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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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Running head: Yield and quality of strawberry fruits irrigated with treated wastewaters

## 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

## 27    **Introduction**

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

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

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

49    Quality in food is a combination of different attributes (e.g. sugars, minerals and bioactive  
50    compounds), which affect organoleptic properties, as well as nutritional and nutraceutical values.  
51    These compounds are susceptible to significant variations, depending on climate conditions and  
52    agronomic practices.<sup>10</sup> 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.<sup>11, 12</sup> 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<sup>13</sup>, whereas on long-term crops, like olive trees, the effect of an extended TWW irrigation increased the level of  $\beta$ -carotene and total tocopherols of olive oil.<sup>3</sup>

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<sup>14</sup>, reasons why strawberry is widely appreciated by consumers. In fact, strawberry covers an important place in the horticultural industry, particularly in the Mediterranean countries<sup>15</sup>, which produce around 1.6 million tons annually, almost 18% of the world production.<sup>5</sup> 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.<sup>16</sup>

Strawberry quality was evaluated through the analysis of sucrose, glucose, and fructose as essential nutritional parameters.<sup>17</sup> 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.<sup>18</sup> Moreover, some phenolic compounds previously highlighted as important constituents of the phenolic fraction of *Fragaria* fruits<sup>19-21</sup> 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

79 significant differences in the nutritional and/or nutraceutical quality of the strawberries obtained; (ii)  
80 the nutritional and nutraceutical quality of the fruits obtained by non-conventional irrigation is high  
81 enough to allow their marketability.

## 82 **Materials and methods**

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

### 85 ***Sample origin***

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

88 Plants were irrigated with four TWWs collected in wastewater treatment plants (WWTPs) managed  
89 by GIDA S.p.A. (Prato, Italy). More in detail, the TWWs derived from the following WWTPs: (i)  
90 “Baciacavallo” (TWW<sub>1</sub>), (ii) “Macrolotto 1” (TWW<sub>2</sub>), (iii) “Macrolotto 2” (TWW<sub>3</sub>), and (iv)  
91 “Calice” (TWW<sub>4</sub>). TW was used as control. WWTPs description is reported in the Section S3 of the  
92 *Supplementary materials*. Physicochemical, chemical, and microbiological parameters reported in the  
93 Italian regulation on wastewater reuse<sup>8</sup> were determined in TWWs and the results are shown in **Table**  
94 **S1** of the *Supplementary materials*, together with data regarding TW, which were taken from a public  
95 database.<sup>22</sup>

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

### 100 ***Extraction of sugars and phenolic compounds***

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

### 104 ***Analysis of sugars***

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

#### 110 ***Analysis of TSP, TMA, RSA and AA***

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

#### 121 ***Analysis of individual phenolic compounds***

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

#### 127 ***Statistical analysis***

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

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

## 135 **Results and discussion**

### 136 *TWWs characterization*

137 **Table S1** of the *Supplementary materials* illustrates the physicochemical, chemical and biological  
138 parameters of the TWWs used in this study, which are foreseen by the Italian Ministerial Decree  
139 185/2003<sup>8</sup>, regulating the wastewater reuse for various applications, including the agricultural  
140 irrigation. **Table S1** includes also the values of these parameters determined in TW, as well as the  
141 limits reported in the M.D. 185/2003. The values determined in TWWs complied the thresholds with  
142 some exceptions. More in detail, among physicochemical and chemical parameters, TSS slightly  
143 exceeded the limit established by the M.D. 185/2003 (i.e. 10 mg/L) for TWW<sub>1</sub> (11±4 mg/L) and  
144 TWW<sub>4</sub> (12±4 mg/L), and ammonia was found just above the legal threshold (i.e. 2 mg/L) in TWW<sub>1</sub>  
145 (2.1±1.3 mg/L). Exceedances of the M.D. 185/2003 limits were much more accentuated and  
146 generalized (i.e. in all the TWWs investigated) for the chloride ion, the mean concentrations of which  
147 were in the range of 258-643 mg/L (legal threshold 250 mg/L). Sodium adsorption ratio (SAR),  
148 although within the limit established by the M.D. 185/2003 (i.e. 10), was also a critical parameter for  
149 TWW reuse in agriculture, being it included from 9.0 (for TWW<sub>3</sub>) to 9.5 (for all the other TWWs).  
150 Conductivity was a further parameter worth to be mentioned, since high values were observed in  
151 TWWs (1322-2428 µS/cm), compared to those considered suitable for crop irrigation.<sup>29</sup> Overall, the  
152 remarkable concentrations of chloride ion, together with the high values of SAR and conductivity,  
153 highlight potential problems in the use of TWWs for irrigation purposes. However, it should be noted  
154 that these waters represents an important source of nutrients for plant growth, since they respectively  
155 contain 5-8 mg/L of nitrogen and 300-900 µg/L of phosphorus.

### 156 *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  $TW > TWW_4 \approx TWW_3 = TWW_2 > TWW_1$ . More in detail, plants irrigated with TWWs showed a reduced fruit production compared to control from 10% with TWW<sub>4</sub> to 26% with TWW<sub>1</sub>, the latter exhibiting by far the highest level of salinity, as measured by electrical conductivity (2428  $\mu\text{S}/\text{cm}$  in TWW<sub>1</sub> vs 1322-1647  $\mu\text{S}/\text{cm}$  in the other TWWs and 872  $\mu\text{S}/\text{cm}$  in TW). Interestingly, a similar reduction in productivity (12-24% depending on the kind of irrigation system) was previously reported for Camarosa strawberry<sup>13</sup> 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  $\mu\text{S}/\text{cm}$ ) of irrigation water.<sup>30</sup> 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<sup>31</sup>, causing a plant water-deficit condition, which inhibited plant growth and productivity.<sup>32</sup> 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.<sup>16</sup>

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<sup>33, 34</sup>, making therefore less critical the impact of irrigation with TWWs.

## 180 **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 <sup>35-38</sup>, 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<sub>2</sub> and TWW<sub>3</sub> 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<sub>1</sub> and TWW<sub>4</sub>, 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<sup>-</sup> concentration exceeding Italian legal limits for wastewater reuse (**Table S1**). In fact, the high Cl<sup>-</sup> 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.<sup>39, 40</sup>

#### ***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<sub>1</sub> (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<sub>5</sub>, COD, N<sub>tot</sub> and P<sub>tot</sub> similar to those of TWWs tested in this study <sup>13</sup>. The comparison between TWW<sub>1</sub> (the most salty TWW) and TW was the only one providing a statistically significant difference ( $p<0.05$ ).

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

210 In contrast to findings obtained for TSP, DPPH, ABTS, and FRAP, irrigation with wastewater  
211 significantly affected TMA values, as total anthocyanins in control fruits (610 mg pelargonidin-3-*O*-  
212 glucoside/100 g d.w.) were up to twofold higher than those found in fruits treated with TWWs (310-  
213 437 mg pelargonidin-3-*O*-glucoside/100 g d.w.). More in detail, the irrigation with TWWs gave rise  
214 in all cases to statistically significant decreases of this parameter compared to control. It is however  
215 remarkable that TSP and TMA concentrations of strawberries produced with TWWs were included  
216 in the range of values reported in literature for Camarosa fruits purchased in the market or produced  
217 in research orchards.<sup>1, 13, 41, 42</sup> Therefore, fruits irrigated with TWWs demonstrated a nutraceutical  
218 quality in line with their marketability.

### 219 *Individual phenolic compounds*

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

228 Similar patterns of relative abundance were highlighted for targeted phenolic compounds, irrespective  
229 of the use of TWWs or TW for irrigation. More in detail, in all samples, PEL-GLU was by far the  
230 most abundant compound (161-343 mg/100g d.w.), accounting for 74-84% and 47-67% of total  
231 individual anthocyanins and total individual phenolic compounds, respectively. Other predominant  
232 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.<sup>1, 43</sup>

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<sub>1</sub> and TWW<sub>4</sub>, and PB2 for TWW<sub>2</sub> and TWW<sub>4</sub>. 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<sub>1</sub> and TWW<sub>4</sub> compared to TW, whereas the use of TWW<sub>2</sub> and TWW<sub>3</sub> 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<sub>1</sub> and TWW<sub>4</sub>, 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<sub>3</sub> 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<sub>3</sub>. The higher PEO-GLU concentrations found in TWW<sub>3</sub> samples also contributed to differentiate them from the others on the PC1 vs PC2 score plot. For TWW<sub>1</sub> and TWW<sub>4</sub> samples, which showed very similar coordinates on PC1, the separation was mainly due to their very different concentrations of KAM-RUT and PHL-

285 GLU, the only two compounds providing very high loadings (in absolute value) on PC2 (**Fig. 1C**).  
286 TW and TWW<sub>2</sub> were the closest samples in the PC1 vs PC2 score plot, reflecting the quite similar  
287 concentrations of most phenolic compounds in fruits from these treatments.  
288 The separation of TW and TWW<sub>2</sub> samples was more evident when PC1 was plotted as a function of  
289 PC3 (EV%=54.0) (**Fig. 1B**). In fact, on this latter component, strawberries irrigated with TWW<sub>2</sub>  
290 strongly differentiated from those grown with TW, due to concentration trends found in these samples  
291 for FER, PB2, CAT and CYA, all of them providing high absolute values of loadings in PC3 (**Fig.**  
292 **1D**). Conversely, TWW<sub>1</sub> and TWW<sub>4</sub> samples grouped together, evidencing very similar behaviours  
293 of the two treatments on PC3 as well. According to the loading plot shown in **Fig. 1D**, fruits irrigated  
294 by TWW<sub>1</sub> and TWW<sub>4</sub> were characterized by high concentrations of QUE-GLU, PB1 and QUE-RUT,  
295 compared to those found in the other samples. It is interesting to note that samples obtained with  
296 TWW<sub>1</sub> and TWW<sub>4</sub>, which are effluents of WWTPs operating on similar mixed domestic-textile  
297 wastewaters and characterized by analogous treatments stages, exhibited very similar score values on  
298 both PC1 (EV%=38.7) and PC3 (EV%=15.3).  
299 The use of CA, as performed by using the squared Euclidean distances of autoscaled concentrations  
300 of targeted analytes (**Figure 2**), confirmed the homogeneous results obtained for replicated samples  
301 within each treatment. In fact, the replicates of each treatment grouped at very high similarity levels  
302 (i.e. TW=77.8%, TWW<sub>1</sub>=79.5%, TWW<sub>2</sub>=81.7%, TWW<sub>3</sub>=85.3%, and TWW<sub>4</sub>=81.1%), which were  
303 much greater than those regarding the other clusters present in the dendrogram (i.e. ≤51.3%).

## 304 **Conclusions**

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

308 The comparative evaluation of the effect of various TWWs characterized by different  
309 physicochemical and chemical properties, allowed for obtaining interesting information that to the  
310 best of our knowledge are provided herein for the first time. Plants grown with TWW<sub>1</sub> appeared to

311 be among the most affected by non-conventional irrigation, displaying the lowest yield, sugar and  
312 TSP concentrations, RSA and AA values, as well as statistically lower TMA content, compared to  
313 control. Interestingly, strawberries irrigated with TWW<sub>2</sub> and TWW<sub>3</sub>, which have common origin and  
314 underwent similar depuration stages, exhibited equivalent quality attributes. Fruits produced by  
315 irrigating plants with TWW<sub>4</sub> showed erratic trends, being among the best for some parameters (e.g.  
316 yield) and among the worst for some others (e.g. TMA).

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

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

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

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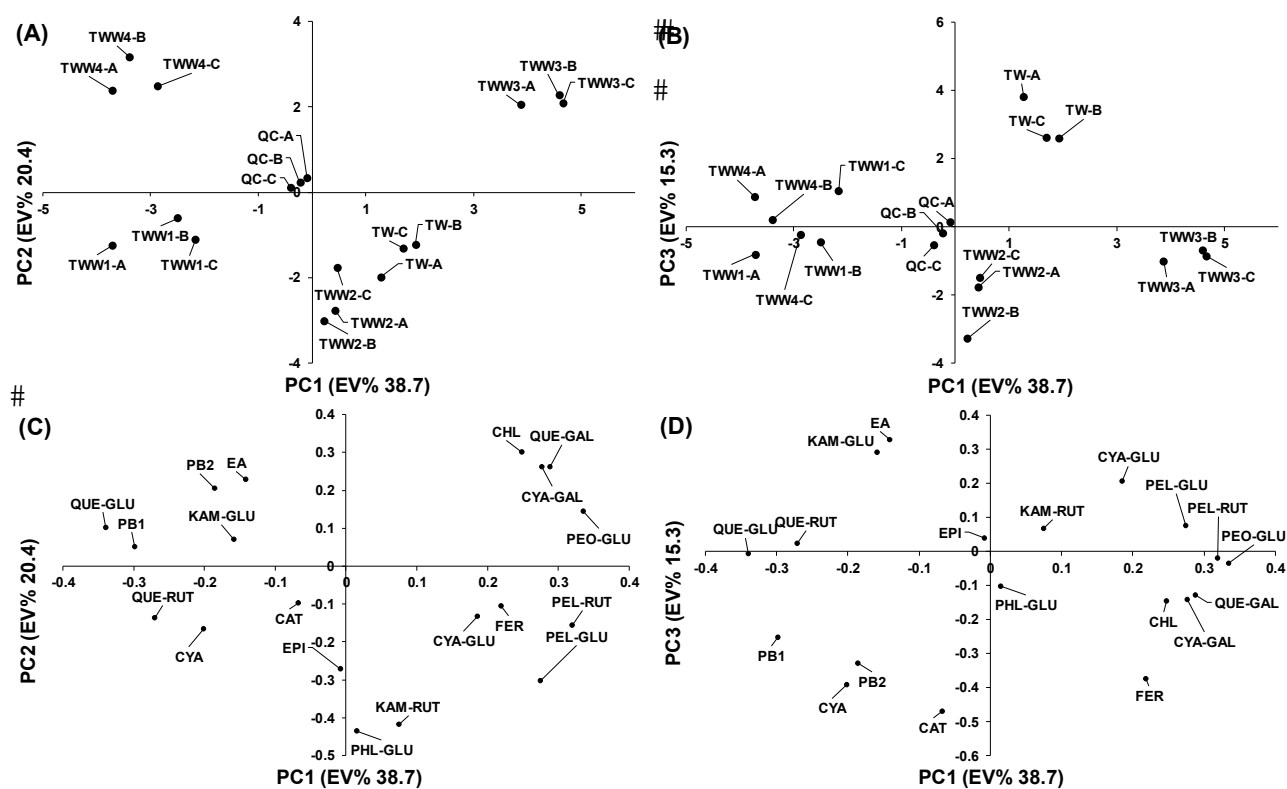
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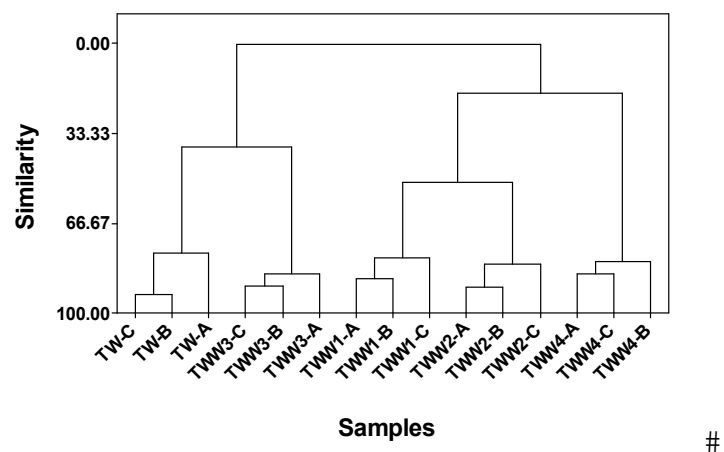


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**Figure 1** – Score (A-B) and loading (C-D) plots of PC1 vs PC2 (EV%=59.1) and PC1 vs PC3 (EV%=54.0). PCA were calculated using autoscaled concentration values of target analytes. EV% = percentage of explained variance. The meaning of abbreviations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



**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<sub>1</sub>, TWW<sub>2</sub>, TWW<sub>3</sub>, and TWW<sub>4</sub>). 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 ( $p<0.05$ ).

<b>Treatment</b>	<b>g f.w./plant</b>
TW	89 (10) a
TWW <sub>1</sub>	66 (7) b
TWW <sub>2</sub>	74 (9) c
TWW <sub>3</sub>	73 (9) c
TWW <sub>4</sub>	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 <sub>1</sub>	TWW <sub>2</sub>	TWW <sub>3</sub>	TWW <sub>4</sub>
<i>Sugars</i>					
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
<i>Phenolic compounds</i>					
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
<i>Antiradical/antioxidant activities</i>					
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

#

**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 <sub>1</sub>	TWW <sub>2</sub>	TWW <sub>3</sub>	TWW <sub>4</sub>
<i>ESI (-)</i>						
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- <i>O</i> -galactoside	QUE-GAL	<0.02*	<0.02*	<0.02*	0.07 (0.01)	<0.02*
Quercetin-3- <i>O</i> -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- <i>O</i> -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- <i>O</i> -rhamnoside	QUE-RHA	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
Kaempferol-3- <i>O</i> -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- <i>O</i> -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'- <i>O</i> -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
<i>ESI (+)</i>						
Peonidin-3- <i>O</i> -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- <i>O</i> -galactoside	CYA-GAL	<0.01*	<0.01*	<0.01*	0.20 (0.02)	<0.01*
Cyanidin-3- <i>O</i> -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- <i>O</i> -glucoside	PEL-GLU	343 (21) a	218 (21) bc	319 (38) ac	293 (24) a	161 (17) b
Pelargonidin-3- <i>O</i> -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.