The Swiss Knife of Analytics

Inspired versatility and reliability – the next era of SFC

To enable customer- and application-specific solutions in the pharmaceutical, chemical and food industries, the Nexera UC supercritical fluid chromatography system is available in multiple configurations. Unique hardware innovations ensure reliable and stable analysis, making the Nexera UC an ideal tool for challenging sample separations. Adding the specificity of MS detection to the versatility of SFC achieves highest sensitivity.

Unparalleled pressure stability to ensure accurate, reproducible data
through unique, low-volume backpressure regulator design

Faster flow rates, higher throughput and less cost per sample
with an environmentally friendly, low-viscosity mobile phase

Automated method scouting workflow
for either LC or SFC method screening

Combination with supercritical fluid extraction
merges quick and easy sample preparation with state-of-the-art chromatographic analysis and highsensitivity detection

www.shimadzu.eu/next-era-SFC

Check for updates

Received: 8 August 2023 | Revised: 26 September 2023 | Accepted: 20 October 2023

DOI: 10.1111/1750-3841.16829

Journal of Food Science WILEY

ORIGINAL ARTICLE

Health, Nutrition, & Food

Nutritionally enriched tomatoes (*Solanum lycopersicum* L.) grown with wood distillate: chemical and biological characterization for quality assessment

Riccardo Fedeli[1] | **Ludovica Marotta**[2] | **Luca Frattaruolo**[3] | **Alice Panti**[1] | **Gabriele Carullo**[2] | **Fabio Fusi**[2] | **Simona Saponara**[1] | **Sandra Gemma**[2] | **Stefania Butini**[2] | **Anna Rita Cappello**[3] | **Andrea Vannini**[1] | **Giuseppe Campiani**[2] | **Stefano Loppi**[1,4]

[1]Department of Life Sciences, University of Siena, Siena, Italy

[2]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy

[3]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Cosenza, Italy

[4]BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, Napoli, Italy

Correspondence
Gabriele Carullo and Giuseppe Campiani, Department of Biotechnology, Chemistry and Pharmacy, University of Siena, 53100 Siena, Italy.
Email: gabriele.carullo@unisi.it and giuseppe.campiani@unisi.it

Abstract: Bio-based products are nowadays useful tools able to affect the productivity and quality of conventionally cultivated crops. Several bio-based products are currently on the market; one of the newest and most promising is the wood distillate (WD) derived from the pyrolysis process of waste biomass after timber. Its foliar application has been widely investigated and shown to promote the antioxidant profile of cultivated crops. WD was used here as additive for the cultivation of tomato (*Solanum lycopersicum* L.) plants. The application improved quality (chemical) parameters, minerals, polyphenols, and lycopene contents of tomato fruits. The extracts of WD-treated and untreated tomatoes have been chemically and biologically characterized. The ^{1}H-NMR and ESI-mass spectrometry analyses of the extracts revealed the presence of different fatty acids, amino acids and sugars. In particular, the WD-treated tomatoes showed the presence of pyroglutamic acid and phloridzin derivatives, but also dihydrokaempferol, naringenin glucoside, cinnamic acid, and kaempferol-3-*O*-glucoside. When tested in cells, the extracts showed a promising anti-inflammatory profile in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Furthermore, the extracts displayed a slight vasorelaxant activity on rat aorta rings (either endothelium-denuded or endothelium-intact) pre-contracted with phenylephrine or potassium chloride.

KEYWORDS
anti-inflammatory activity, lycopene, organic farming, tomato, wood distillate

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2023 The Authors. *Journal of Food Science* published by Wiley Periodicals LLC on behalf of Institute of Food Technologists.

Practical Application: Wood distillate has been used for tomato plant growth. Tomatoes showed improved nutritional parameters, and their extracts displayed antioxidant and anti-inflammatory activities.

1 INTRODUCTION

In May 2020, the European Commission developed the Farm to Fork strategy, a 10-year plan to guide the agricultural transition toward a fair, healthy, and environmentally friendly food system (European Commission, 2023). The achievement of these goals is critical due to the exponential increase in world population and the subsequent need to supply sufficient food globally.

Nowadays, it is urgent to develop alternative agricultural practices to the traditional ones, mainly based on synthetic products, for more efficient and sustainable agriculture (Muhie, 2022).

Recent EU regulations promote the adoption of bio-based solutions for modern agriculture. Bio-based products can improve nutrient use and acquisition efficiency. They include bio-stimulants, biopesticides, organic fertilizers, and natural soil conditioners. Several bio-based stimulants for crop nutrition are currently on the market. Among them, microbial bio-stimulants are available and include mycorrhizal and non-mycorrhizal fungi, bacterial endosymbionts (like *Rhizobium*), and plant growth–promoting rhizobacteria and also substances, such as humic and fulvic acids, protein hydrolysates, and chitosan (du Jardin, 2015).

Worldwide, the concept of circular economy fosters the use of alternative sources, a concept that also applies to agriculture. In this context, one of the most promising bio-stimulant is the wood distillate (WD) that is derived from the pyrolysis process of timber (Grewal et al., 2018). The woody material is burned at very high temperatures (625–775 K) in the absence or poor presence of oxygen. Through this process, two by-products are produced: a solid fraction, the biochar, and a liquid fraction derived from vapor condensation, the WD (Grewal et al., 2018). Both by-products have different applications in agriculture such as remedies to heal soils suffering from various environmental stresses (Fedeli, Alexandrov, et al., 2022), as bio-stimulants (Zhu et al., 2021), and as herbicidal agents for higher plants and cryptogams.

WD has a pungent smell and a color ranging from yellow to reddish-brown with a chemical composition that varies upon the operations and parameters that are set up during the pyrolysis process (Theapparat et al., 2014). More than 200 biologically organic active water-soluble compounds have been detected in WD, including phenols, organic acids, alcohols, and esters (Mu et al., 2006). Due to the presence of these molecules, WD is used in agriculture mainly as a bio-stimulant, though recent studies have shown that it can be used also to increase both biomass and fruit productivity and quality (Fedeli, Vannini, et al., 2022; Fedeli, Vannini, Grattacaso, et al., 2023; Zulkarami et al., 2011). In addition, its environmental safety has been established based on the absence of toxic effects (Fačkovcová et al., 2020a,b; Fanfarillo et al., 2022).

Tomato (*Solanum lycopersicum* L.) is one of the main cultivated crop species in the world mainly due to its nutritive values and the ability of the plant to produce high yields for unit area (Collins et al., 2022). The production of tomatoes worldwide is about 178 M tons per year and ca. 30% is produced in China, whereas Italy accounts for ca. 4% on par with Iran and Egypt. This vegetable is a major food of the Mediterranean diet and contains health-beneficial phytochemicals (e.g., polyphenols, lycopene, and vitamins, mainly of B group).

Several biological properties have been highlighted for tomato, including anti-inflammatory activities and the prevention of chronic diseases (Chaudhary et al., 2018). Seminal studies revealed that the tomato powder is able to significantly increase the diversity and richness of the gut microbiome, avoiding a buildup of Gram-negative bacteria, which can secrete hepatotoxic compounds able to promote liver inflammatory disease and cancer (Xia et al., 2018). Furthermore, on an inflammatory perspective, tomato proved to alter the composition of the gut microbiome by increasing *Flavobacterium* and *Lactobacillus* and decreasing *Oscillospira* populations, thus reducing the gut inflammatory damage in mice (Collins et al., 2022).

Among all the phytochemicals, one of the most essential is lycopene, the supplementation of which with tomatoes has been shown to reduce blood lipids (such as LDL-cholesterol) and systolic blood pressure (Chen et al., 2013). Furthermore, lycopene can alleviate oxidative stress often responsible for cardiovascular diseases (Li et al., 2021).

This research proposes the use of WD for foliar application on tomato plants, aiming at investigating if WD can interfere with tomato yields and with the fruits' quality parameters together with the presence of bioactive compounds. The study is performed by comparing the data obtained from not treated and treated tomatoes (TOM_C and TOM_WD, respectively). To the best of our knowledge, this is the first study reporting the cultivation of

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

 Journal of Food Science WILEY

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

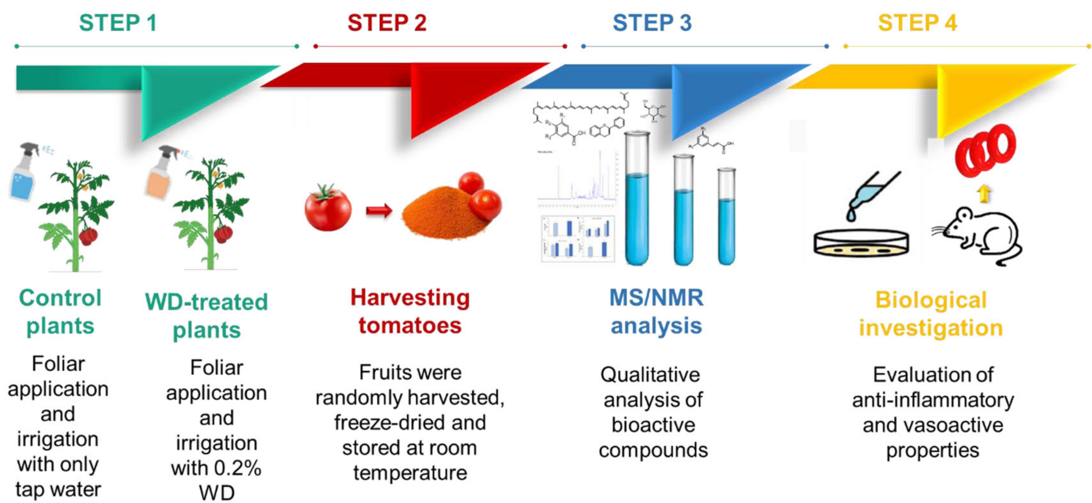


FIGURE 1 Experimental design.

tomatoes with foliar application of WD and the correspondent analysis for their functional properties. Tomato extracts (TOM_Ce and TOM_WDe), obtained by the mean of solid–liquid extraction, were first analyzed for their chemical composition via ESI-mass spectrometry (MS) and ^{1}H NMR analyses, then, in vitro tested to evaluate the anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages and their influence on vasoactivity in thoracic rat aorta rings (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Chemicals

The materials used inlcude Dulbecco's modified Eagle's medium (DMEM), FBS, L-glutamine, penicillin, streptomycin, phenylephrine, acetylcholine, nifedipine, and phenylephrine (Sigma Chimica); sodium nitroprusside (Riedel-De Haen AG). Phenylephrine was solubilized in 0.1 M HCl. Nifedipine was dissolved directly in ethanol and the extracts in DMSO.

2.2 | Experimental design

The planting material, that is, tomato seeds (*S. lycopersicum L.* var. impact), was sown in April 2021 according to a randomized complete block design with 10 blocks (replications) and 2 treatments. Within each block, two 1×1 m^2 plots were placed 3 m apart. One treatment consisted of water alone (control) and the other of 0.2% WD of chestnut (*Castanea sativa*) (BioDea®). At planting, the soil was fertigated with water (control) or 0.2% WD. Fertigation was repeated two more times when the tomato plants reached a height of 15–20 cm and had five to six leaves. The plants were sprayed every 10 days with water (control) or 0.2% WD until the fruits were harvested. In June, the fruits were randomly harvested in the center of the experimental plots and prepared for analysis. Then they were freeze-dried and stored at room temperature prior to analyses.

2.3 | Soluble sugars

Determination of soluble sugar content (i.e., glucose and fructose) was performed according to literature (Fedeli, Vannini, et al., 2022) Approximately 1.5 g of samples were homogenized in 2 mL of deionized water and then centrifuged at 15,000 rpm for 5 min. An aliquot of the supernatant was filtered at 0.45 µm with a syringe filter. Then the filtered extract was analyzed directly using an HPLC (Waters 600 system) equipped with a Waters 2410 refractive index detector. Separation of sugars was achieved using deionized water as the mobile phase, eluted at 0.5 mL/min, and a Waters Sugar-Pak I ion-exchange column (6.5 mm × 300 mm) maintained at 90°C using an external temperature controller (Waters Column Heater Module). Standard curves of pure analytical sugars (>98.0%, Sigma-Aldrich), ranging from 0.1 to 20 mg/mL, were used for determining the concentration of the sugars in the samples.

2.4 | Antioxidant compounds (polyphenols and flavonoids)

Determination of total polyphenol content was performed according to the method described by Henríquez et al. (2010) with minor modifications. Approximately 1 g of the

sample was homogenized in 4 mL of an acetone/water solution (70:30 v/v) and then centrifuged at 4000 rpm for 5 min. An aliquot of the supernatant (500 μL) was added to 3 mL deionized water, 125 μL Folin–Denis reagent (Sigma-Aldrich), 750 μL saturated Na_2CO_3, and 950 μL deionized water. The samples were placed in an oven at 37°C for 30 min, then centrifuged at 4000 rpm for 5 min. Absorbance was read at 765 nm with a UV–Vis spectrophotometer (8453, Agilent). A standard curve of pure gallic acid (>98%, Sigma-Aldrich), ranging from 5 to 20 μg/mL, was used for determining the concentration of polyphenols in the samples. Determination of flavonoid content was performed according to the literature (Loppi et al., 2021). About 1 g of samples were homogenized in 2 mL ethanol/water (80:10 v/v) and then centrifuged at 15,000 rpm for 5 min. An aliquot of the supernatant (500 μL) was added to 45 μL of a 5% $NaNO_2$ solution and 300 μL of deionized water. Then 45 μL of a 10% $AlCl_3$ solution, 300 μL of a 1 M NaOH solution and 300 μL of deionized water were added. After that, the samples were read at 510 nm with a UV–Vis spectrophotometer. A standard curve of pure quercetin (>95%, Sigma-Aldrich), ranging from 5 to 200 μg/mL, was used for determining the concentration of flavonoids in the samples.

2.5 | Lycopene

Determination of lycopene content was performed according to the literature (Olives Barba et al., 2006), with minor modifications. Approximately 1.5 g of tomato fruit was homogenized in 10 mL of a solution composed of hexane/acetone/ethanol (50:25:25 v/v/v). Subsequently, 1.5 mL of deionized water was added and vortexed. An aliquot of the supernatant (1 mL) was taken and dried under vacuum (20°C), and the dry extract was resuspended in 0.4 mL of methanol/acetonitrile/tetrahydrofuran (55:30:15 v/v/v). The mobile phase for HPLC analysis (Perkin Elmer Nelson 3200 Series) consisted of methanol/acetonitrile (90:10 v/v) and 9 mM triethanolamine at a flow rate of 0.9 mL/min, using an RP-C18 column (SUPELCO Kromasil 100A-5u-C18 4.6 mm × 250 mm); absorbance was set at 475 nm, and the running time of the analysis was about 20 min. A standard curve of pure lycopene (>85.0%, Sigma-Aldrich), ranging from 6.25 to 100 μg/mL, was used for determining the concentration of lycopene in the samples.

2.6 | Mineral element

Approximately 200 mg of lyophilized sample was mineralized with a mixture of 3 mL of 70% (v/v) HNO_3 and 0.5 mL of 30% (v/v) H_2O_2, using a microwave digestion system (Milestone Ethos900, Metrohm) at 280°C and 55 bar. The content of Fe, Ca, Mg, Na, K, Cu, Zn, and P was quantified by inductively coupled MS (ICP-MS, Perkin Elmer NexION 350). Analytical quality was measured using NCS DC 73350 certified standard reference material *Poplar leaves*; recoveries were in the range 98%–106%. The precision of analysis was estimated from the coefficient of variation of seven replicates and was always >98%.

2.7 | Extraction of phytochemicals

Six grams of minced tomatoes (TOM_C or TOM_WD) were extracted for 3 h at 65°C with 160 mL of acetone:water (4:1 v/v). The mixture was filtered, and the filtrate was evaporated to dryness under vacuum (Minoggio et al., 2003).

2.8 | Spectroscopic analyses

NMR analyses were conducted on a Varian 300 MHz spectrometer by solubilizing 10 mg of each sample in 600 μL of $CDCl_3$ (Carullo, Governa, et al., 2020). To fully appreciate the different metabolites in the extracts, a further extraction was performed: each sample (TOM_Ce or TOM_WDe) of 20 mg was solubilized in 1 mL of $CDCl_3$, and then this solution was extracted with a hydroalcoholic mixture constituted of 900 μL D_2O and 100 μL MeOD. The two phases were then analyzed in order to better evaluate the chemical composition of the extracts. The assignment of the resonances was performed by analyzing ^{1}H-NMR characteristics and the comparison of tests in the literature (Le Gall et al., 2003; Sobolev et al., 2003). MS analysis was performed applying the following operating conditions: spray voltage (+/−): 4.5 kV; capillary temperature: 200°C; sheath gas (nitrogen) flow rate: ca. 0.75 L/min. MSn product ion experiments were carried out inside the ion trap by isolating the precursor ion and then by applying a supplementary potential for collision-induced dissociations; collision gas: He; collision energy: 24%–30% arbitrary units (a.u.). The m/z window for precursor ion isolation was 1 or 2 u. Samples were introduced via flow injection in the ESI source at a flow rate of 5 μL/min. The Orbitrap Q Exactive Plus (Thermo Fisher) with an electrospray source, operating in positive and negative ion mode, in flow injection, was used for high resolution (30,000 and 140,000 FWHM@ m/z 200) mass spectra and higher energy collisional dissociation MS/MS spectra by using nitrogen as collision gas; collision energy: 18%–30% a.u.

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.9 | Cell cultures

RAW 264.7 murine macrophages, obtained from American Type Culture Collection, were cultured in DMEM (Sigma-Aldrich) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, and 1% penicillin/streptomycin (all from Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% CO_2 (Carullo, Sciubba, et al., 2020).

2.10 | Nitric oxide production assessment

RAW 264.7 macrophages were seeded in 24-well plates at a density of 2×10^5 cells/well and cultured overnight in DMEM. Then, cells were simultaneously exposed for 24 h to 1 µg/mL LPS and to serial dilutions of TOM_Ce or TOM_WDe (concentrations ranging from 12.5 to 200 µg/mL). DMSO-treated cells were used as control. The presence of nitrites, a stable oxidized product of nitric oxide (NO), was assessed in cell culture media by using the Griess reagent, as previously reported (Tundis et al., 2018). More in detail, 100 µL of Griess reagent (Sigma-Aldrich) and the same volume of cell culture medium from each sample were combined, and absorbance at 550 nm was measured by using a BioTek Synergy H1 microplate reader (Agilent Technologies). Cell viability of treated RAW 264.7 cells was assessed by MTT (Sigma-Aldrich) assay and compared to that of untreated cells.

2.11 | qPCR analysis

RAW 264.7 cells were cultured as described above and exposed for 6 h to DMSO or to 1 µg/mL LPS alone or in the presence of 100 µg/mL TOM_Ce or TOM_WDe. Pro-inflammatory cytokine determination was executed as described in Mazzotta et al. (2019). Total cellular RNA was extracted using TRI-Reagent (Sigma-Aldrich), following manufacturer's recommendations. RNA purity and integrity were assayed spectroscopically and by gel electrophoresis. Reverse transcription of RNA to cDNA was achieved by reverse transcription, by using OneScript Plus cDNA Synthesis Kit (Applied Biological Materials Inc.), following manufacturer's procedure. Gene expression analyses of tumor necrosis factor alpha (TNF-α) interleukin 1 beta and interleukin 6 (IL-6) were performed using the platform Quant Studio3 Real-Time PCR System (Life Technologies) using PowerUp™ SYBR™ Green Master Mix (Life Technologies), according to manufacturer's recommendations. The results were normalized using glyceraldehyde 3-phosphate dehydrogenase mRNA levels. Relative mRNA levels were calculated using the $\Delta\Delta Ct$ method comparing to control group. All the primers used for amplifications are listed in Table S1.

2.12 | Animal care statement

The Animal Care and Ethics Committee of the University of Siena and the Italian Department of Health (7DF19.N.TBT) approved all the procedures, in strict accordance with the European Union Guidelines for the Care and the Use of Laboratory Animals (European Union Directive 2010/63/EU). An isoflurane (4%) and O_2 gas mixture was used to anaesthetize male Wistar rats (260–370 g; Charles River Italia) with Fluovac (Harvard Apparatus), before heparinization, decapitation, and exsanguination. The thoracic aorta was immersed in a modified Krebs–Henseleit solution (KHS) and processed as detailed below (Campiani et al., 2021; Carullo et al., 2022).

2.13 | Aorta ring preparation

Rings (3–4 mm wide) were cut from the thoracic aorta and mounted, under a passive tension of 1 g, in organ baths filled with KHS (composition in mM: 118 NaCl, 4.75 KCl, 1.19 KH_2PO_4, 1.19 $MgSO_4$, 25 $NaHCO_3$, 11.5 glucose, 2.5 $CaCl_2$, gassed with carboxygen; pH 7.4) for isometric tension recordings, using a digital PowerLab data acquisition system (PowerLab 8/30; ADInstruments). Rings responding to 0.3–0.6 µM phenylephrine and 60 mM KCl were considered viable (Carullo et al., 2019, 2023; Carullo, Ahmed, Fusi, et al., 2020). When necessary, the endothelium was removed by gently rubbing the lumen of the ring with a forceps tip. This procedure was validated by adding 10 µM acetylcholine at the plateau of phenylephrine-induced contraction: a relaxation greater than 70% or less than 10% denoted the presence or absence of functional endothelium, respectively (Fusi et al., 2000).

2.14 | Phenylephrine- and KCl-induced contractions

Each extract was added cumulatively to aorta rings stimulated by 0.3–0.6 µM phenylephrine, 25–35 mM or 60 mM KCl. Nifedipine (10 µM) and/or sodium nitroprusside (100 µM) were added at the end of each experiment to test smooth muscle viability. The response was calculated as a percentage of the initial tension evoked by phenylephrine or KCl (Cuong et al., 2014). Analysis of data was accomplished using LabChart 7.3.7 Pro (PowerLab; ADInstruments) (Carullo, Ahmed, Trezza, et al., 2020; Carullo et al., 2021).

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Mineral content in TOM_C and TOM_WD expressed as mg kg^{-1}.

	TOM_C	TOM_WD
Fe	100.77 ± 9.46[a]	87.29 ± 7.93[a]
Ca	1477.78 ± 87.31[a]	1759.16 ± 183.95[a]
Mg	1932.12 ± 102.68[a]	1975.21 ± 101.53[a]
Na	1111.71 ± 67.02[a]	1246.63 ± 86.00[a]
K	44,047.69 ± 1503.36[a]	41,297.46 ± 2193.98[a]
Cu	12.56 ± 1.02[a]	10.67 ± 0.27[a]
Zn	29.34 ± 2.16[a]	27.15 ± 3.16[a]
P	4688.31 ± 297.93[a]	3558.07 ± 98.67[b]

Note: Data are presented as mean ± standard deviation. Different letters indicate statistically significant ($p < 0.05$) differences between treatments.
Abbreviations: Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Na, sodium; P, phosphorus; Zn, zinc.

2.15 | Statistical analysis

The normality of data was verified through the Shapiro–Wilk test. All results are presented with mean ± standard error. Statistical differences between control and WD-treated samples were evaluated by the Student *t*-test with a significance level of $p < 0.05$. Statistical analysis was performed using either R software (R Core Team, 2022) or GraphPad Prism 5.04 (GraphPad Software Inc.).

3 | RESULTS AND DISCUSSION

3.1 | Quality parameters

Tomatoes derived from plants treated with WD, TOM_WD showed statistically significant increases in fresh weight (+16.0%), soluble sugar content (i.e., glucose (+32.9%), fructose (+24.4%), and total antioxidant compound content (+27.8%) (i.e., polyphenols (+17.9%), flavonoids (+58.1%)), and lycopene content (+51.9%), compared to control, TOM_C (Figure 2). The results revealed a significant increase in both yield and nutritional quality of tomato fruits following the application of 0.2% WD on plants. This behavior is in line with other crops, which showed a positive trend in yield and nutritional quality after WD treatment. This increment in terms of individual product weight could be due to the stimulating action of WD on the root system and to the raising of the photosynthetic action of plants, thus stimulating flowering and

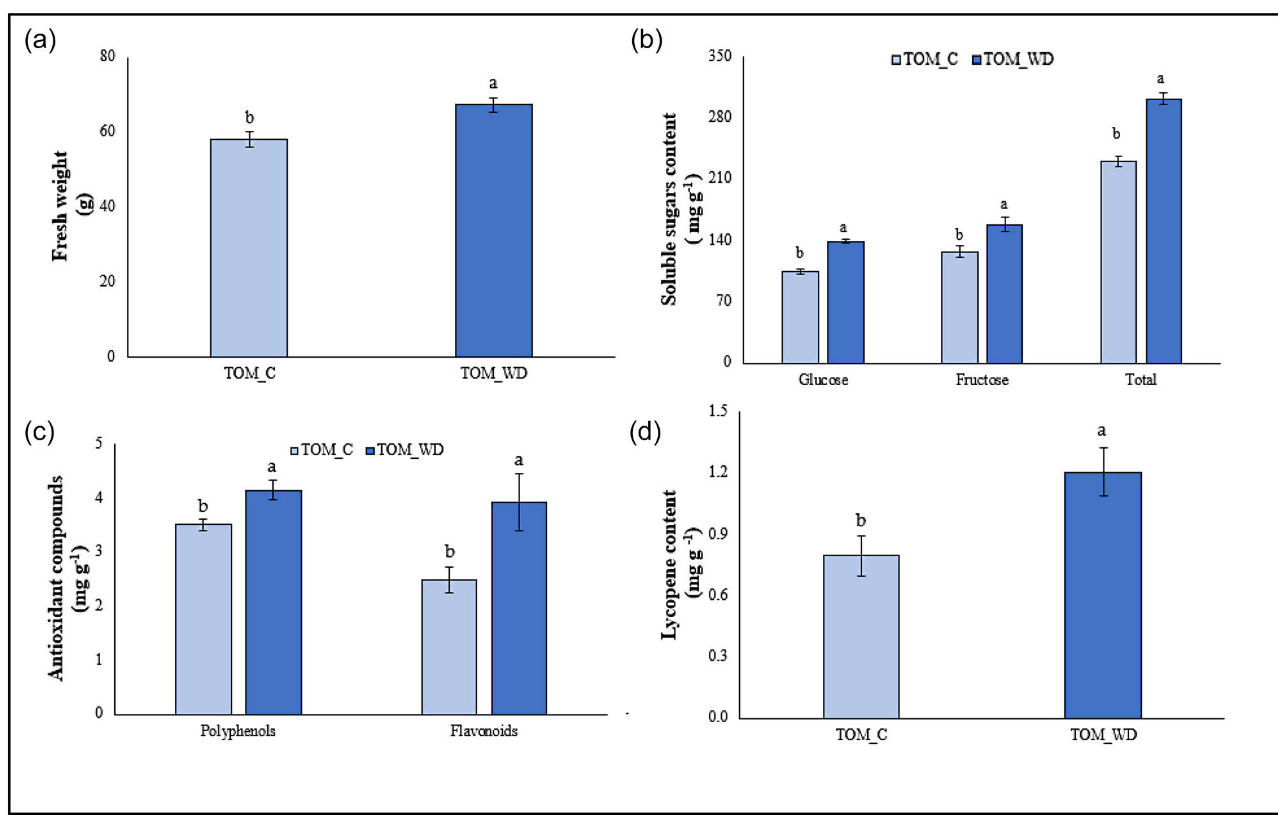


FIGURE 2 Quality parameters for tomato fruits derived from plants irrigated with water (**TOM_C)** and tomato fruits derived from plants treated with wood distillate (WD) **(TOM_WD**): (a) fresh weight; (b) soluble sugar content; (c) antioxidant compound content; (d) lycopene content. Data are presented as mean ± standard deviation. Distinct letters indicate statistically significant differences ($p < 0.05$) among treatments.

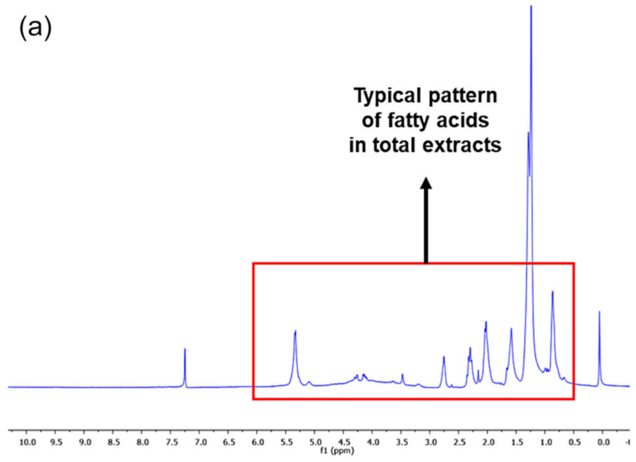
17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

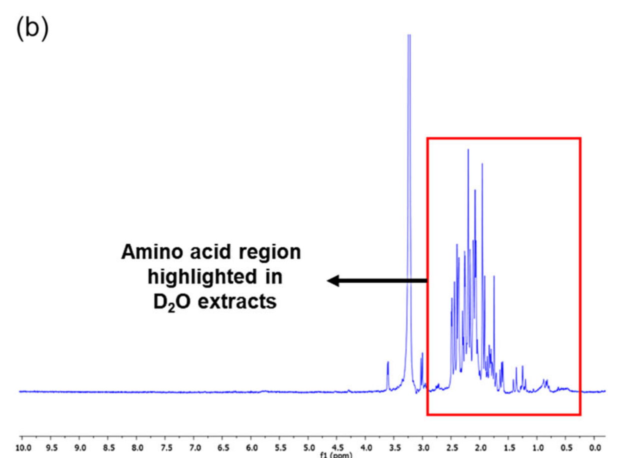
17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

plant growth (Rose et al., 2016). Furthermore, this effect could be influenced by several compounds present in WD, such as alcohols, polyphenols, and acids (Wei et al., 2010). Limited data are available about the impact of WD on the sugar content of crop species; for tomato, it has been shown an enhancement in soluble sugar concentration after a 0.5% WD application (CheonSoon et al., 2006). These studies are consistent with the results reported here; in fact, all three values for glucose, fructose, and total sugars were significantly higher than those of the respective control groups (Jones and Scott, 1983). These data demonstrate that the WD treatment can promote higher sugar content in the product, resulting in a tastier outcome without the need for any genetic editing. Moreover, a higher concentration of polyphenols was observed, as previously reported for chickpea seeds (Fedeli, Vannini, Celletti, et al., 2023), tomato (Rose et al., 2016), and strawberry fruits (Kårlund et al., 2014), following an application of 0.2%–0.5% WD. It is well known that lycopene is one of the most relevant and characteristic molecules of tomato, as it is found in much higher levels than other fruits (i.e., watermelon, pink grapefruit, and apricot). It was also observed that there was an increase in total carotenoid content in tomatoes following an application of 0.5%–1% WD (Ofoe et al., 2022). The raising of antioxidant compound content analyzed could be due to the stimulating effect of WD, called eustress. This practice is very common in the horticultural field. Several methods are used to bring the plant to a mild state of stress that stimulates the generation of antioxidant compounds as a defense mechanism (Baenas et al., 2014). The increase of these compounds, without the use of chemical pesticides or genetic modification, is interesting and useful, as they are essential to prevent oxidative stress and various diseases. No significant difference in the mineral components was observed between TOM_C and TOM_WD, except for the phosphorus (P), which showed a significant reduction in WD-treated tomatoes by 24.1% (Table 1), as already shown in citrus rootstocks treated with 0.4% WD. This reduction may be related to the low pH of WD (Mirsoleimani et al., 2022).

3.2 | Extraction and chemical characterization of the extracts

Starting from 6.02 g of TOM_C or TOM_WD we obtained 0.25 g of TOM_Ce (4.15% *w/w* of extract deriving from TOM_C) and 0.63 g of TOM_WDe (10.4% *w/w* of extract deriving from TOM_WD). The two extracts were stored at−20°C until further analyses. Extracts were diluted in $CDCl_3$ and analyzed through ^1H NMR, revealing a similar pattern in the corresponding spectra (Figure 3a, Figure S1,





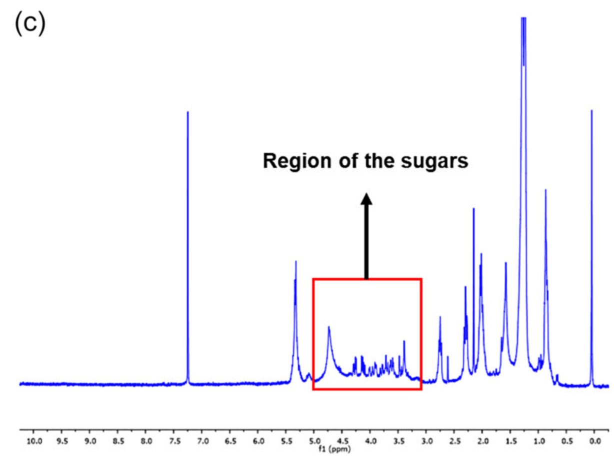


FIGURE 3 Details of ^1H-NMR spectra of TOM extracts. (a) full spectrum in $CDCl_3$. (b) amino acids regions highlighted in D_2O extract. (c) sugars region.

Table 2). In particular, the ^1H NMR spectra of total extracts TOM_WDe and TOM_Ce revealed the typical pattern of fatty acids, such as oleic, linoleic, and linolenic acids. In particular, the visible signals (Figure 3a) showed to be essentially those of olefinic protons (5.33 ppm)

TABLE 2 Resonance assignments with chemical shifts of identified metabolites.

Molecule	^{1}H shift	Multiplicity	Assignment	TOM_Ce	TOM_WDe
Oleic, linoleic, linolenic acids	0.98	s	CH_3	+	+
Acetate	1.91	s	CH_3	+	+
Lycopene	1.95	s	$-CH_3$	+	+
Glutamate	2.03	m	β-CH_2	+	+
Valine	2.23	m	β-CH	+	+
γ-Aminobutyrate	2.29	t	α-CH_2	+	+
GABA	2.32	m	CH_2	−	+
Glutamate	2.34	m	γ-CH_2	+	+
Malate	2.37	dd	β-CH_2	+	+
Citrate	2.66	m	CH_2	+	+
Aspartate	2.78	m	β-CH_2	+	+
Phenylalanine	3.10	m	β-CH_2	+	+
Tyrosine	3.13	m	β-CH_2	+	+
α-D-Glucose	3.52	dd	H2	+	+
Threonine	3.58	m	α-CH	+	+
Tyrosine	3.90		α-CH	+	+
α-Tocopherol	4.18		COH	−	+
β-D-Glucose	4.65	d	H1	+	+
Fatty acids	5.32, 5.34	d	CH_2	+	+
Dihydrokaempferol	6.20	m	ArH	−	+
Naringenin glucoside	6.22	m	ArH	−	+
Cinnamic acid	7.46	m	CH=CH	−	+
Kaempferol-3-*O*-glucoside	8.07	m	ArH	−	+
Trigonelline	8.85	m	ArH	−	+

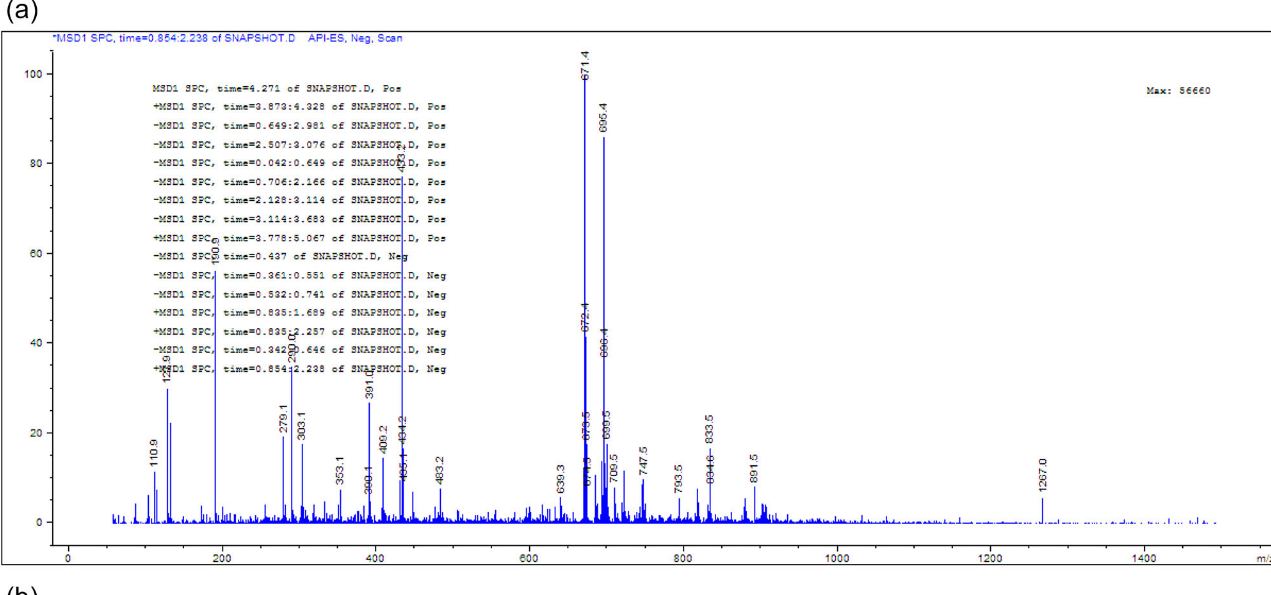
Note: "+" identified in the extract; "−" not identified in the extract.

(Masetti et al., 2017). In these conditions, it was not possible to identify other compounds, so we decided to perform a liquid/liquid extraction on the samples. First, the extracts were solubilized in $CDCl_3$, and then they were partitioned between $CDCl_3$ and D_2O/MeOD, to obtain two separated phases and therefore four different samples that were analyzed through ^{1}H NMR (Figures S2 and S3) (Tomassini et al., 2016). As expected, hydroalcoholic extracts revealed high amounts of acetate, malate, α-tocopherol, sugars like α-D-glucose, β-D-glucose, and different amino acids like valine, threonine, GABA, phenylalanine, tyrosine, glutamate, and aspartate (Figure 3b,c, Table 2) (Sobolev et al., 2003). Other substances identified were lycopene and polyphenols like dihydrokaempferol, naringenin glucoside, and kaempferol-3-*O*-glucoside, but also cinnamic acid, although limited amounts of these compounds can be inferred by the low intensity of diagnostic peaks for assignments (Table 2) (Le Gall et al., 2003). To fully complete the chemical characterization, the extracts have also been subjected to LC–MS analysis operating in ESI mode (Figure 4, Table 3). In particular, the extracts contain similar identifiable metabolites, including derivatives of caffeic acid, chlorogenic acid derivative, caffeic acid hexoside, diacyl phosphoglyceride, diacyl phosphatidyl-myoinositol, hydroxy-phloretin 3′,5′-di-C-hexoside, monoacylglycerol-phosphoserine, caffeoyl monoacyl phosphoglyceride, and diacyl phosphoglyceride. On the other hand, TOM_WDe presented some ions attributable to different metabolites like hydroxyoctanedioic acid, pyroglutamic acid, chlorogenic acid, phloretin 3′,5′-di-C-hexoside. The ESI (+) analysis is not useful to identify secondary metabolites present in tomatoes (Otify et al., 2023).

3.3 | Anti-inflammatory effects of TOM_WDe and TOM_Ce in LPS-stimulated macrophages

In order to evaluate the anti-inflammatory potential of the obtained extracts as well as to highlight any significant

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



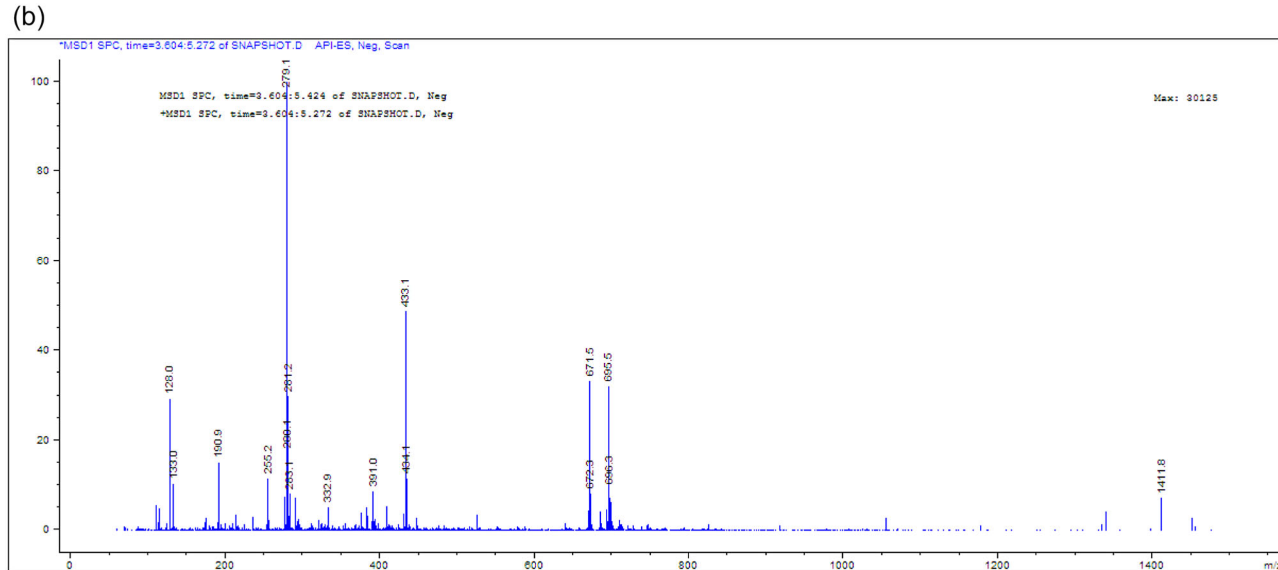


FIGURE 4 ESI (−) fingerprints of TOM_WDe (a) and TOM_Ce (b) extracts.

differences between TOM_WDe and TOM_Ce, their ability to inhibit NO production in LPS-stimulated RAW 264.7 macrophages was evaluated. Indeed, NO is an important gaseous signaling molecule that regulates various physiological and pathophysiological processes, such as the phlogistic response. Its production, during the inflammatory process, is mediated by the inducible nitroxide synthase, the expression of which is modulated by NF-kB transcription factor, a key element in the cellular response to harmful stimuli (Brindisi et al., 2020; Frattaruolo et al., 2019). As shown in Figure 5a, in LPS-stimulated RAW 264.7 cells, both extracts were able to reduce NO production in a concentration-dependent manner, although significantly higher effects were found on cells after TOM_WDe treatment. Indeed, TOM_WDe exhibited inhibitory activity at lower concentrations compared to TOM_Ce, reaching an IC_{50} value equal to 21.47 μg/mL (Figure 5a). Furthermore, MTT assay (Figure 5b) revealed no cytotoxic effect on macrophages, which allows us to exclude any correlation between the observed biological effects and alterations of cell viability, thus confirming the safety of the tested extracts. In order to corroborate the promising anti-inflammatory potential of the extracts, as well as the effectiveness of the WD treatment, the transcription levels of genes encoding different pro-inflammatory cytokines were assessed in LPS-stimulated macrophages after treatment with TOM_WDe and TOM_Ce. The results obtained (Figure 5c) indicated the ability of both extracts to decrease transcript levels of TNF-α, IL-6, and interleukin 1ß, which are important biological mediators in the activation and propagation of the inflammatory response. The comparison between the two different treatments

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 ESI (−) data with putative identification of most significant ions in TOM_Ce and TOM_WDe.

Molecule	*m/z*	Formula	MS/MS	TOM_Ce	TOM_WDe
Pyroglutamic acid	128.03	$C_5H_6NO_3^-$	128	+	−
Hydroxy-octanedioic acid	189.07	$C_8H_{13}O_5^-$	129	−	+
Derivative of caffeic acid	487.12,	$C_{24}H_{23}O_{11}^-$,	133	+	+
	505.13,	$C_{24}H_{25}O_{12}^-$,			
	519.14,	$C_{25}H_{27}O_{12}$,			
	491.26,	$C_{27}H_{39}O_8$,			
	473.25	$C_{27}H_{37}O_7^-$			
Chlorogenic acid derivative	353.08,	$C_{16}H_{17}O_9^-$,	191	+	+
	529.15,	$C_{23}H_{29}O_{14}^-$,			
	691.18,	$C_{32}H_{35}O_{17}^-$,			
	337.09,	$C_{16}H_{17}O_8^-$,			
	515.11,	$C_{25}H_{23}O_{12}^-$,			
	677.15	$C_{34}H_{29}O_{15}^-$			
Octadecadienoic acid derivative	515.29	$C_{26}H_{43}O_{10}^-$	279	+	+
Lipids	723.37,	$C_{33}H_{57}O_{14}^-$,	279	+	+
	595.28,	$C_{27}H_{48}O_{12}P^-$,			
	638.32,	$C_{29}H_{53}NO_{12}P^-$,			
	520.25,	$C_{24}H_{43}NO_9P^-$,			
	476.27,	$C_{23}H_{43}NO_7P^-$,			
	564.32,	$C_{27}H_{51}NO_9P^-$,			
	579.28,	$C_{27}H_{47}O_{11}S^-$,			
	507.26,	$C_{24}H_{44}O_9P^-$,			
	595.25,	$C_{30}H_{44}O_{10}P^-$,			
	529.28,	$C_{27}H_{45}O_{10}^-$,			
	515.3,	$C_{27}H_{47}O_9^-$,			
	433.23,	$C_{21}H_{38}O_7P^-$,			
	477.25,	$C_{21}H_{36}O_7P^-$,			
	447.24,	$C_{22}H_{40}O_7P^-$,			
	461.26,	$C_{23}H_{42}O_7P^-$,			
	693.45,	$C_{39}H_{66}O_8P^-$,			
	833.51,	$C_{43}H_{78}O_{13}P^-$,			
	695.46	$C_{39}H_{68}O_8P^-$			
Caffeic acid hexoside derivative	621.19,	$C_{33}H_{33}O_{12}^-$,	281	+	+
	561.14	$C_{23}H_{13}O_{17}^-$			
Lipids	581.27,	$C_{27}H_{49}O_{11}S^-$,	281	+	+
	597.3,	$C_{27}H_{50}O_{12}P^-$,			
	478.28,	$C_{23}H_{45}NO_7P^-$,			
	566.34,	$C_{27}H_{53}NO_9P^-$,			
	509.28	$C_{24}H_{46}O_9P^-$			
Pyroglutamic acid derivative	632.15	$C_{36}H_{26}NO_{10}^-$	290	−	+
Chlorogenic acid	529.15,	$C_{23}H_{29}O_{14}^-$,	353	−	+
	691.18,	$C_{32}H_{35}O_{17}^-$,			
	677.17,	$C_{31}H_{33}O_{17}^-$,			
	515.11,	$C_{25}H_{23}O_{12}^-$,			
	677.15	$C_{34}H_{29}O_{15}^-$,			

(Continues)

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 (Continued)

Molecule	*m/z*	Formula	MS/MS	TOM_Ce	TOM_WDe
Diacyl phosphoglyceride	669.44	$C_{37}H_{66}O_8P^-$	391	+	+
Diacyl phosphatidyl-myoinositol	833.51	$C_{43}H_{78}O_{13}P^-$	391	+	+
Hydroxy-phloretin 3′,5′-di-*C*-hexoside	613.17	$C_{27}H_{33}O_{16}^-$	433	+	+
Monoacyl-glycerol-phosphoserine	520.25	$C_{24}H_{43}NO_9P^-$	433	+	+
Caffeoyl monoacyl phosphoglyceride	595.25	$C_{30}H_{44}O_{10}P^-$	433	+	+
Phloretin 3′,5′-di-*C*-hexoside *O*-hexoside	759.23	$C_{33}H_{43}O_{20}^-$	639	−	+
Diacyl phosphoglyceride	695.46	$C_{39}H_{68}O_8P^-$	695	+	+

Note: "+" identified in the extract; "−" not identified in the extract.

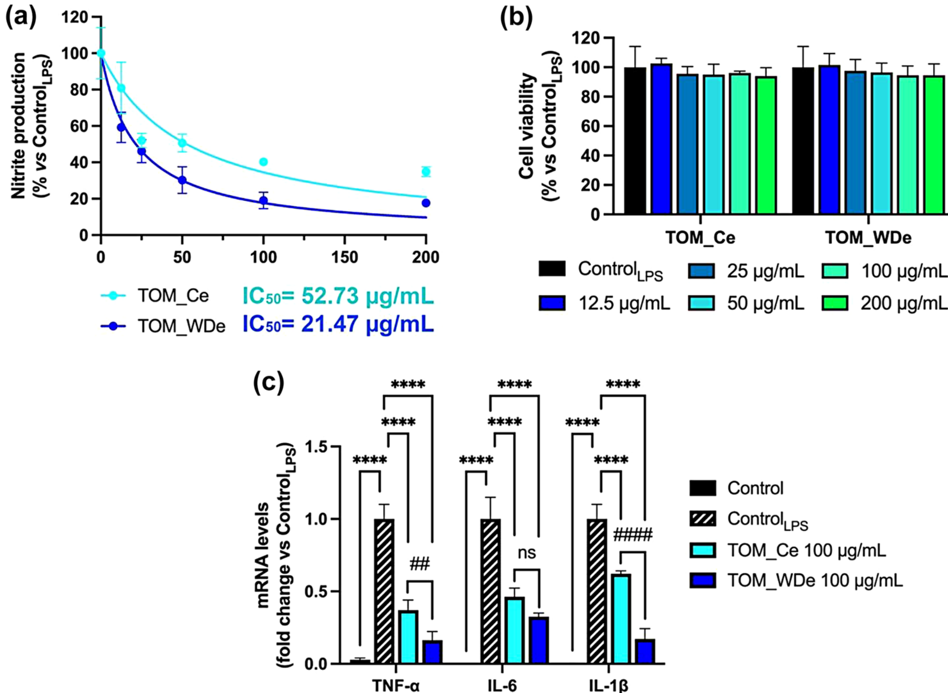


FIGURE 5 Anti-inflammatory potential of TOM_Ce and TOM_WDe in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. (a) Nitrite production assessment in LPS stimulated macrophages after treatment with different concentrations of extracts. IC_{50} values were obtained by nonlinear regression analysis of three independent experiments. (b) Cell viability rating of LPS-stimulated RAW 264.7 cells treated with TOM_Ce and TOM_WDe. (c) qPCR analysis of the transcription levels of genes encoding pro-inflammatory cytokines. ****p value <0.0001 (samples vs. control$_{LPS}$); ns: not significant, ##p value <0.01, ####p value <0.0001 (TOM_WDe vs. TOM_Ce).

highlighted a significant activity of TOM_WDe with respect to TOM_Ce in reducing the expression of TNF-*α* and IL-1ß, further emphasizing that WD treatment is able to increase the biological properties of tomatoes. These results could be explained by the different chemical composition of the two phytocomplexes. Indeed, TOM_WDe is characterized by the presence of different compounds like dihydrokaempferol, naringenin glucoside, cinnamic acid, kaempferol-3-*O*-glucoside, and trigonelline, which were proven, by several previous reports (Chen et al., 2023; Khalili et al., 2018; Zeng et al., 2018) to be endowed with anti-inflammatory action; consequently, they could contribute to the anti-inflammatory activity of the TOM_WDe.

3.4 | Effect of TOM_WDe and TOM_Ce on phenylephrine- or KCl-induced contractions

To investigate the effects of the two extracts on pharmacomechanical coupling and define a role for the endothelium in their vasorelaxant activity, they were assessed in rings,

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 6 Effects of tomato extracts on phenylephrine- and KCl-induced contraction of rat aorta rings. (a) Concentration–response curves for **TOM_WDe** and **TOM_Ce** in endothelium-intact (+endo) or denuded (−endo) rings pre-contracted by 0.3 µM phenylephrine. The steady-state contraction was evoked by phenylephrine and then each extract or an equal volume of vehicle (DMSO) was added cumulatively. In the ordinate scale, relaxation is reported as a percentage of the initial tension induced by phenylephrine. (b) Concentration–response curves for **TOM_WDe** and **TOM_Ce** in endothelium-denuded rings pre-contracted by either 60 mM KCl (K60) or 25–30 mM KCl (K25/30). The steady-state contraction was evoked by KCl and then each extract or an equal volume of vehicle (DMSO) was added cumulatively. In the ordinate scale, relaxation is reported as a percentage of the initial tension induced by KCl. Data points represent the mean ± standard deviation (n = 3–5 rings isolated from at least three animals).

either endothelium-denuded or endothelium-intact, pre-contracted by 0.3–0.6 µM phenylephrine or 25–35 mM or 60 mM KCl. As shown in Figure 6a, neither TOM_WDe nor TOM_Ce affected the vessel tone induced by the α_1 agonist, independently of the presence of a functional endothelium. In order to assess the effect of TOM_WDe and TOM_Ce on electromechanical coupling, which is essentially due to the opening of $Ca_V1.2$ channels, aorta rings were depolarized by either 25–35 mM or 60 mM KCl. When muscle tone reached a plateau, cumulative concentrations of each extract or vehicle were added (Figure 6b). Neither TOM_WDe nor TOM_Ce affected 60 mM KCl-induced contraction. However, both extracts relaxed by about 25%, and the preparations depolarized by 25 mM KCl. This effect was observed only at low concentrations of TOM_WDe, whereas it persisted in the range of concentrations 3–100 µg/mL of TOM_Ce. The pharmacological analysis demonstrated that the two extracts possessed a modest vasodilatory activity, likely ascribable to a K^+ channel opening effect, as it was observed only in rings depolarized with moderate but not high concentrations of K^+ (Gurney, 1994). $Ca_V1.2$ channels, endothelium-derived vasorelaxant factors or α_1 adrenergic receptors seemed not to be affected by the extracts.

4 | CONCLUSION

Overall, the data herein described demonstrated the efficacy of foliar treatment with 0.2% WD in improving the nutritional parameters of tomatoes. This treatment is able to increase total flavonoids and polyphenols in the fruits (TOM_C and TOM_WD). The corresponding extracts (TOM_Ce and TOM_WDe) did not show significant signals in the classical pattern of amino acids and carotenoids. Some substances like glutamic acid, phloridzin derivatives, and naringenin have been identified in TOM_WDe but not in TOM_Ce. TOM_WDe displayed significant nitrite production inhibitory activity without affecting cell viability; this effect is most likely due to the presence of dihydrokaempferol, naringenin glucoside, cinnamic acid, kaempferol-3-*O*-glucoside, and trigonelline, only in this extract. When tested in rat aorta rings, TOM_WDe did not reveal any significant vasorelaxant activity. To sum up, this study shows new efforts for the use of WD in agriculture. In particular, the data herein presented evidence that the use of WD improves the nutritional parameters of the treated tomatoes, fostering anti-inflammatory properties of the extracts. These results deserve further studies for understanding the mechanism by which WD improves the quality parameters of the *S. lycopersicum* L.

AUTHOR CONTRIBUTIONS

Riccardo Fedeli: Conceptualization; investigation; writing—original draft. **Ludovica Marotta**: Investigation; formal analysis. **Luca Frattaruolo**: Methodology; formal analysis; investigation; writing—original draft; writing—review and editing. **Alice Panti**: Validation; formal analysis; methodology. **Gabriele Carullo**: Conceptualization; investigation; writing—original draft;

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

software; formal analysis; supervision. **Fabio Fusi**: Investigation; writing—original draft; software; data curation; formal analysis. **Simona Saponara**: Investigation; writing—original draft; writing—review and editing; formal analysis; supervision. **Sandra Gemma**: Writing—review and editing; visualization; methodology; software; formal analysis; validation. **Stefania Butini**: Investigation; validation; writing—review and editing; software; formal analysis; visualization; methodology. **Anna Rita Cappello**: Investigation; funding acquisition; validation; formal analysis; supervision; writing—review and editing. **Andrea Vannini**: Investigation; validation. **Giuseppe Campiani**: Investigation; funding acquisition; writing—review and editing; visualization; supervision; resources; data curation. **Stefano Loppi**: Project administration; supervision; resources; data curation; investigation; funding acquisition; writing—review and editing; visualization.

ACKNOWLEDGMENTS

The authors thank Francesco Barbagli (BioDea and BioEsperia s.r.l.) who kindly provided the wood distillate.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to declare.

ORCID

Riccardo Fedeli https://orcid.org/0000-0002-8736-1313
Ludovica Marotta https://orcid.org/0000-0002-4004-6463
Luca Frattaruolo https://orcid.org/0000-0002-7018-8454
Alice Panti https://orcid.org/0009-0005-0470-654X
Gabriele Carullo https://orcid.org/0000-0002-1619-3295
Fabio Fusi https://orcid.org/0000-0002-5737-8309
Simona Saponara https://orcid.org/0000-0002-3831-8669
Sandra Gemma https://orcid.org/0000-0002-8313-2417
Stefania Butini https://orcid.org/0000-0002-8471-0880
Anna Rita Cappello https://orcid.org/0000-0001-7824-3561
Andrea Vannini https://orcid.org/0000-0003-3789-6691
Giuseppe Campiani https://orcid.org/0000-0001-5295-9529
Stefano Loppi https://orcid.org/0000-0002-3404-1017

REFERENCES

Baenas, N., García-Viguera, C., & Moreno, D. A. (2014). Elicitation: A tool for enriching the bioactive composition of foods. *Molecules*, *19*, 13541–13563.

Brindisi, M., Bouzidi, C., Frattaruolo, L., Loizzo, M. R., Tundis, R., Dugay, A., Deguin, B., Cappello, A. R., & Cappello, M. S. (2020). Chemical profile, antioxidant, anti-inflammatory, and anti-cancer effects of Italian *Salvia rosmarinus* Spenn. methanol leaves extracts. *Antioxidants*, *9*, 826.

Campiani, G., Cavella, C., Osko, J. D., Brindisi, M., Relitti, N., Brogi, S., Saraswati, A. P., Federico, S., Chemi, G., Maramai, S., Carullo, G., Jaeger, B., Carleo, A., Benedetti, R., Sarno, F., Lamponi, S., Rottoli, P., Bargagli, E., Bertucci, C., … Prasse, A. (2021). Harnessing the role of HDAC6 in idiopathic pulmonary fibrosis: Design, synthesis, structural analysis, and biological evaluation of potent inhibitors. *Journal of Medicinal Chemistry*, *64*, 9960–9988.

Carullo, G., Ahmed, A., Fusi, F., Sciubba, F., Cocco, M. E. D., Restuccia, D., Spizzirri, U. G., Saponara, S., & Aiello, F. (2020). Vasorelaxant effects induced by red wine and pomace extracts of Magliocco dolce cv. *Pharmaceuticals*, *13*, 87.

Carullo, G., Ahmed, A., Trezza, A., Spiga, O., Brizzi, A., Saponara, S., Fusi, F., & Aiello, F. (2020). Design, synthesis and pharmacological evaluation of ester-based quercetin derivatives as selective vascular $K_{Ca}1.1$ channel stimulators. *Bioorganic Chemistry*, *105*, 104404.

Carullo, G., Ahmed, A., Trezza, A., Spiga, O., Brizzi, A., Saponara, S., Fusi, F., & Aiello, F. (2021). A multitarget semi-synthetic derivative of the flavonoid morin with improved in vitro vasorelaxant activity: Role of $Ca_V1.2$ and $K_{Ca}1.1$ channels. *Biochemical Pharmacology*, *185*, 114429.

Carullo, G., Durante, M., Sciubba, F., Restuccia, D., Spizzirri, U. G., Ahmed, A., Cocco, M. E. D., Saponara, S., Aiello, F., & Fusi, F. (2019). Vasoactivity of Mantonico and Pecorello grape pomaces on rat aorta rings: An insight into nutraceutical development. *Journal of Functional Foods*, *57*, 328–334.

Carullo, G., Falbo, F., Ahmed, A., Trezza, A., Gianibbi, B., Nicolotti, O., Campiani, G., Aiello, F., Saponara, S., & Fusi, F. (2023). Artificial intelligence-driven identification of morin analogues acting as $Ca_V1.2$ channel blockers: Synthesis and biological evaluation. *Bioorganic Chemistry*, *131*, 106326.

Carullo, G., Governa, P., Spizzirri, U. G., Biagi, M., Sciubba, F., Giorgi, G., Loizzo, M. R., Cocco, M. E. D., Aiello, F., & Restuccia, D. (2020). Sangiovese cv pomace seeds extract-fortified kefir exerts anti-inflammatory activity in an in vitro model of intestinal epithelium using caco-2 cells. *Antioxidants*, *9*, 54.

Carullo, G., Saponara, S., Ahmed, A., Gorelli, B., Mazzotta, S., Trezza, A., Gianibbi, B., Campiani, G., Fusi, F., & Aiello, F. (2022). Novel labdane diterpenes-based synthetic derivatives: Identification of a bifunctional vasodilator that inhibits $Ca_V1.2$ and stimulates $K_{Ca}1.1$ channels. *Marine Drugs*, *20*, 515.

Carullo, G., Sciubba, F., Governa, P., Mazzotta, S., Frattaruolo, L., Grillo, G., Cappello, A. R., Cravotto, G., Cocco, M. E. D., & Aiello, F. (2020). Mantonico and Pecorello grape seed extracts: Chemical characterization and evaluation of in vitro wound-healing and anti-inflammatory activities. *Pharmaceuticals*, *13*, 97.

Chaudhary, P., Sharma, A., Singh, B., & Nagpal, A. K. (2018). Bioactivities of phytochemicals present in tomato. *Journal of Food Science and Technology*, *55*, 2833–2849.

Chen, J., Song, Y., & Zhang, L. (2013). Effect of lycopene supplementation on oxidative stress: An exploratory systematic review and meta-analysis of randomized controlled trials. *Journal of Medicinal Food*, *16*, 361–374.

Chen, P., Xu, Z., Wang, X., He, J., Yang, J., Wang, J., Chattipakorn, N., Wu, D., Tang, Q., Liang, G., & Chen, T. (2023). Discovery of new cinnamic derivatives as anti-inflammatory agents for treating acute lung injury in mice. *Archiv der Pharmazie*, *356*, 2200191.

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CheonSoon, J., InJue, Y., JongNam, P., JangHun, K., JungPhil, K., SungJae, L., TaeSu, J., & ByoungJun, A. (2006). Effect of wood vinegar and charcoal on growth and quality of sweet pepper. *Korean Journal of Horticultural Science & Technology*, *24*, 177–180.

Collins, E. J., Bowyer, C., Tsouza, A., & Chopra, M. (2022). Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. *Biology 2022*, *11*, 239.

Cuong, N. M., Khanh, P. N., Huyen, P. T., Duc, H. V., Huong, T. T., Ha, V. T., Durante, M., Sgaragli, G., & Fusi, F. (2014). Vascular L-type $Ca2^{+}$ channel blocking activity of sulfur-containing indole alkaloids from *Glycosmis petelotii*. *Journal of Natural Products*, *77*, 1586–1593.

du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3–14.

European Commission. (2023). Farm to fork strategy [Internet document]. European Commission. https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en

Fačkovcová, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., & Loppi, S. (2020a). Uptake of trace elements in the water fern *Azolla filiculoides* after short-term application of chestnut wood distillate (pyroligneous acid). *Plants*, *9*, 1179.

Fačkovcová, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., & Loppi, S. (2020b). Effects of wood distillate (pyroligneous acid) on sensitive bioindicators (lichen and moss). *Ecotoxicology and Environmental Safety*, *204*, 111117.

Fanfarillo, E., Fedeli, R., Fiaschi, T., de Simone, L., Vannini, A., Angiolini, C., Loppi, S., & Maccherini, S. (2022). Effects of wood distillate on seedling emergence and first-stage growth in five threatened arable plants. *Diversity*, *14*, 669.

Fedeli, R., Alexandrov, D., Celletti, S., Nafikova, E., & Loppi, S. (2022). Biochar improves the performance of *Avena sativa* L. grown in gasoline-polluted soils. *Environmental Science and Pollution Research*, *30*, 28791–28802.

Fedeli, R., Vannini, A., Celletti, S., Maresca, V., Munzi, S., Cruz, C., Alexandrov, D., Guarnieri, M., & Loppi, S. (2023). Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea. *Annals of Applied Biology*, *182*, 57–64.

Fedeli, R., Vannini, A., Grattacaso, M., & Loppi, S. (2023). Wood distillate (pyroligneous acid) boosts nutritional traits of potato tubers. *Annals of Applied Biology*, *183*, 135–140.

Fedeli, R., Vannini, A., Guarnieri, M., Monaci, F., & Loppi, S. (2022). Bio-based solutions for agriculture: Foliar application of wood distillate alone and in combination with other plant-derived corroborants results in different effects on lettuce (*Lactuca sativa* L.). *Biology*, *11*, 404.

Frattaruolo, L., Carullo, G., Brindisi, M., Mazzotta, S., Bellissimo, L., Rago, V., Curcio, R., Dolce, V., Aiello, F., & Cappello, A. R. (2019). Antioxidant and anti-inflammatory activities of flavanones from *Glycyrrhiza glabra* L. (licorice) leaf phytocomplexes: Identification of licoflavanone as a modulator of NF-kB/MAPK pathway. *Antioxidants (Basel, Switzerland)*, *8*, 186.

Fusi, F., Marazova, K., Pessina, F., Gorelli, B., Valoti, M., Frosini, M., & Sgaragli, G. (2000). On the mechanisms of the antispasmodic action of some hindered phenols in rat aorta rings. *European Journal of Pharmacology*, *394*, 109–115.

Grewal, A., Abbey, L., & Gunupuru, L. R. (2018). Production, prospects and potential application of pyroligneous acid in agriculture. *Journal of Analytical and Applied Pyrolysis*, *135*, 152–159.

Gurney, A. M. (1994). Mechanisms of drug-induced vasodilation. *Journal of Pharmacy and Pharmacology*, *46*, 242–251.

Henríquez, C., Almonacid, S., Chiffelle, I., Valenzuela, T., Araya, M., Cabezas, L., Simpson, R., & Speisky, H. (2010). Determinación de la Capacidad Antioxidante, contenido de Fenoles totales y Composición Mineral de Diferentes Tejidos de Frutos de Cinco Variedades de Manzana cultivadas en Chile. *Chilean Journal of Agricultural Research*, *70*, 523–536.

Jones, R. A., & Scott, S. J. (1983). Improvement of tomato flavor by genetically increasing sugar and acid contents. *Euphytica*, *32*, 845–855.

Kårlund, A., Salminen, J. P., Koskinen, P., Ahern, J. R., Karonen, M., Tiilikkala, K., & Karjalainen, R. O. (2014). Polyphenols in strawberry (*Fragaria × ananassa*) leaves induced by plant activators. *Journal of Agricultural and Food Chemistry*, *62*, 4592–4600.

Khalili, M., Alavi, M., Esmaeil-Jamaat, E., Baluchnejadmojarad, T., & Roghani, M. (2018). Trigonelline mitigates lipopolysaccharide-induced learning and memory impairment in the rat due to its anti-oxidative and anti-inflammatory effect. *International Immunopharmacology*, *61*, 355–362.

Le Gall, G., Colquhoun, I. J., Davis, A. L., Collins, G. J., & Verhoeyen, M. E. (2003). Metabolite profiling of tomato (*Lycopersicon esculentum*) using 1H NMR spectroscopy as a tool to detect potential unintended effects following a genetic modification. *Journal of Agricultural and Food Chemistry*, *51*, 2447–2456.

Li, N., Wu, X., Zhuang, W., Xia, L., Chen, Y., Wu, C., Rao, Z., Du, L., Zhao, R., Yi, M., Wan, Q., & Zhou, Y. (2021). Tomato and lycopene and multiple health outcomes: Umbrella review. *Food Chemistry*, *343*, 128396.

Loppi, S., Fedeli, R., Canali, G., Guarnieri, M., Biagiotti, S., & Vannini, A. (2021). Comparison of the mineral and nutraceutical profiles of elephant garlic (*Allium ampeloprasum* L.) grown in organic and conventional fields of valdichiana, a traditional cultivation area of Tuscany, Italy. *Biology*, *10*, 1058.

Masetti, O., Ciampa, A., Nisini, L., Sequi, P., & Dell'Abate, M. T. (2017). A multifactorial approach in characterizing geographical origin of Sicilian cherry tomatoes using 1H-NMR profiling. *Food Research International*, *100*, 623–630.

Mazzotta, S., Frattaruolo, L., Brindisi, M., Ulivieri, C., Vanni, F., Brizzi, A., Carullo, G., Cappello, A. R., & Aiello, F. (2019). 3-Amino-alkylated indoles: Unexplored green products acting as anti-inflammatory agents. *Future Medicinal Chemistry*, *12*, 5–17.

Minoggio, M., Bramati, L., Simonetti, P., Gardana, C., Iemoli, L., Santangelo, E., Mauri, P. L., Spigno, P., Soressi, G. P., & Pietta, P. G. (2003). Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Annals of Nutrition and Metabolism*, *47*, 64–69.

Mirsoleimani, A., Najafi-Ghiri, M., Boostani, H. R., & Farrokhzadeh, S. (2022). Relationships between soil and plant nutrients of citrus rootstocks as influenced by potassium and wood vinegar application. *Journal of Soils and Sediments*, *23*, 1439–1450.

Mu, J., Yu, Z., Wu, W., & Wu, Q. (2006). Preliminary study of application effect of bamboo vinegar on vegetable growth. *Forestry Studies in China*, *8*, 43–47.

Muhie, S. H. (2022). Novel approaches and practices to sustainable agriculture. *Journal of Agriculture and Food Research*, *10*, 100446.

Ofoe, R., Qin, D., Gunupuru, L. R., Thomas, R. H., & Abbey, L. (2022). Effect of pyroligneous acid on the productivity and nutritional quality of greenhouse tomato. *Plants*, *11*, 1650.

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Olives Barba, A. I., Cámara Hurtado, M., Sánchez Mata, M. C., Fernández Ruiz, V., & López Sáenz De Tejada, M. (2006). Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry*, *95*, 328–336.

Otify, A. M., Ibrahim, R. M., Abib, B., Laub, A., Wessjohann, L. A., Jiang, Y., & Farag, M. A. (2023). Unveiling metabolome heterogeneity and new chemicals in 7 tomato varieties via multiplex approach of UHPLC-MS/MS, GC–MS, and UV–Vis in relation to antioxidant effects as analyzed using molecular networking and chemometrics. *Food Chemistry*, *417*, 135866.

Rose, H., Benzon, L., & Lee, S. C. (2016). Potential of wood vinegar in enhancing fruit yield and antioxidant capacity in tomato. *Korean Journal of Plant Resources*, *29*, 704–711.

Sobolev, A. P., Segre, A., & Lamanna, R. (2003). Proton high-field NMR study of tomato juice. *Magnetic Resonance in Chemistry*, *41*, 237–245.

Theapparat, Y., Chandumpai, A., Leelasuphakul, W., Laemsak, N., & Ponglimanont, C. (2014). Physicochemical characteristics of wood vinegars from carbonization of *Leucaena leucocephala*, *Azadirachta indica*, *Eucalyptus camaldulensis*, *Hevea brasiliensis* and *Dendrocalamus asper*. *Agriculture and Natural Resources*, *48*, 916–928.

Tomassini, A., Sciubba, F., Cocco, M. E. D., Capuani, G., Delfini, M., Aureli, W., & Miccheli, A. (2016). 1H NMR-based metabolomics reveals a pedoclimatic metabolic imprinting in ready-to-drink carrot juices. *Journal of Agricultural and Food Chemistry*, *64*, 5284–5291.

Tundis, R., Frattaruolo, L., Carullo, G., Armentano, B., Badolato, M., Loizzo, M. R., Aiello, F., & Cappello, A. R. (2018). An ancient remedial repurposing: Synthesis of new pinocembrin fatty acid acyl derivatives as potential antimicrobial/anti-inflammatory agents. *Natural Product Research*, *33*, 162–168. https://doi.org/10.1080/14786419.2018.1440224

Wei, Q., Ma, X., & Dong, J. (2010). Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut tree branches. *Journal of Analytical and Applied Pyrolysis*, *87*, 24–28.

Xia, H., Liu, C., Li, C. C., Fu, M., Takahashi, S., Hu, K. Q., Aizawa, K., Hiroyuki, S., Wu, G., Zhao, L., & Wang, X. D. (2018). Dietary tomato powder inhibits high-fat diet-promoted hepatocellular carcinoma with alteration of gut microbiota in mice lacking carotenoid cleavage enzymes. *Cancer Prevention Research*, *11*, 797–810.

Zeng, W., Jin, L., Zhang, F., Zhang, C., & Liang, W. (2018). Naringenin as a potential immunomodulator in therapeutics. *Pharmacological Research*, *135*, 122–126.

Zhu, K., Gu, S., Liu, J., Luo, T., Khan, Z., Zhang, K., & Hu, L. (2021). Wood vinegar as a complex growth regulator promotes the growth, yield, and quality of rapeseed. *Agronomy*, *11*, 510.

Zulkarami, B., Ashrafuzzaman, M., Husni, M. O., & Ismail, M. R. (2011). Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. *Australian Journal of Crop Science*, *5*, 1508–1514.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Fedeli, R., Marotta, L., Frattaruolo, L., Panti, A., Carullo, G., Fusi, F., Saponara, S., Gemma, S., Butini, S., Cappello, A. R., Vannini, A., Campiani, G., & Loppi, S. (2023). Nutritionally enriched tomatoes (*Solanum lycopersicum* L.) grown with wood distillate: chemical and biological characterization for quality assessment. *Journal of Food Science*, 1–15. https://doi.org/10.1111/1750-3841.16829

17503841, 0, Downloaded from https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.16829 by University Of Siena Sist Bibliot Di Ateneo, Wiley Online Library on [14/11/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License