Waste and Biomass Valorization
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Valorization of Waste from Bell Pepper: Chemical Composition and Bioactive Potential

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Abstract
Agri-food waste, especially in the fruit and vegetable sector, presents environmental and economic challenges. The bell pepper industry generates significant waste, offering potential for resource recovery within a circular economy. This study analyzed the nutritional and biochemical composition of waste from green, orange, and red bell peppers (*Capsicum annuum* L.) and evaluated the bioactive potential of hydroethanolic extracts from fruits, stalks, and seeds. Carbohydrates were the main macronutrients, followed by proteins and ashes. Fructose was dominant in orange and red peppers, while glucose prevailed in green peppers. Waste samples contained organic acids, fatty acids, and phenolic compounds, including oxalic and malic acids, polyunsaturated fatty acids, 22 bioactive phenolics, and 34 volatile compounds. Hydroethanolic extracts demonstrated strong antioxidant activity, with green bell pepper waste showing the highest levels. The extracts also exhibited antibacterial effects against *Yersinia enterocolitica* and *Bacillus cereus* and antifungal activity against *Aspergillus brasiliensis*. These findings highlight the potential of bell pepper waste as a rich source of bioactive compounds with applications in food, cosmetics, and pharmaceuticals. Utilizing these by-products promotes sustainability, supports the circular economy, and addresses global waste challenges.

Keywords Agri-food waste · Bell pepper · Phenolic and volatile compounds · Circular economy

Introduction

Food loss and waste remain major global challenges, complicating efforts to build efficient, sustainable agri-food systems and ensure food security and nutrition for all. A substantial portion of food-whether from plant or animal sources-is discarded rather than consumed, despite its nutritional potential [1]. Estimates suggest that nearly one-third of all food produced globally is lost or wasted each year, amounting to around 1.3–1.4 billion tons, a figure expected to rise to 2.6 billion tons by 2025 [2, 3]. This loss is especially prevalent in fresh fruits and vegetables, largely due to post-harvest handling, processing inefficiencies, and consumer habits [4]. Food waste, however, holds considerable potential as a source of bioactive compounds suitable for industrial applications. Beyond prevention, valorizing food waste by repurposing it as natural bioactive ingredients offers a promising, sustainable alternative to synthetic compounds.

Bell peppers(Capsicum annuum L.) are part of the Solanaceae family, which includes numerous crops of high dietary and economic importance [5]. This family comprises over 90 genera and 2,000 species, with around 30 species classified under the genus *Capsicum*. Pepper fruits are commonly consumed in various forms: raw, fermented, as a colorant (paprika), or ground into a spice. They range from large, mild varieties like bell peppers to thin, intensely hot types like cayenne [6]. Key cultivated varieties include both hot and sweet peppers, among them *C. annuum*, *C. baccatum*, *C. frutescens*, *C. pubescens*, and *C. chinense*. *C. annuum*, known for its smooth, glossy appearance [7], most commonly appears in green, yellow, orange, and red colors that

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correspond to different maturation stages and varying levels of carotenoid and chlorophyll synthesis [8]. More unusual colors, such as purple, brown, white, and black, are seen in certain varieties [9]. Botanically classified as a fruit due to its seed-bearing structure, bell peppers are generally treated as non-starchy vegetables in culinary contexts [10]. Nutritionally, bell peppers are a low-calorie food, primarily composed of water and carbohydrates, with moderate dietary fiber content and minimal protein and fat [11]. The nutritional value of bell peppers plays a significant role in consumer health and is influenced by factors such as the fruit's color, variety, growing conditions, postharvest processing, and stage of development [12]. While bell peppers are widely recognized for their nutritional value, health-promoting properties, and appealing sensory characteristics, a substantial proportion of the total production is often lost or discarded during postharvest handling and processing. Such losses are mainly due to visual imperfections, mechanical damage, or noncompliance with market standards, resulting in the generation of significant amounts of agri-food waste. Postharvest losses represent a major challenge in bell pepper production, accounting for approximately 25–35% of the total yield [13]. This wasted produce holds significant potential as a source of valuable bioactive compounds. Key bioactives in bell peppers, including capsaicinoids, phenolic compounds, carotenoids (provitamin A), and essential vitamins such as C and E, are linked to various biological activities that can aid in the prevention and treatment of several acute and chronic diseases [14].

Thus, the aim of this work is to valorize the waste-green, orange, and red fruits of *C. annuum*, including their seeds and stalks, at the post-harvest stage by assessing their nutritional and chemical profiles, concomitantly evaluating the bioactive properties (antioxidant, cytotoxic, and antimicrobial activities) of hydroethanolic extracts from these waste from colored bell pepper fruits, seeds, and stalks.

Material and Methods

Standards and Reagents

High-performance liquid chromatography (HPLC) grade *n*-hexane, 99% acetonitrile and 99.98% ethyl acetate solvents were purchased from Fisher Scientific (Lisbon, Portugal) and analytical grade methanol solvent was purchased from Pronalab (Lisbon, Portugal). The water was treated prior to use by the Milli-Q-Water purification system (TGI Pure Water Systems, Greenville, South Carolina, USA). The standard blend with 37 fatty acid methyl esters (FAME) (standard 47,885-U) was purchased from Sigma (St. Louis, Missouri, USA), as well as other individual fatty acid isomers, sugar standards and organic acid standards. Fetal bovine serum (FBS), *L*-glutamine, Hank's saline solution (HBSS), and DMEM medium (medium for animal cells (Dulbecco Modified Eagle)) were purchased from Hyclone (Logan, Utah, USA). Acetic acid, ellipticin, sulforodamine B (SRB), trichloroacetic acid (TCA) and Tris were supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Mueller-Hinton agar (MHB) and Malt Agar Broth (MAB) were obtained from Biolab® (Hungary). The compound *p*-Iodonitrotetrazolium chloride (INT) was purchased from Panreac Applichem (Barcelona, Spain).

Samples

Fruits of orange, red, and green waste bell peppers (*C. annuum* var. *grossum*) were supplied by a local producer in Bragança, Portugal. The fruits, seeds, and stalks were carefully separated, cleaned to remove foreign materials, frozen, lyophilized, milled using a Foss Knifetec^{TM} 1095 mill at a controlled temperature of 20 °C, and stored until further analysis.

Extracts Preparation

The hydroethanolic extracts from waste from colored bell pepper fruits including their seeds and stalks, were performed for the analysis of the phenolic composition and bioactive properties, as previously described [15]. Briefly, 1 g of each sample was subjected to an extraction of 1 h (25 ℃ at 150 rpm) twice with 40 mL of ethanol/water (80:20; *v/v*) and then filtered through Whatman No. 4 paper. The ethanol of the combined extracts was removed using a rotary evaporator (Büchi R-210, Flawil, Switzerland) and the extract was frozen and lyophilized (FreeZone 4.5 model 7,750,031, Labconco, Kansas City, MO, USA) for further analysis.

Proximate Composition

The proximate composition of fruits of orange, red, and green waste bell peppers was evaluated following AOAC methods [16]. In summary, crude protein content was determined using the macro-Kjeldahl method (N × 6.25) with an automatic distillation and titration system (model Pro-Nitro-A, JP Selecta, Barcelona). Crude fat content was measured using Soxhlet extraction, where a known sample weight (~3 g) was extracted with petroleum ether for 7 h. Ash content was determined by incinerating samples at 550 ± 5 °C. Total carbohydrates were estimated by difference using the formula: Total carbohydrates = 100 − (g

ash + g proteins + g fat). The energy content was calculated using the equation: Energy (kcal/100 g) = 4 × (g proteins + g carbohydrates) + 9 × (g fat).

Hydrophilic Compounds

Free Sugars

Free sugars were analyzed using high-performance liquid chromatography with a refractive index detector (HPLC-RI; Knauer, Smartline 1000 and Smartline 2300 systems), following the method outlined [17]. Briefly, 1.0 g of dried sample powder was mixed with 5 mg/mL melezitose as an internal standard (IS) and extracted using 40 mL of 80% aqueous ethanol at 80 °C for 90 min. The mixture was centrifuged at 3500 rpm for 10 min, and the supernatant was concentrated at 60 °C under reduced pressure. The extract was defatted by washing three times with 10 mL of ethyl ether, then further concentrated at 40 °C. The final residue was dissolved in 5 mL of water and filtered through 0.2 µm Whatman nylon filters. Peak identification and quantification were performed by comparing retention times (Rt) with authentic standards and calibration curves. Data processing was carried out using Clarity Software (Data Apex, Prague, Czech Republic), and results were expressed as grams per 100 g of dry weight (g/100 g dw).

Organic Acids

The organic acid profile of the waste from colored bell pepper fruits was analyzed using ultra-fast liquid chromatography coupled with a photodiode array detector (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan). Samples (2 g) were extracted by stirring with 25 mL of meta-phosphoric acid at 25 °C and 150 rpm for 25 min, followed by filtration through Whatman No. 4 paper [18]. Before analysis, the extract was further filtered through 0.2 µm nylon filters. Compounds were separated using a reverse-phase 18 SphereClone column (Phenomenex) (5 µm, 250 × 4.6 mm i.d.), maintained at 35 °C. A 3.6 mM sulfuric acid solution was used as the eluent at a flow rate of 0.8 mL/min. Each compound was identified by comparing chromatograms with those of commercial standards, and quantification was performed by correlating peak areas, recorded at 215 nm, with calibration curves from the standards. Results were expressed in grams per 100 g of dry weight (g/100 g dw).

Lipophilic Compounds

Fatty Acids

The fatty acid methyl esters (FAME) profile was determined following transesterification of the lipid fraction extracted via Soxhlet, as described by the authors [19]. The analysis was performed using gas–liquid chromatography with flame ionization detection on a YOUNG IN Chromass 6500 GC system (Gyeonggi, South Korea), equipped with a split/splitless injector set at 250 °C (split ratio of 1:80), a flame ionization detector set at 260 °C, and a Zebron-FAME column (30 m × 0.25 mm i.d. × 0.20 µm df; Phenomenex, Lisbon, Portugal). FAMEs were identified and quantified by comparing the relative retention times of sample peaks with those of commercial standards (standard mixture 47,885-U, Sigma, St. Louis, MO, USA). Data were recorded and processed using Clarity DataApex 4.0 software (Prague, Czech Republic), with results expressed as the relative percentage of each fatty acid.

Tocopherols

Tocopherols were analyzed using a method previous outlined [19], employing an HPLC system coupled with a fluorescence detector (P-2020; Jasco, Japan), set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. In brief, 100 µL of BHT solution in hexane (10 mg/mL) and 400 µL of an internal standard (tocol; 50 µg/mL in hexane) were added to 500 mg of the sample, which was then homogenized with 4 mL of methanol by vortex mixing for 1 min. After adding 4 mL of hexane and vortex mixing for an additional minute, 2 mL of a saturated NaCl aqueous solution was added, and the mixture was homogenized and centrifuged at 3500 rpm for 5 min. The clear upper layer was transferred to a vial, and the sample was re-extracted twice with hexane.

The combined extracts were evaporated to dryness under nitrogen, and the residue was dissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulfate, and filtered through 0.2 µm Whatman nylon filters before being transferred to a dark injection vial. The tocopherol isoforms were separated on a Polyamide II normal-phase column (250 mm × 4.6 mm i.d.) from YMC Waters (Japan), operated at 30 °C. The mobile phase consisted of hexane and ethyl acetate (7:3, v/v) at a flow rate of 1 mL/min, with a 20 µL injection volume. Tocopherols were identified by comparing chromatograms with those of authentic standards, and quantification was based on fluorescence signal response, using tocol as an internal standard. Results were expressed in milligrams per 100 g of dry weight (mg/100 g dw).

Pigments

The content of pigments was evaluated using a method described by the authors [20]. Briefly, the samples (~500 mg) were vigorously shaken with 10 mL of acetone/hexane mixture (4:6, *v/v*) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance was measured at 453, 505, 645 and 663 nm, and the contents of carotenoids (β-carotene and lycopene), chlorophyll a, and chlorophyll b were expressed in mg per 100 g of dw, using the equations:

$$\beta - \text{carotene}\left(\frac{\text{mg}}{100\ \text{mL}}\right) = 0.216 \times A663 - 1.220 \times A645 - 0.304 \times A505 + 0.452 \times A453$$

$$\text{Lycopene}\left(\frac{\text{mg}}{100\ \text{mL}}\right) = -0.0458 \times A663 + 0.204 \times A645 - 0.304 \times A505 + 0.452 \times A453$$

$$\text{Chlorophyll } a\left(\frac{\text{mg}}{100\ \text{mL}}\right) = 0.999 \times A663 - 0.0989 \times A645$$

$$\text{Chlorophyll } b\left(\frac{\text{mg}}{100\ \text{mL}}\right) = -0.328 \times A663 + 1.77 \times A645$$

Phenolic Compounds

Phenolic compounds were analyzed in lyophilized waste from colored bell pepper fruits, stalks, and seeds hydroethanolic extracts, which were redissolved in an ethanol/water solution (80:20; *v/v*) to a final concentration of 10 mg/mL. Analysis was carried out on a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) with a DAD detector (280 and 370 nm) coupled to an electrospray ionization mass detector (LC-DAD-ESI/MS^n). Chromatographic separation was achieved using a Waters Spherisorb S3 ODS-2 C18 column (3 µm, 4.6 mm × 150 mm; Waters, Milford, MA, USA) at 35 °C.

The gradient elution solvents were 0.1% formic acid in water and acetonitrile. For mass detection in negative mode, a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) with an electrospray ionization source (ESI) was utilized. Phenolic compounds were identified based on their chromatographic behavior, spectral data, and UV–Vis masses, compared against standard compounds or literature data using Xcalibur® software (ThermoFinnigan, San Jose, CA, USA). Quantitative analysis employed calibration curves from UV signals of standard compounds, or the most similar standards when commercial versions were unavailable. Detailed operating conditions, identification, and quantification procedures [15]. The results are reported in mg per g of extract (mg/g extract).

Volatile Compounds

Waste from colored bell pepper fruits (100 mg) were extracted with ethanol (80%, 1 mL) in an ultrasonic bath for 1 h. After centrifugation for 10 min at 1500 rpm, the supernatant was filtered through 0.2 pm cellulose filters (Agilent Technologies, Santa Clara, California, USA) and stored at 4 ºC until use. All the analyses were performed in triplicates. Agilent 8890 gas chromatography (GC) System with mass selective detector (MSD-5977B; Agilent Technologies, USA), configured with an automated sample extraction and enrichment platform (Centri®, Markes International Ltd., Bridgend, UK), was used for profiling volatile compounds in samples, following the methodology described by the authors [21]. HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) (Agilent Technologies, USA) is used for chromatographic separations and Helium (99.999%, The Linde Group, Ireland) as a carrier gas at a flow rate of 1.6 mL/min. The temperature of the column was programmed linearly from 40 to 300 °C at a rate of 20 °C/min and then held isothermally at 240 °C for 10 min.

The detector temperature was set to 270 °C and the transfer line temperature was heated at 280 °C. Electron Ionization (EI) source set to 280 °C and the mass spectra were collected in positive EI mode (+ 70 eV). In a split mode (20:1), an ethanol extract (1 µL) was injected with a split flow of 24 mL/min. The analyses were carried out in SCAN mode, with the compounds being tracked between 45 and 500 amu. Compounds in samples were identified by comparison of their mass spectra and retention times with the NIST05 library. Amounts were expressed as a relative percentage (%) for each sample independently.

Bioactive Properties

Antioxidant Activity

The antioxidant activity of the hydroethanolic extracts obtained from waste from colored bell pepper fruits, including their stalks and seeds, was re-dissolved in distilled water and subjected to dilutions from 10 to 0.1562 mg/mL. Lipid peroxidation inhibition in porcine brain homogenates was evaluated by the decrease in TBARS. Brains were obtained from the pig (*Sus scrofa* Linnaeus), dissected and homogenized in ice-cold

Tris–HCl buffer (pH 7.4, 20 mM) and centrifuged at 3000 *g* for 10 min. The color intensity of the malondialdehyde-thiobarbituric acid (MDA–TBA) complex was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated by using the following formula: $[(A-B)/A]\times 100\%$, in which A and B refer to the absorbance of the control and the sample solutions, respectively. The results were expressed in EC_{50} values (μg/mL with the sample concentration accounting for 50% of the antioxidant activity [21]. Trolox was used as a positive control.

Antimicrobial Activity

The antibacterial activity of the hydroethanolic extracts was evaluated using the broth microdilution method coupled with the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay. The extracts were tested against five Gram-negative bacteria, namely, *Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* subsp (ATCC 13076), *Yersinia enterocolitica* (ATCC 8610), and three Gram-positive bacteria, namely *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (ATCC 25923). These bacterial strains (food contaminants) were purchased at Frilabo, Porto, Portugal. Additionally, the extracts were also tested against clinical isolates obtained from hospitalized patients in various departments at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). Five Gram-negative bacteria *Escherichia coli* (VRU12881), *Proteus mirabilis* (VRU17684), *Klebsiella pneumoniae* (VRI17214), *Pseudomonas aeruginosa* (VRU14123), and *Morganella morganii* (VRU14272), and three Gram-positive bacteria, among them *Enterococcus faecalis* (VRU14041), *Listeria monocytogenes* (VRU17684), and methicillin-resistant *Staphylococcus aureus* (MRSA; VRI17654) were tested. The bacteria were incubated at 37 ºC in an appropriate fresh medium for 24 h before analysis to maintain the exponential growth phase.

The results were presented as minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs). Regarding food contaminants, streptomicin was used as a positive control for all bacterial strains, methicillin for *S. aureus* and ampicillin for all Gran-negative bacteria tested, *L. monocytogenes* and *S. aureus*. For clinical isolates, ampicillin was used as a positive control for all bacterial strains, imipenem for all Gram-negative bacteria tested and *L. monocytogenes* and Vancomycin for *Enterococcus faecalis* and MRSA (Supplementary material Table SM1).

For antifungal activity, two fungal strains, namely *Aspergillus brasiliensis* (ATCC 16404) and *Aspergillus fumigatus* (ATCC 204305) were used. Commercial fungicide ketoconazole (Frilabo, Porto, Portugal), was used as positive control. The results were presented as MIC and minimum fungicidal concentration (MFC).

Statistical Analysis

All assays were conducted in triplicate ($n=3$), with results expressed as mean ± standard deviation (SD). Statistical analysis was performed at a 5% significance level using SPSS software (IBM SPSS Statistics for Windows, Version 23.0; IBM Corp., Armonk, NY, USA). Differences among samples were assessed by one-way analysis of variance (ANOVA), with means compared using Tukey's HSD test ($p=0.05$). For comparisons between only two samples, a Student's *t*-test was applied.

It should be noted that all analyses were conducted using technical replicates derived from a single batch of bell pepper waste. Future research should include biological replicates from different batches to strengthen reproducibility and the broader applicability of the findings.

Results and Discussion

Proximate Composition

This study investigated the nutritional potential of waste from colored bell pepper fruits (*C. annuum* var. *grossum*) as a source of valuable compounds for industrial applications by comprehensively analyzing the nutrient profile of waste green, orange, and red bell peppers. Statistically significant differences ($p<0.05$) were observed across most nutritional components, which are expressed in Table 1. The colored-waste peppers exhibited high moisture content, with green peppers having the highest levels (92.5 ± 0.5% fw), followed by orange (90.7 ± 0.5% fw) and red (89.4 ± 0.9% fw), like findings by Ozgur et al. [22]. Carbohydrates represented the primary macronutrient across all samples, with red peppers displaying the highest concentration (88.20 ± 0.06 g/100 g dw). Comparatively, Olatunji et al. [23] reported lower carbohydrate levels, whereas Cisternas-Jamet et al. [24] noted similar values, highlighting the influence of genotype and environmental factors in the nutritional profile of this agriculture products. Protein content, in turn, was highest in the orange peppers (11.8 ± 0.3 g/100 g dw), corroborating previous findings [23, 25]. Ash content was highest in red peppers (11.2 ± 0.8 mg/100 g dw), though values reported by Olatunji et al. [23] and Cisternas-Jamet et al. [24] were slightly elevated. Finally, the lipid content followed a similar pattern, with red peppers showing the highest values (1.88 ± 0.02 g/100 g dw), consistent with Cisternas-Jamet et al. [23], Kim et al. [25] and El-Ghorab et al. [26]. The highest energy values were found in red peppers (409.35 ± 0.07 kcal/100 g dw), whereas Olatunji et al. [23] and Kefale et al. [27] reported comparatively lower values. Variability in these results is likely due to differences in

Table 1 Proximate composition and hydrophilic compounds of waste-colored bell peppers fruits (mean ± SD; $n = 3$)

Proximate composition	Waste-colored bell peppers		
	Orange	Red	Green
Moisture (%, fw)	90.7 ± 0.5^{b}	89.4 ± 0.9^{bc}	92.5 ± 0.5^{a}
Fat (g/100 g dw)	1.51 ± 0.09^{c}	1.88 ± 0.02^{a}	1.81 ± 0.05^{ab}
Proteins (g/100 g dw)	11.8 ± 0.3^{a}	$9.9 \pm 0.1b^{c}$	11 ± 1^{ab}
Ash (mg/100 g dw)	10.2 ± 0.4^{ab}	11.2 ± 0.8^{a}	10 ± 1^{bc}
Carbohydrates (g/100 g dw)	86.7 ± 0.3^{bc}	88.20 ± 0.06^{a}	87.4 ± 0.8^{ab}
Energy (kcal/100 g dw)	407.5 ± 0.3^{bc}	409.35 ± 0.07^{a}	409.0 ± 0.2^{ab}
Free sugars (g/100 g dw)			
Fructose	21.1 ± 0.7^{a}	17.1 ± 0.9^{b}	8.25 ± 0.02^{c}
Glucose	15.0 ± 0.7^{a}	14.2 ± 0.7^{b}	10.03 ± 0.07^{c}
Sucrose	2.0 ± 0.4^{ab}	2.1 ± 0.1^{a}	1.31 ± 0.03^{c}
Trehalose	0.63 ± 0.04^{a}	0.47 ± 0.07^{b}	0.32 ± 0.01^{c}
Total	39 ± 2^{a}	34 ± 2^{b}	19.91 ± 0.10^{c}
Organic acids (g/100 g dw)			
Oxalic acid	6.07 ± 0.01^{a}	5.68 ± 0.01^{b}	5.42 ± 0.03^{c}
Malic acid	5.99 ± 0.01^{b}	5.22 ± 0.02^{c}	8.29 ± 0.02^{a}
Shikinic acid	0.78 ± 0.02^{c}	0.95 ± 0.01^{b}	1.27 ± 0.06^{a}
Ascorbic acid	1.40 ± 0.01^{c}	$1.46 \pm 0.01b^{c}$	1.63 ± 0.01^{a}
Total	14.24 ± 0.01^{b}	13.32 ± 0.04^{c}	16.62 ± 0.08^{a}

In each line, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters

ripening stage, cultivation conditions, and postharvest practices, factors noted to impact nutrient profiles in bell peppers [28]. This comprehensive nutrient analysis underscores the potential of waste bell peppers as a rich source of bioactive compounds, suitable for incorporation as natural ingredients in various industrial applications.

Hydrophilic Compounds

Free Sugars

Soluble sugars significantly influence the flavor and acceptability of plant foods, making them essential components of agricultural products [29]. Table 1 presents the free sugars identified in waste from colored bell pepper fruits, showing statistically significant differences ($p < 0.05$) across fructose, glucose, sucrose, and trehalose concentrations. In waste-orange and red peppers, fructose was the predominant sugar (21.1 ± 0.7 g/100 g dw and 17.1 ± 0.9 g/100 g dw, respectively), followed by glucose (15.0 ± 0.7 g/100 g dw and 14.2 ± 0.7 g/100 g dw), sucrose (2.0 ± 0.4 g/100 g dw and 2.1 ± 0.1 g/100 g dw), and trehalose (0.63 ± 0.04 g/100 g dw and 0.47 ± 0.07 g/100 g dw). In contrast, waste-green peppers had glucose as the primary sugar (10.03 ± 0.07 g/100 g dw), followed by fructose (8.25 ± 0.02 g/100 g dw), sucrose (1.31 ± 0.03 g/100 g dw), and trehalose (0.32 ± 0.01 g/100 g dw). Additionally, total free sugars were highest in waste-orange peppers (39 ± 2 g/100 g dw, Fig. 1), followed by red (34 ± 2 g/100 g dw) and green peppers (19.0 ± 0.1 g/100 g dw).

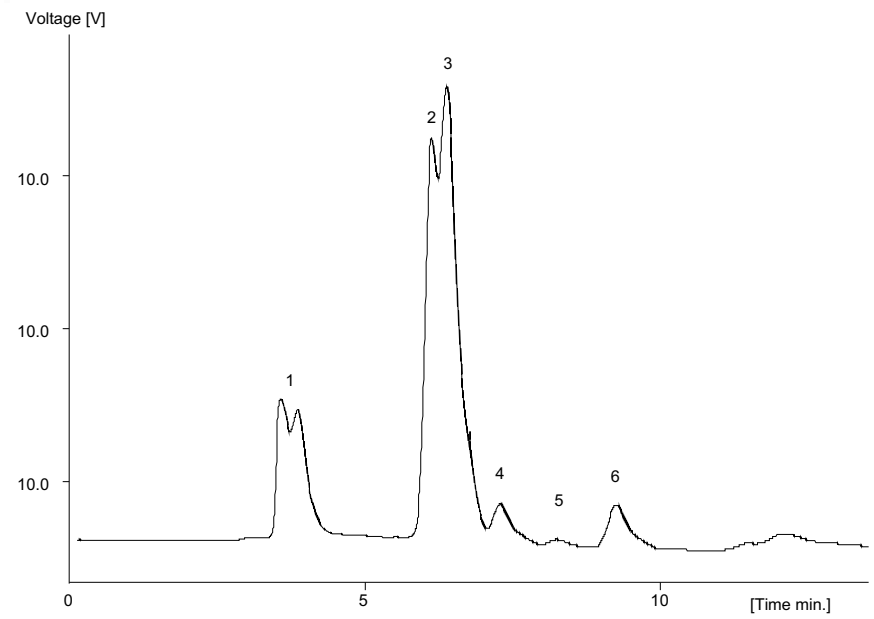

Fig. 1 Free sugars profile of waste from colored orange bell peppers fruit. 1-Mobile phase, 2-fructose, 3-glucose, 4-sucrose, 5-trehalose, 6-internal standard

These findings align with Zamljen et al. [30], who reported similar fructose, glucose, and sucrose levels in red *C. annuum*. However, Kim et al. [31] found higher fructose and glucose contents in both red and green peppers, suggesting that sugar concentrations may vary due to genotype, geographical origin, agricultural practices, environmental factors, or ripeness level. Generally, sugars in peppers increase with ripening, peaking at maturity [32], this happens because, during the ripening process of peppers, starches and other complex carbohydrates are converted into simple sugars like glucose and fructose. This process is driven by enzymes that break down polysaccharides into smaller sugars, resulting in a sweeter taste as the fruit reaches maturity. Additionally, photosynthesis continues to supply energy to the fruit, further contributing to the increase in sugar levels over time.

Organic Acids

Organic acids are essential for both nutritional and sensory quality in foods and serve as versatile food additives, including roles as preservatives, acidity regulators, and antioxidants with numerous applications [33]. Table 1 Presents data on the organic acids identified in waste from colored bell pepper fruits, with significant differences ($p < 0.05$) observed among oxalic, malic, shikimic, and ascorbic acids across all samples. In waste-orange and red bell peppers, oxalic acid was the most abundant organic acid (6.07 ± 0.01 g/100 g dw and 5.68 ± 0.01 g/100 g dw, respectively), followed by malic acid (5.99 ± 0.01 g/100 g dw and 5.22 ± 0.02 g/100 g dw). For waste-green bell peppers, malic acid was the main organic acid (8.29 ± 0.02 g/100 g dw), followed by oxalic acid (5.42 ± 0.03 g/100 g dw). Ascorbic acid was most concentrated in waste-green bell peppers (1.63 ± 0.01 g/100 g dw), followed by red (1.46 ± 0.01 g/100 g dw), and orange (1.40 ± 0.01 g/100 g dw) samples. Shikimic acid had the lowest concentrations overall, with the highest levels in green peppers (1.27 ± 0.06 g/100 g dw). Total organic acid content was highest in waste-green peppers (16.62 ± 0.08 g/100 g dw), followed by orange (14.24 ± 0.01 g/100 g dw) and red peppers (13.32 ± 0.04 g/100 g dw). These findings show higher oxalic and malic acid levels than those reported by Kye et al. [34], who found lower concentrations in orange and red "conical type" peppers (1.40 ± 0.03 g/100 g dw and 1.99 ± 0.02 g/100 g dw for oxalic, and 3.94 ± 0.13 g/100 g dw and 3.89 ± 0.13 g/100 g dw for malic acid, respectively). They also reported higher ascorbic acid content in these varieties, particularly in orange peppers (13.6 ± 0.4 g/100 g dw). Ozgur et al. [22] similarly found lower ascorbic acid in red and green peppers compared to the present study. The concentration of organic acids in bell peppers is influenced by factors such as growing region, climate, species, and ripening stage, which likely account for the variations observed between studies [33].

Lipophilic Compounds

Fatty Acids

Although lipids and fatty acids constitute a minor fraction of the edible portion of bell peppers, they contribute significantly to the fruit's structure, likely facilitating the solubility of bioactive compounds, vitamins, and carotenoids [24]. In this study, fifteen fatty acids were identified in waste from colored bell pepper fruits, with notable statistical differences ($p < 0.05$) between fatty acid classes in all analyzed samples (Table 2). Linoleic acid (C18:2n6c) emerged as the most abundant fatty acid across samples, with average concentrations of 44.3 ± 0.01% in waste-red, 43.9 ± 0.1% in green, and 42.8 ± 0.95% in orange bell peppers. Palmitic acid (C16:0) was next in abundance, with the highest level observed in orange peppers (21.0 ± 0.1%), followed by red (20.61 ± 0.01%) and green (18.4 ± 0.5%) varieties. α-Linolenic acid (C18:3n3) was most prevalent in green peppers (19.4 ± 0.2%), followed by orange (17.0 ± 0.5%) and red (14.88 ± 0.01%) varieties. These findings align with those reported by Saini et al. [35], who recorded similar linoleic, palmitic, and linolenic acid levels in orange and red varieties of sweet pepper (*C. annuum* L.). Similarly, Martinez et al. [36]. Found comparable concentrations of linoleic (41.47 ± 0.09% dw in red and 39.79 ± 0.36% in green), palmitic (17.09 ± 0.14% in red and 19.56 ± 0.23% in green), and linolenic acids (17.08 ± 0.03% in red and 21.69 ± 0.19% in green) across *C. annuum* varieties. In terms of classification, polyunsaturated fatty acids (PUFAs) were the predominant class across all waste from colored bell pepper fruits, with the highest levels found in green peppers (63.65 ± 0.03%, Fig. 2), followed by orange (60 ± 1%) and red (59.4 ± 0.1%). Saturated fatty acids (SFAs) followed, with the orange variety presenting the highest concentration (37 ± 1%), followed by green (34.9 ± 0.4%) and red (34.3 ± 0.1%). Monounsaturated fatty acids (MUFAs) were present in much lower concentrations in all samples. These observations are consistent with findings by Saini and Keum [35] and Martinez et al. [36], which also identified PUFAs as the dominant class and MUFAs as the least represented among *C. annuum* varieties. While bell peppers are not typically considered a significant source of dietary lipids, their high levels of polyunsaturated fatty acids—particularly linoleic and linolenic acids—may have beneficial effects on human health by potentially reducing risks associated with cardiovascular disease and type II diabetes [37].

Tocopherols

The tocopherol analysis of waste from colored bell pepper fruits in this study identified four isoforms: α-, β-, γ-, and

Table 2 Lipophilic compounds of waste-colored bell peppers fruits (mean ± SD; $n = 3$)

Fatty acids (%)	Waste-colored bell peppers		
	Orange	Red	Green
Lauric (C12:0)	0.51 ± 0.01	0.84 ± 0.01	0.116 ± 0.004
Myristic (C14:0)	2.1 ± 0.1	2.80 ± 0.01	1.41 ± 0.01
Pentadecylic (C15:0)	0.175 ± 0.004	0.15 ± 0.01	0.16 ± 0.01
Palmitic (C16:0)	21.0 ± 0.1	20.61 ± 0.01	18.4 ± 0.5
Palmitoleic (C16:1)	0.68 ± 0.01	1.81 ± 0.01	0.52 ± 0.02
Margaric (C17:0)	0.36 ± 0.03	0.28 ± 0.01	0.48 ± 0.01
Stearic (C18:0)	5.6 ± 0.1	5.20 ± 0.01	4.9 ± 0.1
Oleic (C18:1n9c)	2.4 ± 0.2	4.50 ± 0.01	1.19 ± 0.01
Linoleic (C18:2n6c)	42.8 ± 0.9	44.3 ± 0.01	43.9 ± 0.1
α-linolenic (C18:3n3)	17.0 ± 0.5	14.88 ± 0.01	19.4 ± 0.2
Arachidic (C20:0)	1.09 ± 0.08	1.94 ± 0.01	2.88 ± 0.08
Eicosadienoic (C20:2)	0.41 ± 0.01	0.237 ± 0.001	0.36 ± 0.01
Behenic (C22:0)	2.1 ± 0.7	1.40 ± 0.01	2.18 ± 0.03
Tricosylic (C23:0)	0.74 ± 0.02	0.307 ± 0.001	0.56 ± 0.01
Lignoceric (C24:0)	3.07 ± 0.05	0.73 ± 0.01	3.86 ± 0.07
SFA	37 ± 1^{a}	34.3 ± 0.1^{bc}	34.9 ± 0.4^{b}
MUFA	3.1 ± 0.2^{b}	6.31 ± 0.01^{a}	1.71 ± 0.01^{c}
PUFA	60 ± 1^{b}	59.4 ± 0.1^{bc}	63.65 ± 0.03^{a}
Tocopherols (mg/100 g dw)			
α-tocopherol	11.4 ± 0.3^{a}	9.45 ± 0.09^{b}	7.24 ± 0.01^{c}
β-tocopherol	1.21 ± 0.02^{a}	0.76 ± 0.02^{b}	0.40 ± 0.01^{c}
γ-tocopherol	0.44 ± 0.01^{ab}	0.48 ± 0.01^{a}	0.39 ± 0.01^{c}
δ-tocopherol	0.12 ± 0.01^{b}	0.19 ± 0.01^{a}	0.06 ± 0.01^{c}
Total	13.1 ± 0.4^{a}	10.88 ± 0.07^{b}	8.10 ± 0.01^{c}
Pigments (mg/100 g dw)			
β-carotene	44.9 ± 0.3^{a}	16 ± 4^{b}	5.8 ± 0.3^{c}
Lycopene*	nd	7.5 ± 0.1^{a}	0.46 ± 0.06^{b}
Chlorophyll *a*	0.22 ± 0.09^{c}	0.45 ± 0.03^{b}	15.4 ± 0.8^{a}
Chlorophyll *b*	0.61 ± 0.01^{c}	1.2 ± 0.7^{b}	5.6 ± 0.7^{a}

nd–not detected. In each line, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters. Mean statistical differences obtained by *t*-Student test

δ-tocopherols, with significant differences ($p < 0.05$) across the varieties (Table 2). Among these, α-tocopherol was the most abundant in all samples. Waste-orange bell peppers exhibited the highest concentration (11.4 ± 0.3 mg/100 g dw), followed by red (9.45 ± 0.09 mg/100 g dw) and green (7.24 ± 0.01 mg/100 g dw) peppers. This isoform, known for its strong vitamin E and antioxidant properties, is considered the most active among tocopherols. β-tocopherol was also most prominent in waste-orange peppers (1.21 ± 0.02 mg/100 g dw), followed by red (0.76 ± 0.02 mg/100 g dw) and green (0.40 ± 0.01 mg/100 g dw) varieties. Regarding γ-tocopherol, waste-red peppers had the highest levels (0.48 ± 0.01 mg/100 g dw), with orange (0.44 ± 0.01 mg/100 g dw) and green (0.39 ± 0.01 mg/100 g dw) varieties following. δ-tocopherol had the lowest concentrations across all samples, with red peppers containing 0.19 ± 0.01 mg/100 g dw, orange peppers 0.12 ± 0.01 mg/100 g dw, and green peppers 0.06 ± 0.01 mg/100 g dw. Total tocopherol content was highest in waste-orange peppers (13.1 ± 0.4 mg/100 g dw), followed by red (10.88 ± 0.07 mg/100 g dw) and green (8.10 ± 0.01 mg/100 g dw) varieties. These findings align partially with those of Kye et al. [34], who reported slightly higher α-tocopherol levels (13.6 ± 0.4 mg/100 g) in orange peppers along with β-tocopherol (1.14 ± 0.02 mg/100 g dw), γ-tocopherol (0.818 ± 0.002 mg/100 g dw), and δ-tocopherol (1.41 ± 0.01 mg/100 g dw). For red peppers, these authors also observed higher tocopherol levels, with red peppers showing the highest total tocopherol content, differing from our findings where orange peppers ranked highest. Similarly, Bhandari et al. [38] reported slightly elevated values for red peppers, including α-tocopherol (17.48 mg/100 g dw), β-tocopherol (3.6 mg/100 g dw), γ-tocopherol (4.53 mg/100 g), and δ-tocopherol (2.7 mg/100 g dw). These variations may be attributed to the specific physicochemical properties of the waste from colored bell pepper fruits analyzed, as well as environmental, genetic factors, ripeness, harvest timing, postharvest handling, processing, and storage conditions.

Pigments

The pigment analysis of waste from colored bell pepper fruits presented in Table 2 revealed the presence of four major pigments: β-carotene, lycopene, chlorophyll a, and chlorophyll b, with statistically significant differences ($p < 0.05$) observed among the varieties. β-Carotene emerged as the dominant carotenoid across all samples. This fat-soluble pigment is valued for its numerous biological functions and wide applications in food, pharmaceuticals, cosmetics, and textiles [39]. Waste-orange bell peppers exhibited the highest β-carotene concentration (44.9 ± 0.3 mg/100 g dw), followed by red (16 ± 4 mg/100 g dw) and green (5.8 ± 0.3 mg/100 g dw) varieties. Compared to the current findings, Kye et al. [34] reported lower β-carotene concentrations in orange (3.14 ± 0.26 mg/100 g dw) and red (4.46 ± 0.35 mg/100 g dw) pepper fruits. Similarly, Guil-Guerrero et al.[40] found reduced levels in orange (1.88 ± 2.9 mg/100 g dw), red (9.45 ± 1.17 to 13.48 ± 1.53 mg/100 g dw), and green (0.02 ± 0.02 to 2.8 ± 0.3 mg/100 g dw) varieties compared to this study, which may be related to the maturation state of the fruits. Lycopene, in turn, was most abundant in waste-red bell peppers (7.5 ± 0.1 mg/100 g dw), followed by waste-green peppers (0.46 ± 0.06 mg/100 g dw), this being undetectable in waste-orange peppers. Previous studies, such as Ozgur et al. [22], reported higher lycopene levels in red (12.39 ± 0.02 mg/100 g dw) and green

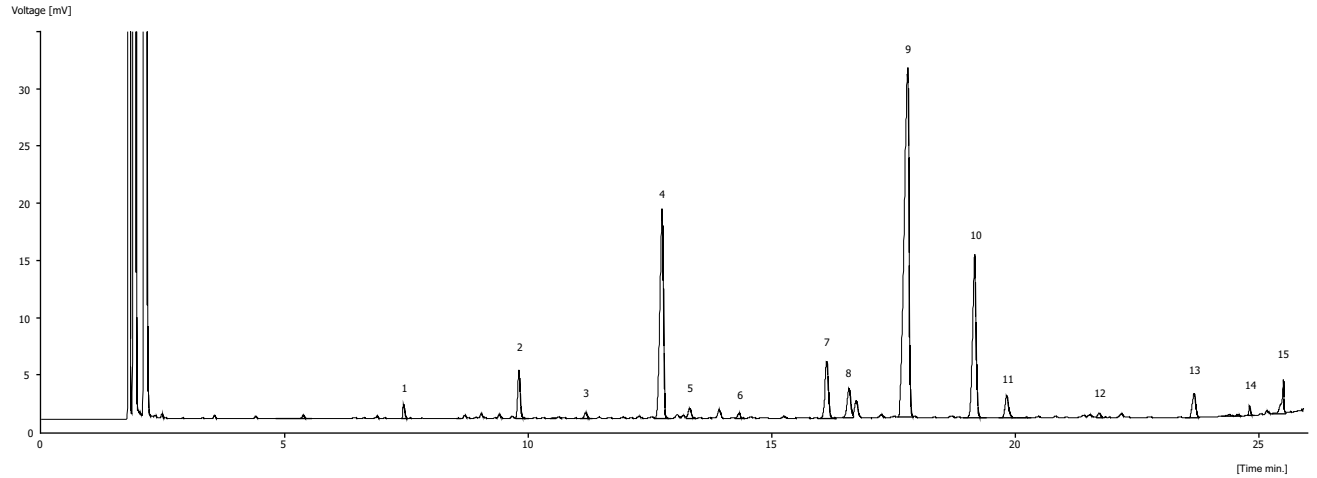

Fig. 2 Fatty acids profile of waste from colored green bell peppers fruit. 1-C12:0, 2-C14:0, 3-C15:0, 4-C16:0, 5-C16:1, 6-C17:0, 7-C18:0, 8-C18:1n9c, 9-C18:2n6c, 10-C18:3n3, 11-C20:0, 12-C20:2, 13-C22:0, 14-C23:0, 15-C24:0

(17.16 ± 0.01 mg/100 g dw) peppers. Interestingly, Razola-Díaz et al. [41] detected lycopene in orange bell peppers (*C. annuum* var. Kioto) from two companies, with concentrations ranging between 0.026–0.082 mg/100 g dw, contrasting with the absence observed in the present study. Regarding chlorophyll a and chlorophyll b, waste-green bell peppers displayed the highest concentrations (15.4 ± 0.8 mg/100 g dw for both pigments), followed by red (0.45 ± 0.03 mg/100 g dw for chlorophyll a and 1.2 ± 0.7 mg/100 g dw for chlorophyll b) and orange (0.22 ± 0.09 mg/100 g dw for chlorophyll a and 0.61 ± 0.01 mg/100 g dw for chlorophyll b) varieties. Żurawik et al. [42] in their study on antioxidant properties of Polish and Bulgarian pepper cultivars (*C. annuum*), reported higher chlorophyll concentrations in green peppers (chlorophyll a: 5.25–29.25 mg/100 g dw; chlorophyll b: 2.3–17.5 mg/100 g dw) and colored varieties (chlorophyll a: 0.73–1.83 mg/100 g dw; chlorophyll b: 0.78–2.64 mg/100 g dw) compared to the present findings. Variations in carotenoid profiles and concentrations among species may be attributed to factors such as growing conditions, maturity stage, and postharvest processing practices [43].

Phenolic Compounds

The tentative identification of the phenolic compounds found in the waste from colored bell pepper fruits, seeds, and stalks hydroethanolic extracts, as well as the retention times (Rt), maximum absorbance (λ_{max}), pseudomolecular ion ([M-H]$^-$), main ion fragments (MS^2) (Table SM2), and quantification of each compound are presented in Table 3. Individual phenolic compounds were tentatively identified according to the data presented and in comparison, with available standard compounds and/or existing literature. Among the twenty-two compounds spotted, seven were phenolic acids, fourteen were flavonoids, and one was stilbene. Regarding phenolic acids and considering the parameters below, the compounds were tentatively identified by comparing their retention times and UV spectrum with available standards as vanillic acid hexoside (peak 1; λ_{max}, 287; [M-H$^-$] at *m/z* 329), syringic acid hexoside (peak 2; λ_{max}, 285; [M-H]$^-$ at *m/z* 359), hydroxysinaptic acid hexoside (peak 3; λ_{max}, 281; [M-H]$^-$ at *m/z* 403), *p*-coumaric acid hexoside (peak 4; λ_{max}, 291; [M-H]$^-$ at *m/z* 325), syringic acid rhamnoside (peak 6; λ_{max}, 281; [M-H]$^-$ at *m/z* 361), ferulic acid hexoside (peak 7; λ_{max}, 330; [M-H]$^-$ at *m/z* 355), and finally synaptic acid hexoside (peak 8; λ_{max}, 330; [M-H]$^-$ at *m/z* 385).

Flavonoids were the most abundant group, with luteolin derivatives predominating, followed by apigenin and quercetin derivatives in their glycosidic and isomeric forms. Identified flavonoids included luteolin-6,8-di-*C*-glucoside (peak 5; λ_{max}, 330; [M-H]$^-$ at *m/z* 609), apigenin-6,8-di-C-glucoside (peak 9; λ_{max}, 339; [M-H]$^-$ at *m/z* 593), quercetin-glucopyranoside-rhamnopyranoside (peak 10; λ_{max}, 339; [M-H]$^-$ at *m/z* 609), luteolin-*C*-pentosyl-*C*-hexoside Isomer I and II (peaks 11 and 12; λ_{max}, 330; [M-H]$^-$ at *m/z* 579), apigenin-6-*C*-glucoside-8-*C*-arabinose (schaftoside and isoschaftoside) (peaks 13 and 14; λ_{max}, 335; [M-H]$^-$ at *m/z* 563), dimethylluteolin-apioside Isomer I and II (peaks 15 and 16; λ_{max}, 330 and 331, respectively; [M-H]$^-$ at *m/z* 447), apigenin-6-*C*-β-D-glucopyranoside-8-C-α-L-arabinopyranoside (peak 17; λ_{max}, 332; [M-H]$^-$ at *m/z* 563), luteolin-*O*-(apiosyl)-hexoside (peak 19; λ_{max}, 345; [M-H]$^-$ at *m/z* 579), luteolin-*O*-(apiosyl-acetyl)-hexoside (peak 20; λ_{max}, 345; [M-H]$^-$ at *m/z* 621), quercetin-3-*O*. rhamnoside (peak 21; λ_{max}, 341; [M-H]$^-$ at *m/z* 447), and luteolin-*O*-(apiosyl-malonyl)-hexoside (peak 22; λ_{max}, 349;

Table 3 Quantification (mg/g extract) of phenolic compounds found in waste-colored bell peppers stalks, fruits, and seeds hydroethanolic extracts (mean ± SD, $n = 3$)

Peaks	Stalk			Fruits			Seeds		
	Orange	Red	Green	Orange	Red	Green	Orange	Red	Green
1	0.209 ± 0.002	0.206 ± 0.002	0.224 ± 0.005	0.105 ± 0.001	tr	0.103 ± 0.001	0.147 ± 0.001	0.161 ± 0.001	0.130 ± 0.001
2	0.015 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	nd	nd	nd	nd
3	nd	nd	nd	0.013 ± 0.001	nd	nd	nd	nd	nd
4	nd	nd	nd	0.008 ± 0.001	nd	0.015 ± 0.001	0.003 ± 0.001	0.002 ± 0.001	0.001 ± 0.001
5	0.058 ± 0.001	0.052 ± 0.001	0.087 ± 0.001	0.044 ± 0.001	0.047 ± 0.001	0.053 ± 0.001	nd	nd	nd
6	nd	nd	nd	0.013 ± 0.001	nd	0.014 ± 0.002	nd	nd	nd
7	0.030 ± 0.001	0.030 ± 0.001	0.030 ± 0.001	0.030 ± 0.001	0.030 ± 0.001	0.030 ± 0.001	0.020 ± 0.001	0.030 ± 0.001	0.030 ± 0.001
8	0.013 ± 0.001	nd	nd	0.014 ± 0.001	nd	0.013 ± 0.001	nd	nd	0.013 ± 0.001
9	0.055 ± 0.001	0.056 ± 0.001	0.096 ± 0.001	0.042 ± 0.001	0.050 ± 0.001	nd	nd	nd	nd
10	0.464 ± 0.002	0.462 ± 0.001	0.464 ± 0.001	0.461 ± 0.001	0.461 ± 0.001	0.462 ± 0.001	nd	nd	nd
11	0.056 ± 0.001	0.05 ± 0.001	0.075 ± 0.001	0.042 ± 0.001	0.044 ± 0.001	nd	nd	nd	nd
12	0.054 ± 0.001	0.049 ± 0.001	0.064 ± 0.001	0.043 ± 0.001	0.044 ± 0.001	nd	0.131 ± 0.002	0.108 ± 0.001	0.100 ± 0.001
13	0.044 ± 0.001	0.042 ± 0.001	0.048 ± 0.001	nd	0.042 ± 0.001	nd	0.085 ± 0.001	0.125 ± 0.001	0.111 ± 0.001
14	nd	0.043 ± 0.001	0.050 ± 0.001	nd	0.043 ± 0.001	nd	nd	nd	nd
15	0.057 ± 0.001	0.048 ± 0.001	0.07 ± 0.01	0.043 ± 0.001	0.044 ± 0.001	nd	nd	nd	nd
16	0.059 ± 0.001	0.046 ± 0.001	0.071 ± 0.001	0.044 ± 0.001	0.044 ± 0.001	0.048 ± 0.001	nd	nd	nd
17	0.049 ± 0.001	0.045 ± 0.001	0.058 ± 0.001	0.042 ± 0.001	0.043 ± 0.001	nd	nd	nd	nd
18	0.114 ± 0.001	0.086 ± 0.001	0.058 ± 0.001	0.468 ± 0.001	0.055 ± 0.001	0.056 ± 0.001	0.398 ± 0.003	0.436 ± 0.006	0.391 ± 0.004
19	0.669 ± 0.003	0.502 ± 0.001	0.72 ± 0.001	0.468 ± 0.001	0.466 ± 0.001	0.442 ± 0.001	nd	nd	nd
20	0.438 ± 0.001	0.435 ± 0.001	nd	nd	0.435 ± 0.001	nd	nd	nd	nd
21	nd	nd	nd	0.464 ± 0.001	0.472 ± 0.001	0.461 ± 0.001	nd	nd	nd
22	0.667 ± 0.006	0.485 ± 0.001	0.741 ± 0.003	0.452 ± 0.001	0.485 ± 0.001	0.435 ± 0.001	nd	nd	nd
TPA	0.266 ± 0.002^{a}	0.249 ± 0.002^{b}	0.269 ± 0.005^{a}	0.197 ± 0.004^{c}	0.044 ± 0.001^{g}	0.174 ± 0.001^{f}	0.177 ± 0.001^{e}	$0.193 \pm 0.001^{c,d}$	0.174 ± 0.001^{f}
TF	$2.67 \pm 0.01^{a,b}$	2.315 ± 0.004^{e}	2.55 ± 0.02^{c}	2.14 ± 0.01^{f}	2.72 ± 0.01^{a}	1.90 ± 0.01^{h}	0.216 ± 0.002^{f}	0.233 ± 0.003^{d}	$0.211 \pm 0.001^{f,g}$
TS	0.114 ± 0.01^{d}	0.086 ± 0.001^{e}	0.057 ± 0.001^{f}	0.468 ± 0.001^{b}	0.055 ± 0.001^{f}	0.056 ± 0.001^{f}	0.398 ± 0.003^{c}	0.86 ± 0.01^{a}	0.391 ± 0.004^{c}
TPC	3.05 ± 0.02^{a}	2.65 ± 0.01^{d}	2.873 ± 0.02^{c}	2.81 ± 0.01^{b}	2.82 ± 0.01^{b}	2.13 ± 0.001^{e}	$0.79 \pm 0.01^{f,g}$	0.86 ± 0.01^{f}	$0.776 \pm 0.004^{f,g}$

TPA- Total Phenolic Acids, TF- Total Flavonoids, TS- Total Stilbene, TPC- Total Phenolic compounds. In each line, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters. All compounds were quantified using standards, namely vanillic acid (peak 1) ($y = 29751x - 28{,}661$, $R^2 = 0.9987$; LOD = 0.20 µg/mL; LOQ = 0.59 µg/mL), syringic acid (peaks 2 and 6) ($y = 53993x + 4671.4$, $R^2 = 0.998$; LOD = 0.50 µg/mL; LOQ = 0.98 µg/mL), sinapic acid (peaks 3 and 8) ($y = 197.337x + 30{,}036$; $R^2 = 0.999$; LOD = 0.17 µg/ml; LOQ = 1.22 µg/ml), *p*-coumaric acid (peak 4) ($y = 301{,}950x + 6966.7$; $R^2 = 0.999$; LOD = 0.68 µg/ml; LOQ = 1.61 µg/ml), luteolin-6-C-glucoside (peaks 5, 9, 11–17) ($y = 4087.1x + 72{,}589$, $R^2 = 0.999$, LOD = 0.86 µg/mL; LOQ = 1.67 µg/mL, ferulic acid (peak 7) ($y = 633126x - 185{,}462$, $R^2 = 0.9990$; LOD = 0.20 µg/mL; 1.01 µg/mL), quercetin-3-*O*-glucoside (peak 10, 21) ($y = 34843x - 160{,}173$; $R^2 = 0.9998$; LOD = 0.21 µg/mL; LOQ = 0.71 µg/mL), resveratrol (peak 17) ($y = 54835x - 29{,}986$; $R^2 = 0.9936$; LOD = 0.21 µg/mL; LOQ = 0.82 µg/mL), and apigenin-7-*O*-glucoside (peaks 19–20, 22) ($y = 10683x - 45{,}794$; $R^2 = 0.999$; LOD = 0.10 µg/mL; LOQ = 0.53 µg/mL)

$[M\text{-}H]^-$ at *m/z* 665) by comparing their retention times and UV spectrum with available standards. Icariside E5 (peak 17; λ_{max}, 259; $[M\text{-}H]^-$ at *m/z* 521) was the only stilbene identified in all of the analyzed parts of waste from colored bell pepper fruits.

The quantitative analysis of phenolic compounds in hydroethanolic extracts from waste from colored bell pepper fruits, salks, and seeds revealed distinct profiles among the different parts and varieties. Waste-orange bell pepper stalks had the highest total phenolic content (3.05 ± 0.02 mg/g, Figure 3) among all samples. Luteolin-*O*-(apiosyl)-hexoside (0.502–0.72 mg/g extract) and luteolin-*O*-(apiosyl-malonyl)-hexoside (0.485–0.741 mg/g extract), in turn, were most prevalent in the stalks and fruits, while Icariside E5 (0.391–0.436 mg/g extract) predominated in the seeds, which overall contained the lowest total phenolic levels. Abdalla et al. [44] reported similar findings in fresh yellow, red, and Balady green bell peppers, where phenolic acids and flavonoids were the main classes of compounds. However, they identified hesperidin as the most abundant phenolic in red and green varieties (1513.13 and 1065.65 µg/g extract, respectively), followed by compounds like catechin, pyrogallol, luteolin-7-glucose, *p*-OH-benzoic acid, apigenin-6-rhamnose-8-glucose, rutin, naringin, quercetin, ellagic acid, and various benzoic acids. Additionally, their study identified simple phenols and free flavonoids, indicating a broader phenolic diversity compared to the current research. These differences can be attributed to genetic, soil,

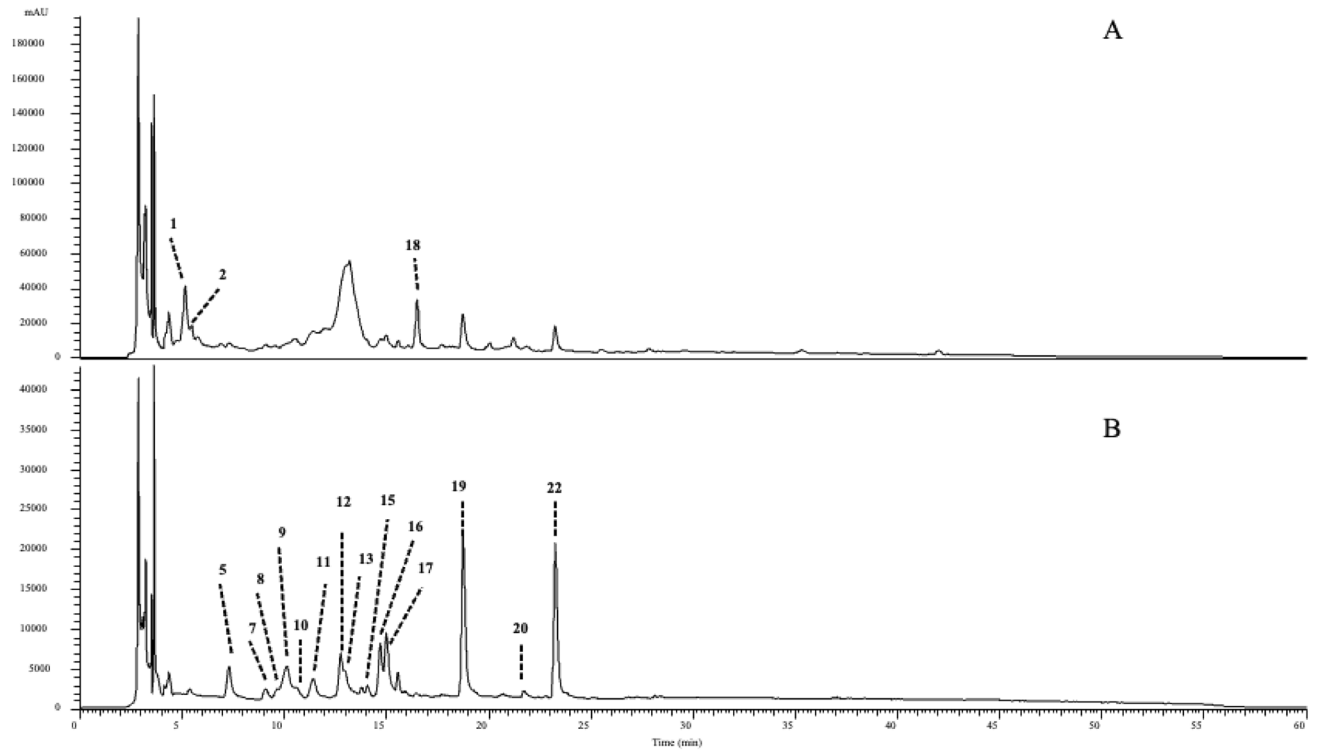

Fig. 3 Phenolic profile of hydroethanolic extracts of waste from colored orange bell pepper stalks, recorded at 280 nm **A** and 370 nm **B**. The peaks are identified in Table SM2

and climatic factors, as well as the use of waste bell peppers in the present study, which were in an advanced maturation stage. Maturation is a critical factor influencing polyphenol accumulation, as noted by Shaha and colleagues [45], who suggested that the phenolic profile and concentrations in peppers are predominantly determined by the fruit's maturation state. This could explain both the phenolic composition and the reduced overall quantities observed in this study. Similarly, Razola-Diaz et al. [41] identified 38 phenolic compounds in whole yellow bell peppers, including phenolic acids and flavonoids. Phenolic acids dominated, accounting for 66–93% of the total profile, with feruloyl glucose, sinapic acid-*O*-hexoside, and vanillic acid 1-*O*-D-glucopyranosylester being the most abundant. Among flavonoids, kaempferol-3-(3″-acetyl-alpha-L-arabinopyranosyl)-glucoside was prominent, alongside substantial amounts of quercetin, luteolin, and apigenin derivatives. These variations also highlight the influence of genetic, environmental, and maturation factors on phenolic profiles.

Specifically, extracts rich in phenolic compounds and natural pigments could be explored as natural antioxidants or colorants in food formulations, as active ingredients in nutraceuticals or dietary supplements, and as bioactive components in cosmetic products due to their antioxidant and antimicrobial properties.

Volatile Compounds

The tentative identification of volatile organic compounds (VOCs) in hydroethanolic extracts of waste from colored bell pepper, along with their retention times (Rt) and quantification, is detailed in Table 4. A total of 34 VOCs were detected, including eight heterocyclic compounds, seven acids, six esters, three alcohols, two amines, two alkanes, and one compound per each of the following classes: acetal, aldehyde, ether, glyceride, ketone, and ketose. The VOC profile varied across the different varieties of waste from colored bell pepper fruits, influenced by their biochemical characteristics and maturation stages. In waste-red bell peppers, dihydroxyacetone (9.49%), glyceraldehyde (6.20%), 4, 5, 7-trihydroxyoct-2-enoic acid (5.52%), methyl pyruvate (5.46%), and methyl 2-[(2-methoxy-2-oxoethyl)amino] acetate (5.46%) were the most abundant VOCs. Waste-orange bell peppers had dihydroxyacetone (7.86%) as the primary volatile compound, followed by methyl pyruvate (6.93%), 4, 5, 7-trihydroxyoct-2-enoic acid (5.52%), and methyl 2-[(2-methoxy-2-oxoethyl) amino] acetate (5.46%). In waste-green bell peppers, glyceraldehyde (8.94%), methyl pyruvate (7.14%), thymine (4.65%), and N-(ethoxycarbonyl)-glycine ethyl ester (4.29%) were dominant. A study by Forero et al [46]. on VOCs in green and red chili peppers (*C. annuum* var. *glabriusculum)* revealed similarities with the present findings, identifying esters, aldehydes,

Table 4 Volatile compounds found in waste-colored bell peppers fruits hydroethanolic extracts (mean ± SD, $n = 3$)

No	Retention time	Compound name	Class	Molecular formula	Relative percentage (%)		
					Orange	Red	Green
1	4.75	1,1-Dimethoxy-propane	Acetal	C5H12O2	3.20^{c}	4.13^{a}	3.67^{b}
2	4.98	Glyceraldehyde*	Aldehyde	C3H6O3	nd	6.20	8.94
3	5.72	Methyl pyruvate	Ester	C4H6O3	6.93^{b}	5.46^{c}	7.14^{a}
4	5.86	2-Hydroxyethyl acetate	Alcohol	C4H8O3	nd	nd	2.07
5	5.89	Dihydroxyacetone	Ketose	C3H6O3	7.86^{b}	9.49^{a}	5.10^{c}
6	5.94	N-Ethyl-4-methyl-2-pentanamine	Amine	C8H19N	nd	nd	2.66
7	6.04	(S)−1,3-Butanediol	Alcohol	C4H10O2	7.86	nd	nd
8	6.26	1,2-Cyclopentanedione	Ketone	C5H6O2	2.37c	3.07^{a}	2.94^{b}
9	6.97	2-Hydroxy-γ-butyrolactone*	Ester	C4H6O3	2.01	nd	2.94
10	6.97	2-Propen-1-ol	Alcohol	C3H6O	nd	2.85	nd
11	7.56	1,2-Dihydro-3H-1,2,4-triazol-3-one	HC	C2H3N3O	nd	nd	2.18
12	7.58	3-Methyl-2,5-piperazinedione	HC	C5H8N2O2	nd	nd	2.18
13	7.76	Thymine	HC	C5H6N2O2	3.39^{c}	4.07^{b}	4.65^{a}
14	8.39	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one*	HC	C6H8O4	nd	2.61	2.51
15	8.61	(S)−5-Hydroxymethyl-2[5H]-furanone*	HC	C5H6O3	nd	2.35	2.18
16	8.72	(S)-(+)−2′,3′-Dideoxyribonolactone	HC	C5H8O3	nd	2.08	nd
17	8.89	N-Vinylacetamide	Acid	C4H7NO	nd	2.51	nd
18	8.91	N-Acetyl-cyclobutylamine	Amine	C6H11NO	2.94	nd	nd
19	9.13	Glycerol α-monoacetate	Glyceride	C5H10O4	3.61	nd	nd
20	10.59	2-Oxo-butanoic acid	Acid	C4H6O3	4.38	nd	nd
21	10.59	2-Methyl-3-propyloxaziridine	HC	C5H11NO	4.38	nd	nd
22	10.76	2,4(3H,5H)-Furandione	HC	C4H4O3	nd	nd	2.02
23	10.96	Methyl (E.4S)−4-amino-5-methylhex-2-enoate	Ester	C7H13NO2	2.49	nd	nd
24	10.96	(3-Methyl-4-oxobut-2-enyl) acetate	Ester	C7H10O3	2.50	nd	nd
25	11.46	Hexadecane	Alkane	C16H34	nd	nd	2.28
26	11.48	N-(Ethoxycarbonyl)-glycine ethyl ester	Ester	C7H13NO4	nd	nd	4.29
27	11.52	2,5-Diethoxy-3-methyl-3-vinylhexane	Alkane	C13H26O2	nd	3.42	nd
28	11.52	Dipropyl ether	Ether	C6H14O	nd	2.41	nd
29	11.61	4,5,7-Trihydroxyoct-2-enoic acid	Acid	C8H14O5	5.52	nd	nd
30	11.62	Methyl 2-[(2-methoxy-2-oxoethyl)amino]acetate	Ester	C6H11NO4	5.46	nd	nd
31	11.69	3-Deoxy-D-mannonic acid	Acid	C6H12O6	nd	2.41	nd
32	11.69	N.N-Dimethylformamide	Acid	C3H7NO	nd	2.26	nd
33	14.27	(Z,Z)−9,12-Octadecadienoic acid	Acid	C18H32O2	2.35	2.26	nd
34	14.30	(Z,Z,Z)−9,12,15-Octadecatrienoic acid	Acid	C18H30O2	2.21^{b}	2.41^{a}	2.06^{c}

Volatile organic compounds identified in the samples considering relative content above 2.00%; RT – retention time; HC – heterocyclic compound; nd – not detected. In each line, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD) test, and are indicated by different letters. * Mean statistical differences obtained by *t*-Student test

ketones, alcohols, and acids in significant proportions. However, their study highlighted esters such as hexyl isopentanoate, limonene, and hexyl isohexanoate as major constituents, differing from the dominant compounds identified in this study. Another investigation by Korkmaz et al. [47] on sun-dried ripe pepper fruits (*C. annuum* cv. *Inan* 3363) identified 136 volatile compounds, predominantly terpenoids, aldehydes, alcohols, ketones, esters, and acids, with α-limonene, acetic acid, and n-butyl isobutyrate among the major components. Variations in VOC composition and content are influenced by species, cultivation conditions, and fruit development stages, as suggested by Qiu et al. [48] VOCs significantly contribute to the aroma and flavor quality of bell peppers, as shown in a study by Zhang et al. [49] on wrinkled green bell peppers, where 2-isobutyl-methoxypyrazine was a key aroma compound. In the current study, heterocyclic compounds were prevalent, including 1, 2-dihydro-3H-1, 2, 4-triazol-3-one, 3-methyl-2,

5-piperazinedione, and 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one. Heterocyclic compounds are valued for their strong and complex odors, making them significant as flavoring agents.

Bioactive Properties

Antioxidant and Cytotoxic Activities

Data on the antioxidant and cytotoxic activities of hydroethanolic extracts from waste from colored bell pepper fruits, stalks, and seeds are presented in Table 5. The extracts exhibited varying antioxidant capacities, with statistically significant differences ($p < 0.05$) observed among the different parts and varieties. In the stalk extracts, the waste-green bell peppers demonstrated the highest antioxidant activity, with the lowest EC_{50} value (0.36 ± 0.01 mg/mL), followed by red (0.37 ± 0.01 mg/mL) and orange (0.49 ± 0.02 mg/mL) varieties. For fruit extracts, waste-green bell peppers again showed the lowest EC_{50} value (0.44 ± 0.02 mg/mL), followed by red (0.95 ± 0.02 mg/mL) and orange (1.85 ± 0.05 mg/mL) varieties. Seed extracts from waste-green bell peppers also stood out, with the lowest EC_{50} value (0.21 ± 0.01 mg/mL), followed by red (0.86 ± 0.05 mg/mL) and orange (0.89 ± 0.02 mg/mL) varieties. These results highlight that all parts of the waste-green bell peppers exhibited the highest antioxidant activity across the analyzed samples. These findings align with a study by Thupairo et al. [50] who reported green bell peppers having the highest antioxidant activity among green, red, orange, and yellow varieties using DPPH, FRAP, and ORAC methods. This was attributed to higher ascorbic acid and phenolic contents, particularly *p*-coumaric and ferulic acids, in green and red peppers. In contrast, Chávez-Mendoza et al. [51] demonstrated slightly higher antioxidant activity in ethanolic extracts of red bell peppers compared to green and orange varieties using the DPPH assay. Martí et al. [52] however, found no significant differences in antioxidant activity between green and red peppers using the ABTS method. These variations may be due to the different methodologies employed, which target distinct free radicals and mechanisms, as well as differences in *Capsicum* cultivars. Stalk and seed extracts generally showed higher antioxidant potential compared to fruit extracts. This is consistent with findings by Chen et al. [53], who observed stronger antioxidant activity in red pepper stalk extracts than fruit extracts, attributed to higher concentrations of phenolic compounds like chlorogenic and *p*-coumaric acids in the firsts. Similarly, Sora et al. [54] demonstrated that seed extracts had higher antioxidant capacities than pulp extracts in bell peppers, associating this with the higher total phenolic content in seeds (409 mg GAE/g) compared to pulp (119 mg GAE/g).

Table 5 Antioxidant activity of waste-colored bell peppers fruits including their seeds and stalks hydroethanolic extracts

Antioxidant activity	TBARS
Stalk	
Orange	0.49 ± 0.02^{e}
Red	0.37 ± 0.01^{g}
Green	0.36 ± 0.01^{gh}
Fruits	
Orange	1.85 ± 0.05^{a}
Red	0.95 ± 0.02^{b}
Green	0.44 ± 0.02^{f}
Seeds	
Orange	0.89 ± 0.02^{c}
Red	0.86 ± 0.05^{d}
Green	0.21 ± 0.01^{i}

EC_{50} values (mg/mL, mean ± SD, $n = 3$): extract concentration corresponding to a 50% of antioxidant activity. Positive control – trolox (0.0054 ± 0.0003). In each colunm, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters

Antimicrobial Activity

The antibacterial activity of the hydroethanolic extracts prepared from waste from colored bell pepper fruits and their byproducts were evaluated using Gram-positive and Gram-negative bacteria categorized as food contaminants and clinical isolates. The results obtained from the evaluation are presented in Table 6. The results were expressed as the minimum inhibitory (MIC) and bactericidal (MBC) concentrations. The antibacterial assessment of hydroethanolic extracts from waste from colored bell pepper fruits, stalks, and seeds against food contaminants revealed notable inhibitory effects, particularly from fruit extracts. These extracts inhibited most bacterial strains, although *E. cloacae* and *P. aeruginosa* were less susceptible, requiring higher concentrations (MIC > 10 mg/mL). The strongest antibacterial activity was observed against *Y. enterocolitica* (MIC = 5 mg/mL for all samples), *B. cereus* (MIC = 5 mg/mL for orange and green peppers), and *S. aureus* (MIC = 5 mg/mL for all samples). Stalk extracts also showed activity against *Y. enterocolitica*, *B. cereus*, and *S. aureus*, with better results for orange and red peppers (MIC = 10 mg/mL), and excellent inhibition of *L. monocytogenes* by all varieties (MIC = 5 mg/mL). Seed extracts demonstrated antibacterial effects against *Y. enterocolitica* (red peppers, MIC = 2.5 mg/mL), *B. cereus* (orange peppers, MIC = 10 mg/mL), *L. monocytogenes* (red and green peppers, MIC = 10 mg/mL), and *S. aureus* (orange peppers, MIC = 5 mg/mL; red peppers, MIC = 10 mg/mL). None of the extracts were bactericidal up to the maximum

Table 6 Antibacterial activity of waste-colored bell peppers fruits including their seeds and stalks hydroethanolic extracts against food contaminants and clinical isolates strains

Gram-negative bacteria						Gram-positive bacteria		
Food contaminants	*E. cloacae*	*E. coli*	*P. aeruginosa*	*S. enterica*	*Y. enterocolitica*	*B. cereus*	*L. monocytogenes*	*S. aureus*
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Stalks								
Orange	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	5/> 10	10/> 10
Red	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	5/> 10	10/> 10
Green	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	5/> 10	> 10/> 10
Fruits								
Orange	> 10/> 10	10/> 10	> 10/> 10	10/> 10	5/> 10	5/> 10	10/> 10	5/> 10
Red	> 10/> 10	10/> 10	> 10/> 10	10/> 10	5/> 10	10/> 10	10/> 10	5/> 10
Green	> 10/> 10	10/> 10	> 10/> 10	10/> 10	5/> 10	5/> 10	10/> 10	5/> 10
Seeds								
Orange	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	> 10/> 10	5/> 10
Red	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	2.5/> 10	> 10/> 10	10/> 10	10/> 10
Green	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	> 10/> 10	10/> 10	> 10/> 10
Clinical isolates	*E. coli*	*K. pneumoniae*	*M. morganii*	*P. mirabilis*	*P. aeruginosa*	*E. faecalis*	*L. monocytogenes*	*MRSA*
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Stalks								
Orange	> 10/> 10	> 10/> 10	10/> 10	> 10/> 10	> 10/> 10	5/> 10	5/> 10	> 10/> 10
Red	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	5/> 10	10/> 10
Green	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	> 10/> 10
Fruits								
Orange	10/> 10	> 10/> 10	10/> 10	> 10/> 10	> 10/> 10	5/> 10	10/> 10	5/> 10
Red	> 10/> 10	> 10/> 10	10/> 10	> 10/> 10	> 10/> 10	5/> 10	10/> 10	5/> 10
Green	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	10/> 10
Seeds								
Orange	> 10/> 10	> 10/> 10	10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10
Red	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10
Green	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	> 10/> 10	10/> 10	10/> 10	> 10/> 10

MIC values correspond to the minimum extract concentration that inhibits the bacterial growth for the maximum tested concentration of 10 mg/mL; MBC – minimum bactericidal concentration; MRSA – Methicillin-resistant *Staphylococcus aureus*

concentration tested (10 mg/mL), and their activity was inferior to that of positive controls (streptomycin, methicillin, and ampicillin, Table SM1).

For clinical bacterial isolates, fruit extracts exhibited superior inhibitory capacity compared to stalk and seed extracts. The best results were against *E. faecalis* and MRSA, with orange and red peppers achieving MIC = 5 mg/mL. Conversely, *K. pneumoniae, P. mirabilis*, and *P. aeruginosa* were less susceptible (MIC > 10 mg/mL). Stalk extracts were effective against *L. monocytogenes* (orange and red peppers, MIC = 5 mg/mL), *E. faecalis* (orange peppers, MIC = 5 mg/mL), *M. morganii* (orange peppers), and MRSA (red peppers). Seed extracts were active against *M. morganii* (orange peppers), *E. faecalis* (green peppers), *L. monocytogenes* (red and green peppers), and MRSA (orange and red peppers). Notably, orange pepper stalk extracts, orange and red pepper fruit extracts, and orange pepper seed extracts showed better inhibitory effects against *M. morganii* than the positive control ampicillin. However, none of the extracts surpassed the efficacy of controls (ampicillin, imipenem, and vancomycin, Table SM1) against other clinical strains and were not bactericidal up to 10 mg/mL. Previous studies support these findings. Loizzo et al. [55] reported inhibition of *S. aureus* and *L. monocytogenes* by extracts from various *C. annuum* varieties, with MIC values ranging between 12.5 and 25 mg/mL for certain red pepper types. Ekom et al. [56] highlighted the antimicrobial activity of red bell peppers, with the Kahta variety showing low MICs against *S. aureus,* demonstrating that methanolic extracts of *C. annuum* fruits inhibited all tested bacteria in a wound model, with MIC as low as 64 µg/mL. These authors attributed the antibacterial activity of bell peppers to their phenolic, flavonoid, and tannin contents, which disrupt bacterial processes such as biofilm formation, ATPase activity, and membrane integrity,

causing leakage of cellular components like nucleic acids, sugars, and proteins.

The antifungal activity of hydroethanolic extracts from waste from colored bell pepper fruits, stalks, and seeds was evaluated against two fungal strains, *A. brasiliensis* and *A. fumigatus*, as summarized in Table 7. The results demonstrated that all tested extracts exhibited antifungal properties against both strains. *A. brasiliensis* was the most susceptible, particularly to the fruit extracts. Waste-red bell pepper extracts showed the strongest inhibitory effect (MIC = 1.25 mg/mL), followed by orange (MIC = 2.5 mg/mL) and green varieties (MIC = 5 mg/mL). For stalk extracts, waste-red bell peppers also had the best activity (MIC = 2.5 mg/mL), while orange and green varieties displayed similar activity (MIC = 5 mg/mL). Among seed extracts, waste-red and green bell peppers provided the strongest antifungal effect (MIC = 2.5 mg/mL), with waste-orange bell peppers following (MIC = 5 mg/mL). Regarding *A. fumigatus*, all extracts demonstrated inhibitory activity (MIC = 10 mg/mL). However, none of them were fungicidal against either fungal strain up to the maximum concentration tested (10 mg/mL). Additionally, their antifungal activity did not surpass the positive control, ketoconazole. These findings align with prior research. Añibarro-Ortega et al. [57] reported significant antifungal activity from Holland and Italian bell pepper extracts, which effectively inhibited *A. fumigatus* (MIC = 1.54 and 1.52 mg/mL, respectively) and performed better than positive controls against other fungal strains. López-Muñoz et al. [58] noted the antifungal activity of ethanolic red bell pepper fruit extracts (*C. annuum*) against *F. andiyasi* and *Cochliobolus* spp., attributing this to phenolic compounds that disrupt fungal cell walls, ultimately leading to cell death. Similarly, Hajji Hedf et al. [59] demonstrated antifungal potential of *C. annuum* seed extracts, which significantly inhibited the growth of *Botrytis cinerea*, a pathogen responsible for gray mold disease in tomato crops.

Table 7 Antifungal activity from waste-colored bell pepper fruits including their seeds and stalks hydroethanolic extracts

Fungal strains	*A. brasiliensis* MIC/MFC	*A. fumigatus* MIC/MFC
Stalks		
Orange	5/> 10	10/> 10
Red	2.5/> 10	10/> 10
Green	5/> 10	10/> 10
Fruits		
Orange	2.5/> 10	10/> 10
Red	1.25/> 10	10/> 10
Green	5/> 10	10/> 10
Seeds		
Orange	5/> 10	10/> 10
Red	2.5/> 10	10/> 10
Green	2.5/> 10	10/> 10
Ketoconazole	0.06/0.125	0.5/1

MIC – Minimum inhibitory concentration; MFC – Minimum fungicidal concentration

The valorization of bell pepper waste aligns closely with the principles of the circular economy, which seek to minimize resource loss and promote the sustainable reuse of agricultural by-products. By demonstrating that discarded fruits, seeds, and stalks retain considerable levels of bioactive compounds and exhibit significant antioxidant and antimicrobial activities, our results highlight their potential as valuable raw materials for high-value applications. These waste fractions could be utilized for the extraction of phenolic compounds and natural pigments for use in the food, nutraceutical, and cosmetic industries, or as bio-based ingredients in the formulation of functional products. Integrating such valorization strategies into the production chain could not only reduce postharvest losses and environmental impact but also generate new economic opportunities, contributing to a more sustainable and resource-efficient agri-food system.

The valorization of bell pepper waste presents significant potential for scalability, as large quantities of residues are generated during agricultural and processing activities. These processes, including the extraction of bioactive compounds, composting, and conversion into biofuels or bioplastics, can be integrated into existing agro-industrial systems [60]. Economically, the utilization of such residues as low-cost raw materials reduces disposal costs and generates new revenue streams through the production of high-value compounds such as natural pigments and antioxidants [61]. Environmentally, these valorization strategies reduce organic waste accumulation, minimize greenhouse gas emissions, and promote sustainable resource use, thereby aligning with circular economy principles [62].

Conclusions

The increasing waste generated by the agri-food industry and the limited availability of natural resources have prompted research into recovering bioactive compounds from food residues to reduce environmental impacts and food loss. Waste from colored bell pepper, discarded due to market imperfections, present a valuable opportunity for sustainable valorization. These peppers demonstrate a rich proximal and biochemical composition, including fructose, glucose, oxalic acid, malic acid, polyunsaturated fatty acids, α-tocopherol, β-carotene, chlorophyll a, phenolic acids, flavonoids, and a diverse range of volatile organic compounds (VOCs). Their hydroethanolic extracts show promising bioactive properties,

including antioxidant, antibacterial, and antifungal activities, particularly in green and red bell peppers. Extracts from fruits exhibited the strongest antibacterial activity, especially against *Y. enterocolitica*, *B. cereus*, *S. aureus, E. faecalis*, and MRSA, though none were bactericidal. Antifungal activity was observed, with *A. brasiliensis* being the most susceptible, particularly to red pepper extracts, though no fungicidal effects were recorded.

These findings indicate that waste from colored bell peppers could be used as natural preservatives in the food industry or as sources of bioactive compounds for pharmaceutical and cosmetic applications. Valorizing such waste supports the circular bioeconomy, promoting resource efficiency and a more sustainable food system.

To the best of our knowledge, this is the first study to provide an integrated evaluation of fruits, seeds, and stalks from three color varieties of waste from bell peppers, combining advanced chromatographic and spectrometric techniques (HPLC, GC–MS, LC-DAD-ESI/MS^n) with a comprehensive assessment of phenolic compounds, antioxidant, and antimicrobial activities, including tests against clinical isolates.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interests The authors have no relevant financial or non-financial interests to disclose.

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