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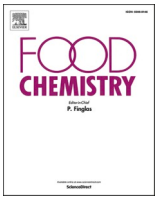

Tomato pomace food waste from different variants as a high antioxidant potential resource

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ABSTRACT

Pomaces obtained from three San Marzano tomato genotypes including the wild type (WT), *Sun Black* (SB), and *colorless fruit epidermis* (CL) were dried at 50 °C and analyzed for nutritional composition, total polyphenol (TPC), flavonoid (TFC) content, polyphenol qualitative profile, total antioxidant capacity (TAC), and antimicrobial activity. Commercial dried tomato powder (CTRP) was included as a control. No differences were detected nutritionally, in TPC and antimicrobial activity, but significant changes were observed for TFC and TAC, underlying variation in the phenolic profile. SB pomace (SBP) had the highest TFC and TAC. LC-HRMS analysis showed a flavonoid-enriched profile in SBP besides the exclusive presence of anthocyanins, with petanin and negretein as the most abundant. Among flavonoids, quercetin-hexose-deoxyhexose-pentose, naringenin, and rutin were the major. Overall, we showed the potential of dried tomato pomace, especially SBP, as an extremely valuable waste product to be transformed into a functional ingredient, reducing the food industry waste.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated vegetables in the world, with a worldwide production of over 189 million tons in 2021, of which about 18 million tons were produced in the European Union (EU) (FAOSTAT, 2021). According to EUROSTAT, Italy is the EU's largest tomato producer with 6.64 million tons produced in 2021 (i.e., 36.7% of the EU total production) (European Commission - DG AGRI E2, 2022). Tomato fruit is a key component of the Mediterranean diet which can be consumed as a fresh or processed product. Major processed tomato products include paste, sauces, puree, ketchup, or canned tomato. More than half of the total tomato production is estimated to be processed generating globally million tons of by-products including pomace (i.e., a mix of seeds and peels), seeds, vascular tissues, and peels. These byproducts still contain several healthy molecules, and they could be revalorized by adopting them as functional ingredients in a way that perfectly matches the circular economy concept. Previous studies reported that tomato peel is among the major food sources of carotenoids, mainly lycopene, and ß-carotene, which level can reach up to five-fold the concentration found in tomato flesh (Szabo et al., 2018), especially in the ripened tomato. In fact, during the ripening stage, various physiological reactions are involved including the synthesis and storage of flavonoids, mainly naringenin chalcone, rutin, and kaempferol-3-rutinoside, as well as carotenoids. These phytochemicals accumulate predominantly in the tomato peel, as their biosynthetic pathway is not active in the fruit flesh (Ballester et al., 2009), and here, they act as compounds to protect against biotic and abiotic stresses. Thus, several studies attempted to incorporate tomato peel and pomace waste as functional ingredients to produce value-added and nutritionally enriched foods including pasta, bread, sausages, and cookies with improved carotenoid content, dietary fiber and minerals levels, and antioxidant activity (Szabo et al., 2018).

Although tomato is stated as a good source of antioxidants in the human diet and defined as the greatest source of carotenoids as well as food containing a medium level of flavonoids (Borguini et al., 2009), it lacks anthocyanins (structurally anthocyanidins glycosides) a class of flavonoids responsible for the red, purple, and blue color of vegetables and fruits. The dietary consumption of anthocyanins has been associated

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with a broad range of health-promoting effects, including antioxidant and antimicrobial activities, benefits on neurological health, and protection against various non-communicable diseases (Khoo et al., 2017). Considering its worldwide spread consumption, efforts to anthocyanin enrichment of tomatoes were performed. Strategies based on non-transgenic conventional breeding approaches were adopted to introduce into cultivated tomato genetic background a combination of alleles affecting the chlorophylls, carotenoids, flavonoids, and polyphenols metabolic pathways. As a result, a set of tomato variants producing fruits with different colors due to the accumulation of specific classes of compounds was obtained. Among these, the purple tomato line called *Sun Black* (SB) and the *colorless fruit epidermis* (CL) mutant line can be mentioned. The first one is characterized by the combination of two alleles (*Aft* from the wild tomato species *S. cheesmaniae*, and *atv* allele from cultivated tomato) leading to the production of fruits with a purple skin color, due to the biosynthesis and accumulation of anthocyanins. The purple pigmentation is limited to the peel and the greater the sunlight exposure, the greater the purple intensity, while the flesh still preserves a red color (Blando et al., 2019; Mazzucato et al., 2013). The CL genotype derives from the monogenic, recessive mutant allele *y* causing a down-regulation of the flavonoid pathway, resulting in the lack of naringenin chalcone accumulation in the peel that hence, appears transparent, giving a pink color to the fruit (Ballester et al., 2009).

According to their organoleptic and physical-chemical properties, tomato varieties are adopted for fresh or processing consumption. San Marzano (SM) is one of the most popular Italian tomato landraces marked as EU Protected Denomination of Origin, particularly suitable for processing and production of tomato sauce. Considering the commercial importance of SM tomato type not only for the Italian food industry but for the global one, several breeding programs and genetic studies were performed on this tomato genotype leading to the selection of a repertoire of tomato fruit variants in the traditional SM background, phenotypically differing for the color of the fruits, including the SB and CL lines (Dono et al., 2020). As previously stated, the flesh of these latter mutant lines is not affected likewise the quality of their processing products. However, industrial processing leads to the loss of functional compounds present in the peel. Hence, alternative use of the SB tomato pomace or peel could enhance the transformation and use of this functional variety and appear promising and affordable for the tomato processing industry helping to reduce costs and environmental concerns. The alternative use of the tomato pomace/peel perfectly fits into the "Farm to Fork" (F2F) European Commission strategy, which aims to halve food waste by 2030.

An additional purpose of the F2F strategy is the chemical pesticides reduction by 50% and by 2030, encouraging the use of biopesticides which include several natural plant products such as terpenes, polyphenols and flavonoids, alkaloids, cyanogenic glucosides with antifeedant, attractant, nematicide, fungicide, repellent, insecticide, and insect growth regulator bioactivity (Souto et al., 2021). In this context, previous works highlighted an improved shelf-life of traditional breeding or genetic engineering anthocyanin-enriched tomatoes, due to the anthocyanin accumulation which reduced susceptibility to the fungal *Botrytis cinerea*, one of the most important postharvest pathogens (Bassolino et al., 2013).

Hence, in this work, three SM genotypes including the wild type (WT), SB, and CL were processed by an in-house method simulating the industrial tomato sauce production. The obtained waste pomace was dried at a relatively low temperature (50 °C) to get a storable product. Experimental samples WT, SB, and CL were compared to commercial dried whole tomato powder (named control powder, CTRP) used as an external control, to understand the potential nutritional and antioxidant advantages of these experimental matrices as ingredients compared to the conventional one. Proximate and phytochemical composition, as well as antioxidant activity, were assessed to evaluate the potential functional features of each sample after the treatment. Additionally, the possible function as biopesticides against *Pseudomonas syringae* and *Fusarium graminearum* was also tested.

2. Material and methods

2.1. Plant materials and growth

WT SM tomato genotype and the two genetic variants (SB and CL) were used. For each line, 22 plants were grown at the Experimental Farm of the University of Tuscia at Viterbo, Lazio, Italy (42°25′07″ N, 12°06′34″ E). Plants were arranged in twin rows and grown with standard agronomic practices for fresh market tomatoes. All lines were grown on tutors with weekly removal of lateral shoots.

2.2. Fruit collection and peel preparation

At full ripening, the fruits produced by each line were collected, counted, weighted, and processed to produce tomato pomace. For all the varieties the fruits were considered completely ripe when at least 90% of the fruit surface had the classic mature red color in agreement with Cantwell (2010). For the SB genotype, the developmental stage was gathered by inspecting the blossom end which usually lacks anthocyanin accumulation, according to Blando et al. (2019). The harvesting was performed at four timings (8th, 16th, 29th of August and 6th of September 2022). To produce dried tomato pomace, fruits were washed and dissected, and the pomace was separated from the pulp by using a domestic extractor. The pulp was discarded, and the pomace was dried in a static oven at 50 °C, overnight. Dried pomaces were immediately collected, weighted, and ground by the IKA® A11 basic Analytical mill. The tomato pomace powders (WTP, wild-type powder; CLP, colorless powder; SBP, Sun Black powder) were stored at −20 °C. CTRP (Erbologica Amazonas Andes) was purchased at the local market.

2.3. Proximate composition

Proximate composition was determined on two biological replicates for each line, according to standard procedures of AOAC International (Association of Official Analysis Chemists International (AOAC, 2006). Specifically, crude protein content (conversion factor, 6.25) was estimated using the Kjeldahl method (AOAC 2001.11) (SpeedDigester K-425 and Distillation Unit K-350, BÜCHI, Labortechnik, AG, Switzerland). Crude fat (AOAC 920.39) was extracted using a Soxhlet Extraction System B-811 (BÜCHI, Labortechnik, AG, Switzerland) with petroleum ether as solvent. Ash was determined in a muffle furnace at 550 °C for 4 h (AOAC 923.03). Total carbohydrates were determined by difference (i.e., 100 – (g [protein + fat + ash] in 100 g of dry weight (DW) sample). The energy value was calculated on DW, using the Atwater factor, as follows:

Energy value (Kcal) = (%Protein×4) + (%Fat×9) + (% Carbohydrate×4).

2.4. Extracts' preparation for polyphenol compounds and antioxidant activity determination

For the analyses of the TPC, TFC, and TAC, three biological and two technical replicates were processed and analyzed. Samples were extracted according to Costantini et al. (2014). Briefly, experimental powders were extracted overnight in the dark with 80% aqueous methanol (1:25, *w/v*). Then, the samples were centrifuged at 5000 ×*g* (ALC PK121R centrifuge; Bodanchimica s.r.l., Cagliari, Italy) for 10 min at 4 °C. The supernatants were collected and stored at −80 °C until further processing.

2.5. Total phenolic compound content (TPC)

The TPC was determined using the Folin-Ciocalteu standard method as modified by (Costantini et al., 2014) and adapted for 96-well plates

and an automatic reader. Briefly, 30 μL of deionized water was added to 10 μL of methanolic extract, 10 μL of Folin-Ciocalteau reagent, and 200 μL of 30% Na_2CO_3. After 30 min at room temperature (RT), the absorbance of the mixture was measured at 725 nm in the automatic reader (Infinite 2000, Tecan, Salzburg, Austria). A gallic acid standard curve was prepared and the results were expressed as mg of gallic acid equivalents (GAE)/g of DW of the sample.

2.6. Total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method described by (Qin et al., 2010) and adapted for 96-well plates and an automatic reader. Briefly, the appropriate dilution of extracts (50 μL) was mixed with 130 μL of 95% ethanol, 10 μL of 10% aluminum chloride hexahydrate ($AlCl_3$), 10 μL of 1 M potassium acetate (CH_3COOK). After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm in the automatic reader (Infinite 2000, Tecan, Salzburg, Austria). The total flavonoid contents were expressed as mg rutin equivalents (RE)/g of DW of the sample.

2.7. Flavonoid and anthocyanin profile determination by LC-HRMS analysis

Phenolic compounds were extracted from 15 mg of tomato pomace powders using 0.75 mL 75% methanol (*v/v*) + 0.1% formic acid spiked with 0.5 mg/L formononetin as internal standard (IS), as previously described (Dono et al., 2022). Three biological and two technical replicates were processed and analyzed. Samples were centrifuged at 20,000 ×*g* at RT, and the supernatant was filtered to PTFE-Filter tubes (0.22 μm pore size) for the Liquid Chromatography-High Resolution Mass Spectrometry (LC–PDA–HRMS) analysis, using a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). LC separation was carried out using a C18 Luna reverse-phase column (100 × 2.1 mm, 2.5 μm; Phenomenex, Macclesfield, UK) and an elution system consisting of (A) water (0.1% formic acid, *v/v*) and (B) acetonitrile (0.1% formic acid, *v/v*). LC parameters were as previously described (Demurtas et al., 2023) and ESI-HRMS parameters were set as described in Diretto et al. (2017). Metabolite identification was performed by comparing chromatographic and MS spectra with authentic standard if available, and reference spectra (Dono et al., 2022; Sulli et al., 2021). Identified metabolites were categorized according to their respective class. Relative levels of accumulation of investigated metabolites were measured and normalized relative to the internal standard, thus expressed as Fold-IS. All chemicals and solvents were LC-MS grade quality (CHROMASOLV®, Merck).

2.8. Total antioxidant capacity (TAC) determination

The total antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP), 2,2′ -azino-bis (3-ethyl- benzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging activity assays, and Cupric Reducing Antioxidant Capacity (CUPRAC), as follows. FRAP assay was performed using the method described by Costantini et al. (2014). Briefly, 160 μL of FRAP assay solution (20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer, pH 3.6) was prepared daily, mixed with 10 μL of the sample, standard, or blank, and dispensed into each well of a 96-well plate. The absorbance was measured at 595 nm at 37 °C in an automatic reader (Infinite 2000, Tecan, Salzburg, Austria), after 30 min of incubation. The results were expressed as mmol Fe^{2+} equivalents/g of DW of the sample.

The $ABTS^{\bullet+}$ radical scavenging activity was evaluated by the OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit (ABTS) (Cell Biolabs INC.) following the manufacturer's instructions. The absorbance was recorded at 405 nm in an automatic reader (Infinite 2000, Tecan, Salzburg, Austria). A standard curve for Trolox was prepared and the antioxidant capacity was expressed as μmol of Trolox equivalents (TE)/g of DW. The CUPRAC assay was performed according to the method described by Farinon et al. (2022) adapted for 96-well plates and an automatic reader (Infinite 2000, Tecan, Salzburg, Austria).

2.9. Biopesticides activity evaluation

Antimicrobial activity against the bacterium *P. syringae* and the fungus *F. graminearum* strain 3827 were tested on two biological and three technical replicates, using agar diffusion assay according to (Valgas et al., 2007). The bacterial strain was cultured on LB media (Luria-Bertani agar) and fungus on Potato Dextrose media (PDA) at 25 °C.

Methanol extracts of tomato powder were evaporated to dryness in a rotary evaporator system (BUCHI, Switzerland) at 35 °C and 122 mbar. The dried extracts were suspended in DMSO 20%, to obtain final concentrations of 50 mg/mL, sterilized via filtration through a 0.22 μm membrane filter, and stored at −20 °C. For the antimicrobial assays, the dried extracts were diluted in sterile condition to a concentration of 1 mg/mL and 2 mg/mL. Streptomycin sulfate and cycloheximide were used as positive controls for bacteria and for eukaryotic microbial strains, respectively, at a concentration of 1 mg/mL; a solution of DMSO/H_2O (0.8%) was used as a negative control.

24 h-old bacterial inoculum was uniformly spread all over the surface of LB sterile agar Petri dishes using a sterile cotton swab. Mycelium plugs (Ø 8 mm) of 5-day-old *F. graminearum* were placed in the center of the plates containing PDA. After the inoculation of the test microorganism, wells (Ø 6 mm) were created under sterile conditions in the agar to dispense extracts and positive and negative controls (20 μL/well). Plates were incubated at 25 °C for 24 h for bacterium and 5 days for fungus.

2.10. Statistical analysis

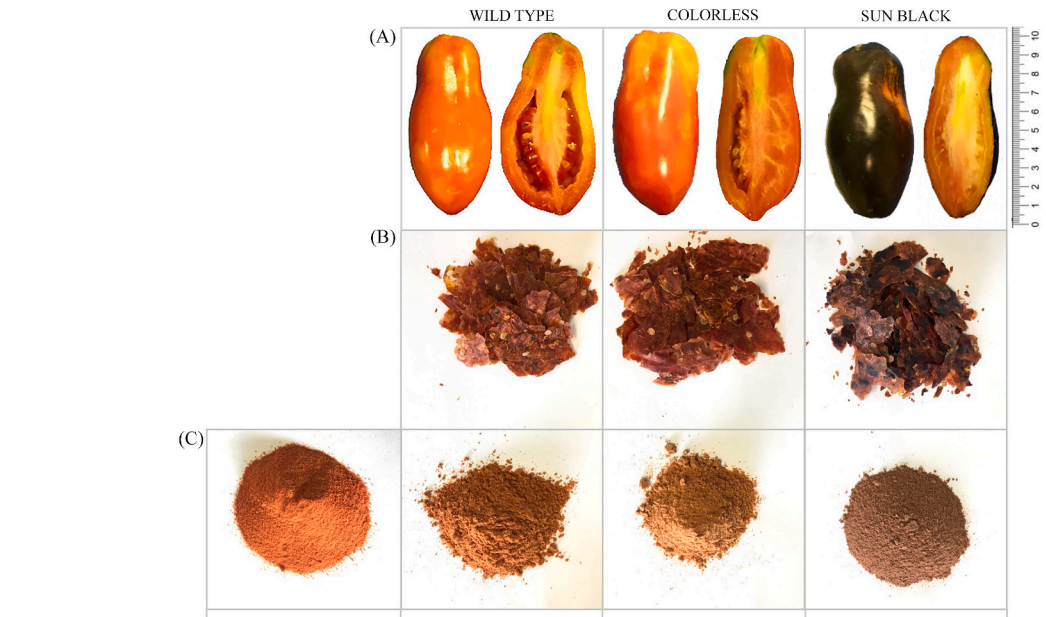
As for proximate composition and spectrophotometric assays, analysis of variance (ANOVA) was performed with the XLSTAT 2023.1.1 (Addinsoft SARL, New York, USA) software, and Tukey's post hoc test was used to describe statistical differences between means. As regards the LC-HRMS analysis, the complete dataset including all replicates was considered to perform a principal component analysis (PCA) carried out by using the factoextra package implemented in R software (version 4.1.1) (https://github.com/kassambara/factoextra/issues, February 13, 2023). Then, ANOVA followed by Tukey's post hoc test was applied using SPSS software (version 23) to assess significant differences among samples in the level of the identified compounds. For all analyses, differences were considered significant at the $p \leq 0.05$ level.

3. Results and discussion

3.1. Fruit, pomace, and powder appearance and yield

The fruits of the analyzed genotypes were collected during four harvests performed in August and September 2022. Phenotypically, for all the samples the SM typical traits were clearly evident (Fig. 1A) (Dono et al., 2020). Although the flesh was red for all three genotypes, the external fruit color differed according to the mutations. When compared to the SM WT, differences were particularly evident for the SB line, and less perceptible for the CL genotype, even if the pink-like color is visible, especially at the stylar pole (Fig. 1A). The same fruit color variation persisted in the pomace and powder (Fig. 1B, C), suggesting that a different phenolic profile exists among the samples.

With regards to fruit production, among the genotypes, SB resulted as the most productive with a total of 7463 g of collected fruits and an average of 166 harvested fruits, whereas WT was the least productive (1883 g and 37 harvested fruits on average) (Table S1). The whole amount of fresh pomace produced per genotype ranged from 403 g in the WT line to 1108 g in the SB line, delivering up to 21% of waste.



(D)

	CTRP	WTP	CLP	SBP
Protein[1]	15.56 ± 0.19[a]	13.41 ± 0.04[b]	13.96 ± 0.62[b]	13.08 ± 0.32[b]
Fat	0.03 ± 0.01[c]	0.83 ± 0.23[ab]	1.11 ± 0.22[a]	0.62 ± 0.06[b]
Carbohydrate[2]	74.13 ± 0.69[c]	79.76 ± 0.24[ab]	77.87 ± 0.70[b]	80.17 ± 0.06[a]
Ash	10.28 ± 0.48[a]	6.01± 0.03[c]	7.05 ± 014[b]	6.12 ± 0.20[c]
KCal/100 g	358.58 ± 1.86[b]	380.12 ± 1.25[a]	377.36 ± 1.67[a]	378.64 ± 0.48[a]
kJ/100 g	1502.05 ± 7.77[b]	1590.41 ± 5.25[a]	1578.86 ± 6.96[a]	1584.22 ± 1.99[a]

[1] Conversion factor: 6.25
[2] As difference (*i.e.*, 100 – (g [protein + fat + ash] in 100 g of dry weight sample)

Fig. 1. Fruits, dried pomaces, powders, and proximate compositions of the three tomato lines used in this study. (A) Wild type, colorless, and Sun Black San Marzano whole fruits and longitudinal sections at the red ripe stage. The scale bar (10 cm) is reported on the right. (B) Wild type, colorless, and Sun Black San Marzano pomaces after extraction and drying at 50 °C. (C) Commercial dried whole tomato powder (control) and tomato powders are produced by grinding the dried pomaces of wild type, colorless, and Sun Black San Marzano lines. (D) Proximate composition (g/100 g DW) and energy value (Kcal/100 g and kJ/100 g DW) of control powder (CTRP), wild type powder (WTP), colorless powder (CLP), and Sun Black powder (SBP). Data represents mean ± standard deviation of $n = 2$ biological replicates. Different letters indicate significant differences ($p \leq 0.05$), according to one-way analysis of variance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Considering the weight of the whole harvested fruits and the fresh pomace weight, a lower trend in the pomace yield was observed in the mutant CL and SB lines (16.8% and 14.8%, respectively) compared to the WT (21.3%). Anyway, the pomace yield after drying was similar among the analyzed tomato lines (Table S1).

3.2. Proximate composition

The proximate composition of CTRP, WTP, CLP, and SBP was determined, and the data are reported in Fig. 1D. All these products showed a predominance of carbohydrates followed by proteins, ash, and fat. All the experimental samples showed statistically comparable levels of proteins (between 13.08 and 13.96 g/100 g DW), while the commercial sample CTRP, showed the highest amount (15.56 g/100 g DW), although the values were numerically close to each other. Instead, more distant values between the control CTRP and the experimental matrices were found for the ash and the total lipids. The explanation for the commercial CTRP's higher ash level is due to the presence of silicon dioxide food additive, as an anti-caking agent. Similarly, the CTRP's lowest fat level might be due to specific technological processes and/or to silicon dioxide presence. Anyway, the observed results for the experimental tomato pomace powders are in accordance with the literature data (Azabou et al., 2020).

3.3. Total phenolic and flavonoid content

The TPC and TFC were assessed for all the samples and the control powder (Fig. 2A, B). The TPC level ranged from 10.38 ± 0.22 mg GAE/g DW in the CTRP to 11.27 ± 1.69 mg GAE/g DW in the SBP (Fig. 2A). Although SBP showed a slightly higher value (+5%) in comparison to the other samples, not statistically differences were observed among the analyzed specimens. Blando et al. (2019) reported a mean value of 5.8 mg GAE/g DW in the SB whole tomato fruit at the ripening stage. In the present work, the SB tomato pomace instead of the whole fruit was for the first time analyzed and the greater concentration of polyphenols in the tomato peel than the flesh explains the higher TPC value we found. Literature data concerning the TPC in tomato pomace are rather conflicting (Abbasi-Parizad et al., 2021; Aksoylu Özbek et al., 2020; Bao et al., 2020; Perea-Domínguez et al., 2018; Plaza et al., 2023; Vorobyova et al., 2022). Differences in the TPC could be related to genetic variability (e.g., the tomato genotype from which the pomace is generated) as well as environmental conditions, including soil mineral composition, agricultural management, biotic and abiotic stresses that occurred during the plant growth (Heimler et al., 2017). Moreover, processing conditions for waste production, as well as the extraction solvent can further affect the level of polyphenols, by improving their extraction or preserving them from oxidative degradation (Silva et al., 2019; Vorobyova et al., 2022).

For TFC, the lowest flavonoid level was found in the CLP sample,

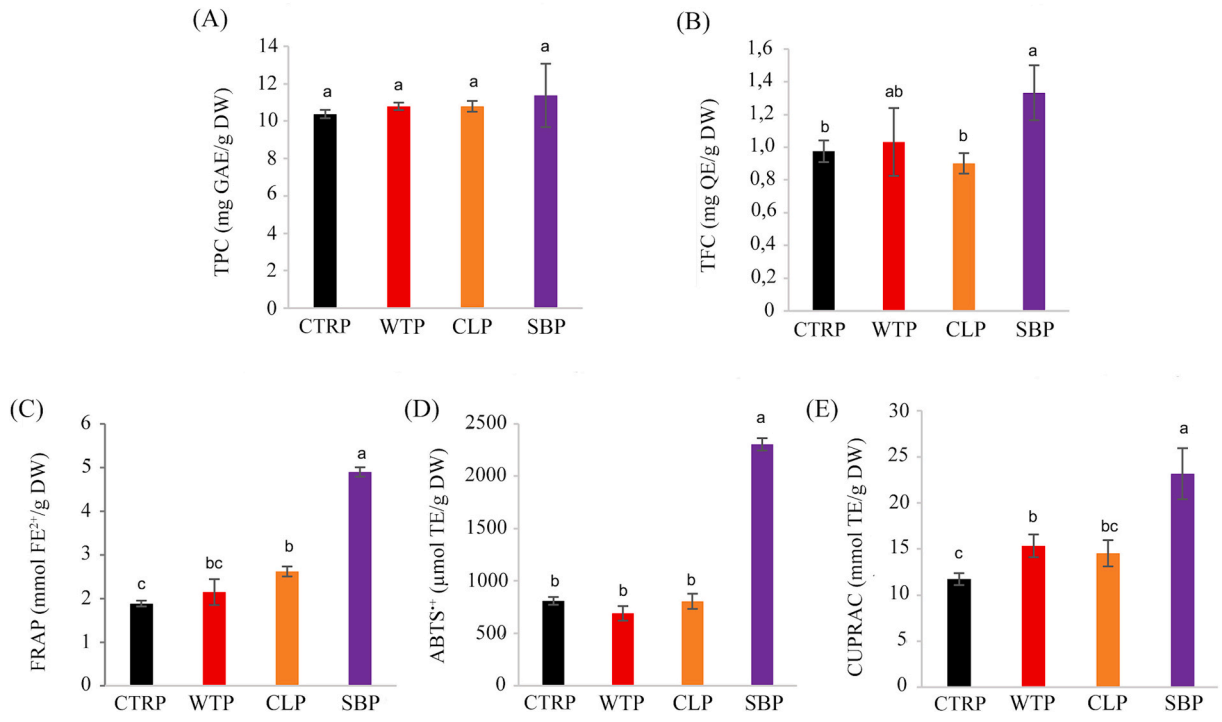


Fig. 2. Antioxidant profiles of commercial dried whole tomato powder (control) and tomato powder produced by pomaces of wild type, colorless, and Sun Black San Marzano lines. (A) Total phenolic content (TPC) (mg GAE/g DW); (B) Total flavonoid content (TFC) (mg QE/g DW); (C) Ferric Reducing Antioxidant Power Assay (FRAP) (mmol Fe^{2+}/g DW); (D) $ABTS^{\bullet+}$ radical scavenging activity (µmol TE/g DW); (E) Cupric ion reducing antioxidant power (CUPRAC) (µmol TE/g DW). Data represents mean ± standard deviation of $n = 3$ biological replicates and n = 2 technical replicates. Different letters indicate significant differences ($p \leq 0.05$), according to one-way analysis of variance. CTRP: Control Powder; WTP: Wild Type Powder; CLP: Colorless Powder; SBP: Sun Black Powder; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; TE: Trolox Equivalent; DW: dry weight.

whereas SBP was the one with the highest value (Fig. 2B). The SBP TFC amount resulted in 16.5%, 23.3%, and 36.5% significantly higher than WTP, CTR, and CLP, respectively, indicating an enrichment of flavonoids in this matrix. A similar outcome was reported by Blando et al. (2019) for the whole fruit of a SB line with a genetic background different from SM. The significantly higher TFC value of the SBP agrees with the genetic ability of this genotype to synthesize and accumulate in the peel anthocyanins, compounds belonging to the flavonoid family. On the other hand, the lowest TFC found in the CLP sample is consistent with the lack of the naringenin chalcone accumulation in the skin due to the *y* mutation (Ballester et al., 2009). Saleh et al. (2021) found that drying treatment performed at 45 °C on the peel of different fruits increased both the TPC and TFC values. This was attributed to the inactivation of polyphenols oxidase enzymes by heating, resulting in the inhibition of polyphenols degradation, as well as to the release of bound phenolics leading to an improvement of their bioaccessibility. In our case, the same thermal treatment was performed on the three tomato pomace samples, therefore the differences found for the TFC values are supposed to be ascribed to genetic features rather than to processing, highlighting the higher functionality of SB by-product. This evidence highlights the economic and health benefits of employing this by-product in functional and nutraceutical preparations instead of discarding a matrix still rich in bioactive compounds.

3.4. LC-HRMS analysis of phenolic compounds

Considering the health effects ascribed to phenolic and flavonoid compounds, the polyphenol profile of the three tomato pomaces under study was assessed by LC-HRMS analysis, to better characterize the specific composition of each sample and evaluate any potential functionality of these matrices as a value-added food ingredient.

More in detail, a total of 56 phenolic compounds were identified and grouped into 14 subclasses (Table S2).

Sample separation according to their phenolic content distribution was evaluated by the PCA. The first two dimensions covered 69.8% of the total variance, with the first (PC1) and the second (PC2) dimensions explaining 51.2% and 18.2% of the variance, respectively (Fig. S1A). Among the analyzed compounds, dicaffeoyl-quinic acid (4,5-) (4), p-coumaroyl quinic acid (8), tricaffeoyl quinic acid (9), caffeic acid glucoside iso3 (13), hydrocinnamic acid hexose (17), kaempferol rutinoside (26), quercetin dihexose pentose deoxyhexose (32), and rutin (38) were the main factors discriminating the analyzed samples, each of them accounted for 3.4% of the total variation of the PC1, while quercetin hexose (34) and kaempferol hexose-dehoxyhexose pentose (24) were the main discriminating factors along the second dimension, accounted for 8.5% and 7.4%, respectively of the total variation of the PC2 (Fig. S1B). All these compounds belong to the classes of phenolic acids, quinic acids and derivatives, and flavonoids that have been previously described as phenolic compounds commonly found in the tomato peel and pomace (Aksoylu Özbek et al., 2020; Bao et al., 2020; Perea-Domínguez et al., 2018). The PCA scatter plot clearly showed three groups of samples clustered according to their phenotypes (Fig. 3A). Specifically, SBP specimens localized in the positive dials of the PC1, at high positive values indicating an enrichment in the phenolic content, while CLP samples were placed in the negative dials of the PC1 and PC2, resulting the ones with the lowest phenolic content, compared to both WTP and SBP. Concerning the WTP samples, the PCA showed that they set up a cluster placed in the negative dials of PC1, and in the positive dials of PC2, indicating an intermediate phenolic profile compared to SBP and CLP. The paucity of polyphenols in CLP and their abundance in SBP is also shown by the Venn diagram (Fig. 3B) with 9 out of the 56 identified compounds being detected both in the WTP and SBP, but not in CLP (compounds 19, 23, 25, 28, 29, 41, 46, 47, 48). These compounds are flavonoids related to the naringenin chalcone, as

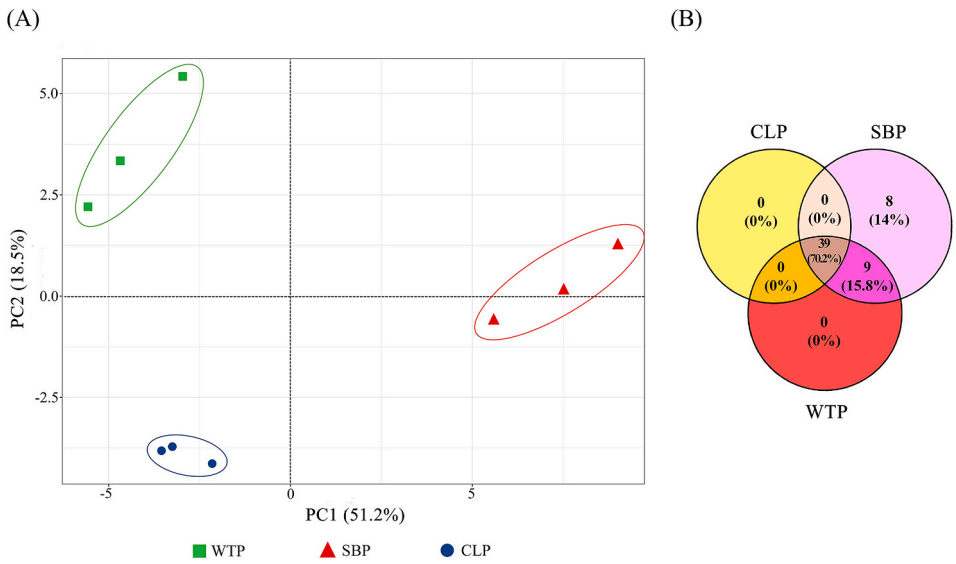


Fig. 3. Principal component analysis (PCA) and Venn diagram of phenolic compounds identified in the tomato pomace powders of wild type, colorless, and Sun Black San Marzano tomatoes by LC-HRMS analysis. (A) Scatter plot of the first (PC1) and the second (PC2) dimensions showing the variability of 59 phenolic compounds identified in WTP, CLP, and SBP according to the LC-HRMS analysis. Analyzed samples (n = 3 biological replicates) are represented by different colored symbols as indicated in the legend. (B) Venn diagram for WTP, CLP, and SBP. Numbers indicate the amount and the relative percentage of analyzed polyphenols shared among the three analyzed samples. WTP: wild-type powder; CLP: colorless powder; SBP: Sun Black powder.

is shown in Fig. S2 reporting the relative amount of compounds identified in the pomace powders. This outcome reflects the genetic inability of the CL tomato variant to synthesize naringenin chalcone, one of the major flavonoids in tomato fruit skin, as well as the precursor of most flavonoids, including kaempferol and quercetin (Fig. 4) (Ballester et al., 2009). By contrast, the relative level of the flavonoid precursor phenylalanine (1) was found significantly higher in CLP, especially than in SBP (+82%) (Figs. S2, 4), most likely as a result of the downregulation of the flavonoid biosynthetic pathway.

An additional eight compounds were exclusively found in SBP (Fig. 3B). They were all encompassed into the anthocyanins subclass (compounds 49–56) (Fig. S2). The same anthocyanins were previously detected by Su et al. (2016) in the transgenic purple tomato, as well as in the peel of cherry SB tomato where petunidin-3-coumaroyl-rutinoside-5-glucoside (54), also commonly called petanin, and malvidin-3-coumaroyl-rutinoside-5-glucoside (51), namely negretein, were identified as the most abundant anthocyanins (Blando et al., 2019), accordingly with our results. Petanin is known as the major anthocyanin of the black goji fruit and was described for its cytoprotective effect due to its strong antioxidant activity, mainly exserted by radical scavenging and up-regulating intracellular antioxidant enzymes (Tang et al., 2017). Negretein was described to have a role in the protection against major cardiovascular risk factors (Juturu, 2014). Interestingly, in the SBP, petanin, and negretein represented 53.8% and 14.2% of the total anthocyanins, respectively, similar to the percentages found by Blando et al. (2019) in the SB cherry tomato freeze-dried peel, indicating that our processing method and heat treatment did not affect the SBP anthocyanins composition.

Among the anthocyanins, only delphinidin (48) was detected in the WTP other than SBP, but not in CLP, and its relative content was significantly higher in the WTP than SBP (12.07 ± 1.16 Fold-IS vs 5.28 ± 1.60 Fold-IS) (Fig. S2). According to the anthocyanin biosynthesis pathway (https://www.kegg.jp/pathway/map00942, online access March 18, 2023), delphinidin is one of the six primary anthocyanidins (Khoo et al., 2017) acting as a link between the flavonoid and anthocyanin biosynthesis pathway (Fig. 4) and serves as precursor for the synthesis of other two primary anthocyanidins: malvidin and petunidin. The higher level of delphinidin in WTP than SBP could be ascribed to a higher metabolization of this anthocyanidin in their derivatives in SB tomato. This assertion would agree with the exclusive presence of delphinidin derivatives (i.e., petunidin and malvidin derivatives) detected in SBP (Fig. 5).

Among flavonoids, rutin (38), naringenin (40) and their derivatives (42, 43), quercetin hexose-deoxyhexose pentose (35), quercetin 3-O-glucosyl-rutinoside (20), quercetin hexose (34), and kaempferol rutinoside (26) were found to be predominant in all the analyzed samples with the SBP containing the highest level, CLP the lowest one, and WTP an intermediate amount (Fig. S2). These flavonoids were already reported as the most abundant flavonoids in tomato pomace and peel (Abbasi-Parizad et al., 2021; Perea-Domínguez et al., 2018; Valdez-Morales et al., 2014). Noteworthy, the levels of naringenin, kaempferol rutinoside, and rutin were markedly higher in SBP in comparison to WTP (1.3-fold, 1.8-fold, 2-fold higher, respectively) and especially, CLP (31-fold, 2.5-fold, 4-fold higher, respectively) (Fig. S2). This feature, together with the presence of anthocyanins only in SBP, confers to this by-product a remarkable functionality, since the wide range of health-related properties of these compounds. Indeed, naringenin was demonstrated to have a protective action against cardiovascular diseases, especially in already compromised patients (Salehi et al., 2019); whereas kaempferol rutinoside was found to exert neuroprotective and anti-inflammatory effect by preventing the decrease of the mitochondrial membrane potential, inhibiting the production of intracellular ROS, and suppressing the expression of inflammatory-related genes through the inhibition of the NF-kB pathway (Hwang et al., 2019); finally, rutin was shown to have antibacterial, anticancer, anti-inflammatory, and cardioprotective activities (Patel & Patel, 2019).

Altogether, the LC-HRMS data demonstrated that SM tomato pomace dried at 50 °C contains several bioactive compounds regardless of the genetic variants. However, when the SB variant is used, the resulting pomace waste appears to have a pronounced abundance of these healthy compounds, making it particularly promising as a functional value-added food ingredient.

3.5. Total antioxidant capacity (TAC)

Considering the valuable level of antioxidant compounds as polyphenols, found in the tomato pomaces, the TAC of WTP, CLP, and SBP was assessed and compared with the CTRP, to understand if the

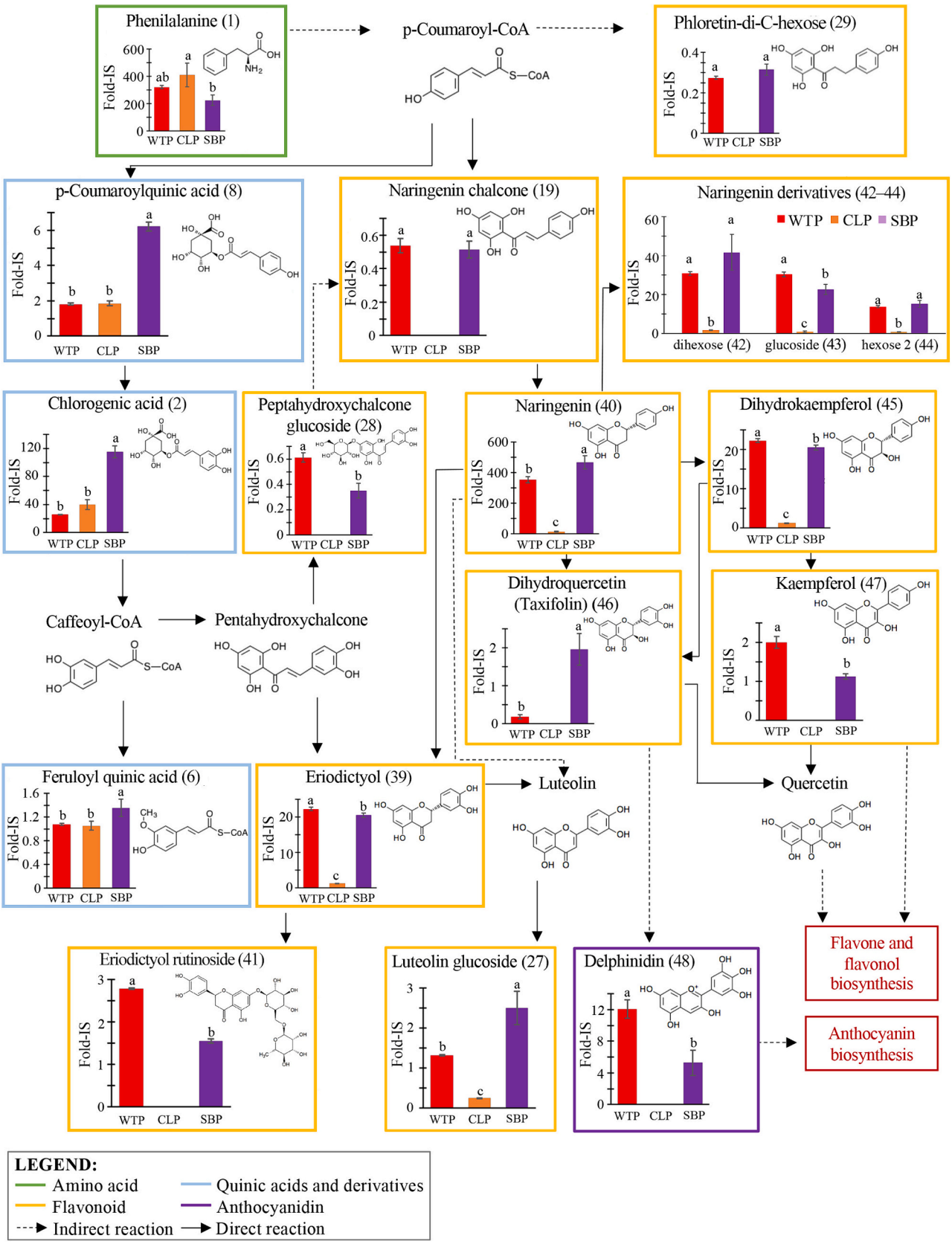


Fig. 4. Schematic representation of the biosynthetic pathway of polyphenols, including phenylalanine, quinic acids and derivatives, and flavonoids. The pathway routes involving quinic acids and derivatives, and flavonoids identified in this study are reported. The detected compounds are surrounded by a frame colored according to their class, as stated in the legend. Levels of each detectable metabolite, expressed as internal standard (formononetin) fold (Fold-IS), are represented in the histograms. Different letters indicate significant differences ($p \leq 0.05$), according to one-way analysis of variance. WTP: wild-type powder; CLP: colorless powder; SBP: Sun Black powder.

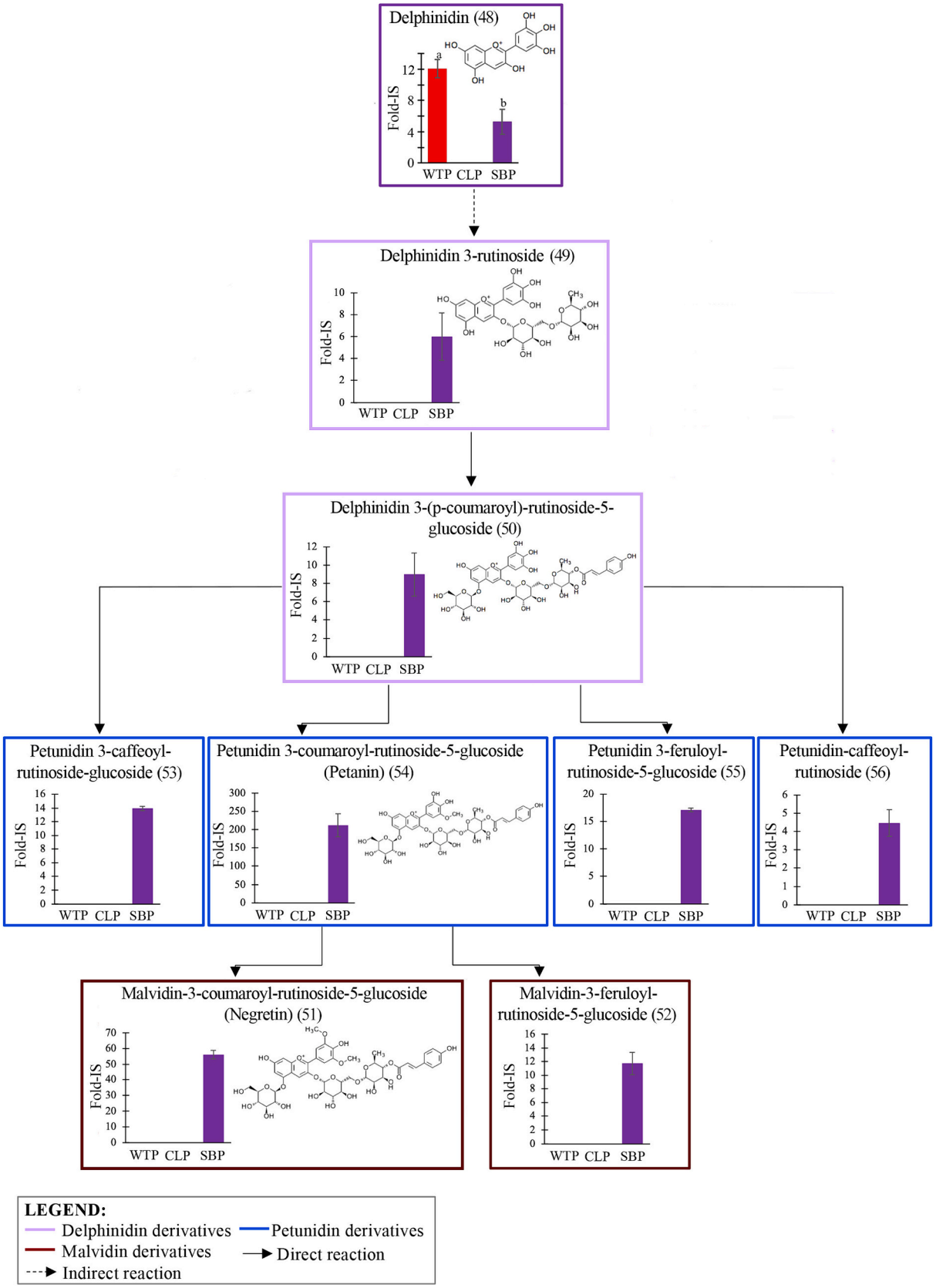


Fig. 5. Schematic representation of the biosynthetic pathway of anthocyanins. The pathway routes involving the anthocyanin compounds identified in this study are reported. The detected identified compounds are surrounded by a frame colored according to their class, as stated in the legend. Levels of each detectable metabolite, expressed as internal standard (formononetin) fold (Fold-IS), are represented in the histograms. Different letters indicate significant differences ($p \leq 0.05$), according to one-way analysis of variance. WTP: wild-type powder; CLP: colorless powder; SBP: Sun Black powder.

quantitative and qualitative detected differences in the phenolic profiles of the analyzed matrices could have significant impact on their antioxidant function. To obtain a better evaluation of the overall antioxidant capacity of the samples, three assays including FRAP, $ABTS^{\bullet+}$, and CUPRAC differing in the chemistry and the kind of reactive species or radicals employed, were adopted.

For all three assays, a similar trend was observed, showing the SBP as the matrix with the significantly higher FRAP, $ABTS^{\bullet+}$, and CUPRAC values, followed by WTP and CLP showing comparable values. The lowest level was observed for the CTRP (Fig. 2C, D, E). Specifically, the $ABTS^{\bullet+}$ antiradical/scavenging activity of SBP was 185% and 233% higher than CTRP and WTP, respectively; the reducing power was 160% and 128% higher than CTRP and WTP, respectively, when measured by the FRAP assay, and 98% and 51% higher than CTRP and WTP, respectively, when measured by the CUPRAC method. The highest TAC detected for SBP reflects its higher flavonoid content as well as the presence of anthocyanins, lacking in the other samples, as demonstrated by LC-HRMS analysis. Indeed, since FRAP detected hydrophilic antioxidants, whereas $ABTS^{\bullet+}$ and CUPRAC assays pointed out both hydrophilic and lipophilic ones, the similar trend obtained from all three assays indicated that are mostly the hydrophilic instead of lipophilic antioxidants as the flavonoids and anthocyanins, to exert the major antioxidant activity of the samples.

Differences concerning the genotype, processing conditions, extraction methods, and analytical procedure used for the TAC evaluation, make the comparison with the literature rather difficult. Considering only the literature data based on the same analytical methods we used, the obtained results for WTP TAC were up to 2-fold (for FRAP) and 355-fold higher (for $ABTS^{\bullet+}$) compared to the value found by P. A. Silva et al. (2019) in Brazilian tomato pomace samples. Even Valdez-Morales et al. (2014) detected for the Saladette genotype tomato peel an $ABTS^{\bullet+}$ value 170-fold lower than that we found for WTP. Regarding the SBP, to the best of our knowledge, there is no study investigating the TAC of SB tomato pomace; Blando et al. (2019) assessed the antioxidant capacity of the cherry SB whole fruit, obtaining a lower value than our by $ABTS^{\bullet+}$ assay, but the comparison between SB and WT whole fruit showed that the SB $ABTS^{\bullet+}$ value was 200% more than the WT, according to our outcomes. The major $ABTS^{\bullet+}$ value for the SBP could be related to the accumulation of flavonoids and anthocyanins in the peel, making more concentrated the antioxidants compounds and, thus, their antioxidant activity in the pomace extract, in comparison to the whole fruit extract. More in general, our results demonstrated that the polyphenols found in the tomato pomace by-product, mainly including flavonoids and in the SBP anthocyanins too, exert a relevant antioxidant function, significantly higher than that provided by the commercial tomato powder, even when the pomace is produced from the CL tomato mutant, lacking in certain flavonoids. Of interest, if pomace is obtained from SB tomato, rich in anthocyanins besides flavonoids, the pomace functionality is much more evident.

3.6. Biopesticides activity

There is a present need in agriculture to provide novel alternatives for the control of phytopathogen-caused illnesses, particularly those of fungal and bacterial origin. A wide range of fungi of agronomic relevance cause losses in cultivation, post-harvest, storage, and distribution. It is known that phenolic compounds, besides having antioxidant properties, can also exert antimicrobial activity. Particularly, flavonoids and anthocyanins have been demonstrated to be the major polyphenols able to effectively inhibit the growth of bacteria and fungi by damaging the microorganism cell wall through the reaction between the cell wall lipids and amino acids, and polyphenols alcoholic moiety (Khoo et al., 2017). Considering the valuable flavonoids amount we found in tomato pomaces, and especially the anthocyanins occurrence in SBP, we decided to evaluate the antimicrobial activity of the experimental pomaces against phytopathogens that cause serious crop losses as the fungus *F. graminearum* and the gram-negative bacterium *P. syringae*. More specifically, concentrations of 2 and 1 mg/mL were used in a first screening phase, to test the antimicrobial potential of the extracts by evaluating their growth inhibition capacity on the selected microorganisms. The obtained results did not detect inhibitory activity in any of the extracts analyzed, showing a growth inhibition halo only around the positive control wells, containing the antibiotic (Figs. S3, S4). Previous studies conducted on human pathogenic bacterial strains have shown acceptable antimicrobial activity (MIC lower than 2 mg/mL) of tomato pomace, but only against gram-positive strains: in gram-negative strains, the antimicrobial activity was found just at very high concentrations (10 mg/mL). This was attributed to the lower sensitivity of gram-negative bacteria to natural extracts due to the physical structure of their cell wall (Szabo et al., 2019). Hence, the lack of inhibitory activity against the phytopathogen *P. syringae* we have found could be related to both the relatively low concentration of extracts we tested and the nature of the bacteria. There are not many studies regarding the antifungal activity of tomato residues against phytopathogens of the *Fusarium* genus. Kim et al. (2019) observed a fair activity of acetone extract of tomato whole fruits against *Fusarium oxysporum* but at MIC = 2.50 mg/mL and identified linolenic and caffeic acids as the main antifungal compounds found in the tomato leaves extract as well as in the whole red tomato fruit even if in a lower concentration. Although no activity was found at the concentrations tested here, different extraction methods and/or solvents, as well as higher concentrations can be adopted to further evaluate their potential activity against phytopathogens of agronomic interest.

4. Conclusions

The findings obtained from this work demonstrated that SM tomato pomace of WT, CL, and SB lines provided a valuable amount of bioactive compounds in comparison to the whole tomato powder available on the market. Among these, flavonoids are the most representative compounds in all three experimental pomaces, responsible for significantly higher antioxidant properties than the commercial powder. Major antioxidant features than CTRP were obtained also for the CLP sample through its lower flavonoid content due to the genetic inability to produce the naringenin chalcone derivatives, highlighting that, despite this, CLP by-product also possesses better functional properties than the CTRP. When the tomato pomace is produced from SM SB fruits, even greater functional properties were observed, thanks to its ability to produce and store anthocyanins in the peel, the tomato pomace's major constituent. Indeed, the occurrence of anthocyanins conferred to SBP the highest antiradical activity and reducing power.

To the best of our knowledge, this is the first study comparing the nutritional and functional properties of tomato pomace and commercial tomato powder, as well as investigating the functional properties of SB tomato pomace. The present results highlight that the experimental pomaces could be CTRP nutritionally comparable ingredients, but potentially more functional due to the high antioxidant content, as well as virtuous from a circular economy perspective. According to our results and considering the high quantity of tomato pomace (in this study, up to 21%) that can be obtained by tomato processing and its valuable healthy properties, efforts in promoting its valorization and reusing as a value-added food ingredient are very advisable.

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CRediT authorship contribution statement

Barbara Farinon: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Martina Felli:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Maria Sulli:** Methodology, Validation, Writing – review & editing. **Gianfranco Diretto:** Methodology, Resources, Writing – review & editing. **Daniel V. Savatin:** Resources, Supervision, Writing – review & editing. **Andrea Mazzucato:** Resources, Supervision, Writing – review & editing. **Nicolò Merendino:** Resources, Supervision, Writing – review & editing. **Lara Costantini:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AM is participating as inventors in owing the trademark "Sun Black" and the rights on the registered variety "Solenero." The ownership is the University of Tuscia, Viterbo, Italy.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139509.

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