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The effect of fertilization with microfiltered liquid digestate on the quality parameters of Citrus fruits

Angela Castellano[1], Roberta Selvaggi[2]*, Paolo Mantovi[3], Daniela Spina[2], Manal Hamam[2] and Gioacchino Pappalardo[2]

[1]Mediterranean Nutraceutical Extracts (Medinutrex) srls, Catania, Italy, [2]Department of Agriculture, Food and Environment, University of Catania, Catania, Italy, [3]Centro Ricerche Produzioni Animali (CRPA) SpA, Reggio Emilia, Italy

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EDITED BY
Marzia Ingrassia,
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REVIEWED BY
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Marian Butu,
National Institute of Research and Development for Biological Sciences (NIRDBS), Romania

*CORRESPONDENCE
Roberta Selvaggi
✉ roberta.selvaggi@unict.it

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Nowadays, the adoption of sustainable agricultural practices, including the reduction of synthetic fertilizers, has become a challenge for the agriculture sector. In this experimental work, the effect of the liquid fraction of digestate (by-product of the anaerobic digestion process) as a fertilizer was evaluated. The aim of the research was to verify to which extent digestate can affect growth and quality parameters of orange fruits, comparing the results to those obtained for fruits grown on soil treated with conventional mineral fertilizers. To assess the effectiveness of the treatments, different qualitative and quantitative parameters of Citrus fruits were measured. In particular, the results showed slight differences between the two treatments, suggesting that digestate may be used for the production of high-quality fruits. Moreover, in some orchards, the Citrus fruits of the plants treated with digestate showed a higher concentration of health-promoting compounds, such as vitamin C, flavonoids, phenolic content, when compared to the control group. Thus, digestate can be considered an optimal source of plant nutrients and can be used as a crop growth promoter, since it represents an effective strategy for reducing the mineral fertilizers input.

KEYWORDS

digestate, orange juice, fertilization, HPLC, flavonoids, ascorbic acid

1. Introduction

The genus Citrus, native to subtropical Asia, belongs to the subfamily Aurantioideae and order of Sapindales of the Rutaceae family (Agouillal et al., 2017). Nowadays, citrus make up the largest sector of the world's fruit production with more than 100 million tons produced every year (Li et al., 2006). Citrus fruits are a great source of naturally occurring nutrients, such as sugars, organic acids, vitamin C and flavonoids, which only in recent years, have attracted increasing attention thanks to their nutritional and beneficial effects on human health (Turner and Burri, 2013). Among all citrus crops, oranges account for more than half of world citrus production and are the most widely traded fruits, followed by mandarins, limes and lemons and grapefruits (Food Agriculture Organization, 2017). In Europe, Italy is the second orange producer after Spain. Italian orange production is concentrated in the Mediterranean area, in particular in Sicily and Calabria, whose production accounts for ∼63 and 19% of the total national production, respectively (Bettini, 2018). As a perennial evergreen tree, citrus requires water and nutrients throughout the year for higher orchard efficiency (Davies and Albrigo, 1994). The quality of Citrus fruits is influenced by several factors, among which fertilization plays a key role. Growers can modulate fruit quality development with modifications of the cationic (K, Ca, and Mg) or anionic (N, P, and S) composition of the soil solution. In the last century, the increasing growth of world population, followed by a higher demand for food, has led farmers to rely almost exclusively on synthetic mineral fertilizers. The advantages of these chemicals are unquestionable, since they can boost crop production, allowing farmers to grow more food on less land. On the other hand, synthetic mineral fertilizers are responsible of many environmental issues, contributing for instance to the eutrophication of freshwater systems and coastal areas and causing pollution of soil, groundwater and air (Lado et al., 2018).

In this context, to reverse the trend of massive use of synthetic fertilizers, the role of the digestate can be very important. It is an organic soil improver obtained at the end of the anaerobic digestion process. Digestate is rich in stable organic matter and fertility elements such as nitrogen, phosphorus and potassium, and for this reason it can be used as fertilizer on major agricultural crops (Pappalardo et al., 2018; Giuseppe et al., 2020). This substrate, compared to the initial biomass fed into anaerobic digestion plants, is more homogeneous and has a higher moisture content. This happens as a consequence of the dry matter biological degradation operated by the bacteria contained in the digesters, which are responsible for biogas production (Nkoa, 2014). Moreover, digestate retains the main fertility elements (macro and virtually all meso- and trace elements) together with the portion of the less degradable organic carbon that has not been converted into methane or CO_2; for this reason, it is more stable when it returns to the soil (Hans and Eder, 2013). In fact, anaerobic digestion results into a reduction of less stable organic matter, but does not decrease the nitrogen, phosphorus and potassium supply of the initial biomass (Valenti et al., 2018, 2020).

The use of digestate as fertilizer represents an important agronomic strategy not only because of the presence of fertility elements but also because of the possibility to close the carbon and nutrient cycles. The latter figures among the key principles of sustainable agriculture which brings back the centrality of matter recovery as a means of sustaining agricultural production (Murano et al., 2021; Pappalardo et al., 2022). In the last years, the effects of digestate on soil quality have been widely investigated (Alburquerque et al., 2012; Muscolo et al., 2017; Doyeni et al., 2021), bringing considerable socio-economic and environmental benefits for all the agricultural system. But, to the best of our knowledge, only few experimental works (Morra et al., 2021; Panuccio et al., 2021) have investigated how it can affect fruit quality for pluriannual crops. So, we set up an experiment to measure how much the usage of digestate can affect the quality parameters of citrus fruits.

Ascorbic acid, total phenols, flavonoids and other physicochemical parameters of citrus treated with digestate were compared to those obtained for fruits treated with conventional mineral fertilizers, in the same farm.

To this purpose, orange fruits (Citrus sinensis, cv Washington Navel and Tarocco Scirè) were collected in three different farms located in Sicily. For each farm we have distinguished two adjacent fields. All the cultivation conditions were the same, but we changed the fertilizers used: in the experimental field, only digestate was spread for the yearly fertilization; while, in the conventional one, only synthetic fertilizers were used.

Then, the fresh squeezed citrus juices obtained by the different field were analyzed to determine physicochemical and antioxidant parameters, such as the content of ascorbic acid, total phenolics, flavonoids and others. It is important to underline that the comparison of fruit quality parameters was made on citrus collected in the same farm, meaning that the statistical analysis regards those differences determined by the treatment (conventional or digestate) only.

2. Technical information on microfiltered digestate

It is well-known that the use of biomass for agro-energy purposes leads, through the anaerobic digestion process, to the production of biogas. It is much less known, however, that digestate is the by-product of this anaerobic digestion process and it is a product that contains the main elements of soil fertility, making it suitable as a fertilizer on the main agricultural crops.

The agronomic use of digestate as fertilizer is important for the contribution of fertility elements to replace synthetic fertilizers. It is also important for the possibility of closing the carbon and nutrients cycle which are key factors for understanding a sustainable agriculture based on the recovery and the reuse of waste from the production process (Jin et al., 2022).

After the digestate production process in biogas plants, usually, its mechanical separation is carried out. This phase allows above all to obtain two fractions of the digestate: a liquid one called clarified or "pumpable" and a solid one called solid or "palable" (Giuseppe et al., 2020). This separation is due both to a greater efficiency in managing the digestate at company level and to its agronomic uses.

The two fractions generated from the separation process have a very distinct fertilizing power. The solid fraction is called "palable" because of its dry matter content higher than 20% that gives it greater consistency. It presents nitrogen in organic form and a nitrogen / phosphorus ratio shifted in favor of phosphorus (Peng et al., 2020). It has a greater amount of organic matter than the liquid fraction. In the agronomic field it is the most suitable fraction to be used as a soil improver and it represents a valid substitute for manure, helping to maintain the soil's organic matter supply. This fraction can be used whenever a slow-acting organic fertilizer is needed, capable of slowly transferring the nutrients to the soil (Zeng et al., 2022).

The liquid fraction is "pumpable" because it has a low amount of dry matter. It has a lower amount of organic matter and a higher content of nitrogen in the ammoniacal form, which can represent up to 70–90% of the total nitrogen and a nitrogen/phosphorus ratio shifted in favor of nitrogen (Peng et al., 2020). It is a ready-to-use fertilizer, capable of quickly releasing nutrients to crops. Moreover, thanks to the significant ease of infiltration into the soil immediately after the spreading, the distribution of the liquid fraction of the digestate can reduce ammonia emissions into the atmosphere with a shallow burial (Möller, 2015). The burial technique also reduces the odor impact caused by the digestate injection, avoiding annoyance to local inhabitants (Orzi et al., 2018).

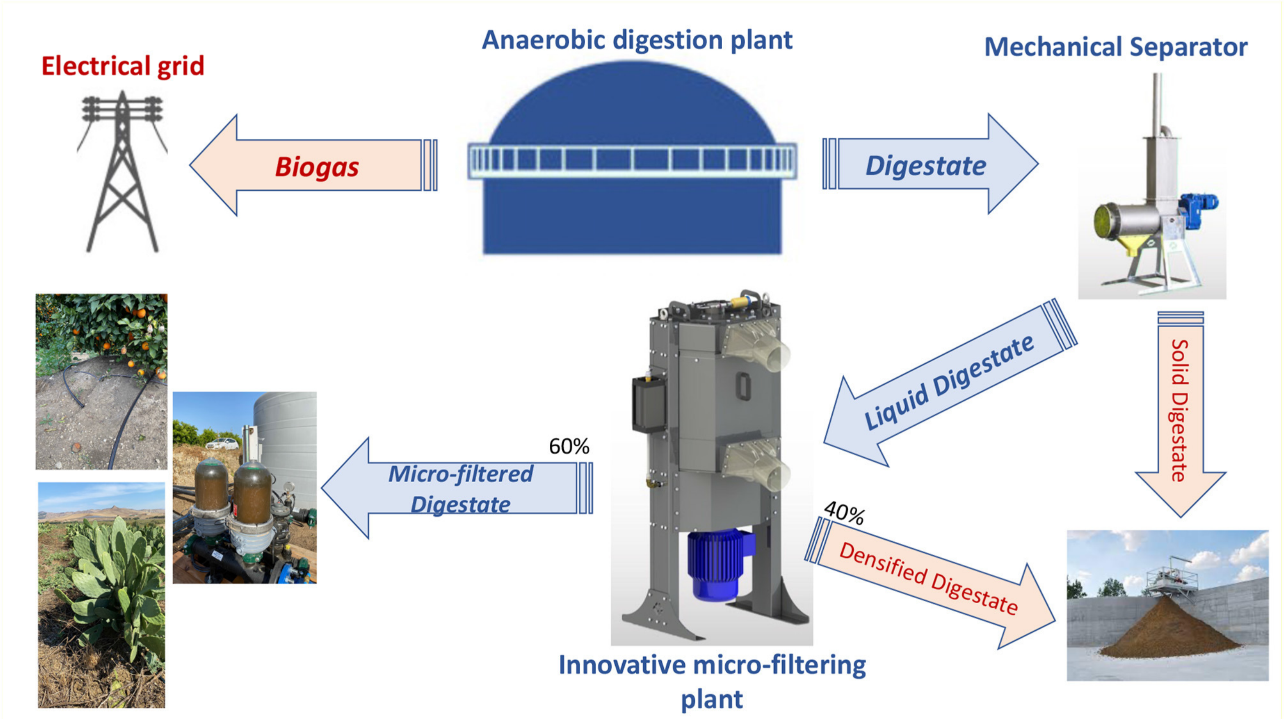

FIGURE 1
Micro-filtered digestate production process.

Alongside the aforementioned traditional forms of digestate, in this paper we have considered a further innovative form of digestate known as "microfiltered digestate." As shown in Figure 1, the microfiltered digestate is obtained from the liquid digestate fraction, subjected to a micro-filtration process within an innovative plant known as a "micro-filter."

It is a mechanical separation of the liquid fraction, without any pre-treatment.

The innovative experimental microfiltration plant shown in Figure 1 allows to obtain a microfiltered liquid phase that can be used in fertigation with driplines, ensuring the maximum use efficiency of nutrients and water contained in it. The microfilter allows, in fact, to exclude from the microfiltered phase the particles larger than 50 microns which could occlude the drip labyrinths of the dripline system (Manetto et al., 2022).

In this experimental condition, the microfiltered digestate represents about 60% of the liquid digestate inside the microfilter and retains, on average, 1.5–8% dry matter. Within this solution there are many chemical compounds useful for crop fertilization, the most important of which is undoubtedly nitrogen in ammoniacal form, in the percentage of 70–90% of the total dissolved nitrogen.

Microfiltered digestate is produced in order to provide it in fertigation on permanent crops (e.g., citrus, olive trees, vines, and forage opuntia) with a dripline system, after to be stored in tanks near the fertigation site.

Currently, fertigation with digestate mixed with irrigation water is a practice not yet widespread. That is because the chemical-physical characteristics of the digestate, even if already clarified with the solid-liquid separation treatment, cause clogging problems of the dispensers with considerable worsening of the quality and efficiency of the overall operation.

The densified fraction obtained after the innovative micro-filtration is semi-solid, with a relatively high dry matter content, usually above about 20 percent. Usually, this fraction is mixed to the liquid pumpable fraction of the digestate.

The best way to use this microfiltered fraction consists in the distribution systems that temporally allow the contributions to coincide as much as possible with the demands of the crops, maximizing the use efficiency of nutrients and water.

Typically, in Mediterranean area, digestate is uncompetitive with chemical fertilizers because it is scattered throughout the territory, resulting in huge efforts to collect and transport them. The principal barrier to logistic chain is its transportation cost and not its chemical characteristics. Maximum travel distances are highly variable and are strongly dependent on the logistic solution adopted.

In addition, the digestate microfiltration can be the key factor for: (a) optimizing the management of the digestate, expanding its calendar and the possibility of spreading; (b) enhancing in a "particular way" the liquid fraction of the digestate while reducing the use of mineral fertilizers; (c) reducing the problems related to the emissions of odors, ammonia, greenhouse gases, the loss of nitrates to the water (maximizing the efficiency of the use of nutrients); (d) reducing the incidence of transportation costs as it is used in high-income perennial crops.

TABLE 1 Physicochemical composition of the liquid microfilterd digestate used as fertilizer.

TS (g/kg)	VS (g/kg)	TKN (mg/kg)	N-NH_4^+ (mg/kg)	P tot (mg/kg)	K tot (mg/kg)	TSS (g/L)
53.7	27.6	6,266	4,219	846	4,542	40.4

TS, total solids; VS, volatile solids; TKN, total kjeldahl nitrogen; N-NH_4^+, ammonium nitrogen; P tot, total phosphorous; K tot, total potassium; TSS, total suspended solids.

3. Materials and methods

3.1. Experimental design

From March to October, the study was carried out in three different citrus orchards, situated in Eastern Sicily (Italy). Two treatments were compared: (1) plant fertilization managed according to the conventional mineral fertilization applied in the orchard (Control) and (2) plant fertilization based on the microfiltered liquid fraction of digestate. Both experimental and conventional plots were 1 hectare wide each.

The digestate used in this experimental study derives from anaerobic fermentation of mixed agricultural biomasses. Specifically, the biomass mix fed into the anaerobic fermenters consists of 50 percent livestock effluent (poultry manure, chicken manure, cattle manure and cattle slurry), 10 percent triticale silage, 5 percent whey, 20 percent pulp and 15 percent olive pomace and vegetable water. Thus, it is a typical diet in the Mediterranean area where many by-products of agricultural and agro-industrial supply chains are available for energetical purposes.

After a solid-liquid separation process, the clarified portion of the digestate was selected. Before being applied in the orchards, through drip irrigation, the liquid underwent a microfiltration step by means of an innovative SEPCOM microfilter already tested for this scope (Mantovi et al., 2020). The physicochemical characteristics of the microfiltered digestate used as fertilizer are reported in Table 1.

The experimental activity was carried out in three orchards of Citrus sinensis, involved in an European Union-funded research project. To date, there are no other fields employing microfiltered digestate on permanent crops, because this is a copyrighted technology not yet developed.

In farm 1 and farm 3, a common variety of orange (cv Washington Navel) was cultivated, while in Farm 2 a pigmented variety (cv Tarocco Scirè) was harvested. The age of the citrus groves under study varied among the case studies. In farm 1 there are trees of about 28 years old, in farm 2 there are 12-year-old trees, and farm 3 is characterized by younger trees (about 5 years old). It has to be noted that all plots, prior to the use of digestate, used to be fertilized with synthetic fertilizers following a fertilization plan in which we substituted digestate according to nitrogen content.

Table 2 shows the fertilization plan followed for each orchard. The substitution of traditional chemical fertilizers with digestate was studied for each farm separately, considering all the typical individual orchards conditions (age of the plants, cultivar, number of plants per hectare, and others).

Fruits were harvested in the period between January and April 2022, when the commercial size and optimal parameters were reached. In order to represent the studied field adequately, the hectare (sample plot) was divided into 5 subplots and, from each of them, 25 mature fruits were harvested. From a total of 125 fruits, 25 citrus were randomly selected. Fruits were immediately stored at 4°C and, few days later, hand squeezed for the determination of physicochemical and nutraceutical parameters.

The results were obtained by comparing the data pertaining to fruits harvested in the control field (conventional fertilization) and the experimental field (digestate fertilization), within the same farm. This means that the trees, belonging to the same farm, have the same characteristics when it comes to variety, age, and cultivation techniques.

3.2. Chemicals and instrumentation

Folin–Ciocalteu reagent (FCR), sodium carbonate (Na_2CO_3), DMSO and meta-phosphoric acid were purchased from Carlo Erba Reagents (Italy); gallic acid and hesperidin were purchased from Glentham Life Science (United Kingdom), ethanol, hydrochloric acid, sodium hydroxide, L-ascorbic acid, sodium fluoride (NaF) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma Aldrich (Milan, Italy). HPLC–MS grade solvents (Carlo Erba Chemicals, Italy) were used for chromatography and all other reagents were of analytical grade.

3.3. Determination of physico-chemical parameters

3.3.1. Color

The color of the peel as well as the pulp of the fruits was measured using a precision colorimeter (NR60CP, Shenzhen 3nH Technology Co, LTD, China) based on the CIELAB color space represented by L*, a*, b*, c* and hue values (Giuseppe et al., 2020). Measurements were taken at two different points on equatorial area of each fruit.

3.3.2. Juice yield

For the determination of juice yield, 25 fruits were analyzed. An analytical scale was used to weight both the whole fruits and then the residual peels, obtained after the hand squeezing.

The percentage of juice content was calculated by dividing the difference of total weight and peel weight by the total fruit weight. By multiplying this number by 100, it was possible to get the percentage.

3.3.3. Total soluble solids

The total soluble solids content was measured through a digital refractometer (ATAGO RX-5000). The determination was carried out by placing a fruit juice drop in the sample area of

TABLE 2 Fertigation plans with traditional chemical fertilizers.

	Chemical fertilizer	Nitrogen		Phosphorous		Potassium	
	kg/ha*	Content (%)	kg/ha*	Content (%)	kg/ha*	Content (%)	kg/ha*
Farm 1							
April	400	9.0	36.0	1.0	4.0	5.0	20.0
June	600	9.0	54.0	1.0	6.0	5.0	30.0
September	600	9.0	54.0	1.0	6.0	5.0	30.0
Total			144.0		16.0		80.0
*400 plants per hectare.							
Farm 2							
February	18.15	40.0	7.3				
March	330	30.0	99.0				
April	264					25.0	66.0
April**	54.12	13.0	7.0			46.0	24.9
May	165			54.0	89.1		
May**	18.15	10.5	1.9				
June	330	30.0	99.0				
June**	54.12	13.0	7.0			46.0	24.9
July	99			54.0	53.5		
Total			221.2		142.6		115.8
*330 plants per hectare. **Foliar treatment of fertigation (not substituted by digestate).							
Farm 3							
March	500	9.0	45.0	1.0	5.0	1.0	5.0
April	50	3.0	1.5			25.0	8.3
April**	250	5.0	12.5	2.0	5.0	2.0	5.0
May**	250	5.0	12.5	2.0	5.0	2.0	5.0
June	500	6.0	30.0	2.0	10.0	1.0	5.0
June	50	5.2	2.6				
September**	250	5.0	12.5	2.0	5.0	2.0	5.0
September	100					52.0	52.0
October	500	5.0	25.0	2.0	10.0	1.3	6.5
Total			141.6		40.0		91.8

*500 plants per hectare.
**Foliar treatment of fertigation (not substituted by digestate).

the refractometer to obtain the values of the °Brix concentration readable on the display. The observed Brix degree was then corrected for temperature using the appropriate scale (Kimball, 1991).

3.3.4. pH and total titratable acidity

The pH value was measured with a pH meter (pH700, EUTECH Instruments). Prior to the analysis, the pH electrode was calibrated using technical buffers (pH = 4.00 and pH = 7.00). The electrode was dipped into the samples and rinsed with distilled water before proceeding from one solution to the other. The values appeared on the display unit of the instrument were recorded only one stabilized in order to ensure accuracy. TA was measured by titration of 1 g of juice, diluted with distilled water, with NaOH 0.1 M, using phenolphthalein as indicator. The result was expressed as percentage of citric acid (Kimball, 1991).

3.4. Evaluation of antioxidant compounds and antioxidant activity

3.4.1. Ascorbic acid

The quantification of Vitamin C in orange juice was performed by means of HPLC analysis (Shimadzu Prominence L2C-20AD and SPD-20A) (Rapisarda and Intelisano, 1996). Prior to the determination of the ascorbic acid, the pulp was centrifugated at

15,000 rpm for 30 min at a temperature comprised between 4 and 10°C. The supernatant was filtered using Miracloth paper and 5 mL of this solution were diluted to 50 mL with a 3% solution of metaphosphoric acid. After passing through a 0.45 µm PTFE membrane filter, 20 µL of the sample were injected into the HPLC instrument. For the analysis, a RP-C18 Luna (Phenomenex, 4.6 × 250 mm) column, kept at 30°C, was used. A solution of ortophosphoric acid 0.02 M was used as mobile phase and the flow rate was 1 mL/min. The photodiode array detector was set at 260 nm. The results were expressed as mg of ascorbic acid on 100 mL of juice, by a calibration curve derived from solutions at different concentrations of ascorbic acid.

3.4.2. Total phenolic content

The total phenolic content was evaluated using the Folin–Ciocalteu assay (Singleton et al., 1999). The juice was centrifugated at 15,000 rpm (IEC CL10 Centrifuge, Thermoscientific) for 30 min at a temperature comprised between 4 and 10°C. The supernatant was filtered by using Miracloth paper and 500 µL of the latter were diluted to 10 mL with distilled water. Then, 1 mL of the aqueous solution was added in a flask, together with 5 mL of 10% Folin-Ciocolteau reagent. After 5 min, the mixture was filled up with a solution of Na_2CO_3 (7.5% w/v), agitated and stored in a dark place for 2 h. Afterward, the absorbance was measured at 765 nm using a spectrophotometer (Shimadzu UV-1800). The results were expressed as mg of gallic acid equivalents on L of juice, by a calibration curve.

3.4.3. Flavonoids

For the measurement of the phenolic compounds typical of sweet oranges, i.e., narirutin, hesperidin and didymin, the juice was pre-treated as follows: 10 mL of the flesh were centrifuged with 3,000 rpm for 5 min at 4°C (IEC CL10 Centrifuge, Thermoscientific). Then, 5 mL of the supernatant were diluted to 10 mL with DMSO. Afterwards, 1 mL of the solution was re-diluted with Mobile Phase A (HPLC water+ 0.3% formic acid). Finally, this solution was filtered with a 0.45 µm PTFE membrane filter and injected into the HPLC instrument.

HPLC analyses were carried out by means of Shimadzu Prominence LC-20AD and SPD-20A system, consisting of a quaternary pump, a column temperature control oven and a photodiode array detector. 20 µL of sample were injected into the RP-C18 Luna (Phenomenex, 4.6 × 250 mm) column. The column was kept at 30°C and the flow rate was 1 mL/min. The photodiode array detector was set at 280 nm. A binary gradient composed of water containing 0.3% of formic acid (PHASE A) and acetonitrile containing 0.3% of formic acid (Phase B) was used for the separation. The gradient elution was determined as follows: 0 min: 5% B; 10 min: 20% B; 50 min: 28% B; 60 min: 43% B; 70 min, isocratic for 5 min, followed by re-equilibrating the column to initial conditions (Amenta et al., 2015).

Quantification of phenolic compounds was carried out at 280 nm using external standard method. The phenolic compounds were identified by comparing the retention times with those of the corresponding standards. Calibration curves were obtained using the commercial standard of hesperidin, showing regression coefficients (R2) above 0.999. The results are expressed as mg of hesperidin on L of juice.

3.4.4. Anthocyanins

The anthocyanins content in the orange juice was determined through a spectrophotometer method: 2.5 mL of juice were diluted to 25 mL using a mixture of 95% ethanol and 37% HCl. After the centrifugation of the solution at 3,000 rpm for 5 min, absorbance of the mixture was measured at 535 nm (Shimadzu UV-1800) (Di Giacomo et al., 1989). The results are expressed as mg of cyanidin 3-glucoside on L of juice.

3.4.5. ORAC assay

For the determination of the antioxidant activity of orange juices, the oxygen radical absorbance capacity (ORAC) assay was performed using a Spectrofluorimeter (Perkin Elmer Wallac 1420). The assay (Nagy et al., 1977) consisted in the initial extraction of the lyophilised test samples (0.5 g) at ambient temperature with 25 mL of 80% methanol containing 2 mmol/L NaF for 4 h under stirring and away from light. All samples were dissolved in phosphate buffer solution (pH 7.4). The results are recorded as micromoles of Trolox equivalents per g of dry weight (µmol TE/g DW).

3.4.6. Statistical analysis

All measurements were repeated three times and the values of the data are expressed as arithmetic mean ± standard deviation. One-way analysis of variance (ANOVA) has been performed, and Tukey's test was run to assess the significance of the differences between samples and control samples. A p-value < 0.05 is considered statistically significant.

4. Results

The physicochemical and nutraceutical parameters of citrus harvested in the three different orchards are presented in Table 3. Data are grouped according to the fertilization regime (Control with chemical fertilizers vs. Digestate). Moreover, the results of ORAC assay are reported in a graph (Figure 2).

With regards to Farm 1, results showed that the treatment of the soil with digestate increased the juice yield as well as the titratable acidity. On the contrary, the total soluble solids were higher in the juice obtained from citrus conventionally fertilized (15.31° Brix vs. 12.26° Brix). Moreover, it can be observed that the content of ascorbic acid and flavanones in the juice, both determined by HPLC analysis, was negatively affected by the use of digestate as soil amendment (71.12 vs. 64.11 mg/100 mL and 434.3 vs. 269.6 mg/L, respectively).

The same trend can be observed for total polyphenols, determined using the Folin-Ciocalteau assay, whose values were 89.3 mg/L for the juice treated with liquid digestate and 103.6 mg/L for the flesh derived from conventional treatment, respectively.

Finally, no significant pH variations were observed when conventional fertilizers and digestate were used as amendments.

TABLE 3 Physicochemical parameters, bioactive compounds concentration and antioxidant activity evaluated in *Citrus sinensis* varieties.

Parameters	Farm 1		Farm 2				Farm 3			
	Single harverst		First harverst		Second harverst		First harverst		Second harverst	
	Control	Digestate	Control	Digestate	Control	Digestate	Control	Digestate	Control	Digestate
Juice yield (%)	42.21 ± 8.35	47.41 ± 0.85	50.96 ± 0.57	54.69 ± 5.22	58.32 ± 1.80**A**	55.30 ± 1.99**B**	53.88 ± 0.44 **A**	46.07 ± 1.31**B**	47.43 ± 2.02**A**	35.92 ± 1.31**B**
L* peel	60.69 ± 0.65	62.18 ± 0.63	65.91 ± 0.26	63.56 ± 1.03	62.45 ± 0.23	62.34 ± 0.97	65.91 ± 0.26	64.96 ± 0.68	61.74 ± 1.51	62.89 ± 1.28
a* peel	38.17 ± 0.02	36.85 ± 1.46	32.14 ± 0.30	37.17 ± 2.65	38.86 ± 0.42	38.55 ± 0.96	37.17 ± 2.65	32.12 ± 1.65	29.51 ± 1.05	26.85 ± 2.74
b* peel	57.69 ± 0.05	58.82 ± 0.64	42.08 ± 2.08**A**	60.77 ± 0.69**B**	59.45 ± 0.08	60.09 ± 1.40	42.08 ± 2.08**A**	63.98 ± 0.05**B**	55.91 ± 1.30	58.25 ± 2.51
c* peel	69.20 ± 0.05	69.48 ± 1.32	53.00 ± 1.80**A**	71.32 ± 1.94**B**	71.05 ± 0.28	71.45 ± 0.71	53.00 ± 1.80**A**	71.63 ± 0.68**B**	62.58 ± 1.41	64.19 ± 2.99
h* peel	56.49 ± 0.01	57.89 ± 0.73	52.61 ± 1.07	58.54 ± 1.51	58.32 ± 2.35	57.29 ± 1.27	52.61 ± 1.07**A**	63.35 ± 1.22**B**	62.30 ± 0.47	65.24 ± 1.93
L pulp	54.04 ± 0.36	54.71 ± 1.81	44.61 ± 0.95**A**	41.42 ± 0.04**B**	42.31 ± 2.72	45.72 ± 2.56	44.61 ± 0.95**A**	52.02 ± 0.88**B**	57.33 ± 2.22	64.74 ± 1.41
a* pulp	8.60 ± 0.35	6.65 ± 0.66	9.65 ± 0.68	11.95 ± 1.61	9.47 ± 0.99	8.28 ± 0.23	6.68 ± 0.67	11.95 ± 1.61	9.89 ± 1.22	12.09 ± 0.23
b* pulp	27.75 ± 1.90	24.78 ± 1.28	14.66 ± 0.13	10.78 ± 1.62	13.40 ± 1.99	14.48 ± 4.88	25.40 ± 1.29**A**	10.78 ± 1.62**B**	46.54 ± 3.46	53.31 ± 1.50
c* pulp	29.13 ± 1.90	25.79 ± 1.34	17.71 ± 0.66	16.19 ± 2.20	16.54 ± 2.16	16.82 ± 4.38	26.27 ± 1.42**A**	16.19 ± 2.20**B**	47.78 ± 3.40	54.67 ± 1.50
h* pulp	72.61 ± 0.25	75.32 ± 1.06	56.27 ± 2.37**A**	41.52 ± 1.02**B**	53.76 ± 1.77	58.74 ± 8.55	75.25 ± 0.69**A**	41.52 ± 1.02**B**	77.23 ± 0.42	77.25 ± 0.30
pH	3.52 ± 0.05	3.57 ± 0.01	3.45 ± 0.15	3.78 ± 0.14	3.63 ± 0.01**A**	3.54 ± 0.01**B**	3.98 ± 0.01**A**	3.94 ± 0.01**B**	4.13 ± 0.01**A**	3.97 ± 0.01**B**
TA (% citric acid)	0.98 ± 0.03**A**	1.13 ± 0.02**B**	0.94 ± 0.03**A**	0.69 ± 0.02**B**	0.87 ± 0.01**A**	0.96 ± 0.01**B**	0.62 ± 0.01**A**	0.55 ± 0.02**B**	0.49 ± 0.01	0.52 ± 0.02
TSS (° Brix)	15.31 ± 0.12**A**	12.26 ± 0.08**B**	12.50 ± 0.57	12.82 ± 0.41	13.10 ± 0.01**A**	12.73 ± 0.01**B**	12.19 ± 0.01**A**	11.95 ± 0.02**B**	13.77 ± 0.02**A**	13.31 ± 0.01**B**
Vitamin C (mg/100 mL)	71.12 ± 5.21**A**	64.11 ± 1.87**B**	57.37 ± 2.03	59.84 ± 4.24	57.11 ± 0.16**A**	56.39 ± 0.08**A**	39.58 ± 0.73**A**	44.36 ± 0.49**B**	27.64 ± 0.43**A**	36.79 ± 0.10**B**
TPC (mg/L)	103.6 ± 0.2**A**	89.3 ± 1.3**B**	91.1 ± 0.1	106.6 ± 0.1	87.2 ± 0.7**A**	83.4 ± 0.7**B**	76.5 ± 0.7**A**	83.1 ± 0.7**B**	96.5 ± 0.1**A**	117.7 ± 0.4**B**
Total flavanones (mg/L)	434.40 ± 5.6**A**	269.57 ± 6.4**B**	134.99 ± 3.41	148.95 ± 5.31	205.00 ± 2.8**A**	167.60 ± 4.8**B**	188.00 ± 3.39	185.53 ± 3.58	334.97 ± 2.7**A**	432.46 ± 4.0**B**
Narirutin (mg/L)	206.68 ± 1.59	123.80 ± 1.70	41.78 ± 1.10	37.30 ± 1.84	43.50 ± 2.12	30.55 ± 2.19	39.30 ± 0.42	43.55 ± 0.78	45.13 ± 0.18	64.37 ± 0.52
Hesperidin (mg/L)	173.93 ± 2.83	120.68 ± 1.87	83.54 ± 1.36	100.43 ± 1.73	146.45 ± 0.64	126.45 ± 0.35	140.60 ± 2.83	132.65 ± 2.33	275.35 ± 1.91	347.90 ± 2.82
Dydimin (mg/L)	53.80 ± 1.27	25.10 ± 2.83	9.68 ± 3.41	11.23 ± 1.74	15.05 ± 0.07	10.60 ± 2.26	8.10 ± 0.14	9.34 ± 0.47	14.48 ± 0.67	20.19 ± 0.69
Anthocyanins (mg/L)	–	–	205.0 ± 1.4**A**	317.5 ± 3.5**B**	369.0 ± 2.8**A**	146.5 ± 2.1**B**	–	–	–	–
ORAC (µmol TE/g DW)	1,652 ± 247	1,916 ± 308	2,455 ± 210	2,587 ± 103	2,001 ± 94	2,112 ± 104	1,692 ± 59	1,871 ± 74	2,122 ± 33	2,026 ± 77

L* = lightness, h* = hue, a*, b*, and c* = color coordinates, TA, titratable acidity; TSS, total soluble solids; TPC, total phenolic content; ORAC, oxygen radical absorbance capacity. Results expressed as Mean ± standard deviation. Different letters mean statistical differences between samples ($p < 0.05$).

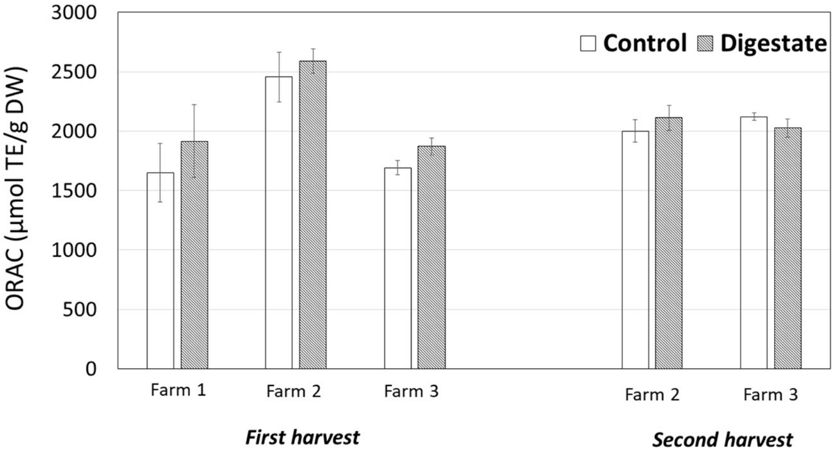

FIGURE 2
Antioxidant activity of flesh orange juice obtained from citrus harvested in the three different farms. The data are expressed as mean ± SD; $p \leq 0.05$.

The same can be said for CIELab indices for peel and pulp and antioxidant activity determined using ORAC assay (Figure 2).

With regards to Farm 2, since two harvests occurred, there is the need to consider them separately.

The first harvest resulted in fruits with most of the physicochemical parameters being comparable between the two treatments. In particular, the values of juice yield, pH, solid soluble showed no significant differences when fruits fertilized with digestate were compared to those treated with conventional chemicals.

Also, HPLC analysis for the determination of vitamin C content and flavanones concentration gave results not significantly different between the two studied groups.

On the other hand, a significant difference was observed for titratable acidity, that was higher in the flesh obtained from citrus treated conventionally (0.94 vs. 0.69%), and for the total phenolic content which, on the contrary, resulted positively affected when liquid digestate was used as fertilizer (106.6 vs. 91.1 mg/L).

In the measurements of fruits color, significant differences in the indices were observed between the two groups of samples. The peel of citrus fertilized with liquid digestate showed higher b*, and c* values, resulting in a brighter yellow color. When it comes to pulp, L* and h* indices resulted significantly different, with lower values for citrus treated with digestate. For the determination of antioxidant activity, ORAC assay was performed and the obtained results showed no significant differences between the two studied groups (Figure 2).

Since citrus fruits harvested in Farm 2 belong to the cv Tarocco Scirè (Blood Oranges), also the determination of anthocyanins was carried out as well. These values were significantly different between the two studied groups: it was found a higher concentration of anthocyanins was found in the juice obtained from oranges treated with liquid digestate (317 vs. 205 mg/L).

The second harvest occurred in Farm 2 showed similar flesh yields as well as comparable CIElab coordinates of peel and pulp, when the two groups investigated were compared.

Moreover, titratable acidity values for citrus grown with digestate fertilization were higher in comparison to conventionally fertilized citrus (0.96 vs. 0.87%). On the contrary, other physicochemical parameters, such as pH and total soluble solids, resulted in lower values for the juice obtained from fruits treated with microfiltered digestate.

Spectrophotometric determinations, i.e., total polyphenols content and anthocyanins concentration, showed higher values for fruits treated with conventional fertilizers. The same trend was followed by total flavanones concentration (205 vs. 167.6 mg/L). No significant differences were observed for antioxidant activity, determined by means of ORAC assay (Figure 2).

For the last farm involved in the study, Farm 3, two harvests were carried out.

The first one resulted in fruits and flesh with physicochemical parameters significantly different between the two groups investigated. In particular, the juice yield, the pH of the juice as well as total soluble solids were higher in the samples grown in the soil fertilized with chemicals. On the other hand, the content of vitamin C in fruits picked from trees treated with digestate was significantly different and higher compared to those harvested in the conventionally fertilized orchard (44.36 vs. 39.58 mg/100 mL). The concentration of total flavanones, calculated as the sum of the three most representative flavonoids, as well as the antioxidant activity, did not differ significantly, showing comparable figures (Figure 2).

Moreover, differences in the CIElab coordinates values, in particular peel parameters, such as b*, c*, and h* values, were observed, resulting in higher values for the digestate group. With regards to the pulp color coordinates, all of the parameters were significantly different when compared between the two soil treatments.

In the second harvest, significantly different results were obtained: the flesh yield was lower for fruits treated with digestate compared to the one obtained for citrus treated conventionally (35.92 vs. 47.43%). Lower values of pH and total soluble solids of the juice were obtained for oranges treated with liquid digestate.

Significant differences were noticed among the fruits in terms of secondary metabolites. Fruits collected from plants grown on soil amended with liquid digestate showed the highest amount of vitamin C (36.79 vs. 27.64 mg/100 mL) and total polyphenols (117.7 vs. 96.5 mg/L). The same trend was emerged for flavanones concentration (432.46 vs. 334.95 mg/L), among which hesperidin was found to be the most abundant flavanone in both groups.

Finally, significant differences in the pulp color were detected: L*, a*, b*, and c* values were higher in the fruits grown on trees treated with digestate compared to those irrigated conventionally. The values of the antioxidant capacity obtained using the ORAC assay showed no significant differences between the two groups (Figure 2).

5. Discussion

In this research work, two different fertilizers (conventional mineral fertilizer and digestate) and their effects on physicochemical parameters of citrus fruits, were compared with the aim to test further potential benefits of an innovative environmentally friendly fertilization strategy.

Table 3 reports the characteristic parameters for the quality of fruits collected in three different farms. Among these markers, titratable acidity, expressed as % citric acid and total soluble solids, reported as °Brix degrees are two of the most important ones. The acids content in juices tends to decrease along with the maturation of citrus fruit, mostly because of the use of these compounds as respiratory substrates, as well as, for the synthesis of new substances. Values of acidity recorded in the present study ranged from 0.49 (Farm 3) to 1.13% of citric acid (Farm 1). Another change that can be observed in the juice during fruits' development is sugar accumulation. In this regard, total soluble solids values were determined since they represent the main index of sugar content in the flesh. The values obtained for TSS ranged from 11.95 (Farm 3) to 15.31 (Farm 1). The trend for acid content and sugars in the current study is in agreement with the aforementioned statement (Liao et al., 2019), showing that the highest titratable acidity values are accompanied by the lowest sugars content values.

The pH values of the juices were within the normal range (3.52–4.13). As one would expect, the pH values are lower in the juices with more acidity.

Another index of citrus quality is the external citrus peel color, that is also generally used as a selection criterion throughout the supply and consumer chain (Selvaggi et al., 2023). In the current study, the color parameters obtained, quantitatively defined into three dimensions of hue, chroma, and lightness, showed variations between the two studied groups (digestate and conventional fertilization). Researchers (Byers and Perry, 1992; Saija et al., 1998; Pallottino, 2010) attribute the changes in rind color to the ripeness process, during which chlorophyll concentration decreases and carotenoid content increases.

In this study, the Vitamin C (ascorbic acid) values of oranges grown in different fertilization conditions were compared. Orange juices analyzed in this experimental study showed values of vitamin C comprised between 36.79 (Farm 3) to 64.11 mg/100 mL (Farm 1) when the flesh came from fruits (Del Amor et al., 2008) treated with digestate, whereas the ascorbic acid content was found to range from 27.64 to 71.12 mg/100 mL for the flesh obtained from citrus fruit treated with commercial fertilizers. The values found in this study confirm what was obtained by other authors (Martí et al., 2009; Marti et al., 2015; Rapisarda et al., 2010; Chanson-Rolle et al., 2016).

Moreover, being as the dominant group of flavonoids in citrus, flavanones concentration has been determined. In this experimental work, the concentration of three flavanones, namely narirutin, hesperidin, and didymin, has been quantified.

The results obtained for fruits collected during the first harvest in Farm 2 and during the second harvest in Farm 3 suggested that, the use of digestate, stimulated plant-resource reallocation from primary metabolism to secondary metabolite production, driving the synthesis of flavanones. In particular, citrus collected in Farm 2 during the first harvest showed an increase of flavanones' concentration by nearly 10%, while an increment by 29% was observed for fruits collected during the second harvest in Farm 3. These results were in agreement with previous studies reporting higher levels of secondary metabolites in organic carrots (Sharma et al., 2012), sweet peppers (Del Amor et al., 2008) and tomatoes (Panuccio et al., 2021).

In every tested juice, hesperidin represents the most abundant flavanone, with values comprised between 85.38 and 347.9 mg/L. While narirutin was the second most abundant flavanone in juices (values range from 30.56 to 206.60 mg/L), dydimin was the minor flavonoid identified in all studied juices (values ranged from 8.09 to 53.79 mg/L). The data obtained for the purpose of the present study are in agreement with those available in the scientific literature (Mondello et al., 2000; Gattuso et al., 2007; Selli, 2007), in which the orange juices tested presented a similar flavanones' profile.

Another class of flavonoids that has been investigated in the present research work is anthocyanins, the water-soluble pigments responsible for the cyanic color of fruits and vegetables. Since these pigments can be found only in blood oranges, their content was determined only for citrus collected in Farm 2 (cv Tarocco Scirè), whose values ranged from 146.5 to 369.0 mg/L, coherently with the results that can be found in literature (Rapisarda et al., 2000; Fabroni et al., 2016).

6. Conclusions

The present study highlighted the possibility of using the liquid fraction of digestate as a soil supplement to obtain multiple benefits to orange growth and quality. The most important fruit parameters of citrus quality, which depend also on nutrient supply, were evaluated and, for most of the physicochemical determinations, comparable results between the two groups were obtained with no negative effects on quality parameters when digestate was used as fertilizer.

On the contrary, in some cases (first harvest in Farm 2 and second harvest in Farm 3), the content of bioactive compounds investigated, i.e., vitamin C, total flavanones and total polyphenols, was positively affected by the use of digestate as soil amendment.

Considering the overall agronomic performances, digestate can be a useful option for replacing synthetic fertilizers, avoiding the negative effects associated with conventional mineral fertilizers. Since this environmentally friendly soil amendment holds huge potential, there is the intention to assess it in future experiments on other crops.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization: AC, RS, and PM. Data curation and methodology: AC. Formal analysis: DS and MH. Funding acquisition and supervision: GP. Investigation and writing—review and editing: RS and PM. Visualization: RS and GP. Writing—original draft: AC and RS. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

AC was employed by Mediterranean Nutraceutical Extracts (Medinutrex) srls, Catania, Italy and PM was employed by Centro Ricerche Produzioni Animali (CRPA) SpA, Reggio Emilia, Italy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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