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Productivity and nutritional and nutraceutical value of strawberry fruits (*Fragaria x ananassa* Duch.) cultivated under irrigation with treated wastewaters

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Abstract

BACKGROUND. Agriculture represents a productive sector typically characterized by a high water demand, whilst FW availability is a problem of increasing concern in the world and FW resources are becoming insufficient for sustaining agricultural irrigation. The reuse of treated wastewaters (TWWs) for crop irrigation could be an efficient tool of reducing water shortage. Hence, this study evaluated the food quality of *Fragaria* x *ananassa* (cultivar Camarosa) fruits irrigated with four kinds of treated wastewaters (TWWs). Strawberries were analysed for yield, sucrose, fructose, glucose, total soluble polyphenols (TSP), total monomeric anthocyanins (TMA), as well as antiradical and antioxidant capacity. In addition, a targeted quantification of the most representative phenolic compounds of strawberry was performed.

RESULTS. TWWs complied the Italian ministerial decree 185/2003 for wastewater reuse with very few exceptions, mainly represented by chloride concentrations (258-643 mg/L vs a legal threshold of 250 mg/L). The reuse of TWWs reduced fruit yield (10-26%) compared to irrigation with tap water as control. Irrigation with TWWs gave also rise to the decrease of total sugars (14-26%), TSP (2-10%) and TMA (29-49%). Individual phenolic acids, flavonols and flavanols were quite stable in response to the irrigation with TWWs, whereas anthocyanidins significantly decreased.

CONCLUSIONS. Although TWWs negatively affected fruit quality, nutritional and nutraceutical parameters herein determined were in line with data previously reported for strawberries purchased in the market or cultivated in research orchards, thus suggesting that the use of TWWs does not prevent the fruit marketability.

Keywords: fruit yield; sugars; polyphenols; wastewater reuse; circular economy

Introduction

Agriculture represents a productive sector typically characterized by a high water demand. According to the European Environment Agency, a third of the water use in Europe goes to the agricultural sector, most of it for crop irrigation[1], and, as recently pointed out by the United Nations World Water Assessment Program[2], about 70% of worldwide freshwater (FW) withdrawals is used for agricultural irrigation.

On the other hand, limited FW availability is a problem of increasing concern in the world and FW resources are becoming insufficient to efficiently sustain agricultural irrigation, mainly due to climate-related conditions. In fact, water scarcity is in most cases a climate-bound regional problem and affects many areas of the Earth's planet, including Middle East, North Africa[3], but also Southern Europe, including Italy.[4]

The reuse of non-conventional waters for irrigation, such as treated wastewaters (TWWs) of municipal or mixed municipal/industrial origin, could be an efficient tool of reducing water shortage[5], reason why TWWs reuse is becoming a widely adopted practice in agriculture.[2] Moreover, soils and plants can benefit from the fertilizing effect of wastewater.[6] However, TWWs may contain chemical and bacteriological contaminations that can affect crop safety. For this reason, many countries have developed their own regulations in the field of water reuse.[7] For example, in Italy, wastewaters are allowed to be reused for the irrigation of crops intended for both human and animal consumption, whether a number of chemical and biological properties meet the limits established by a specific regulation on wastewater reuse.[8] Moreover, TWWs often exhibit physicochemical and/or chemical properties (e.g. pH, conductivity, sodium and chloride ions), which may negatively affect crop productivity and/or quality.[9]

Quality in food is a combination of different attributes (e.g. sugars, minerals and bioactive compounds), which affect organoleptic properties, as well as nutritional and nutraceutical values. These compounds are susceptible to significant variations, depending on climate conditions and agronomic practices.[10] Generally, the quality of vegetables and fruits irrigated with TWWs has been commonly evaluated through their main pomological parameters related to product marketability, reporting slight differences compared to traditional watering techniques.[11, 12] Conversely, the impact of crop irrigation by TWWs on nutritional and nutraceutical value is poorly described in literature. More in detail, irrigation with TWWs of short-term crops, like strawberries, does not seem to promote significant variations of the principal nutritional and nutraceutical values[13], whereas on long-term crops, like olive trees, the effect of an extended TWW irrigation increased the level of β-carotene and total tocopherols of olive oil.[3]

Among crop species that can be investigated for their quality in response to irrigation with TWWs, strawberry (*Fragaria* x *ananassa* Duch.) is certainly a very attractive fruit due to its unique organoleptic characteristics, as well as overall fruit nutritional and nutraceutical attributes[14], reasons why strawberry is widely appreciated by consumers. In fact, strawberry covers an important place in the horticultural industry, particularly in the Mediterranean countries[15], which produce around 1.6 million tons annually, almost 18% of the world production.[5] However, these countries are notoriously suffering from limited water resources, which clash with the high demand for water to irrigate strawberry.

Based on the aforementioned considerations, in this study, strawberry plants were grown under irrigation with four types of TWWs, characterized by different physicochemical attributes (e.g. different level of salinity), using tap water (TW) as control. More in detail, Camarosa cultivar was selected due to its lower salt tolerance threshold, compared to other varieties.[16]

Strawberry quality was evaluated through the analysis of sucrose, glucose, and fructose as essential nutritional parameters.[17] Total soluble polyphenols (TSP), total monomeric anthocyanins (TMA), as well as radical scavenging and antioxidant activities (RSA and AA) were also analysed, as important nutraceutical attributes.[18] Moreover, some phenolic compounds previously highlighted as important constituents of the phenolic fraction of *Fragaria* fruits[19-21] were determined to further characterize fruit nutraceutical quality under non-conventional irrigation practices. Through this experimental design, the following hypotheses will be verified: (i) the use of different TWWs and TW impart significant differences in the nutritional and/or nutraceutical quality of the strawberries obtained; (ii) the nutritional and nutraceutical quality of the fruits obtained by non-conventional irrigation is high enough to allow their marketability.

Materials and methods

Standards, reagents, solvents and materials used in this study are described in section S1 of the *Supplementary materials*.

Sample origin

Young fridge stored certified *Fragaria* x *ananassa* plants (Camarosa cultivar) were grown outdoor from March to July 2017 (see Section S2 of the *Supplementary materials* for details).

Plants were irrigated with four TWWs collected in wastewater treatment plants (WWTPs) managed by GIDA S.p.A. (Prato, Italy). More in detail, the TWWs derived from the following WWTPs: (i) “Baciacavallo” (TWW_1), (ii) “Macrolotto 1” (TWW_2), (iii) “Macrolotto 2” (TWW_3), and (iv) “Calice” (TWW_4). TW was used as control. WWTPs description is reported in the Section S3 of the *Supplementary materials*. Physicochemical, chemical, and microbiological parameters reported in the Italian regulation on wastewater reuse[8] were determined in TWWs and the results are shown in **Table S1** of the *Supplementary materials*, together with data regarding TW, which were taken from a public database.[22]

Strawberry fruits were harvested when characterized by a red colour all over the fruit. The collected strawberries were transported to the laboratory, gently washed with distilled water, dried with paper towel and finally weighted in order to determine the fruit yield. All fruits from each plant were separately freeze-dried and stored at -20 °C until analysis.

Extraction of sugars and phenolic compounds

Sugars and phenolic compounds were extracted by the same procedure[23], using raffinose, myricetin and petunidin-3-*O*-arabinoside for the evaluation of the apparent recovery.[24] Full details of the extraction procedure are reported in the Section S4 of the *Supplementary materials*.

Analysis of sugars

Fructose, glucose and sucrose were instrumentally determined by liquid chromatography (LC), coupled with evaporative light scattering detection (ELSD) after frontal elution of the extracts on Supelclean LC-18 SPE Tubes. Individual and total sugars were expressed as mg/g d.w. and mmol/g d.w., respectively. Full details of the LC-ELSD analysis are reported in the Section S5 of the *Supplementary materials*, whereas figures of merit of the method are shown in **Table S2**.

Analysis of TSP, TMA, RSA and AA

TSP, TMA, RSA and AA were determined on the extracts using spectrophotometric methods. TSP were analysed according to the Folin-Ciocalteu method [23], using calibration lines prepared with (+)-catechin (see Section S6 of the *Supplementary material* for full details). TMA were determined with the pH differential method[25] using pelargonidin-3-glucoside as reference standard. RSA was determined through the methods based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals.[26, 27] AA was measured through the Ferric Reducing Antioxidant Power (FRAP) assays.[28] Results were expressed as micromoles of Trolox equivalents per gram of fruit on a dry weight basis (μmol of Trolox/g d.w.). TSP and TMA were used for the evaluation of the relative recovery percentage of sequential extractions (see Sections S7 of the *Supplementary material*).

Analysis of individual phenolic compounds

Selected individual phenolic acids, chalcones, flavanols, flavonols and anthocyanins were analysed by LC hyphenated with electrospray ionization (ESI) triple quadrupole tandem mass spectrometry (MS/MS), using a Shimadzu (Kyoto, Japan) chromatographic system coupled with a 5500 QTrap™ mass spectrometer (Sciex, Ontario, Canada). Full details of LC-MS/MS analysis of targeted phenolic compounds are reported in the Section S8 of the *Supplementary material*.

Statistical analysis

The analysis of variance, the non-parametric Games-Howell test for multiple comparison of the mean concentration values and the Pearson correlation test were performed by using Minitab®17.1.0 (Minitab Inc., State College, PA, USA). Principal component analysis (PCA) and cluster analysis

(CA) were performed using Minitab®17.1.0. Quality control of PCA was carried out on the mix of extracts of strawberry fruits grown under irrigation with the four investigated TWWs and TW as control (QCs), verifying if object scores are close to the origin of new coordinates in the principal component (PC) plot.

Results and discussion

TWWs characterization

Table S1 of the *Supplementary materials* illustrates the physicochemical, chemical and biological parameters of the TWWs used in this study, which are foreseen by the Italian Ministerial Decree 185/2003[8], regulating the wastewater reuse for various applications, including the agricultural irrigation. **Table S1** includes also the values of these parameters determined in TW, as well as the limits reported in the M.D. 185/2003. The values determined in TWWs complied the thresholds with some exceptions. More in detail, among physicochemical and chemical parameters, TSS slightly exceeded the limit established by the M.D. 185/2003 (i.e. 10 mg/L) for TWW_1 (11±4 mg/L) and TWW_4 (12±4 mg/L), and ammonia was found just above the legal threshold (i.e. 2 mg/L) in TWW_1 (2.1±1.3 mg/L). Exceedances of the M.D. 185/2003 limits were much more accentuated and generalized (i.e. in all the TWWs investigated) for the chloride ion, the mean concentrations of which were in the range of 258-643 mg/L (legal threshold 250 mg/L). Sodium adsorption ratio (SAR), although within the limit established by the M.D. 185/2003 (i.e. 10), was also a critical parameter for TWW reuse in agriculture, being it included from 9.0 (for TWW_3) to 9.5 (for all the other TWWs). Conductivity was a further parameter worth to be mentioned, since high values were observed in TWWs (1322-2428 µS/cm), compared to those considered suitable for crop irrigation.[29] Overall, the remarkable concentrations of chloride ion, together with the high values of SAR and conductivity, highlight potential problems in the use of TWWs for irrigation purposes. However, it should be noted that these waters represents an important source of nutrients for plant growth, since they respectively contain 5-8 mg/L of nitrogen and 300-900 µg/L of phosphorus.

Fruit yield

Table 1 illustrates the fruit yield obtained with the four TWWs and TW (control). The irrigation with TWWs influenced fruit yield, resulting in a general decrease of productivity. The trend of fruit yield followed the order TW>TWW_4≈TWW_3=TWW_2>TWW_1. More in detail, plants irrigated with TWWs showed a reduced fruit production compared to control from 10% with TWW_4 to 26% with TWW_1, the latter exhibiting by far the highest level of salinity, as measured by electrical conductivity (2428 µS/cm in TWW_1 vs 1322-1647 µS/cm in the other TWWs and 872 µS/cm in TW). Interestingly, a similar reduction in productivity (12-24% depending on the kind of irrigation system) was previously reported for Camarosa strawberry[13] irrigated with a TWW, which displayed a conductivity comparable to TWWs used in this study. A stronger yield reduction (38-63%) was highlighted in various *Fragaria x ananassa* varieties in response to increasing conductivity levels (from 700 to 2500 µS/cm) of irrigation water.[30] These findings evidenced the presence of a stress condition in strawberry plants irrigated with TWWs, in agreement with the aforementioned higher levels of chloride, SAR and conductivity in TWWs than in TW (**Table S1**). Salinity may compromise the plant water ability absorption, since ions in soil solution force plant to further lower its water potential to maintain a proper water supply from soil[31], causing a plant water-deficit condition, which inhibited plant growth and productivity.[32] Moreover, a specific toxicity of chloride ion may contribute to the yield reduction observed in this study, since chloride concentrations as high as 150 mg/L exhibited toxicity towards Camarosa strawberries with significant effects on fruit production.[16]

The yield could be further compromised if long-term irrigation with saline TWWs is carried out. However, it should be considered that the soilless cultivation of horticultural products, including strawberry, usually involves plants and substrate replacement every two vegetative cycles (i.e. one-two years) owing to the decrease of the production performances observed after this period[33, 34], making therefore less critical the impact of irrigation with TWWs.

Sugars

Table 2 shows the concentrations of fructose, glucose and sucrose determined in strawberry fruits obtained under irrigation with TW and TWWs. Sugar levels found herein were in line with the range elsewhere reported for Camarosa fruits purchased in the market or cultivated in soilless systems in research orchards [35-38], thus demonstrating that, from this viewpoint, the fruit quality is high enough to guarantee their marketability. However, significant variations ($p<0.05$) were observed among treatments. In fact, fruits irrigated with TWWs showed significantly lower values of individual and total sugar compared with control fruits. Fruits grown under irrigation with TWW_2 and TWW_3 showed comparable concentrations of total sugars, fructose and glucose. The greater influence on sugar concentrations of the irrigation by TWWs was highlighted for strawberries obtained with TWW_1 and TWW_4, which displayed the lowest individual and total sugar values (i.e. 1.98 and 2.06 mmol/g d.w., respectively), approximately 25% lower than control fruits. The lower abundance of individual sugars in strawberry fruits might be ascribable to the salinity of the TWWs used for irrigation, which showed Cl^- concentration exceeding Italian legal limits for wastewater reuse (**Table S1**). In fact, the high Cl^- concentrations could have caused a water deficit in the strawberry plants. The reduction of carbohydrates was probably linked to the consumption of photoassimilates for osmotic adjustment, as previously reported for fruits of strawberry plants cultivated in soils characterized by high NaCl contents.[39, 40]

TSP, TMA, RSA and AA

Mean values of TSP, TMA, DPPH-RSA, ABTS-RSA, and FRAP-AA determined in strawberry fruits in response to the irrigation with TW and TWWs are shown in **Table 2**.

The treatments exhibited quite similar TSP concentrations, being the highest variation (about 10%) observed between TWW_1 (2521 mg catechin/100 g d.w.) and control (2807 mg catechin/100 g d.w.). This trend was also found elsewhere on Camarosa strawberries irrigated with a tertiary TWW characterized by conductivity and concentrations of BOD_5, COD, N_{tot} and P_{tot} similar to those of TWWs tested in this study [13]. The comparison between TWW_1 (the most salty TWW) and TW was the only one providing a statistically significant difference ($p<0.05$).

The antiradical and antioxidant activity parameters behaved in a very similar way to the TSP, showing a significant linear correlation each other (r=0.923-0.988, $p<0.05$) and with TSP itself (r=0.903-0.960, $p<0.05$).

In contrast to findings obtained for TSP, DPPH, ABTS, and FRAP, irrigation with wastewater significantly affected TMA values, as total anthocyanins in control fruits (610 mg pelargonidin-3-*O*-glucoside/100 g d.w.) were up to twofold higher than those found in fruits treated with TWWs (310-437 mg pelargonidin-3-*O*-glucoside/100 g d.w.). More in detail, the irrigation with TWWs gave rise in all cases to statistically significant decreases of this parameter compared to control. It is however remarkable that TSP and TMA concentrations of strawberries produced with TWWs were included in the range of values reported in literature for Camarosa fruits purchased in the market or produced in research orchards.[1, 13, 41, 42] Therefore, fruits irrigated with TWWs demonstrated a nutraceutical quality in line with their marketability.

Individual phenolic compounds

Table 3 shows the concentrations of targeted phenolic compounds (i.e. principal phenolic acids, chalcones, flavanols, flavonols and anthocyanins) herein used as further indicators of the quality of strawberry fruits obtained by irrigation with TW and TWWs. **Table 3** also provides abbreviations of targeted analytes, which are used below. In the whole set of treatments, the majority of target analytes showed a signal-to-noise ratio higher than 10, being therefore successfully quantified. CHL, QUE-GAL, and CYA-GAL were determined only in fruits produced with TWW_3, although at very low concentrations (≤0.31 mg/100 g d.w.). Moreover, CAF, QUE, QUE-RHA and PHL were never quantified in the investigated samples.

Similar patterns of relative abundance were highlighted for targeted phenolic compounds, irrespective of the use of TWWs or TW for irrigation. More in detail, in all samples, PEL-GLU was by far the most abundant compound (161-343 mg/100g d.w.), accounting for 74-84% and 47-67% of total individual anthocyanins and total individual phenolic compounds, respectively. Other predominant compounds were PB2 (29-54 mg/100 g d.w.), CAT (40-49 mg/100 g d.w.), EA (15-26 mg/100 g d.w.), CYA (11-17 mg/100 g d.w.), CYA-GLU (9-12 mg/100 g d.w.), and PEL-RUT (20-40 mg/100g d.w.). Literature data related to Camarosa strawberries obtained in soilless systems using fresh water for irrigation, confirmed this trend.[1, 43]

A general concentration increase was evidenced for non-anthocyanin phenolic compounds in fruits treated with TWWs compared to those irrigated with TW (**Table 3**). These differences were statistically significant only in few cases, such as PB1 for TWW_1 and TWW_4, and PB2 for TWW_2 and TWW_4. However, when the total concentration of these compounds was considered, the increase was remarkable (percentage increase of 15-29%) and statistically significant in all cases.

Conversely, individual anthocyanins evidenced a concentration decrease in response to the use of TWWs in almost all cases. In particular, PEL-GLU and PEL-RUT were significantly lower in fruits produced with TWW_1 and TWW_4 compared to TW, whereas the use of TWW_2 and TWW_3 did not provide statistically significant reductions in concentration. A slight concentration decrease was observed for CYA-GLU in response to the use of all TWWs, but the differences were not statistically significant. An opposite behaviour was found for CYA, which was more abundant in fruits produced under irrigation with TWWs. Total concentration of the quantified anthocyanins followed the trend of the predominant individual anthocyanins (i.e. the two pelargonidins), being it statistically lower in fruits irrigated with TWW_1 and TWW_4, compared to TW. Interestingly, the sum of the concentrations of targeted individual anthocyanins represented a significant percentage (about 70-90%, depending on the sample considered) of total anthocyanins spectrophotometrically determined as TMA (see **Table 1**). Hence, the group of individual anthocyanins herein selected seems to give a representative picture of the whole set of anthocyanins occurring in strawberry fruits. In this regard, it should be noted that total concentrations of individual anthocyanins showed some correlation with TMA values ($r=0.795$, $p=0.108$).

Multivariate analysis

In order to summarize the set of information obtained from the analysis of phenolic compounds in the 18 strawberry samples (including QCs), and to highlight more easily the effects of the irrigation with TWWs and TW, a multivariate elaboration of the autoscaled original data was performed by means of PCA and CA. These data elaborations included the 19 phenolic compounds quantified in at least one strawberry sample.

As shown in **Table S5** (Section S9 of the *Supplementary material*) four principal components (PCs), characterized by eigenvalues > 1 and accounting for percentages of explained variances (EV%) of 38.7%, 20.4%, 15.3% and 10.0%, were obtained (total EV%=84.4). However, the contributions of each variable to the four significant PCs were not well differentiated, since only few variables evidenced remarkable differences among the four components in terms of absolute values of loadings. More in detail, the highest differences among loadings within a same PC have been highlighted for (i) QUE-GLU, PEO-GLU and PEL-RUT in PC1, (ii) KAM-RUT and especially PHL-GLU in PC2, (iii) FER, CAT, PB2, and CYA in PC3, (iv) KAM-GLU and CYA-GLU in PC4 (see **Table S5**).

Figure 1 illustrates the score plots of PC1 vs PC2 (**Fig. 1A**) and PC1 vs PC3 (**Fig. 1B**), both accounting for a cumulative EV% >50%, as well as the corresponding loading plots (**Fig. 1C** and **Fig. 1D**). In both score plots QCs were very close to the origin of the coordinates, indicating the high accuracy and precision of the entire analytical procedure. Moreover, in both graphs, replicated samples showed quite similar score values, thus evidencing the homogeneous results obtained within each treatment.

The five investigated samples were well discriminated in the PC1 vs PC2 space (EV%=59.1), thus highlighting the different influence exerted by irrigation waters on the expression of the phenolic secondary metabolism of strawberries (**Fig. 1A**). More in detail, the separation of TWW_3 samples was due to their positive and high scores on PC2 and especially PC1, which are in turn related to CHL, QUE-GAL, and CYA-GAL concentrations (**Fig. 1C**). In this regard, it should be noted that these analytes were detected only in strawberries irrigated with TWW_3. The higher PEO-GLU concentrations found in TWW_3 samples also contributed to differentiate them from the others on the PC1 vs PC2 score plot. For TWW_1 and TWW_4 samples, which showed very similar coordinates on PC1, the separation was mainly due to their very different concentrations of KAM-RUT and PHL-

GLU, the only two compounds providing very high loadings (in absolute value) on PC2 (**Fig. 1C**). TW and TWW_2 were the closest samples in the PC1 vs PC2 score plot, reflecting the quite similar concentrations of most phenolic compounds in fruits from these treatments.

The separation of TW and TWW_2 samples was more evident when PC1 was plotted as a function of PC3 (EV%=54.0) (**Fig. 1B**). In fact, on this latter component, strawberries irrigated with TWW_2 strongly differentiated from those grown with TW, due to concentration trends found in these samples for FER, PB2, CAT and CYA, all of them providing high absolute values of loadings in PC3 (**Fig. 1D**). Conversely, TWW_1 and TWW_4 samples grouped together, evidencing very similar behaviours of the two treatments on PC3 as well. According to the loading plot shown in **Fig. 1D**, fruits irrigated by TWW_1 and TWW_4 were characterized by high concentrations of QUE-GLU, PB1 and QUE-RUT, compared to those found in the other samples. It is interesting to note that samples obtained with TWW_1 and TWW_4, which are effluents of WWTPs operating on similar mixed domestic-textile wastewaters and characterized by analogous treatments stages, exhibited very similar score values on both PC1 (EV%=38.7) and PC3 (EV%=15.3).

The use of CA, as performed by using the squared Euclidean distances of autoscaled concentrations of targeted analytes (**Figure 2**), confirmed the homogeneous results obtained for replicated samples within each treatment. In fact, the replicates of each treatment grouped at very high similarity levels (i.e. TW=77.8%, TWW_1=79.5%, TWW_2=81.7%, TWW_3=85.3%, and TWW_4=81.1%), which were much greater than those regarding the other clusters present in the dendrogram (i.e. ≤51.3%).

Conclusions

Strawberry was a responsive fruit model for investigating the effect of irrigation with TWWs on fruit quality, which is an important aspect, currently not yet investigated in-depth, of the issue of wastewater reuse in agriculture.

The comparative evaluation of the effect of various TWWs characterized by different physicochemical and chemical properties, allowed for obtaining interesting information that to the best of our knowledge are provided herein for the first time. Plants grown with TWW_1 appeared to be among the most affected by non-conventional irrigation, displaying the lowest yield, sugar and TSP concentrations, RSA and AA values, as well as statistically lower TMA content, compared to control. Interestingly, strawberries irrigated with TWW_2 and TWW_3, which have common origin and underwent similar depuration stages, exhibited equivalent quality attributes. Fruits produced by irrigating plants with TWW_4 showed erratic trends, being among the best for some parameters (e.g. yield) and among the worst for some others (e.g. TMA).

This research also investigated for the first time a wide number of individual phenolic compounds as quality indicators of non-conventional irrigation strategies, providing further important information. Concentrations of phenolic acids, flavonols and flavanols slightly increased with worsening the quality of TWW used for irrigation, whereas anthocyanins showed in almost all cases an opposite trend.

Overall, these results showed that nutritional and nutraceutical attributes of strawberry fruits are strongly related to the quality of the water used for irrigation. However, the nutritional and nutraceutical attributes of the fruits obtained by non-conventional irrigation seem to be in line with strawberry marketability, even considering the fruits with the lowest quality attributes. It is remarkable that these results have been obtained by using TWWs with high SAR and conductivity values and chloride concentrations more than double than the maximum recommended for reuse in agriculture, and the Camarosa cultivar, which is considered very sensitive to the salinity of the irrigation water. In this regard, the presence in TWWs of significant concentrations of nutrients may has played an important role in the achievement of fruit nutritional and nutraceutical quality similar to the one elsewhere observed for strawberries grown under conventional irrigation.

Accordingly, the reuse of TWWs in the agricultural sector may represent a valuable strategic solution for water saving (especially in countries experiencing water scarcity) suitable to increase the sustainability of soilless agricultural production, without losing fruit quality attributes and in full accordance with the principles of circular economy.

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ReferencesLiterature:

1. Chaves VC, Calvete E and Reginatto FH, Quality properties and antioxidant activity of seven strawberry (Fragaria x ananassa duch) cultivars. *Scientia horticulturae* **225**:293-298 (2017).
2. Jaramillo MF and Restrepo I, Wastewater reuse in agriculture: A review about its limitations and benefits. *Sustainability* **9**:1734 (2017).
3. Bedbabis S, Rouina BB, Mazzeo A and Ferrara G, Irrigation with treated wastewater affected the minor components of virgin olive oil from cv. Chemlali in Tunisia. *European Food Research and Technology* **243**:1887-1894 (2017).
4. Pedrero F, Camposeo S, Pace B, Cefola M and Vivaldi GA, Use of reclaimed wastewater on fruit quality of nectarine in Southern Italy. *Agricultural Water Management* **203**:186-192 (2018).
5. FAOSTAT, *Food and Agriculture Organization of United Nations*. http://www.fao.org/faostat/en/#data/QC.
6. Perulli GD, Bresilla K, Manfrini L, Boini A, Sorrenti G, Grappadelli LC and Morandi B, Beneficial effect of secondary treated wastewater irrigation on nectarine tree physiology. *Agricultural Water Management* **221**:120-130 (2019).
7. Bixio D, Thoeye C, De Koning J, Joksimovic D, Savic D, Wintgens T and Melin T, Wastewater reuse in Europe. *Desalination* **187**:89-101 (2006).
8. Ministerial Decree 185/2003 of Italian Republic, "Regulation containing technical standards for wastewater reuse in implementation of article 26, paragraph 2, of Legislative Decree 152/1999". (2003).
9. Christou A, Maratheftis G, Eliadou E, Michael C, Hapeshi E and Fatta-Kassinos D, Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity. *Agriculture, ecosystems & environment* **192**:105-114 (2014).
10. Wang ZH, Li SX and Malhi S, Effects of fertilization and other agronomic measures on nutritional quality of crops. *Journal of the Science of Food and Agriculture* **88**:7-23 (2008).
11. Cirelli G, Consoli S, Licciardello F, Aiello R, Giuffrida F and Leonardi C, Treated municipal wastewater reuse in vegetable production. *Agricultural Water Management* **104**:163-170 (2012).
12. Bedbabis S, Trigui D, Ahmed CB, Clodoveo ML, Camposeo S, Vivaldi GA and Rouina BB, Long-terms effects of irrigation with treated municipal wastewater on soil, yield and olive oil quality. *Agricultural Water Management* **160**:14-21 (2015).
13. Christou A, Maratheftis G, Elia M, Hapeshi E, Michael C and Fatta-Kassinos D, Effects of wastewater applied with discrete irrigation techniques on strawberry plants' productivity and the safety, quality characteristics and antioxidant capacity of fruits. *Agricultural Water Management* **173**:48-54 (2016).
14. Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B and Battino M, The strawberry: composition, nutritional quality, and impact on human health. *Nutrition* **28**:9-19 (2012).
15. Chandler CK, Folta K, Dale A, Whitaker VM and Herrington M, Strawberry, in *Fruit breeding*. Springer, pp 305-325 (2012).
16. Suarez DL and Grieve CM, Growth, yield, and ion relations of strawberry in response to irrigation with chloride-dominated waters. *Journal of plant nutrition* **36**:1963-1981 (2013).
17. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F and Veberic R, Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *Journal of food science* **77**:C1064-C1070 (2012).
18. Milivojević J, Maksimović V, Nikolić M, Bogdanović J, Maletić R and Milatović D, Chemical and antioxidant properties of cultivated and wild Fragaria and Rubus berries. *Journal of Food Quality* **34**:1-9 (2011).

19. Cerezo AB, Cuevas E, Winterhalter P, Garcia-Parrilla M and Troncoso A, Isolation, identification, and antioxidant activity of anthocyanin compounds in Camarosa strawberry. *Food Chemistry* **123**:574-582 (2010).
20. Del Bubba M, Checchini L, Chiuminatto U, Doumett S, Fibbi D and Giordani E, Liquid chromatographic/electrospray ionization tandem mass spectrometric study of polyphenolic composition of four cultivars of Fragaria vesca L. berries and their comparative evaluation. *Journal of Mass Spectrometry* **47**:1207-1220 (2012).
21. La Barbera G, Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Chiozzi RZ and Laganà A, Comprehensive polyphenol profiling of a strawberry extract (Fragaria× ananassa) by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry. *Analytical and bioanalytical chemistry* **409**:2127-2142 (2017).
22. Publiacqua S.p.A., Monitoraggio sistemi acquedottistici (in Italian), Ed (2019).
23. Doumett S, Fibbi D, Cincinelli A, Giordani E, Nin S and Del Bubba M, Comparison of nutritional and nutraceutical properties in cultivated fruits of Fragaria vesca L. produced in Italy. *Food Research International* **44**:1209-1216 (2011).
24. Scordo CVA, Checchini L, Renai L, Orlandini S, Bruzzoniti MC, Fibbi D, Mandi L, Ouazzani N and Del Bubba M, Optimization and validation of a method based on QuEChERS extraction and liquid chromatographic–tandem mass spectrometric analysis for the determination of perfluoroalkyl acids in strawberry and olive fruits, as model crops with different matrix characteristics. *Journal of Chromatography A* (2020).
25. Ancillotti C, Ciofi L, Pucci D, Sagona E, Giordani E, Biricolti S, Gori M, Petrucci WA, Giardi F and Bartoletti R, Polyphenolic profiles and antioxidant and antiradical activity of Italian berries from Vaccinium myrtillus L. and Vaccinium uliginosum L. subsp. gaultherioides (Bigelow) SB Young. *Food chemistry* **204**:176-184 (2016).
26. Brand-Williams W, Cuvelier M-E and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology* **28**:25-30 (1995).
27. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine* **26**:1231-1237 (1999).
28. Benzie IF and Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry* **239**:70-76 (1996).
29. Bauder TA, Waskom R, Sutherland P, Davis J, Follett R and Soltanpour P, Irrigation water quality criteria. *Service in action; no 0506* (2011).
30. Ferreira JF, Liu X and Suarez DL, Fruit yield and survival of five commercial strawberry cultivars under field cultivation and salinity stress. *Scientia Horticulturae* **243**:401-410 (2019).
31. Maksimović I, Putnik-Delić M, Gani I, Marić J and Ilin Ž, Growth, ion composition, and stomatal conductance of peas exposed to salinity. *Central European Journal of Biology* **5**:682-691 (2010).
32. Parihar P, Singh S, Singh R, Singh VP and Prasad SM, Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research* **22**:4056-4075 (2015).
33. An C, Yoon H, Hwang Y, Hwang HJ and Shon GM, Effects of reuse of organic substrate on growth and yield of strawberry in soilless culture, in *XXVII International Horticultural Congress-IHC2006: International Symposium on Advances in Environmental Control, Automation 761*, Ed, pp 521-526 (2006).
34. Diara C, Incrocci L, Pardossi A and Minuto A, Reusing greenhouse growing media. *Acta Horticulturae* **927**:793-800 (2012).
35. Akhatou I and Fernández Recamales Á, Influence of cultivar and culture system on nutritional and organoleptic quality of strawberry. *Journal of the Science of Food and Agriculture* **94**:866-875 (2014).
36. Tozzi F, Del Bubba M, Petrucci WA, Pecchioli S, Macci C, García FH, Nicolás JJM and Giordani E, Use of a remediated dredged marine sediment as a substrate for food crop cultivation: Sediment characterization and assessment of fruit safety and quality using strawberry (Fragaria x ananassa Duch.) as model species of contamination transfer. *Chemosphere* **238**:124651 (2020).
37. Akhatou I and Fernández-Recamales An, Nutritional and nutraceutical quality of strawberries in relation to harvest time and crop conditions. *Journal of agricultural and food chemistry* **62**:5749-5760 (2014).
38. Kafkas E, Koşar M, Paydaş S, Kafkas S and Başer K, Quality characteristics of strawberry genotypes at different maturation stages. *Food Chemistry* **100**:1229-1236 (2007).
39. Keutgen A and Pawelzik E, Modifications of taste-relevant compounds in strawberry fruit under NaCl salinity. *Food chemistry* **105**:1487-1494 (2007).

40. Awang Y, Atherton J and Taylor A, Salinity effects on strawberry plants grown in rockwool. II. Fruit quality. *Journal of Horticultural Science* **68**:791-795 (1993).

41. Ubeda C, Callejón R, Hidalgo C, Torija M, Troncoso A and Morales M, Employment of different processes for the production of strawberry vinegars: Effects on antioxidant activity, total phenols and monomeric anthocyanins. *LWT-Food Science and Technology* **52**:139-145 (2013).

42. Alonzo-Macías M, Cardador-Martínez A, Mounir S, Montejano-Gaitán G and Allaf K, Comparative study of the effects of drying methods on antioxidant activity of dried strawberry (Fragaria Var. Camarosa). *Journal of Food Research* **2**:92-107 (2013).

43. Hernanz D, Recamales ÁF, Meléndez-Martínez AJ, González-Miret ML and Heredia FJ, Assessment of the differences in the phenolic composition of five strawberry cultivars (Fragaria× ananassa Duch.) grown in two different soilless systems. *Journal of agricultural and food chemistry* **55**:1846-1852 (2007).

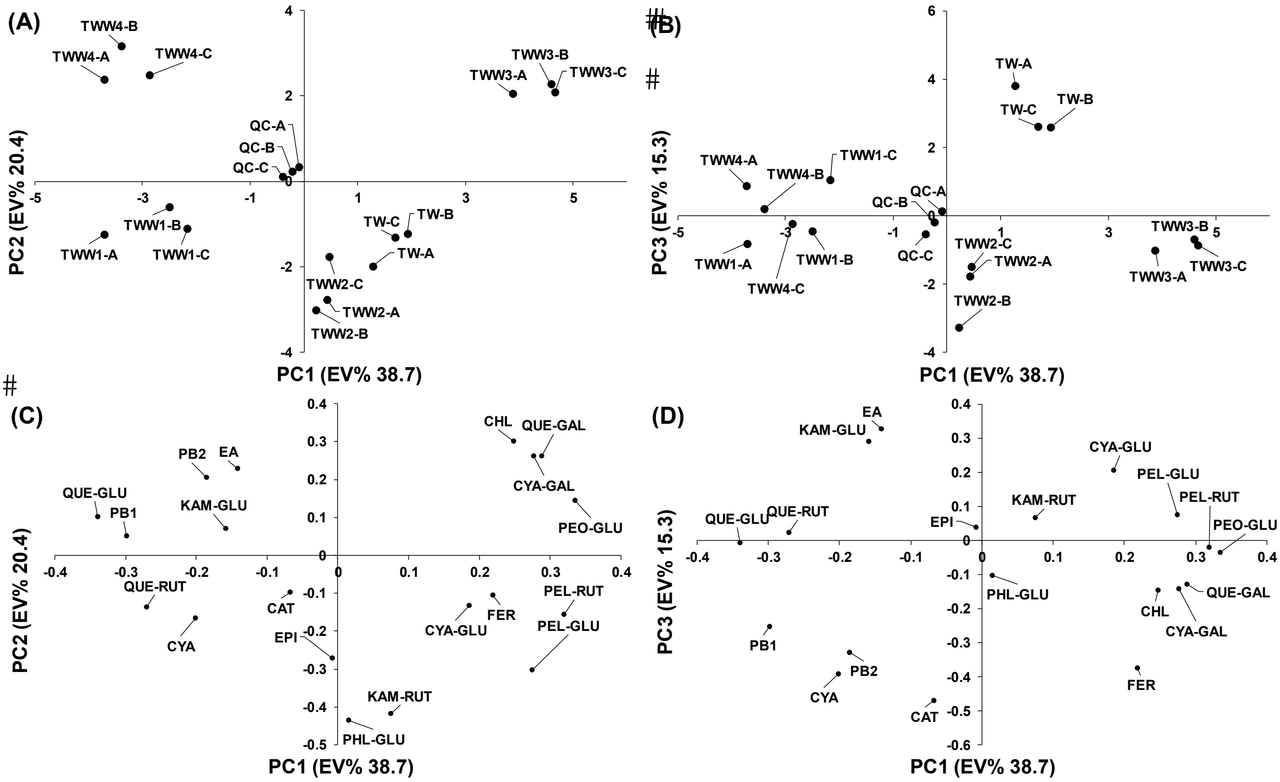

Figure 1 – Score (A-B) and loading (C-D) plots of PC1 vs PC2 (EV%=59.1) and PC1 vs PC3 (EV%=54.0). PCA were calculated using autoscaled concentration values of target analytes. EV% = percentage of explained variance. The meaning of abbreviations used in loading plots are reported in Table 3.

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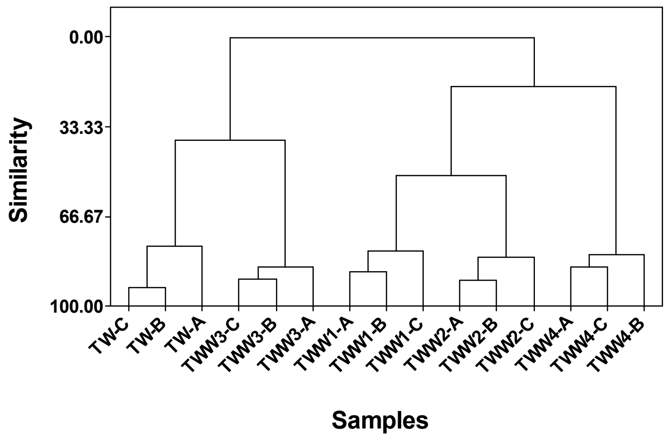

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Figure 2 – Dendrogram of similarity of the fifteen investigated strawberry samples, calculated on the basis of squared Euclidean distances of autoscaled concentration values of the 19 phenolic compounds detected in Camarosa fruits grown under irrigation with tap water (TW) and the four different treated wastewaters (TWW_1, TWW_2, TWW_3, and TWW_4). Letters A, B, and C refer to the analysis of independent samples obtained with a same TWW.#

Table 1 – Mean values and standard deviation (n=63-66, depending on the treatment) of the fruit yield of strawberry plants irrigated with TWWs and TW as control. The yield is expressed as grams of fruit fresh weight per plant (g f.w./plant). Values with different letters are statistically different according to the Games-Howell multicomparison test (p<0.05).

Treatment	**g f.w./plant**
TW	89 (10) a
TWW_1	66 (7) b
TWW_2	74 (9) c
TWW_3	73 (9) c
TWW_4	80 (10) c

Table 2 – Mean values (n=3) and standard deviations (in bracket) of individual (mg/g d.w.) and total sugars (mmol/g d.w.), total soluble polyphenols (TSP, mg Catechin/100 g d.w.), total monomeric anthocyanins (TMA, mg pelargonidin-3-*O*-glucoside/100 g d.w.), antiradical and antioxidant activities as measured by DPPH, ABTS and FRAP methods (μmol Trolox/g d.w.) in strawberry plants irrigated with TW and TWWs. Within the same row, different letters mean statistically significant differences according to the Games-Howell multicomparison test (p<0.05).

Parameters	TW	TWW_1	TWW_2	TWW_3	TWW_4
Sugars					
Fructose	207 (2) a	146 (2) b	162 (5) c	167 (6) c	153 (3) d
Glucose	182 (2) a	132 (2) b	148 (5) cd	155 (2) c	140 (4) d
Sucrose	184 (2) a	156 (1) b	174 (3) c	191 (5) a	157 (9) b
Total sugars	2.67 (0.03) a	1.98 (0.02) b	2.20 (0.08) d	2.32 (0.05) d	2.06 (0.07) b
Phenolic compounds					
TSP	2807 (151) a	2521 (170) b	2680 (173) ab	2683 (71) a	2770 (149) a
TMA	610 (43) a	399 (62) bc	437 (26) b	393 (56) bc	310 (30) c
Antiradical/antioxidant activities					
DPPH	311 (25) a	263 (15) b	272 (19) ab	278 (23) ab	311 (8) a
ABTS	376 (46) a	316 (29) a	333 (29) a	345 (21) a	365 (42) a
FRAP	426 (46) a	355 (9) b	372 (11) b	396 (11) ab	408 (23) ab

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Table 3 – Mean values and standard deviation (n=3), of selected phenolic acids, chalcones, flavanols, flavonols and anthocyanins (mg/100 g d.w.) in strawberry irrigated with TW and TWWs. Within the same row, different letters mean statistically significant differences according to the Games-Howell multicomparison test (*p*<0.05).

Compounds	Abbreviation	TW	TWW_1	TWW_2	TWW_3	TWW_4
ESI (-)						
Chlorogenic acid	CHL	0.04^{*}-0.08^{**}	0.04^{*}-0.08^{**}	0.04^{*}-0.08^{**}	0.31 (0.03)	0.04^{*}-0.08^{**}
Caffeic acid	CAF	$<0.03^{*}$	$<0.03^{*}$	$<0.03^{*}$	$<0.03^{*}$	$<0.03^{*}$
Ferulic acid	FER	3.9 (0.3) ab	4.4 (0.3) ab	5.3 (0.6) ab	5.5 (0.5) a	3.6 (0.4) b
Ellagic acid	EA	24 (2) ab	26 (2) a	15 (1) b	22 (2) ab	26 (3) ab
Quercetin	QUE	$<0.004^{*}$	$<0.004^{*}$	$<0.004^{*}$	$<0.004^{*}$	$<0.004^{*}$
Quercetin-3-*O*-galactoside	QUE-GAL	$<0.02^{*}$	$<0.02^{*}$	$<0.02^{*}$	0.07 (0.01)	$<0.02^{*}$
Quercetin-3-*O*-glucoside	QUE-GLU	1.3 (0.1) a	1.8 (0.2) ab	1.4 (0.1) a	1.2 (0.2) a	1.8 (0.1) b
Quercetin-3-*O*-rutinoside	QUE-RUT	0.79 (0.09) a	1.0 (0.1) a	0.82 (0.07) a	0.69 (0.04) a	0.9 (0.1) a
Quercetin-3-*O*-rhamnoside	QUE-RHA	$<0.02^{*}$	$<0.02^{*}$	$<0.02^{*}$	$<0.02^{*}$	$<0.02^{*}$
Kaempferol-3-*O*-glucoside	KAM-GLU	1.9 (0.3) a	2.2 (0.2) a	1.5 (0.1) a	1.7 (0.2) a	2.0 (0.2) a
Kaempferol-3-*O*-rutinoside	KAM-RUT	1.39 (0.09) a	1.4 (0.1) a	1.3 (0.1) a	1.1 (0.1) ab	0.75 (0.08) b
Procyanidin A2	PA2	$<0.11^{*}$	0.11^{*}-0.26^{**}	$<0.11^{*}$	0.11^{*}-0.26^{**}	$<0.11^{*}$
Procyanidin B1	PB1	4.5 (0.3) a	6.1 (0.5) b	5.4 (0.4) ab	4.8 (0.8) ab	5.9 (0.4) b
Procyanidin B2	PB2	29 (2) a	41 (5) ab	47 (3) b	40 (4) ab	54 (7) b
Phloretin	PHL	$<0.05^{*}$	$<0.05^{*}$	$<0.05^{*}$	$<0.05^{*}$	$<0.05^{*}$
Phloretin-2'-*O*-glucoside	PHL-GLU	2.1 (0.3) ab	2.3 (0.3) ab	2.4 (0.2) a	1.8 (0.2) ab	1.49 (0.09) b
(+)-Catechin	CAT	40 (5) a	48 (4) a	49 (5) a	45 (3) a	44 (3) a
(-)-Epicatechin	EPI	0.86 (0.06) a	0.89 (0.08) a	0.89 (0.09) a	0.81 (0.05) a	0.79 (0.09) a
Total		109 (3) a	135 (7) b	130 (3) b	125 (3) b	141 (10) b
ESI (+)						
Peonidin-3-*O*-glucoside	PEO-GLU	0.111 (0.008) a	0.081 (0.006) bd	0.092 (0.006) ab	0.18 (0.02) c	0.070 (0.006) d
Cyanidin	CYA	10.7 (0.7) ac	16 (1) b	17 (1) b	12 (1) c	14 (2) abc
Cyanidin-3-*O*-galactoside	CYA-GAL	$<0.01^{*}$	$<0.01^{*}$	$<0.01^{*}$	0.20 (0.02)	$<0.01^{*}$
Cyanidin-3-*O*-glucoside	CYA-GLU	12 (1) a	8.8 (0.6) a	10.2 (0.8) a	10.0 (0.7) a	9.5 (0.7) a
Pelargonidin-3-*O*-glucoside	PEL-GLU	343 (21) a	218 (21) bc	319 (38) ac	293 (24) a	161 (17) b
Pelargonidin-3-*O*-rutinoside	PEL-RUT	40 (3) a	29 (3) bc	39 (4) ac	44 (5) a	20 (4) b
Total		406 (18) a	272 (24) b	385 (36) a	359 (22) a	205 (20) b

*Method detection limit. **Method quantification limit.