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ARTICLE

Influence of Different Organic Fertilizers on Quality Parameters and the δ^{15}N, δ^{13}C, δ^{2}H, δ^{34}S, and δ^{18}O Values of Orange Fruit (*Citrus sinensis* L. Osbeck)

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To investigate the influence of different types of fertilizers on quality parameters, N-containing compounds, and the δ^{15}N, δ^{13}C, δ^{2}H, δ^{34}S, and δ^{18}O values of citrus fruit, a study was performed on the orange fruit cv. 'Valencia late' (*Citrus sinensis* L. Osbeck), which was harvested in four plots (three organic and one conventional) located on the same farm. The results demonstrated that different types of organic fertilizers containing the same amount of nitrogen did not effect important changes in orange fruit quality parameters. The levels of total N and N-containing compounds such as synephrine in fruit juice were not statistically different among the different treatments. The δ^{15}N values of orange fruit grown under fertilizer derived from animal origin as well as from vegetable compost were statistically higher than those grown with mineral fertilizer. Therefore, δ^{15}N values can be used as an indicator of citrus fertilization management (organic or conventional), because even when applied organic fertilizers are of different origins, the natural abundance of ^{15}N in organic citrus fruit remains higher than in conventional ones. These treatments also did not effect differences in the δ^{13}C, δ^{2}H, δ^{34}S, and δ^{18}O values of fruit.

KEYWORDS: Stable isotope; compost; manure; citrus; 'Valencia late' orange

INTRODUCTION

In recent years, growing interest among consumers regarding safer and healthier foods has prompted firms to extend the supply of organic products. Organic cultivation systems exclude the use of synthetic fertilizers, pesticides, and insecticides and usually lead to increased antioxidant components in crops (*1*, *2*). In European countries, EC Regulation 834/07 set organic management practices and provided a standard that regulates the right to label food as organic. The complete traceability of organic products at all stages of production, processing, and marketing is assessed by a certification entity. Inspection of documents and sampling for pesticide analysis can be made to control the organic origin of products. However, no methods are available to evaluate soil fertility management, which is the source of nutrients under organic agricultural regimes. Therefore, unscrupulous farmers could apply synthetic nitrogen to the soil, for example, by fertigation. In this case, fraud is difficult to detect.

Recent studies have shown that different fertilizer types applied to soil, such as composted and uncomposted animal or vegetable waste, green manure, and mineral fertilizers, influence plant nitrogen isotope composition (i.e., the ratio ^{15}N/^{14}N, expressed as δ^{15}N) of crops (*3*−*5*). The values of δ^{15}N in synthetic nitrogen fertilizers usually range between −2 and +2‰ (*6*), whereas the different origins of organic fertilizers do not permit the assignment of a narrow range of N isotope ratio to these fertilizers. Usually, a higher value of δ^{15}N found in organic fertilizers is reflected in the δ^{15}N level of N-containing compounds of vegetables (*7*−*10*). Given these findings, the use of δ^{15}N value as a screening tool to differentiate between organically and conventionally grown crops has been suggested. Recently, a study carried out on citrus fruit sampled in commercial organic and conventional farms has demonstrated that the δ^{15}N of pulp proteins and amino acids of juice can be considered as markers to distinguish organic fruit from conventional ones (*11*).

Due to biotic and abiotic processes that occur during the production or composting of organic fertilizers, the fractionation of ^{15}N contributes to different δ^{15}N values for these fertilizers (*12*, *13*). Thus, the different organic fertilizers used as N sources can differentially affect the global δ^{15}N value of plant biomass.

Moreover, values of the isotope ratio ^{13}C/^{12}C (expressed as δ^{13}C) in plant tissues have also been found to indicate certain types of crop management practices (*14*). This is not due to the type of applied fertilizer because mineral and organic fertilizers have the same δ^{13}C values (*15*). The supply of N may affect δ^{13}C via effects on the rate of photosynthesis, and so the source of N may have a direct influence via non-RuBisCo carboxylation and an indirect influence via effects on water use efficiency (*16*).

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Lower δ^{13}C values were found in some organic vegetables in comparison to their conventional counterparts (*17*); this fact has often been explained by highlighting the higher microbial activity in organic fields, which produces higher soil respiration with lower δ^{13}C. Another explanation could be that with higher N availability, as in conventional crops, rates of photosynthesis may increase, followed by lower discrimination of the enzyme RuBisCo against $^{13}CO_2$ (*16*). However, organic potatoes (*10*), tomatoes (*4*), and peppers (*9*) did not show δ^{13}C values significantly lower than their conventional counterparts. Very low δ^{13}C (i.e., less than −35‰) were instead found in crops grown in greenhouses due to the presence of CO_2 from heating of the greenhouse using CH_4 (*18*).

Different leaf water δ^{18}O values (^{18}O/^{16}O ratio) were hypothesized between organic and conventional cultivations (*17*) due to differences in plant density and growth rates influencing evapotranspiration and, therefore, the δ^{18}O leaf water composition (*19*). The same trend can be hypothesized for plant δ^{2}H on the basis of the significant correlation existing normally between δ^{18}O and δ^{2}H (*20*, *21*). Moreover, the ratio ^{34}S/^{32}S (δ^{34}S) was presumed to be different because of the different δ^{34}S values of organic and mineral fertilizers (*17*). However, the sulfates of synthetic fertilizers derive both from sulfuric acid produced from metal sulfides, sulfurous gases, and native S (with a δ^{34}S range from about −5 to + 12‰) and from marine evaporites (δ^{34}S values from +10 to +35‰) (*15*), which show a large δ^{34}S range that should overlap that of organic fertilizers.

To investigate the influence of different organic fertilizers on quality parameters, N-containing compounds, and the δ^{15}N, δ^{13}C, δ^{2}H, δ^{18}O, and δ^{34}S values of citrus fruits, a study was performed on orange fruit cv. 'Valencia late' harvested in four plots (three organic and one conventional) located on the same farm.

MATERIALS AND METHODS

Experimental Design. Orange fruit (*Citrus sinensis* L. Osbeck, cv. 'Valencia late') were collected over a three-year period (2004−2006) from an experimental field located at the Palazzelli (Siracusa, Italy) experimental farm of the CRA-Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (Acireale, Italy). The orchard soil had a sandy loam texture (IUSS), subalkaline pH (7.8), and high CEC (65 mequiv 100^{-1}g of dry soil). Four treatments were carried out on 360 uniform 35-year-old trees of 'Valencia late' grafted onto sour orange rootstock and distributed in a randomized-block experimental design with three replicates. Each plot consisted of 30 trees, and all determinations were performed on fruit collected from 8 trees per plot. The control plots were fertilized by mineral fertilizers (MF) and did not receive any organic carbon. Organic plots were fertilized using two different composts, obtained from citrus byproduct (CB) and from livestock waste (LW), respectively. A third plot was fertilized with poultry manure (PM), which is one of the most widespread organic fertilizers in Italy. The organic plots have been under organic management since 1996. In each of these treatments, the total amounts of applied nitrogen were the same. Total N determination in soil of different plots (0−40 cm depth) was carried out before fertilization (February 2004), the first year of the experimental trial, and the values obtained did not show significant differences (MF, 1760.39 ± 233.31 mg/kg; CB, 2069.90 ± 367.52 mg/kg; LW, 2060.79 ± 267.55 mg/kg; PM, 1963.24 ± 272.04 mg/kg). Details on fertilizer composition, δ^{15}N values, and the annual application of different fertilizers are reported in **Table 1**. In all plots, either for mineral fertilizer or organic treatments, weed control was mechanical and insect control was assured by mineral oil application. Fertilization was applied in early spring, and no pruning residual recycling was adopted. The irrigation system was sprinkler localized.

Table 1. Types of Fertilizers, Chemical Composition, and Doses Used in Organic and Conventional Experimental Plots (2004−2006)

type of fertilizer and chemical composition	dose of fertilizer (kg/tree)	δ^{15}N (‰)
mineral fertilizer (MF)		2.45 ± 0.23
NPK (20−10−10)	2.35	
superphosphate	0.73	
K_2SO_4	0.55	
citrus byproduct compost (CB) (N, 1.4%; P, 1.8%; K, 0.5%; SO, 0.8%)	32.87	8.18 ± 0.15
livestock manure compost (LW) (N, 1.3%; P, 1.2%; K, 1.2%; SO, 29.0%)	35.50	8.65 ± 0.17
poultry manure (PM) (N, 3.5%; P, 3.0%; K, 3%; SO, 3.5%)	14.33	8.59 ± 0.34

Physicochemical Analysis. Fruit quality was assessed on a sample of 50 fruits collected at fruit maturity from 4 trees (2 samples per plot). Physicochemical parameters (i.e., firmness, fruit weight, juice yield, peel thickness, titrable acidity, and total soluble solids) were measured using standard methods (*22*), whereas color analysis was evaluated as peel and flesh CIE *L***a***b** values using the Minolta CR-300 chroma meter. Ascorbic acid was measured using a HPLC system (Waters, Milford, MA) equipped with a Waters 484 UV detector (*23*). Briefly, 10 mL of juice was brought to volume (100 mL) with a solution of 3.0% metaphosphoric acid. The sample was centrifuged to 5000 rpm for 20 min and filtered using a 0.45 μm cartridge prior to HPLC injection. The column was a 250 mm × 4.6 mm i.d., 5 μm, Hypersil ODS (Phenomenex, Torrance, CA), and the solvent system was composed of 0.02 M phosphoric acid at a flow rate of 1.0 mL/min. Detection was performed at 260 nm. Total N in soil and in fruit juice was determined by Kjeldahl's method using an AutoKjeldahl Unit K-370 apparatus (Buchi, Flawil, Switzerland). Synephrine content was determined by the HPLC method described by Rapisarda et al. (*11*) After SPE through a strong cationic exchange (SCX) cartridge (Varian Mega Bond Elut SCX), synephrine was eluted by methanol containing concentrated NH_4OH (85:15 v/v) and the solution evaporated (35 °C, low pressure). The extract was diluted with a solution containing 0.1% of H_3PO_4 and injected into the HPLC, after filtering with a 0.45 μm membrane. Separation was performed on an analytical Symmetry C-18 5 μm column (150 × 4.6 mm i.d.) (Waters), using the same HPLC equipment described above. The mobile phase consisted of CH_3CN/H_2O (35:65) containing 0.01% H_3PO_4 and 0.3% sodium dodecyl sulfate (SDS). The flow rate in the column was 1 mL/min, and the detection wavelength was set at 230 nm.

Stable Isotope Ratio Analysis (SIRA). Measurement of the ^{15}N/^{14}N ratio of pulp (δ^{15}N P), amino acids of juice (δ^{15}N AA), as well as δ^{13}C of pulp and amino acids of juice (δ^{13}C AA) were conducted following the methods described by Bricout and Koziet (*24*) and Kornexl et al. (*25*), respectively, with slight modification. ^{15}N/^{14}N isotopic ratio analyses of different fertilizers were carried out in triplicate (*15*). For the measurement, an isotope ratio mass spectrometer (Delta plus XP ThermoFinnigan, Bremen, Germany) equipped with an elemental analyzer (EA Flash 1112 ThermoFinnigan) was used. The ratio ^{34}S/^{32}S was measured in the pulp fruit using the same instruments on a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau/Germany) coupled to a GVI 2003 or a GVI Isoprime IRMS (GV Instruments Ltd., Manchester, U.K.) for the simultaneous determination of C, N, and S isotopic ratios. The ratio ^{2}H/^{1}H was analyzed in the pulp fruit using the same isotope ratio mass spectrometer (Delta plus XP ThermoFinnigan) following pyrolysis in a high-temperature conversion/elemental analyzer (TC/EA ThermoFinnigan) of the sample. The operating conditions are detailed in previous publications (*21*, *26*). The ^{18}O/^{16}O ratio of juice water was analyzed in CO_2 according to the water equilibration method described in the ENV 12141 method (Isoprep 18 VG ISOGAS − IRMS SIRA II VG ISOGAS). The values were expressed in δ‰ against international standards (Vienna−Pee Dee Belemnite for δ^{13}C, air for δ^{15}N, Vienna−Standard Mean Ocean Water for δ^{18}O, V-CDT for δ^{34}S) (*26*). The isotopic values were calculated against working in-house standards (mainly casein), calibrated against international reference materials, including L-glutamic acid USGS 40 (IAEA-International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA), and sugar IAEA-CH-6 (IAEA) for ^{13}C/^{12}C and L-glutamic acid USGS 40 for ^{15}N/^{14}N measurement. The ^{34}S/^{32}S was calibrated against a casein reference material with an assigned value (δ^{34}S = 4.4‰) and IAEA

Table 2. Quality Parameters of Organically and Conventionally Grown 'Valencia late' Orange Fruit[a]

parameter	MF	CB	LW	PM
fruit yeld (kg/tree)	138.37 ± 29.38	138.85 ± 30.10	139.73 ± 25.37	137.19 ± 37.37
firmness (kg)	5.41 ± 1.04	5.04 ± 0.74	4.91 ± 1.17	4.96 ± 1.18
fruit weight (g)	173.22 ± 14.78	177.84 ± 16.71	179.98 ± 20.05	175.11 ± 20.08
central axis (mm)	10.71 ± 1.81	10.64 ± 1.79	9.96 ± 1.58	10.68 ± 1.69
peel thickness (mm)	5.48 ± 0.48	5.35 ± 0.46	5.23 ± 0.62	5.51 ± 0.56
juice yield (%)	49.59 ± 5.60	50.51 ± 5.99	50.83 ± 4.95	52.07 ± 4.90
titrable acidity (% citric acid)	1.16 ± 0.11 a	1.11 ± 0.13 b	1.17 ± 0.10 a	1.15 ± 0.10 a
total soluble solids (%)	10.27 ± 0.52 a	9.91 ± 0.51 b	10.25 ± 0.45 a	10.31 ± 0.34 a
ascorbic acid (mg/100 mL)	49.34 ± 7.58 b	52.00 ± 5.41 a	50.09 ± 7.23 ab	49.98 ± 7.01 ab
*L** peel	66.59 ± 1.82 a	66.58 ± 1.99 a	64.73 ± 4.58 b	64.97 ± 4.22 b
*a** peel	23.6 ± 4.53 a	23.16 ± 5.11 ab	20.51 ± 5.98 c	22.16 ± 6.15 b
*b** peel	63.85 ± 3.96 a	63.56 ± 4.32 a	58.34 ± 13.55 b	58.53 ± 13.25 b
*L** flesh	54.69 ± 2.80	55.14 ± 2.84	54.33 ± 2.41	53.63 ± 1.85
*a** flesh	8.56 ± 1.10 ab	7.88 ± 1.11 b	8.43 ± 1.01 ab	9.14 ± 1.26 a
*b** flesh	22.14 ± 1.52	21.84 ± 1.36	22.08 ± 0.94	22.05 ± 0.91

[a] Means within the same row of different managed plots followed by different letters are significantly different at $p \leq 0.05$.

S-1 silver sulfide standard. The $^2H/^1H$ values were corrected against the same casein reference material with an assigned value of δ^2H, according to the comparative equilibration technique (*21*). The uncertainty (2σ) of measurements was ±0.3‰ for the $\delta^{13}C$ and $\delta^{15}N$ of pulp as well as for the $\delta^{18}O$ in juice water, ±0.8‰ for $\delta^{34}S$, and ±3‰ for δ^2H.

Statistical Analysis. Statistical analysis of the results was carried out using the STATSOFT 6.0 program (Vigonza, Padova, Italy). The statistical differences were evaluated by variance analysis (ANOVA), and means separation was conducted using the Tukey test.

RESULTS AND DISCUSSION

Table 2 summarizes three-year average yields and the physicochemical parameters found in the fruit collected in the plots treated with different fertilizers. No significant difference in fruit yield among treatments was observed. This demonstrated that the different fertilizers did not affect the yield in all experiments. For most of the fruit quality parameters, no statistically significant differences emerged among the fruits obtained using the four types of fertilizers. Titrable acidity (TA) and total soluble solids (TSS) were lower in the plot treated with CB compost, whereas in the same plot, higher ascorbic acid values than those for mineral fertilizer plot were observed. No significant differences were found in ascorbic acid content among orange fruit grown in soil amended with organic fertilizers. The difference found between ascorbic acid content in fruit produced with CB compost and mineral fertilizers might be related to the specific type of organic fertilizers applied, characterized by lower mineralization rates, which are probably due to lignocellulosic content (*27*) and, consequently, slow N release to the soil. In fact, it is known that the vitamin C concentration in fruits and vegetables is inversely correlated with the nitrogen availability in these crops (*1*, *28*). Howerver, further studies on leaf total N and other N-containing compounds content, as well as the N mineralization process in soil, are in progress to find an explanation for this phenomenon.

Carotenoids are the most abundant pigments in flavedo and the flesh of mature Valencia orange fruit, and their content can be affected by different factors such as hormones, light, temperature, and fertilizers (*29*). Significant differences were observed in peel color data with higher values of *L**, *a**, and *b** in conventionally grown and CB compost plot grown fruit. The green−red coordinate *a** measured on fruit of the LW-treated plot was the lowest, whereas no differences were found in the *L** and *b** parameters of the fruit grown in PM and LW. In other words, organic or conventional farming does not affect the flesh color of the orange for the most part. Only the values of the green−red coordinate *a** of the flesh of oranges grown in CB and PM plots were statistically different.

Table 3. Parameters Linked to Nitrogen Metabolism in Organically and Conventionally Grown 'Valencia late' Orange Fruit[a]

treatment	synephrine (mg/L)	$\delta^{15}N$ P (‰)	$\delta^{15}N$ AA (‰)	N (mg/L)
MF	22.90 ± 2.58	4.64 ± 0.35 C	4.22 ± 0.43 D	904.44 ± 7.42
CB	23.48 ± 3.51	6.74 ± 0.70 B	6.27 ± 0.77 C	873.79 ± 4.10
LW	22.95 ± 3.38	8.95 ± 0.38 A	8.48 ± 0.39 B	922.81 ± 5.11
PM	22.13 ± 4.30	8.45 ± 0.61 A	7.88 ± 0.58 A	888.18 ± 5.83

[a] Means within the same column of different managed plots followed by different letters are significantly different at $p \leq 0.001$.

No significant differences between orange fruit from organic and conventional production were observed with respect to N content (**Table 3**). Therefore, the same amount of N supplied in organically and conventionally grown citrus crops produced no differences in the N content of fruit juice.

Synephrine is an N-containing alkaloid belonging to the ephedrine group that is present in citrus leaves and fruit (*30*, *31*). It represents 3−4% of the total soluble nitrogen in the juice and constitutes an end product of the metabolism of this element. Furthermore, its concentration remains stable during fruit ripening because the synephrine content in fruit is not affected by fruit maturity stage. Results showed that the different cultivation methods employed produced no differences in synephrine content of the juice (**Table 3**) in contrast to the results that Rapisarda et al. (*11*) obtained from sampling 28 organic and conventional fruit from farms situated in the citrus area of eastern Sicily. The difference in synephrine content between organically and conventionally grown orange fruit found by Rapisarda et al. could be due to a diverse amount of N applied in commercial orchards as well as to the degree of synchronization between N release and N uptake by crops. Thus, the fertilization regimens may be sufficient to produce differences between treatments in levels of N-containing compounds. With regard to our experiment, no differences were observed in synephrine content between organic and conventional fruit with equal levels of total N applied to the different plots.

Orange fruit from organically managed plots had statistically higher $\delta^{15}N$ P and $\delta^{15}N$ AA ($p \leq 0.001$) values as compared to the conventional ones (**Table 3**). Among organic fertilizer treatments, fruits from plots fertilized with animal compost and manures showed nitrogen isotopic ratios higher than those of plots fertilized with vegetable compost and mineral fertilizer. This difference may be attributed to higher $\delta^{15}N$ in fertilizers of animal origin due to N isotopic fractionation that occurs during the

Table 4. δ^{13}C (Pulp) and δ^{13}C AA (Amino Acids of Juice), $\delta\,^{2}$H (Pulp), $\delta\,^{34}$S (Pulp), and $\delta\,^{18}$O (Juice) Values in Organically and Conventionally Grown 'Valencia late' Orange Fruit

treatment	δ^{13}C pulp (‰)	δ^{13}C AA (‰)	δ^{2}H pulp (‰)	δ^{34}S pulp (‰)	δ^{18}O juice (‰)
MF	−25.87 ± 0.67	−27.11 ± 0.56	−33.55 ± 6.22	10.37 ± 1.21	3.68 ± 0.79
CB	−26.01 ± 0.40	−27.45 ± 0.75	−33.51 ± 4.64	10.48 ± 1.10	3.65 ± 0.78
LW	−25.94 ± 0.50	−27.11 ± 0.44	−36.30 ± 6.46	10.56 ± 1.20	3.79 ± 0.79
PM	−25.99 ± 0.62	−27.08 ± 0.63	−34.80 ± 6.61	10.14 ± 1.22	3.61 ± 0.66

animal metabolic pathway of nitrogen (*32*) and/or composting (*6*). In addition, the NH_3 volatilization process in these amendments tends to increase the δ^{15}N of the N remaining in the soil, consequently resulting in an isotopic enrichment (^{15}N) of the plant (*33*). Conventionally grown orange fruit had δ^{15}N P and δ^{15}N AA values of 4.64 and 4.22‰, respectively, demonstrating a soil nitrogen isotopic contribution with respect to N found in inorganic fertilizer ($\delta^{15}N = 2.45$‰).

The δ^{13}C values of pulp and amino acids of juice (δ^{13}C AA) of fruit grown in different fertilized plots (**Table 4**) were found to fall into a narrow range (−25.87 and −27.45‰), and they were consistent with the values found in C3 plants (*34*). Fruit from different fertilized plots showed similar δ^{13}C values. Therefore, plant C isotope composition was not affected by organic or synthetic fertilization. The same results were found for organically and conventionally grown tomato (*4*) and pepper (*9*).

Biological processes, such as assimilation and metabolism by plants, may also alter stable H, S, and O isotope ratios (*34*). Thus, we investigated how the different fertilizers used influenced plant metabolism and consequently these bulk stable isotope ratios.

The δ^{2}H and δ^{34}S of pulp as well as the δ^{18}O values of fruit juice were not significantly different in the four plots. Therefore, the different fertilizer treatments did not cause H, S, and O isotope discrimination in orange fruit.

In conclusion, the results of this study demonstrated that different types of organic fertilizers did not determine important changes in orange fruit quality parameters. The levels of total N and N-containing compounds in fruit, such as synephrine, were not statistically different among the diverse treatments. Despite this, significant differences in the δ^{15}N values of fruit from organically and conventionally grown fruit were found. The δ^{15}N values of orange fruit grown under fertilizer of animal origin and vegetable compost were higher than those grown with mineral fertilizer. This could suggest that the application of organic fertilizers of different origin determines an increase in the δ^{15}N values of orange fruit. The same treatments did not determine differences in the δ^{13}C, δ^{2}H, δ^{34}S, and δ^{18}O values of the fruit, pointing out that these parameters are not suitable indicators of the type of fertilizer applied but can be used as indicators of the geographical origin of the product (*34*).

Our results support the possibility of using δ^{15}N as a marker for differentiating organically grown and conventionally grown orange fruit. However, follow-up studies involving a larger number of cases will bring together data sets in order to find out whether there are systematic differences in the nitrogen isotope compositions of the organic and conventionally grown orange fruit that could be used to detect fraud.

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