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Research article

Nutritional enhancement of processed tomatoes through sustainable cultivation with effective microorganisms

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ABSTRACT

Tomatoes play a pivotal role in the Mediterranean diet and are of substantial significance in the Italian agro-food industry, particularly in the production of industrially processed products. This study investigates the application of Effective Microorganisms (EM) technology for tomato cultivation, aiming to augment the nutritional quality of processed tomatoes within the paradigm of sustainable agricultural practices. Eight samples of preserved tomatoes, cultivated with and without EM technology, underwent analysis for antioxidant activity (ABTS and DPPH assays) and total polyphenol content (Folin-Ciocalteu method) to assess the effects of the corroborant on the final products. The examination of the nutritional profile of industrially processed tomatoes grown with EM technology revealed a marked improvement in antioxidant activity, particularly in the lipophilic extract ($p < 0.05$), and for the majority of samples also in the methanolic extract ($p < 0.05$). Most samples also showed a notable increase in total polyphenol content, with statistically significant differences in several cases ($p < 0.05$).

Food safety analyses, including genotoxicity (*Vicia faba* Micronucleus test) and residual metals/pesticides, were implemented on EM-treated samples, which are the primary focus of this study, considering the intended use of the samples for food production. The results confirmed the safety of the EM-treated products for human consumption. Combined with the enhanced nutritional profile, these findings underscore the potential of EM technology to improve the quality of industrially processed tomatoes, promoting a shift towards sustainable and nutritionally enriched agricultural practices.

1. Introduction

Our world is currently facing a complex crisis, where environmental degradation, hunger, poverty, and instability are increasingly interconnected. These global challenges have been exacerbated by the COVID-19 pandemic and ongoing geopolitical conflicts, as outlined in the United Nations' *Sustainable Development Goals Report – 2022*. Agriculture is particularly vulnerable, with cultivable land shrinking due to urbanization, industrial expansion, and environmental damage, including pollution and climate change [1,2].

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As arable land decreases, the pressure to meet the global food demand continues to rise, driven by a growing world population. Traditionally, intensive agricultural practices relying on chemical fertilizers and pesticides have been employed to maximize yields. However, these practices have significant environmental costs, contributing to biodiversity loss, climate change, and soil degradation [3–5]. Excessive use of agrochemicals leads to long-term soil infertility, while residues of pesticides and heavy metals remain in the environment, posing threats to human health and food safety [6–11].

In light of these challenges, the need for sustainable agricultural techniques that enhance crop yields while minimizing environmental harm is more pressing than ever. An important focus is the development of eco-friendly approaches that not only improve productivity but also enhance the nutritional quality of agricultural products, particularly after industrial processing.

One promising strategy involves the use of beneficial microorganisms, particularly Plant Growth-Promoting Bacteria (PGPB) and fungi. These microorganisms act as biofertilizers, phytostimulators, and biocontrol agents, enhancing plant productivity and resilience. Among their key traits, nitrogen fixation plays a crucial role in plant nutrition and is carried out by symbiotic bacteria belonging to genera such as *Bradyrhizobium, Allorhizobium, Rhizobium, Frankia, Azoarcus, Mesorhizobium,* and *Burkholderia*. Additionally, PGPB contribute to plant growth through the synthesis of gibberellins (GAs), a function observed in species from genera such as *Acinetobacter, Azospirillum, Gluconobacter, Bacillus,* and *Herbaspirillum* [12–14]. PGPB consortia are especially effective in promoting plant health and mitigating external stressors that affect crops [15]. Multi-strain microbial inoculants, as opposed to single strains, have been shown to significantly improve the yield and quality of vegetables, especially under challenging growing conditions [16]. This approach aligns with the current research focus on the synthetic design of multifunctional microbial consortia in sustainable agriculture [17].

Tomatoes play a central role in both the global agri-food industry and human nutrition. Known for their versatility, rich nutrient profile, and health benefits, tomatoes are a major source of bioactive compounds, including carotenoids, vitamins, and polyphenols. These compounds provide strong antioxidant properties and are associated with a reduced risk of chronic diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders [18–21]. Even after industrial processing, tomatoes retain much of their nutritional value, making them a crucial part of the Mediterranean diet [22,23]. The global demand for processed tomato products—such as canned tomatoes, sauces, and pastes—continues to grow, reinforcing the importance of ensuring the nutritional quality of these products.

Industrial processing of tomatoes plays a crucial role in extending their shelf life and enhancing their usability. However, the various stages of processing can impact the nutritional content, especially the bioactive compounds that confer health benefits [24]. Therefore, there is growing interest in optimizing both cultivation methods and processing technologies to improve the nutritional value of industrially processed tomato products [25,26].

The Campania region, particularly the Agro Sarnese-Nocerino area, is one of Italy's most important centers for tomato cultivation and processing, thanks to its favorable climate and soil conditions [27]. Our experimentation was conducted in the Sarno river basin area, one of the most exploited regions for tomato cultivation, despite being known for its high levels of agrochemicals and heavy metals due to industrial pollution and agricultural practices [28–30].

Recent studies, such as those by Tommonaro et al., have examined the potential of *Effective Microorganisms* (EM) technology, developed by EM Schweiz AG (Arni BE, Switzerland), to improve tomato crops. EM technology, which includes a consortium of beneficial microorganisms such as lactic acid bacteria, yeasts, actinomycetes, and fungi, has shown promise in increasing yields and improving the quality of tomatoes, including higher carotenoid and polyphenol content, as well as enhanced antioxidant activity [31]. These findings suggest that EM technology could play a significant role in sustainable agriculture, particularly in enhancing the nutritional quality of crops.

The present study aims to evaluate the potential of EM technology to enhance the nutritional quality of industrially processed tomato products, focusing on antioxidant activity and polyphenol content. Our research compares tomato preserves made from tomatoes grown with EM technology to those cultivated with traditional methods, without EM. By examining these preserves, we hope to assess how eco-sustainable cultivation methods can positively impact the nutritional profile of industrially processed products, aligning with the broader goal of promoting sustainable agriculture.

For the EM-treated samples, we conducted comprehensive food safety analyses, including tests for heavy metals and agrochemical residues, as these samples were intended for food production. This focus was particularly important given the widespread cultivation of tomatoes in areas like the Sarno river basin, which faces environmental challenges due to contamination with agrochemicals and heavy metals. This contamination is primarily linked to agricultural activities, such as pesticide use, and industrial processes, including tomato processing and tannery operations.

In conclusion, this research seeks to assess the feasibility of integrating EM technology into tomato cultivation to enhance the nutritional quality of processed products. The study aims to contribute to the growing body of research focused on sustainable agricultural practices, offering a potential solution to improve the nutritional value of industrially processed tomatoes.

2. Materials and methods

2.1. Chemicals

All solvents and reagents used for the extraction of bioactive compounds, as well as for the determination of antioxidant activity and total polyphenol content, were purchased from Sigma-Aldrich (Milan, Italy). In particular, the following chemicals were employed in this study: diethyl ether (≥99.8 %, GC, ACS reagent), methanol (≥99.9 %, GC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (≤100 %), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (≥98.0 %, HPLC), Folin–Ciocalteu reagent (1.9–2.1 N), and sodium carbonate (≥99.5 %, ACS reagent, anhydrous).

In addition, for the assessment of genotoxic effects using the *Vicia faba* micronucleus assay, specific reagents were employed for sample fixation and staining procedures. The fixative solution consisted of a 1:3 (v/v) mixture of glacial acetic acid and ethanol. Glacial acetic acid (99.8 %, Romil, Cambridge, UK) was diluted with distilled water to obtain a 45 % (v/v) working solution. Ethanol (≥99.8 %, GC; purchased from Sigma-Aldrich, Milan, Italy) was used as received. Hydrolysis of the root tips was performed using a 3.7 % hydrochloric acid (HCl) solution, freshly prepared by diluting a 1 N stock solution (Scharlau, Barcelona, Spain) with distilled water. For chromatin visualization, Schiff's reagent (Sigma-Aldrich, St. Louis, MO, USA) was used in the staining step.

For the determination of metal content, all reagents used for sample digestion were purchased from Carlo Erba (Milan, Italy), including ultrapure hydrochloric acid (32–35 %, RS – trace analysis) and ultrapure nitric acid (65–67 %, RS – trace analysis). Calibration curves were established using a multielement standard for ICP-MS (100 mg/L, Ultra Scientific, Bologna, Italy). For quality control purposes, Certified Reference Material 1573a (tomato leaves) was used.

For the determination of pesticide residues, all reagents employed for sample preparation were purchased from Sigma-Aldrich (Milan, Italy). Specifically, acetone and hexane (both ≥99.9 %, GC), anhydrous sodium sulfate (≥99.5 %, ACS reagent), and silica gel (high-purity grade, pore size 60 Å, particle size 2–22 μm) were used. A standard solution of deuterated PAHs (100 mg/L, Ultra Scientific, Bologna, Italy) was used as an internal standard, and a mixture of PAHs (2000 mg/L, Ultra Scientific, Bologna, Italy) was used for calibration curve construction.

2.2. Sample descriptions and application of EM technology

Tomato plants of various varieties were grown in the Sarno river basin region (Italy; 40.7705°N, 14.6430°E), an area well known for its extensive tomato production, favored by optimal climatic conditions. The selected tomato varieties—*Vesuvius Piennolo* (Cherry) Tomato, *Corbara* (Cherry) Tomato, *Kiros*, an improved ecotype of *San Marzano* tomato, *Datterino Giallo* (Cherry) Tomato, and *Goldy F1* Yellow Tomato—were chosen for their excellent nutritional properties and adaptability to processing into preserves, as well as their commercial importance for the Campania region. Despite its agricultural importance, this region is also affected by contamination from agrochemicals and heavy metals, posing potential risks of pollutant transfer to crops. Previous studies have extensively documented the geochemical distribution of these hazardous elements and their potential impact on human exposure [28–30].

The experimental field was divided into two groups: a control group cultivated using conventional agricultural practices and a second group where EM technology was applied.

Conventional agricultural practices included the use of leguminous plants for nitrogen fixation, crop rotation, and the periodic application of composted manure [32]. This manure contained approximately 9 % mineral components (nitrogen, phosphorus, potassium, and magnesium), 38 % organic carbon, and 11 % humic acids. It was incorporated into the soil every two weeks as an organic fertilizer.

For the section of the experimental field dedicated to EM cultivation, Bokashi—a fermented organic substrate enriched with a liquid solution of Effective Microorganisms (EMa)—was applied before transplantation. Two weeks prior to planting, Bokashi was evenly spread across the soil surface and incorporated into the upper 6 cm of soil at a rate of 200 g per square meter. Upon transplanting, the tomato plants received an additional treatment with EMa, diluted at a 1:500 (v/v) ratio with water, which was sprayed onto the plants weekly, following the manufacturer's recommendations. These guidelines are available for download on the manufacturer's website and have also been included in the technical datasheet provided as mmc1. All tomato cultivars received the same fertilization and irrigation treatments to ensure uniform growing conditions.

Mature tomatoes were harvested at full ripeness, ensuring uniformity in developmental stage and the absence of mechanical damage. These tomatoes were processed into preserved products by Inserbo Srl (Angri, SA, Italy) adapting standard industrial procedures to the small-scale production of a limited number of samples exclusively for research purposes in this study. The processed tomato samples analyzed in this study are listed in Table 1. Details regarding the selected tomato varieties and industrial processing techniques are available in the mmc2.

The comparison between preserves derived from tomatoes cultivated with and without EM technology was designed to evaluate the potential benefits of microbial inoculation on the final nutritional and qualitative properties of processed tomato products.

Table 1
Description of the samples subject to this study, analyzed as processed products by Inserbo srl (Angri-SA, Italy).

Sample	Sample description
1-TVC	Typical Vesuvian tomato conserve
2-VPC	Vesuvius *Piennolo* cherry tomato conserve
3-SCC	Smashed *Corbara* cherry tomato
4-SVC	Smashed Vesuvius *Piennolo* cherry tomato
5-BNF	Bio tomato natural fillet
6-YDP	Yellow *Datterino* tomato puree
7-YFP	Yellow *Fiano* tomato puree
8-SYC	Smashed yellow cherry tomato

2.3. Bioactive compound extraction

To analyze bioactive components, 100–250 g of each processed tomato sample was homogenized using a blender. The homogenates were subjected to centrifugation at 13,848×*g* for 20 min. The supernatants, representing the hydrophilic fraction, were collected and stored at −20 °C for future analyses. These supernatants were not the subject of the present study and were preserved solely for potential future investigations.

Separate aliquots of the remaining pellet were extracted separately to obtain different bioactive fractions. The extraction with diethyl ether (1:2 w/v) under constant stirring in the dark for 20 min, repeated three times using fresh solvent, yielded the "lipophilic fraction." Similarly, the extraction with methanol (1:2 w/v) under agitation in the dark for 30 min, also repeated three times, yielded the "methanolic fraction." The obtained extracts, hereafter referred to as "lipophilic fraction" and "methanolic fraction," were collected, evaporated to dryness using a rotary evaporator in the dark at temperatures below 40 °C, and stored at −20 °C until analysis [27].

2.4. Determination of antioxidant activity

The antioxidant capacity of the samples was assessed using two spectrophotometric assays. The DPPH assay was used to evaluate the antioxidant activity of the methanolic fraction, which is rich in polyphenols, by measuring its ability to neutralize the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical. The ABTS assay was employed to determine the antioxidant capacity of the lipophilic fraction, which is rich in carotenoids, based on its capacity to scavenge the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation [23]. Results were expressed as mg of Trolox equivalents per 100 g of processed product.

2.5. Determination of total polyphenol content

Total polyphenol content was quantified using the Folin-Ciocalteu method on the methanolic fraction. The reaction mixture consisted of 50 μL of Folin-Ciocalteu reagent, a variable volume of sample extract (ranging from 10 to 50 μL), and 800 μL of deionized water. After a 1-min incubation, 100 μL of 20 % sodium carbonate solution was added, followed by thorough mixing. The final volume was adjusted to 1 mL with deionized water.

Quercetin was used as the calibration standard. Samples were incubated at room temperature for 2 h before measuring absorbance at 765 nm using a GENESYS™ 150 Vis/UV–Vis Spectrophotometer (Thermo Fisher Scientific). Total polyphenol content was expressed as mg of quercetin equivalents per 100 g of processed product.

2.6. Genotoxicity with Vicia faba

The *Vicia faba* Micronucleus test was used to evaluate the genotoxicity of the different tomato sauces obtained with EM technology. Seeds of *V. faba* were used in order to evaluate genotoxicity according to ISO (2013). Macrophyte dry seeds were soaked for 24 h in distilled water, then were exposed (n = 5) on filter paper Whatman n. 1 imbibed with 25 g of testing tomato sauces in triplicate in Petri dishes.

After 96h incubating in the dark at 22 ± 2 °C, the primary root tips of germinated seed were fixed overnight in Carnoy's solution (1:3 acetic acid: ethanol), then were cut and hydrolyzed in 1 N HCl at 60 °C for 10 min. The root tips were stained in Schiff's Reagent using Feulgens method, and squashed on microscope slides. The interphase cells were scored for micronucleus frequencies at 1000 × magnification and 5 $*10^3$ cells from *V. faba* seeds were counted using the online version of ImageJ [33].

2.7. Determination of metals

Metal analysis was performed on various tomato samples obtained using EM technology, in triplicate after acid digestion. Approximately 2 g of the homogenized samples were subjected to microwave-assisted oxidative acid digestion with a mixture of hydrochloric acid and nitric acid (6:2 v/v) at temperatures up to 180 °C and high pressure for 40 min (Mars – CEM, Italy). The mineralized were then recovered with ultrapure and analyzed using ICP-MS, Aurora M90 Bruker, US. A calibration curve for each analyzed element was prepared from a certified standard solution (Ultrascientific, Bologna, Italy). The detection limit (LOD) and limit of quantification (LOQ) for each metal were calculated using the blank method. The average calculated values for LOD and LOQ in the matrix were 0.0005 and 0.002 mg/kg, respectively. Data quality was ensured by certified reference materials, and the recovery percentage was assessed by analyzing the SRM-NIST in triplicate, yielding a range of 70–110 %.

2.8. Determination of pesticides

For pesticide determination, 5.0 g of homogenized tomato samples obtained using EM technology were extracted with a mixture of an acetone-hexane (1:1 v/v) using an ultrasonic extractor (Brason, US). The extract was concentrated under a nitrogen flow to a final volume of 1 mL (Multivap10, Labtech, Italy). The extract was then cleaned using a sodium sulfate anhydrous column, followed by cleaning with silica gel. Afterward, 10 μL of the internal standard (a mixture of deuterated PAHs) was added and the extract was injected into a gas chromatograph (Shimadzu 2010 Plus, Japan) coupled with a mass spectrometer (MS-TQ8030 - Shimadzu, Japan). Pesticide quantification was performed using an external calibration curve based on the internal standards method. The calculated average values for the detection limit (LOD) and limit of quantification (LOQ) in the tomato matrix were 0.003 mg/kg and 0.01 mg/kg, respectively. Data quality and recovery % was estimated by CRM and the recovery was between 70 and 110 %.

2.9. Statistical analysis

Antioxidant activities and total polyphenol content were measured in triplicate for each sample of processed tomato product. Data were expressed as mean value ± standard deviation. Statistical analysis was elaborated with the OriginPro 9.1 software package (OriginLab Corporation, Northampton, USA) where Gaussian distribution was evaluated through different normality tests (Shapiro-Wilk's, Kolmogorov-Smirnov and D'Agostino-K squared) followed by Levene's test for homoscedasticity. Accordingly, parametric (Student's *t*-test) or non-parametric test (Mann-Whitney) were used to assess statistical variations between treated/not treated tomato cultivars. *p* values < 0.05 were considered as statistically significant.

3. Results

3.1. Antioxidant activity

The antioxidant capacity of lipophilic and methanolic fractions of the tomato samples was evaluated by ABTS and DPPH assays, respectively. The results were expressed as mg eq. Trolox 100 g^{-1} of transformed products for both the samples obtained with EM technology and the control samples obtained without EM treatment, to ensure normalization and comparison. Detailed results are provided in the Supplementary Material as mmc3.

For the lipophilic fraction, the antioxidant capacity of EM-treated samples ranged from a minimum of 1.69 ± 0.12 mg eq. Trolox 100 g^{-1} of processed product for 6-YDP $^{+EM}$ to a maximum of 20.61 ± 0.43 mg eq. Trolox 100 g^{-1} of processed product for 3-SCC $^{+EM}$. The control (without EM) samples exhibited antioxidant activity values ranging from 0.51 ± 0.04 mg eq. Trolox 100 g^{-1} of processed product for 6-YDP to 14.30 ± 1.12 mg eq. Trolox 100 g^{-1} of processed product for 3-SCC, showing a similar trend to the EM-treated samples.

The antioxidant activity of the lipophilic fraction of EM-treated tomato preserves was significantly higher compared to conventionally grown tomatoes, as demonstrated in the bar plot in Fig. 1. This improvement is statistically significant, with *p*-values ranging from 5.14×10^{-5} to 4.58×10^{-2}, as detailed in the supplementary material as mmc4.

Regarding the methanolic fraction, which is rich in polyphenols, EM-treated samples showed a minimum antioxidant capacity of 48.55 ± 3.75 mg eq. Trolox 100 g^{-1} of processed product for 7-YFP $^{+EM}$, and a maximum activity of 166.00 ± 7.19 mg eq. Trolox 100 g^{-1} of processed product for 2-VPC $^{+EM}$. Control samples (the industrial tomato preserves obtained without the application of the EM technology) had values ranging from 31.42 ± 7.76 for 5-BNF to 146.48 ± 4.28 for 2-VPC mg eq. Trolox 100 g^{-1} of processed product. As shown in Fig. 2, the average antioxidant activity values of the processed products from traditionally cultivated tomatoes were significantly lower for most samples compared to those obtained using EM technology (p-values ranging from 3.44×10^{-5} to 3.51 ×

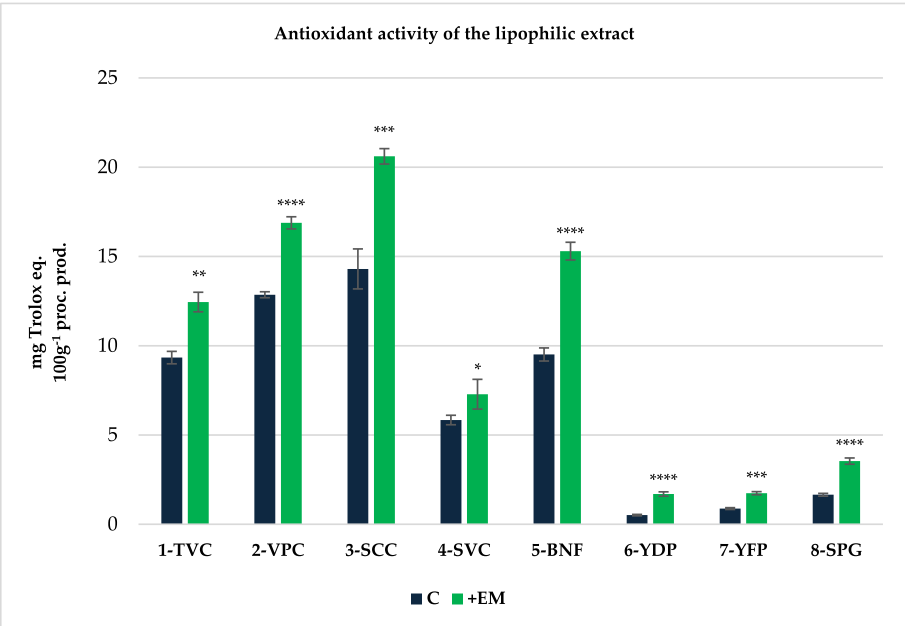

Fig. 1. Bar plot comparing the antioxidant activity of the lipophilic extract of preserves of tomatoes cultivated with (+EM) and without (C) EM technology. *p* values were estimated with Student's t-test and equal variance with Levene's test for Welch correction: ns = $P > 0.05$; * = $0.01 < p < 0.05$; ** = $0.001 < p < 0.01$; *** = $0.0001 < p < 0.001$; **** = $p < 0.0001$.

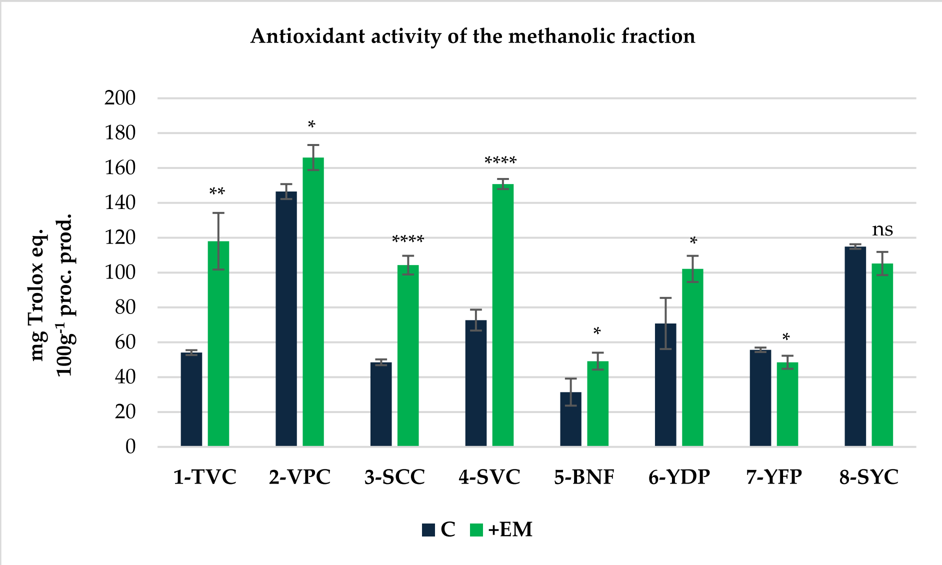

Fig. 2. Bar plot comparing the antioxidant activity of the methanolic fraction of preserves of tomatoes cultivated with (+EM) and without (C) EM technology. *p* values were estimated with Student's t-test and equal variance with Levene's test for Welch correction: ns = $P > 0.05$; * = $0.01<p < 0.05$; ** = $0.001<p < 0.01$; *** = $0.0001<p < 0.001$; **** = $p < 0.0001$.

10^{-2}), indicating an improved nutritional profile induced by the use of Effective Microorganisms.

In addition, no consistent trend was observed between antioxidant capacity of methanolic (DPPH) and lipophilic (ABST) extracts (Supplementary material, mmc5).

3.2. Total polyphenol content

The total polyphenol content of the tomato samples was evaluated according to the Folin-Ciocalteu method. The results, converted into mg Quercetin equivalents 100 g^{-1} of processed products for normalization and comparison purposes, were provided for both the preserves from tomatoes cultivated with EM technology and the equivalent tomato preserves obtained without EM treatment.

EM-treated samples exhibited a minimum total polyphenol content of 4.36 ± 0.21 mg Quercetin equivalents 100 g^{-1} of processed products for 5-BNF^{+EM}, and a maximum of 11.62 ± 0.71 mg Quercetin equivalents 100 g^{-1} of processed products for 4-SVC^{+EM}. In comparison, control samples (industrial tomato preserves produced without EM technology) displayed total polyphenol contents

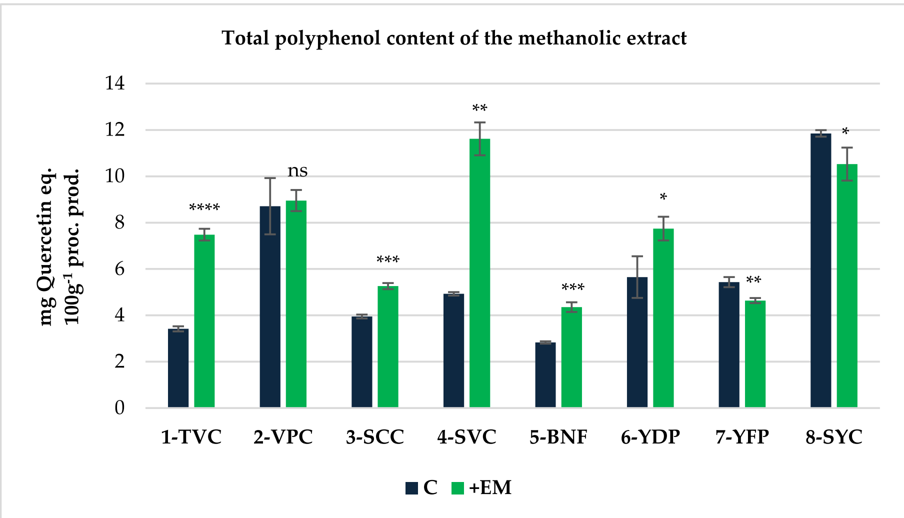

Fig. 3. Bar plot comparing the total polyphenol content of the methanolic extract of preserves of tomatoes cultivated with (+EM) and without (C) EM technology. *p* values were estimated with Student's t-test and equal variance with Levene's test for Welch correction: ns = $P > 0.05$; * = $0.01<p < 0.05$; ** = $0.001<p < 0.01$; *** = $0.0001<p < 0.001$; **** = $p < 0.0001$.

ranging from 2.83 ± 0.05 for 5-BNF to 11.85 ± 0.14 mg Quercetin equivalents 100 g^{-1} of processed products for 8-SYC (Supplementary material, mmc6).

Significant differences were observed in the total polyphenol content between tomato preserves with and without EM, both demonstrating good quality. Importantly, the values for the EM-treated products showed substantial improvement in the majority of samples (p-values between $1,39 \times 10^{-5}$ and $7,60 \times 10^{-1}$), as depicted in Fig. 3.

This result further confirms the ability of EM technology to enhance the levels of polyphenols in tomatoes, with implications extending to the processed products.

Additionally, as it can be seen from the correlation coefficients scatter plot in Fig. 4 and the relative comparison graph in mmc7 (Supplementary material), a high total polyphenol content is usually followed by high antioxidant activity of the methanolic extract. However, the relationship between these values is not always consistent, underscoring the need for multiple methodologies characterizing various bioactive compounds to accurately assess the nutritional quality of food samples.

3.3. Analysis of metals and pesticides

The concentration of metals detected in preserves of tomatoes cultivated with EM technology (Al, Sb, As, Ba, B, Cd, Cr, Fe, Mn, Hg, Ni, Pb, Cu, Se, V, and Zn) are reported in Table 2. Among all analyzed elements, Fe was the most important essential element, with levels between 1.6 and 3.6 mg/kg, followed by Al, B, V, Zn, Mn and Cu, while lower concentrations of Ni, Cr and As are shown in the range between 0.012 and 0.18 mg/kg. The results showed that the levels of Pb and Cd were lower than the detection limit and below the limit imposed by law. The results did not show significant differences on all the samples analyzed. Therefore, the consumption of this vegetable would lead to no health problem.

The results obtained in this work showed that all tomato samples either tested negative or contained residues of about 60 pesticides at levels below the limit of quantification (<0.01 mg/kg). The selection of compounds reflects those most commonly associated with agricultural activity in the investigated area. The complete list of pesticides analyzed is reported in Table 3.

As widely described in the literature, the soils of the Sarno valley are heavily contaminated by organochlorinated pesticides, by DDT and DDE and their isomers with concentrations more than 200-fold the limits imposed the Italian law n. 152 for land for residential (0.01 mg/kg) and industrial (0.1 mg/kg) use [34]. The study proposed by Zeinab A. Abd-Elhaleem, 2020 shows how 36 % of the samples show the presence of some pesticide residues in concentrations between 0.01 and 0.07 mg/kg which are lower than the limits imposed by the European Commission [35]. The absence of pesticides, below the detection limits of the method, confirms the safety of the tomatoes for consumption, ensuring their suitability for use both as fresh produce and for industrial processing.

3.4. Genotoxicity analysis using Vicia faba

The results of the analysis did not reveal any significant differences in micronuclei frequency in *Vicia faba* root tip cells exposed to tomato samples treated with EM technology.

4. Discussion

Over 80 % of global tomato consumption occurs in the form of processed products, particularly canned goods, underscoring their pivotal role in the Mediterranean diet due to their rich antioxidant content, including vitamin C, carotenoids, flavonoids, and hydroxycinnamic acids [18,36]. Our understanding of the effects of various processing operations on the stability and kinetics of phenolic antioxidants has evolved significantly. Contrary to the conventional belief that antioxidants decrease during food processing, recent studies indicate an increase in total antioxidant activity during thermal processing, depending on the antioxidant assay used.

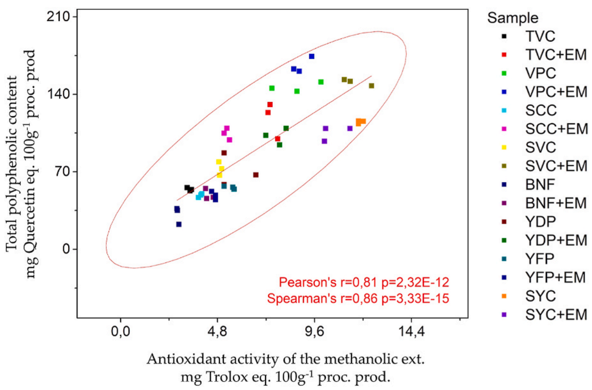

Fig. 4. Scatter plot showing the correlation coefficients for the total polyphenol content (Folin-Ciocalteu test) and the antioxidant activity (DPPH assay) values acquired for the methanolic fraction of the tomato processed products obtained with and without EM treatment. Confidence ellipse value is 95 %.

Table 2
Results of metals (mg/Kg fresh weight) in the sample of preserves of tomatoes cultivated with EM technology.

Sample n°	Al	Sb	As	Ba	B	Cd	Cr	Fe	Mn	Hg	Ni	Pb	Cu	Se	V	Zn
1-TVC $^{+EM}$	1.1	<0.005	0.020	<0.1	2.0	<0.01	0.15	3.2	0.88	<0.002	0.05	<0.003	0.89	<0.03	1.8	1.3
2-VPC $^{+EM}$	1.4	<0.005	0.019	<0.1	1.2	<0.01	0.16	2.9	0.67	<0.002	0.06	<0.003	0.66	<0.03	1.7	1.0
3-SCC $^{+EM}$	1.5	<0.005	0.019	0.2	1.6	<0.01	0.18	3.3	1.0	<0.002	0.08	<0.003	0.70	<0.03	1.7	1.1
4-SVC $^{+EM}$	1.9	<0.005	0.018	<0.1	2.2	<0.01	0.14	1.6	0.68	<0.002	<0.03	<0.003	0.36	<0.03	1.8	0.4
5-BNF $^{+EM}$	1.1	<0.005	0.012	<0.1	2.9	<0.01	0.12	2.6	0.74	<0.002	0.05	<0.003	0.54	<0.03	1.4	0.9
6-YDP $^{+EM}$	2.7	<0.005	0.019	0.2	2.1	<0.01	0.17	2.4	0.79	<0.002	0.08	<0.003	0.48	<0.03	1.3	0.7
7-YFP $^{+EM}$	1.3	<0.005	0.015	<0.1	1.4	<0.01	0.11	2.0	0.49	<0.002	0.05	<0.003	0.49	<0.03	1.4	0.7
8-SYC $^{+EM}$	1.1	<0.005	0.012	<0.1	2.3	<0.01	0.10	1.8	0.33	<0.002	<0.03	<0.003	0.33	<0.03	1.2	0.6
Min	1.1	–	0.012	0.2	1.2	–	0.1	1.6	0.33	–	0.05	–	0.33	–	1.2	0.4
Max	2.7	–	0.020	0.2	2.9	–	0.18	3.3	1	–	0.08	–	0.89	–	1.8	1.3
Mean	1.5	–	0.017	0.2	2.0	–	0.1	2.5	0.7	–	0.06	–	0.56	–	1.5	0.8
SD	0.6	–	0.003	–	0.5	–	0.03	0.6	0.2	–	0.01	–	0.19	–	0.24	0.30
Law Limits[a]	–	–	–	–	–	0.02	–	–	–	–	–	0.05	–	–	–	–

[a] Regulation (CE) n. 915/2023, SD: standard deviation.

Table 3
List of pesticides.

Chloroneb	2,4′-Methoxychlor	Endosulfan I	Malathion
Pentachlorobenzene	Endrinketone	cis-Chlordane	Metolaclor
BHC, alpha	Tetradifon	trans-Nonachlor	Fention
Hexachlorobenzene	Mirex	Chlorfenson	Bromofosmetil
Pentachloroanisole	Dichlorovos	4,4′ DDE	Clorfenvifos
BHC, beta	Diclobenil	Dieldrin	Procimidone
BHC,gamma	Atrazina desetil	2,4′-DDD	Bromofosetil
BHC, delta	Trifluoralin	Endrin	Tetraclorvinfos
Endosulfan ether	Simazine	Ethylan	Oxadixil
Heptachlor	Atrazine	Endosulfan II	Ethion
Pentachlorothioanisole	Terbumenton	4,4′-DDD	4,4′-Methoxychlor
Aldrin	Fonofos	2,4′-DDT	Endosulfansulfate
4,4′-Dichlorobenzophenone	Propizamide	cis-Nonachlor	4,4′-DDT
Fenson	Terbutilazine	Chlorbenside	Alachlor
Isodrin	Terbufos	trans-Chlordane	Metalaxil
Heptachlorepoxide	Tolclofos metile	2,4′-DDE	Fenchlorfos

This highlights the need for comprehensive assessments of antioxidant phytochemicals beyond vitamin C [37].

The industrial transformation of tomatoes into paste involves multiple processing stages that influence the final antioxidant and metabolite profile. Research has increasingly focused on understanding these effects to identify opportunities for improvement. Various techniques, such as hot and cold break methods, induce variations in antioxidant capacities, as assessed by DPPH and ABTS assays, emphasizing the need to optimize the nutritional value of canned products [38].

Carotenoids and lipids undergo chemical changes during tomato processing and storage. These changes, including the isomerization and degradation of carotenoids, are key in preserving the nutritional value of processed products. Lycopene, the dominant carotenoid in tomatoes, is particularly sensitive to processing and storage, and its degradation can reduce its well-known protective effects against chronic diseases. Preservation strategies, such as non-thermal processing methods, micro- and nanoencapsulation, and antioxidant supplementation, aim to preserve bioactive lycopene [39–41].

Thermal stabilization treatments used in tomato-based products, such as pâtés, can also affect aroma and volatile compounds. High temperatures degrade terpenic compounds and trigger the formation of volatile products from lipid oxidation and the Maillard reaction, influencing the sensory qualities of the final product [42].

Despite the widespread consumption of processed tomato products, limited information exists on the bioaccessibility of bioactive compounds during gastrointestinal digestion. Understanding the bioavailability and absorption of compounds like polyphenols and carotenoids is critical to evaluating their health benefits. Research shows that different cooking methods can influence nutrient absorption in the bloodstream, emphasizing the importance of optimizing processing techniques to preserve bioactive compounds [25,42].

Given the integral role tomatoes play in both the agri-food industry and human nutrition, further enhancing the nutritional profile of processed products through sustainable cultivation methods is of increasing interest. Our study explored the use of eco-friendly practices, particularly the application of Effective Microorganisms (EM) technology, to improve the nutritional quality of processed tomato products.

Although the nutritional quality of foods and nutraceutical properties have become topics of great interest, studies specifically evaluating the impact of industrial processing on antioxidant activity remain limited. Some studies have assessed the antioxidant potential of various canned tomato products (Izzo et al., 2022) [43], while others have examined how different processing methods, such as the production of tomato puree and ketchup, influence antioxidant capacity [44]. Additionally, research on home cooking methods, including boiling, baking, and frying, has provided insights into the stability of key antioxidants such as ascorbic acid, total phenolics, and lycopene [45]. However, direct comparisons between these studies and our findings are challenging due to differences in processing techniques, extraction methodologies, and analytical approaches. Our study, by specifically investigating the effects of an eco-sustainable agricultural practice on the antioxidant properties of industrially processed tomato preserves, adds a novel perspective to this field.

The primary aim of this research was to evaluate the effectiveness of EM technology in improving the quality of industrially processed tomato preserves. Our findings show that tomatoes grown using EM technology exhibited significantly higher antioxidant activity and polyphenol content compared to tomatoes cultivated using traditional methods. These results align with previous studies demonstrating the ability of EM technology to enhance plant health and boost secondary metabolite production, such as polyphenols and carotenoids [18]. The results suggest that EM technology can play a crucial role in improving the nutritional quality of processed tomato products, contributing to the development of healthier food options.

This study was conducted in a single geographical region and within a single growing season, and we acknowledge that these conditions inherently limit the generalizability of our findings. The agronomic performance of EM technology, as well as its effects on yield and fruit quality, may vary across seasons and cultivation areas due to multiple environmental factors, including climatic fluctuations, precipitation patterns, and soil heterogeneity—even within the same experimental field. Future multi-season and multi-site studies will therefore be essential to better assess the robustness and broader applicability of EM-based practices under diverse cultivation conditions.

Food safety analyses were conducted on the EM-treated samples to ensure their suitability for human consumption, as the products were intended for food production. These analyses tested for 16 heavy metals, 141 agrochemicals, and genotoxicity. Although the experimental area is known for its suboptimal soils with high levels of agrochemicals and heavy metals, no detectable levels of harmful substances were found in the samples, which is consistent with other studies on tomato cultivation in similar areas [46]. This confirms the safety and nutritional value of the EM-treated products, affirming their potential for industrial food production. Notably, the absence of genotoxic effects observed in *Vicia faba* further supports the conclusion that the use of EM does not induce any biological alterations that could compromise the safety of the final food product.

While the primary focus of our research was on the nutritional benefits of EM technology, the resilience of crops like tomatoes in suboptimal soils offers an interesting avenue for future research. Edible plants such as tomatoes, with their demonstrated ability to grow on suboptimal soils without compromising food safety, could be explored for their potential use in bioremediation strategies. This approach could integrate the environmental and economic benefits of edible crops with remediation efforts, presenting new opportunities for sustainable agricultural practices [47].

In conclusion, our study emphasizes the potential of EM technology to enhance the nutritional quality of processed tomato products through eco-friendly cultivation methods. By focusing on industrially processed tomato preserves, we highlight the critical role that sustainable agricultural practices can play in delivering high-quality, nutritionally enriched foods to consumers. These findings open new pathways for integrating green technologies into modern agriculture, particularly in the agri-food industry, where the demand for healthier, sustainable food products continues to grow.

5. Conclusions

This research marks a significant advancement in enhancing the nutritional quality of industrially processed tomato products. Given the global importance of processed tomato consumption, particularly in the form of canned goods, optimizing their nutritional content through the application of Effective Microorganisms (EM) technology offers promising results, alongside a focus on environmentally sustainable cultivation practices. The data collected from this study demonstrate that while all samples, with or without EM, displayed good overall quality, those treated with EM showed statistically significant improvements in key nutritional metrics.

Our findings strongly suggest that EM technology can elevate the quality parameters of tomato preserves, with notable enhancements in antioxidant activity, both in the lipophilic and methanolic fractions, as well as a marked increase in total polyphenol content. These results are consistent with previous studies and further highlight the positive role of EM technology in promoting not only plant productivity but also the nutritional value of the end products.

The study also included a comprehensive assessment of the food safety of the processed tomato products derived from EM-treated crops. This was an important aspect of our research, as tomatoes are frequently cultivated on suboptimal soils with high levels of pesticides and heavy metals. Ensuring that the final product is safe for consumption underscores the importance of incorporating food safety analyses in the production of nutritionally enhanced, industrially processed goods.

In conclusion, this study contributes meaningful insights to the growing body of research on sustainable agricultural technologies. By focusing on the end products—industrially processed tomato preserves—we emphasize the transformative potential of EM technology in creating healthier, more nutritious tomato-based goods. This work underscores the symbiotic relationship between improving consumer health and advancing ecological sustainability, offering a viable path forward for the agri-food industry in the quest for more sustainable, nutritionally optimized food products.

CRediT authorship contribution statement

Gennaro Roberto Abbamondi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Maria Toscanesi:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Trifuoggi:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Marco Guida:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Serena Iannicelli:** Writing – review & editing, Formal analysis, Data curation. **Carmine Iodice:** Writing – review & editing, Formal analysis, Data curation. **Debora Paris:** Writing – review & editing, Writing – original draft, Visualization, Data curation, Formal analysis. **Rocco De Prisco:** Writing – review & editing, Supervision, Resources, Investigation, Methodology, Conceptualization. **Giuseppina Tommonaro:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability statement

The data presented in this study are available on request from the corresponding authors.

Ethics statement

This study was conducted in accordance with Heliyon's ethical policies, following all relevant regulations and guidelines. Ethical approval and informed consent were not required, as the research did not involve human or animal subjects.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2026.e44537.

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