessary, similar to practices in commercial fields. In both treatments, processing tomatoes were transplanted at the rate of 22500 plants ha⁻¹ (10000 per acre). Transplanting in the organic treatment followed incorporation of a winter legume cover crop consisting of hairy vetch (*Vicia villosa* Roth) and field peas (*Pisum sativum* L.). Transplanting of all plots occurred within a period of 2–4 days in spring, commonly around the middle of April. Prior to incorporation of the cover crop, 9 Mg ha⁻¹ of composted poultry manure currently is top-dressed and incorporated with the cover crop. The amount of N present in cover crops varies from year to year, but, typically, organic plots currently receive between 240 and 260 kg of N ha⁻¹ year in addition to the N fixed by the legume cover crop (24). During the first three cropping cycles, to more rapidly increase soil organic matter levels and soil fertility, 19 Mg ha⁻¹ of composted manure was added to tomato crops. This was reduced after organic matter levels had increased to a near constant level (24). Tomatoes were harvested each year when the field as a whole reached 90% ripe fruit. A commercial tomato harvester was used for main plot harvests. Similar methods of transplanting and plant populations were used for conventional plots as well, and transplanting of all plots occurred within a period of 2–4 days in spring, commonly around the middle of April.

Sampling and Preparation of Plant Material. Immediately prior to harvest of the main plot, samples for the archive were collected from four 3.1 m² subsample areas (subplots) within the larger main plots. Total plant biomass and the yield of the red ripe fruit were determined for each subplot. A random sample of 20 ripe fruits from the four subplots was washed and oven-dried at 60 °C, ground, and stored in glass containers in the dark at 20 °C until use. Samples from conventional and organic plots from even numbered years (1994–2004) were chosen for flavonoid analysis. Three of the subplots ($n = 3$) for each treatment (three organic; three conventional) were analyzed each of the even years (1994–2004). These subplot samples were analyzed in triplicate.

Flavonoid Analysis. Flavonoid analysis followed the general method of Merken and Beecher (25) as modified by Chassy et al. for fresh processing tomatoes (26). Flavonoid glycosides were hydrolyzed to their corresponding aglycones, which were subsequently measured using HPLC. Briefly, 1 g of pestle-pulverized, air-dried tomato sample was combined with 100 mL of 1.6 g/L *tert*-butylhydroquinone, 1.2 N hydrochloric acid, and 50% methanol (MeOH). To this mixture was added 0.5 mL of a 1 mg/mL standard of luteolin as an internal standard. Internal standard recovery was 92–93% for luteolin. Samples were refluxed for 4 h at 100 °C in a 250 mL round-bottom flask. Four hours of reflux time, in 1.2 N hydrochloric acid, was determined as the optimal condition for maximal recoveries of quercetin, kaempferol, and naringenin in these samples. An aliquot was removed, diluted 50:50 v/v with MeOH (200 μ L), and filtered through a 0.5 mL of 25 μ m MC Ultrafree-MC filter (Millipore, Bedford, MA). Flavonoids were sepa-

Table 1. Mean Flavonoid Concentrations in Archived Samples from the LTRAS Experiment (1994–2004) and Single Degree of Freedom Contrasts between Conventional and Organic Farming Systems

flavonoid	mean (SD) (mg g ⁻¹ of DM)		<i>F</i>	<i>p</i>
	conventional	organic		
quercetin	64.6 (2.49)	115.5 (8.0)	108.16	<0.0001
naringenin	30.2 (1.57)	39.6 (1.58)	66.36	<0.0001
kaempferol	32.06 (1.94)	63.3 (5.21)	96.64	<0.0001

rated using a Hewlett-Packard 1090 HPLC equipped with a variable wavelength diode array detector and Chemstation (LC 3D rev. A.08.03) software (Agilent, Palo Alto, CA) monitoring 370 nm. Reversed phase HPLC was performed using a 250 × 4.6 mm i.d., 5 μ m Zorbax XDB-C18 column and a 12.5 × 4.6 mm i.d. 5 μ m Zorbax XDB-C18 precolumn (Agilent). The mobile phase consisted of 0.05% trifluoroacetic acid (TFA) in water (solvent A), 0.05% TFA in MeOH (solvent B), and 0.05% TFA in acetonitrile (solvent C). Separations were effected by a series of linear gradients using a flow rate of 1.0 mL/min as follows: 90–85% A, 6–9% B, 4–6% C, 0–5 min; 85–71% A, 9–17.4% B, 6–11.6% C, 5–30 min; 71–0% A, 17.4–85% B, 11.6–15% C, 30–60 min. The linear ranges of quantitation for quercetin and kaempferol were 0.5–10 and 0.1–10 μ g/mL respectively.

Statistical Analysis. Data were analyzed using SAS software version 9.1 (SAS Institute, Cary, NC). Specifically, PROC Mixed and regression were used to analyze treatment differences and create single degree of freedom contrasts for differences between systems (27).

RESULTS

The mean level of the flavonoids quercetin, naringenin, and kaempferol aglycones (mg g⁻¹ of DM) were significantly higher ($p < 0.001$) in samples from the organic cropping system as compared to samples from the conventional cropping system (Table 1). Quercetin was the most abundant flavonoid in both organic (115.5 ± 8.0 mg g⁻¹ of DM) and conventional (64.6 ± 2.4 mg g⁻¹ of DM) tomatoes. In conventional tomatoes, kaempferol (32.06 ± 1.94 mg g⁻¹ of DM) and naringenin (30.2 ± 1.57 mg g⁻¹ of DM) levels were comparable, whereas levels of kaempferol (63.3 ± 5.21 mg g⁻¹ of DM) were significantly higher than naringenin levels (39.6 ± 1.58 mg g⁻¹ of DM) in organic tomatoes. Absolute differences in the flavonoids increased with time in both systems, with the largest differences observed in the last 4-year period (Figures 2 and 3).

Figure 4 compares the quercetin and kaempferol means to N applications over this 10-year period. Increases in quercetin and kaempferol content appear to be correlated in time with changes in manure application rates, which were reduced in the

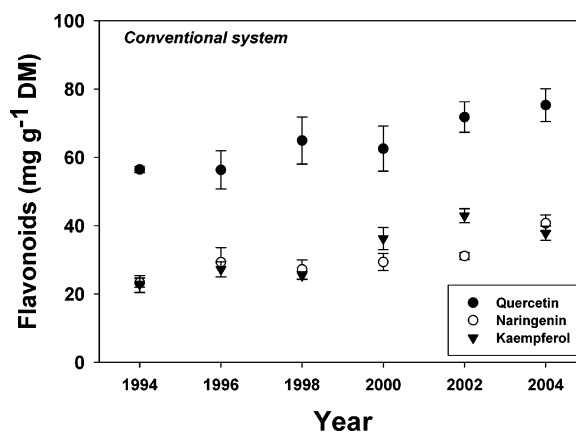


Figure 2. Quercetin (●), naringenin (○), and kaempferol (▼) levels in tomatoes derived from conventional cropping systems at LTRAS from 1994 to 2004 (mg g⁻¹ of DM). Values are given with standard deviations.

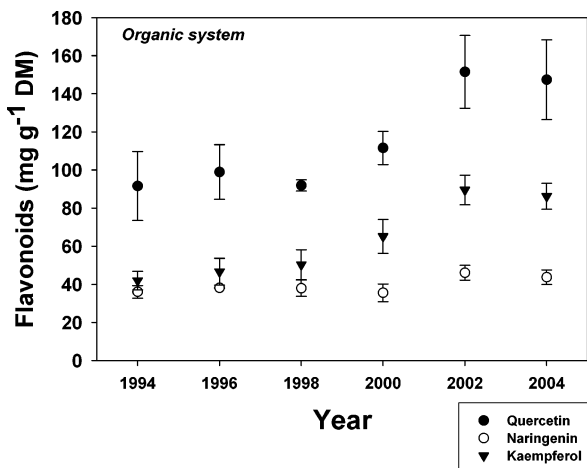


Figure 3. Quercetin (●), naringenin (○), and kaempferol (▼) levels in tomatoes derived from organic systems at LTRAS from 1994 to 2004 (mg g⁻¹ of DM). Values are given with standard deviations.

organic system in 1998 from an average of 45 to 18 Mg ha⁻¹ over the 2-year maize/tomato cycle. Conventional maize/tomato rotation crops received 230 kg of N ha⁻¹ for maize and 160 kg of N ha⁻¹ for tomatoes in each year. Levels of quercetin and kaempferol were highest in years 2000, 2002, and 2004. **Figure 5** shows tomato yield from 1994 to 2004 in organic and conventional plots. On average, yields were not significantly different between the two systems, but there was less year-to-year variation in the organic systems (28, 29).

DISCUSSION

The content of flavonoids appears to have increased over time in the archived tomato samples in both the conventional and organic systems (**Figures 2 and 3**). The rates of increase of quercetin and kaempferol were much lower in the conventional system compared to the organic system. For the conventional system, regression analysis of mean values of flavonoid concentration versus time suggests an increase of 1.9 mg g⁻¹ year⁻¹ for kaempferol (se = 0.34, r² = 0.65) and 2.0 mg g⁻¹ year⁻¹ for quercetin (se = 0.57, r² = 0.43). Naringenin increased at a rate of 1.35 mg g⁻¹ year⁻¹ (se = 0.33, r² = 0.51). Alternatively, these rates of increase can be interpreted as rates of deterioration of flavonoid content in storage with time. During the 10-year period analyzed, farming practices in the conventional system, including N fertilization and the tomato variety grown, did not change, and there was little accumulation of organic matter or other obvious changes in soil quality over that period in conventional plots (24). Therefore, it appears most likely that flavonoids declined slowly with time in storage. The stability of flavonoids has been documented in onions stored for 24 weeks (30) and in apples stored for up to 30 weeks (31). We assume that the rate of decline in storage is identical in both organic and conventional samples. If that is the case, then there is a significant difference in the amount of flavonoids occurring in ripe fruit at harvest between the two systems.

Bongue-Bertlesman and Philips found that N-deficient tomato plants had significantly greater flavonoid content in their leaves (32). Chassy et al. reported significantly higher mean levels of soluble solids, flavonoids, total phenolics, and ascorbic acid in organic tomatoes as compared to their conventional counterparts grown in model plots over a 3-year period (26). However, year-to-year variation in this study was significant, and only the levels of kaempferol were statistically higher in organic tomatoes for all three years (26). Toor et al. also examined the influence of

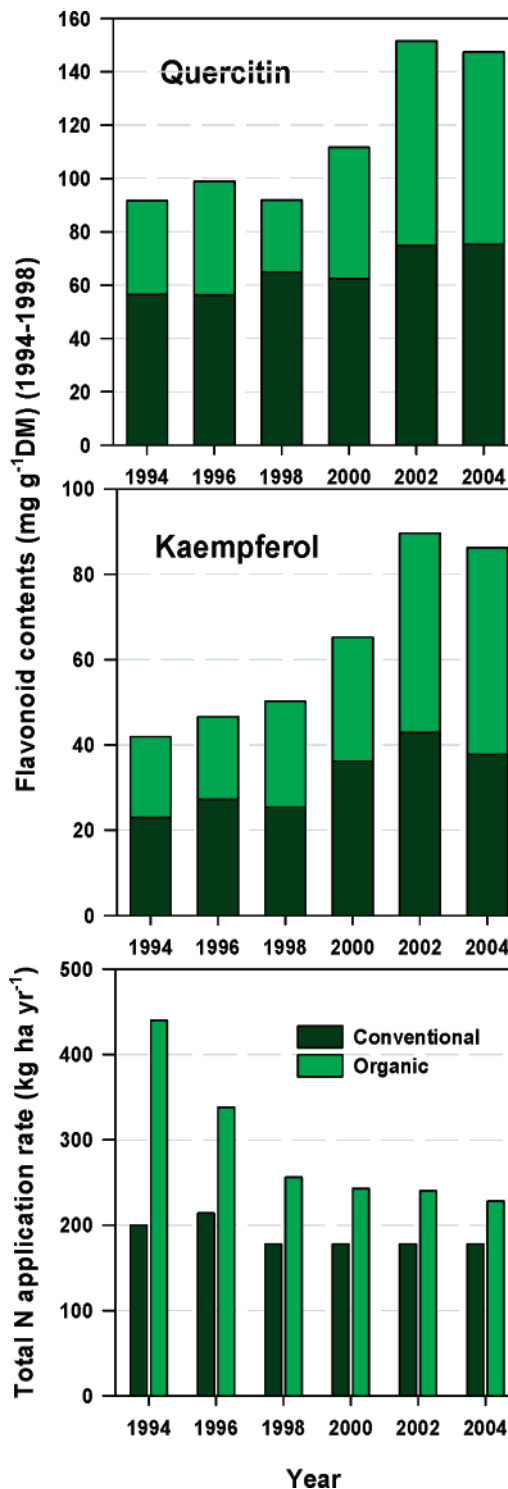


Figure 4. Changes in flavonoid levels averaged over 10 years of the LTRAS trial, and changes in N inputs over time to the tomato systems (1994–2004).

nutrient source on antioxidant components and antioxidant activity of greenhouse-grown tomatoes (11). These tomatoes were grown with mineral nutrient solutions (containing NH₄⁺ and NO₃⁻), chicken manure, and grass-clover mulch. The mean total phenolic and ascorbic acid contents of tomatoes grown using grass-clover mulch (29%) and chicken manure (17.6%) were higher than those of the tomatoes grown with the mineral nutrient solutions and demonstrate that nutrient source can play a role in determining the levels of antioxidants in tomatoes.

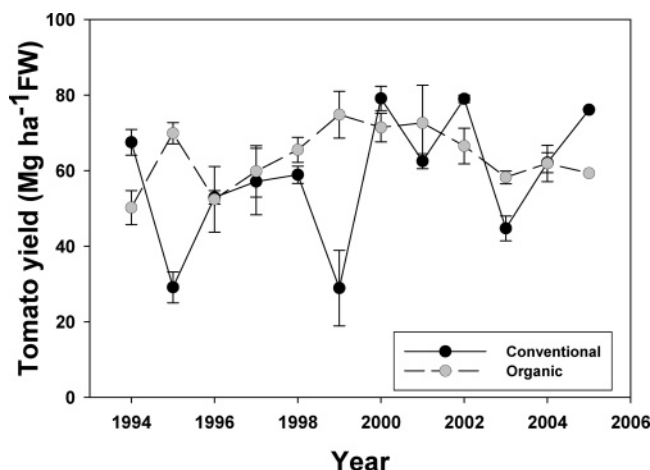


Figure 5. LTRAS tomato yields (Mg of fresh weight) from 1994 to 2004.

Soil organic matter (SOM) levels in the organic system at LTRAS are currently significantly higher in the organic plots than in the conventional ones (18). SOM may have reached a quantitative limit of accumulation by 1997 or 1998 (24, 28), but changes in SOM quality may still be occurring (22). At about the time SOM reached a limit of accumulation, the amount of composted manure applied to organic plots was reduced. Over the 2-year crop rotation (maize/tomato), the amount was reduced from approximately 45 Mg ha⁻¹ on average to 18 Mg ha⁻¹. Similar amounts of N continued to be applied as green manure cover crops grown during the winter season, subject to year-to-year variation in cover crop growth. Figure 4 compares the amounts of quercetin and kaempferol in conventional and organic systems to estimated N applications over the 12-year period. Significant increases in quercetin and kaempferol content appear to be correlated in time with the cumulative effects of changes in manure application rates, resulting in lower total N application amounts to organic plots over the last 6 years as compared to the first 6 years. These changes did not affect LTRAS tomato yield (Figure 5), and they did not consistently affect color or soluble solids, other secondary quality characteristics commonly assessed by tomato processors in fresh samples (29).

Flavonoid content in tomatoes seems to be related to available N (3, 11). Plants with limited N accumulate more flavonoids than those that are well-supplied. If differences in flavonoid content reflect fundamental differences in the behavior of soil N between conventional and organic systems, then the N available to tomatoes late in the season may have declined in organic plots in recent years in response to the cumulative effects of a decrease in compost application rates.

The LTRAS project, a carefully managed long-term research project focusing on cropping systems, is a useful basis for detecting and determining the magnitude of differences in tomato quality. The differences detected appear to be correlated with differences in soil fertility management associated with conventional and organic farming systems. These comparisons are free of confounding factors such as soil type differences, differing and variable crop management histories, uneven amounts of time that systems have been managed organically or conventionally, differing levels of farm management skill among farmers, cultivar differences, irrigation, harvesting, sample handling, and other factors that affect the usefulness of most previous comparisons of fruit quality between conventional and organic farming systems. Instead, differences can be attributed primarily to contrasting soil fertility management practices between the conventional and organic systems. The

two systems produced tomatoes with differing flavonoid contents within individual years, and these differences increased with time. We suggest that it is the behavior and quantity of N in the organic and conventional systems that most strongly influence these differences (11, 30, 33). If so, then overfertilization (conventional or organic) might reduce health benefits from tomatoes.

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