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Linking the chemistry and physics of food with health and nutrition

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ARTICLE

Integrating sustainability and nutrition: comprehensive valorisation of vegetable by-products for bioactive food applications

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Valorising vegetable by-products is a sustainable and innovative strategy to reduce food waste and develop functional ingredients. This study investigated onion peel (Allium cepa L.), kale (Brassica oleracea L. var. Acephala), zucchini (Cucurbita pepo L.), and their combined mixture, materials often discarded as a result of market quality standards. Hydroethanolic extracts and their solid residues were analyzed. The extracts were evaluated for their phenolic content and antioxidant, antidiabetic, and antimicrobial activities. The residues were assessed for their proximate and chemical composition to support their valorization within a zero-waste strategy. Onion peel extract showed the highest phenolic content and antioxidant capacity, with strong α-glucosidase inhibition (IC50: 64.4 µg/mL). Additionally, onion peel and kale extracts showed antimicrobial activity. Among residues, onion peel had the highest dietary fiber (69 g/100 g), and zucchini had the highest protein content (30.7 g/100 g), including essential amino acids. This integrated and scalable approach adds value to both extract and residue, promoting sustainable, circular food systems aligned with the Sustainable Development Goals of the 2030 Agenda.

1. Introduction

The challenges of achieving sustainability in the agri-food sector are highly relevant globally. Issues such as climate change, inefficient use of natural resources, inconsistent annual production, and food waste must be addressed.[1] Food waste is defined by the Food and Agriculture Organization of the United Nations as: *Food waste refers to food appropriate for human consumption being discarded, whether or not after it is kept beyond its expiry date or left to spoil. Often this is because food has spoiled, but it can be for other reasons, such as oversupply due to markets, or individual consumer shopping/eating habits.* [2]

Improper disposal in landfills contributes to greenhouse gas emissions, soil contamination, wasted energy, and additional costs related to transportation and storage, exacerbating issues like hunger and food security.[3]

Fruits and vegetables are responsible for approximately 32% of total food waste, with a substantial loss of essential nutrients for human health.[4] Mitigating this waste and improving the efficient use of these resources aligns with the Sustainable Development Goals (https://sdgs.un.org/goals, accessed on January 28, 2025), particularly Goal 12 "Responsible consumption and production".

According to the FAO statistical database, FAOSTAT (https://www.fao.org/faostat/en/#home, accessed January 29, 2025), global vegetable production in 2022 is estimated at 1 billion tons, of which just over 114 million tons will be lost, and about 1.4 million tons will be processed. During processing, by-products such as peels, seeds, pulp, and stalks are generated, all of which can be reused and valorised. Additionally, a significant volume of vegetable by-products arises from produce that fails to meet strict aesthetic standards for marketing and consumer preferences, which also presents an opportunity for reuse and valorisation.[5] Among these vegetables are onions (*Allium cepa* L.), kale (*Brassica oleracea* L.), and zucchini (*Cucurbita pepo* L.), with onions being one of the world's most important commodities, with a production of about 100 million tons.[6]

The high production of onions is directly related to their routine consumption as an ingredient in cooking, which results in a large generation of by-products such as onion peels. The scientific and industrial community is very interested in finding ways to reuse onion peels due to their rich nutritional value, such as their high content of dietary fiber and phenolic compounds, such as flavonoids, which can be used as food ingredients.[7] Onion peels have the highest flavonoid content than other parts, with quercetin being the major flavonoid found, with highly interesting bioactive properties such as

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antioxidant, antimicrobial, and anti-inflammatory properties, among others.[8]

Phenolic compounds of interest are also found in kale and zucchini by-products. Kale contains quercetin and kaempferol glycosides, as well as ferulic and caffeic acid derivatives as major compounds.[9] Zucchini mainly contains hydroxycinnamic acid derivatives and flavonoids, such as catechins, quercetins, and kaempferol glycosides.[10] These compounds exhibit high biological activity, including antioxidant, anticancer, and antidiabetic effects, in addition to protecting the cardiovascular and gastrointestinal systems.[10-12]

Within the different valorization routes that exist for the reuse of by-products from these vegetables, the extraction of phenolic compounds for potential use as food ingredients and nutraceuticals has been important in the current global scenario.[1] However, a promising and still little explored strategy is the use of waste generated in the extraction process of bioactive compounds. Most studies focus exclusively on extracts as a route for valorizing plant by-products.[13-15] However, the waste from extraction can also be valorized as sources of biopolymers, such as carbohydrates, sugars, dietary fibers, and proteins. These biopolymers, in addition to being biodegradable and derived from renewable sources, have great potential for the formulation of new food ingredients and sustainable materials, contributing to the reduction of waste and the development of environmentally friendly products.[16] Although studies on the extraction of phenolic compounds are widely discussed, the simultaneous valorization of waste for the production of biopolymers remains an area that has not yet been fully explored in the literature, representing an important gap. Combining these two processes can provide sustainable solutions for the food industry, meeting the growing consumer demand for natural and sustainable foods.[5]

Therefore, this study aims to explore valorization strategies for onion, zucchini, and kale by-products, vegetable materials that are commonly discarded, by extracting phenolic compounds and characterizing the resulting residues, with the overarching goal of supporting a zero-waste approach throughout the process. For this purpose, hydroethanolic extracts of each vegetable by-product were obtained to analyze their phenolic profile and bioactive action (antioxidant, antimicrobial, and antidiabetic), while extraction residues were assessed for their proximate composition and chemical characterization to evaluate their potential applications in the food industry.

2. Materials and methods

2.1. Samples

By-products of kale (*Brassica oleracea L.* var. Acephala), zucchini (*Cucurbita pepo* L.), and yellow onion peel (*Allium cepa* L.) were supplied from Campotec S.A., a company located in Torres Vedras, Portugal, in July 2023. The samples used were vegetable by-products that did not meet commercial standards and were discarded, except onions, which used stems, bulbs, and outer and inner onion layers. Initially, the samples were received, washed, frozen, and dehydrated by freeze-drying individually (FreeZone 4.5, Labconco, Kansas City, MO, USA). Afterward, the samples were ground into small particles and stored under refrigeration until extraction. Following extraction, the solutions were filtered, and the resulting extracts were coded as CB (onion peel), CT (zucchini), CG (kale), and MIX (1:1:1 mixture of kale, zucchini, and onion peel). The solid residues retained on the filter were designated as RCB (onion peel residue), RCT (zucchini residue), RCG (kale residue), and RMIX (residue from the mixed extraction).

2.2. Extraction procedure

The extraction process was performed individually for four samples: 1) onion peel; 2) zucchini; 3) kale; and 4) a mixture of onion peel, zucchini, and kale in a 1:1:1 ratio to evaluate the performance of these vegetable by-products together. Ethanol/water (80:20, v/v) was used as the solvent at room temperature, with magnetic stirring (150 rpm) and a solid/liquid ratio of 1:30 g/mL for 2 hours, according to the methodology described by Silva et al.[17] For further analysis, the extract and the extraction residue were dehydrated by freeze-drying (FreeZone 4.5, Labconco, Kansas City, MO, USA) and stored under refrigeration.

2.3. Characterization of hydroethanolic extracts

2.3.1. **Identification and quantification of phenolic compounds profile.** The phenolic compound profiles of CB, CT, and MIX were identified and quantified using a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA). Detection was performed with a diode array detector (DAD) coupled to a mass spectrometer (LTQ XL, Thermo Finnigan, San Jose, CA, USA) operating in negative ion mode. Data acquisition was managed through the Xcalibur® software (Thermo Finnigan, San Jose, CA, USA). The identification of phenolic compounds was performed based on chromatographic behaviour, including retention times, UV-Vis absorption spectra, and MS/MS fragmentation patterns, through comparison with reference standards and literature data. The assignment of sugar moieties was based on characteristic neutral losses observed in MS/MS spectra (e.g., 162 Da for hexosides and 146 Da for deoxyhexoside), and therefore specific sugar identities and linkage positions were not assigned unless confirmed by comparison with authentic standards or were well established in the literature data. For the quantification of phenolic compounds, calibration curves were constructed using appropriate standard compounds, and the results were expressed as mg/g of dry extract. The calibration curves used were quercetin-3-*O*-glucoside ($y = 34843x - 160173$, $R^2 = 0.99$) and quercetin-3-*O*-rutinoside ($y = 9617.8x - 4374.7$, $R^2 = 0.99$).

For the obtention of the CG phenolic compound profile was analyzed by high-performance liquid chromatography coupled to a diode detector and mass spectrometer (HPLC-DAD-ESI-MS/MS) operating under the conditions proposed by Bessada et al.[18]. For that purpose, the hydroethanolic extracts were redissolved in ethanol/water (80:20, v/v) to a final concentration of 10 mg/mL and filtered using a 0.22 µm disposable filter disc. The LC system was coupled to an Orbitrap Exploris 120 mass spectrometer (Thermo Scientific, Hemel Hempstead, UK), equipped with an electrospray ionization source. The mass spectrometer was operated in the negative mode, and parameters were as follows: source voltage, 2.5 kV; sheath gas, 50 a.u. (arbitrary units); auxiliary gas, 10 a.u.; sweep gas, 1 a.u.; capillary temperature, 325 ºC; and vaporizer temperature at 350 °C. Full scan resolving power was 60,000, and data-dependent MS/MS events were collected at a resolving power of 15,000. High-

energy collisional dissociation was conducted with a normalized collision energy of 30%. The monitored mass range was from m/z 100 to 1500. The data analyses and instrument control were performed with Xcalibur 4.6 software (Thermo Fisher Scientific). The identification of compounds was achieved by comparing the obtained retention times, UV-Vis, and mass spectra with those of the available standards. When standards were not available, the compounds were identified based on the fragmentation pattern and data from the literature. The identified compounds were quantified using the calibration curves: kaempferol-3-O-glucoside (y = 27328x + 2683.3, R2 = 0.99) and quercetin-3-O-glucoside (y = 28555x + 3032.3, R2 = 0.99).

2.3.2. **Antioxidant activity assessment.** To measure antioxidant activity, two different colorimetric assays were used: lipid peroxidation inhibition using thiobarbituric acid reactive substances (TBARS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The methodologies followed those detailed by Barros et al.[19], with a slight modification in the DPPH assay, the solvent used for dilution matched that of the extraction process (80:20 ethanol/water v/v). Potassium metabisulfite, a common antioxidant additive, was used as a control. Results were reported as EC50 values (µg/mL), representing the concentration needed to achieve 50% antioxidant efficacy.

2.3.3. **Antimicrobial activity assessment.** The antimicrobial potential of the extract was evaluated using a protocol adapted from Soković and van Griensven [20] and Soković et al.[21] The microorganisms for this assay were sourced from the Mycology Laboratory in the Institute for Biological Research "Siniša Stanković"- National Institute of the Republic of Serbia, University of Belgrade, Serbia. For antibacterial testing, Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030), *Salmonella Typhimurium* (ATCC 13311), and Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (NCTC 7973) were included. Antifungal activity was assessed against six micromycetes: *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), Penicillium funiculosum (ATCC 36839), *Penicillium verrucosum* var. cyclopium (food isolate), and *Trichoderma viride* (IAM 5061). Sodium benzoate (E211) and potassium metabisulfite (E224), common synthetic preservatives, served as positive controls for the antimicrobial tests. Results were expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) in mg/mL, utilizing the microdilution method for quantification.

2.3.4. Antidiabetic activity assessment. The antidiabetic activity was assessed by the inhibition potential of the *α*-glucosidase enzyme, following the method previously described by Cano-Lou et al.[22]. The *α*-glucosidase enzyme was obtained from *Saccharomyces cerevisiae* (Sigma-Aldrich, Barcelona, Spain), and acarbose was used as a positive control. The results were expressed in IC50 values (mg/mL), representing the concentration needed to inhibit 50% of enzyme activity.

2.4. Characterization of solid residue resulting from phenolic compound extraction

Understanding the nutritional value and potential uses of the by-products generated during the phenolic compound extraction process requires characterization of the residue. Furthermore, the combination of valorization routes for phenolic compounds and extraction residues has not been widely explored in the literature. With this, the extraction residue was characterized based on proximate composition (total fat, ash, crude protein, real protein content, and total, soluble, and insoluble dietary fibers) and chemical composition (free sugars, organic acids, and amino acids profile). Four samples were analyzed for this purpose: 1) residue of onion peel extraction (RCB), 2) residue of zucchini extraction (RCT), 3) residue of kale extraction (RCG), and 4) residue from a mixture of onion peel, zucchini, and kale extraction (RMIX).

2.4.1. Proximate composition. For the determination of value-proximate nutritional composition, the methods for total fat (AOAC 989.05), ash (AOAC 935.42), and crude protein content (AOAC 991.02) were performed according to AOAC procedures [23] and as described by Melgar et al.[24] Crude protein was calculated from total nitrogen using a conversion factor of 6.25. Real protein content was determined following the methodology described by Machado et al.[25] The total dietary fibers (TDF), insoluble dietary fibers (IDF), and soluble dietary fibers (SDF) content were determined by the AOAC 991.43 method, using the Kit K-TDFR-200A 04/17 (Megazyme, Ireland).

2.4.2. Chemical composition. The chemical composition of the extraction residues from vegetable by-products was assessed by determining the profile of free sugars, organic acids, and amino acids. Free sugars were analyzed according to the method described by Barros, Pereira, and Ferreira[26], using high-performance liquid chromatography coupled with a refractive index detector (HPLC-RI). Data processing was performed using Clarity 2.4 software (DataApex, Prague, Czech Republic), and identification was based on the retention times of standard sugars. Results were expressed as g/100 g of dry weight (dw).

The profile of organic acids was determined following the procedure outlined by Barros et al.[27], employing ultra-fast liquid chromatography (UFLC; Shimadzu 20A series, Kyoto, Japan) with a photodiode array detector. Identification and quantification were carried out by comparing chromatographic peaks with those of standard organic acids. Results were expressed as mg/100 g of dw.

To determine the total amino acid profile, two hydrolysis methods were performed: alkaline hydrolysis with 4 M KOH (110 ºC, 4h) to determine tryptophan content and acid hydrolysis with 6 M HCl (110 ºC, 24h) to quantify the remaining amino acids, following the methodology previously described by Machado et al.[25] The hydrolysates were analyzed using an HPLC system (Jasco, Japan) equipped with a fluorescence detector (Jasco FP-2020 Plus). An automated derivatization process combining two reagents, OPA/3-MPA and FMOC, via a high-performance autosampler (AS-4150 RHPLC, Jasco, Japan) was performed. Amino acid separation was

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carried out using a ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 µm) from Agilent Technologies (USA).[25]

2.5. Statistical analysis

Statistical analysis was conducted on the antioxidant and antidiabetic activities of the extracts, as well as on the proximate composition and chemical characterization of the extraction residues. All samples were analyzed in triplicate. One-way analysis of variance (ANOVA) was used to compare three or more means. If the assumption of homogeneity of variances was met ($p > 0.05$), Tukey's honestly significant difference (HSD) test was applied to determine which groups differed from each other. For comparisons between two means, Student's t-test was used. Before analysis, normality and homogeneity of variance of the data were checked using the Shapiro-Wilk and Levene tests, respectively. All statistical procedures were performed at a 5% significance level using IBM SPSS Statistics software (version 22.0, IBM Corp, Armonk, NY, USA).

3. Results and discussion

3.1. Phenolic compounds profile of hydroethanolic extracts

The profiles of phenolic compounds of hydroethanolic extracts of onion peel, zucchini, and kale, and the mixture of these matrices were analyzed by LC-DAD-ESI-MS/MS, and the tentative identification and quantification are presented in **Table 1**. Sugar moieties were assigned based on MS/MS fragmentation patterns and are reported according to the level of structural information supported by the data. The chromatograms of the analyzed samples are shown in **Figure 1**.

3.1.1. **Onion peel extract (CB).** Compounds **1** and **2**, with a molecular ion at *m/z* 625, showed MS^2 fragments at *m/z* 463 ($[M-H-162]^-$) and 301 ($[M-H-162-162]^-$), corresponding to the successive loss of two hexose units (162 Da), resulting in the deprotonated aglycone of quercetin ($[M-H]^-$ at m/z 301). This successive loss of hexoses indicates that these hexoses were attached to different carbons in the aglycone.[28, 29] Therefore, these compounds were tentatively identified as quercetin-*O*-hexoside-*O*-hexoside.

Compound **3**, with a deprotonated molecule at *m/z* 639, exhibited fragmentation ions at *m/z* 477, 315, and 300 in MS^2. The fragmentation pattern suggests the combined loss of two hexose units ($[M-H-324]^-$), supported by the high relative abundance of the ion at *m/z* 315. Additionally, a loss of 15 Da was observed in MS^2, which is characteristic of methoxylated flavonoids like isorhamnetin.[30] Thus, the compound was assigned as isorhamnetin-*O*-dihexoside. The same fragmentation pathway, with one hexose less, was observed for compound 6, with $[M-H]^-$ ion at *m/z* 477 and fragments at *m/z* 315 and 300 (MS^2), which was identified as isorhamnetin-*O*-hexoside.[31]

Compound **4**, with a deprotonated molecule at *m/z* 463, was identified as quercetin-3-*O*-glucoside by comparison with a reference standard, based on retention time, UV-Vis spectra, and MS^2 fragmentation pattern. Compound **5** also presented the same molecular ion and the deprotonated aglycone at *m/z* 301 in MS^2. Furthermore, both compounds **4** and **5** exhibited a loss of one hexose unit ($[M-H-162]^-$), which suggests they may be isomers of the same compound. The observed difference in retention time, combined with the confirmation of compound 4 using a reference standard, suggests that compound 5 may differ in the position of the sugar moiety on the aglycone. This structural variation likely accounts for its later elution.[32] Similar findings have been reported, showing that positional isomers of flavonoid glycosides can display different retention times despite having comparable molecular masses and fragmentation patterns.[33] Thus, compound **5** was assigned as quercetin-*O*-hexoside. A similar pattern was also observed in the studies[34,28].

CB presented the highest concentration of total phenolic compounds among all samples, with 24.62 ± 0.05 mg/g extract. Six compounds were tentatively identified, all of them belonging to flavonoids, specifically flavonols. Compounds **2** and **5** were identified as the predominant constituents, corresponding to quercetin-*O*-hexoside-*O*-hexoside (8.2500 ± 0.0003 mg/g extract) and quercetin-*O*-hexoside (12.1 ± 0.1 mg/g extract), respectively. This result highlights the dominance of the derivatives of quercetin glycosides in onion peel, in agreement with previous studies.[30, 35-37] Quercetin is a flavonol widely associated with strong radical-scavenging activity, inhibition of lipid peroxidation, and modulation of inflammatory pathways[38, 39], and these well-documented mechanisms align with the enhanced bioactivity observed (discussed below) for the CB extract in this study.

3.1.2. **Zucchini extract (CT).** The phenolic compound profile of CT led to the identification of five flavonoids, all of them being flavonols. Compounds **1** and **2** presented the same fragmentation pattern, characterized by the cleavage of three sugar units ($[M-H-146-146-162]^-$). This profile indicates the presence of two deoxyhexose moieties, likely rhamnose, and one hexose unit, consistent with previous reports.[40, 41] Aglycones at *m/z* 301 and 285, suggest that these compounds corresponded to quercetin-*O*-dirhaminoside-hexoside and kaempferol-*O*-dirhaminoside-hexoside, respectively.[42, 43] Compound **3** exhibited a deprotonated molecular ion at *m/z* 769, with intense MS^2 fragments at 623, 315, and 300. The fragmentation pattern indicates an initial loss of 146 Da, attributed to a rhamnoside unit, followed by a loss of 308 Da, consistent with a rutinoside moiety. The fragments at *m/z* 315 and 300 are characteristic of the isorhamnetin aglycone, assigning the name isorhamnetin-*O*-rutinoside-rhamnoside. Compound **5** ($[M-H]^-$ *m/z* 623) also presented MS^2 fragments at *m/z* 315 and 300, related to the deprotonated aglycone of isorhamnetin. The observed loss of 308 Da is consistent with a rutinoside group[44], supporting its identification as isorhamnetin-*O*-rutinoside. Compound **4** was identified as quercetin-*O*-rutinoside, also known as rutin, by comparison with an authentic standard, based on retention time, UV spectrum, and MS fragmentation data. The total concentration of these phenolic compounds was 2.350 ± 0.001 mg/g extract.

The same flavonoids were identified in whole zucchini in the study carried out by Iswaldi et al.[42], in the study carried out by Piccolella et al.[45] with *Cucurbita pepo* cv. Lungo Fiorentino, and in the study carried out by Leichtweis et al.[43], except for rutin, where they analyzed different parts of the zucchini.

Table 1. Retention time (Rt), wavelengths of maximum absorption in the UV-vis region (λ_{max}), mass spectra data (m/z), MS^2 fragment ions (in brackets, relative abundances), tentative identification and quantification of the phenolic compounds present in the hydroethanolic extracts of onion peel (CB), courgette (CT), kale (CG) and a mixture of onion peel, courgette and kale by-products (MIX) (mean ± SD).

			CB			
Peak	**Rt (min)**	**λ_{max} (nm)**	**$[M-H]^-$**	**MS^2**	**Assignment**	**Quantification (mg/g extract)**
1	12.64	253, 364	625	463, 301	Quercetin-*O*-hexoside-*O*-hexoside (isomer 1)[A]	0.81 ± 0.02
2	14.24	265, 345	625	463, 301	Quercetin-*O*-hexoside-*O*-hexoside (isomer 2)[A]	8.2500 ± 0.0003
3	15.44	266, 343	639	477, 315, 300	Isorhamnetin-*O*-hexoside-hexoside[A]	0.910 ± 0.003
4	19.06	255, 353	463	301	Quercetin-3-*O*-glucoside[A]	0.73 ± 0.02
5	23.39	253, 365	463	301	Quercetin-*O*-hexoside[A]	12.1 ± 0.1
6	26.03	252, 365	477	315, 300	Isorhamnetin-*O*-hexoside[A]	1.82 ± 0.03
					Total flavonoids	24.62 ± 0.05
					Total phenolic compounds	24.62 ± 0.05
			CT			
Peak	**RT (min)**	**λ_{max} (nm)**	**$[M-H]^-$**	**MS^2**	**Assignment**	**Quantification (mg/g extract)**
1	14.63	353	755	300, 301	Quercetin-*O*-dirhaminoside-hexoside[A]	0.460 ± 0.001
2	16.62	352	739	285, 284	Kaempferol-*O*-dirhaminoside-hexoside[A]	0.4500 ± 0.0003
3	17.06	354	769	623, 315, 300	Isorhamnetin-*O*-rutinoside-rhaminoside[A]	0.4800 ± 0.0002
4	17.86	353	609	301	Rutin[B]	0.4900 ± 0.0001
5	22.13	353	623	315, 300	Isorhamnetin-*O*-rutinoside[A]	0.470 ± 0.02
					Total flavonoids	2.350± 0.001
					Total phenolic compounds	2.350 ± 0.001
			CG			
Peak	**RT (min)**	**λ_{max} (nm)**	**$[M-H]^-$**	**MS^2**	**Assignment**	**Quantification (mg/g extract)**

1	4.02	325	963	284, 285, 609, 801	Kaempferol-3-hydroxyferuloy-sophoroside-7-glucoside[C]	0.56 ± 0.02
2	4.15	268, 331	933	284, 285, 609, 771, 161	Kaempferol-3-*O*-caffeoyl-sophoroside-7-glucoside[C]	0.370 ± 0.002
3	4.34	270, 339	771	299, 300, 301, 446, 625	Quercetin-3-*O*-*p*-coumaroyl sophoroside[D]	0.3300 ± 0.0001
4	4.34	270, 340	993	299, 300, 301, 446, 625, 787	Quercetin-3-*O*-sinapoyl sophoroside-7-glucoside[D]	
5	4.5	268, 331	1139	284, 285, 609, 815	Kaempferol-3-*O*-sinapoyl-caffeoyl-sophoroside-7-*O*-glucoside[C]	0.360 ± 0.001
6	4.5	268, 331	993	299, 300, 301, 462, 625	Quercetin-3-*O*-sophoroside derivative[D]	
7	4.94	269, 331	977	284, 285, 609, 446, 815	Kaempferol-3-*O*-sinapoyl-caffeoyl-glucoside 7-*O*-glucoside[C]	0.680 ± 0.004
8	5.32	269, 331	947	284, 285, 609, 446, 785	Kaempferol-3-*O*-feruloyl-sophoroside-7-glucoside[C]	0.760 ± 0.001
9	5.64	269, 319	917	284,285, 609, 755, 446	Kaempferol-3-*O*-*p*-coumaroyl-shophoroside-7- glucoside[C]	0.220 ± 0.003
10	6.44	269, 331	917	284, 285, 609, 161, 771	Kaempferol-3-*O*-*p*-coumaroyl-caffeioyl-sophoroside[C]	0.390 ± 0.003
11	7.87	269, 336	961	284, 285, 609, 815, 430	Kaempferol-3-*O*-*p*-coumaroyl-synapoyl sophoroside[C]	0.400 ± 0.003
12	7.87	269, 336	1093	284, 285, 609, 771, 430	Kaempferol-3-*O*-feruloyl-coumaroyl-sophorotriose[C]	
13	11.07	266, 347	755	285, 609, 430	Kaempferol-3-*O*-*p*-coumaroyl-sophoroside[C]	0.210 ± 0.001
14	11.82	266, 346	593	283, 285, 430	Kaempferol-rutinoside[C]	0.360 ± 0.003
15	12.1	266, 347	609	284, 285	Kaempferol-3 (7-*O*-glucosyl)-galactoside[C]	0.150 ± 0.002
16	14.81	269, 331	1361	299, 301, 462, 787, 993	Quercetin-3-synapoyl-sophoroside-7-glucoside[D]	0.330 ± 0.001
17	15.68	269, 331	1346	284, 285, 771, 977	Kaempferol-disinapoyl-sophorotriose-7-glucoside[C]	0.390 ± 0.002
18	16.53	nq	1315	284, 285, 771, 977	Kaempferol-sophorotrioside derivative[C]	0.190 ± 0.001
19	17.67	nq	1331	284, 285, 978, 772	Kaempferol-synapoyl-sophorotrioside derivative[C]	0.130 ± 0.002
					Total flavonoids	5.83 ± 0.04
					Total phenolic compounds	5.83 ± 0.04

MIX

Peak	RT (min)	λ_{max} (nm)	$[M-H]^-$	MS^2	Assignment	Quantification (mg/g extract)
1	6.76	271,326	933	771, 609, 285	Kaempferol-3-*O*-caffeoyl-sophoroside-7-glucoside[A]	1.15 ± 0.02
2	9.04	268,333	977	815, 609, 591, 285	Kaempferol-3-*O*-sinapoyl-sophoroside-7-glucoside[A]	0.87 ± 0.01
3	9.91	268,333	947	785, 623, 609, 591, 443, 284	Kaempferol-3-*O*-feruloyl-sophoroside-7-glucoside[A]	0.77 ± 0.01
4	12.77	267,333	755	609, 429, 284, 256	Kaempferol-3-*O*-*p*-coumaroyl-diglucoside[A]	0.59 ± 0.01

5	14.32	265,344	625	463, 301, 179	Quercetin-*O*-hexoside-hexoside (Isomer 1)[A]	2.440 ± 0.001
6	15.07	267,343	625	463, 301	Quercetin-*O*-hexoside-hexoside (Isomer 2)[A]	0.49 ± 0.01
7	17.03	267, 343	769	315, 623	Isorhamnetin-*O*-rutinoside-rhaminoside[A]	0.460 ± 0.003
8	17.83	267,343	609	301, 179	rutin[B]	0.38 ± 0.02
					Total flavonoids	7.15 ± 0.04
					Total phenolic compounds	7.15 ± 0.04

Used curves for quantification of CB, CT and MIX: A - quercetin-3-*O*-glucoside; B - rutin. Used curves for quantification of CG: C - kaempferol-3-*O*-glucoside; D - quercetin-3-*O*-glucoside.

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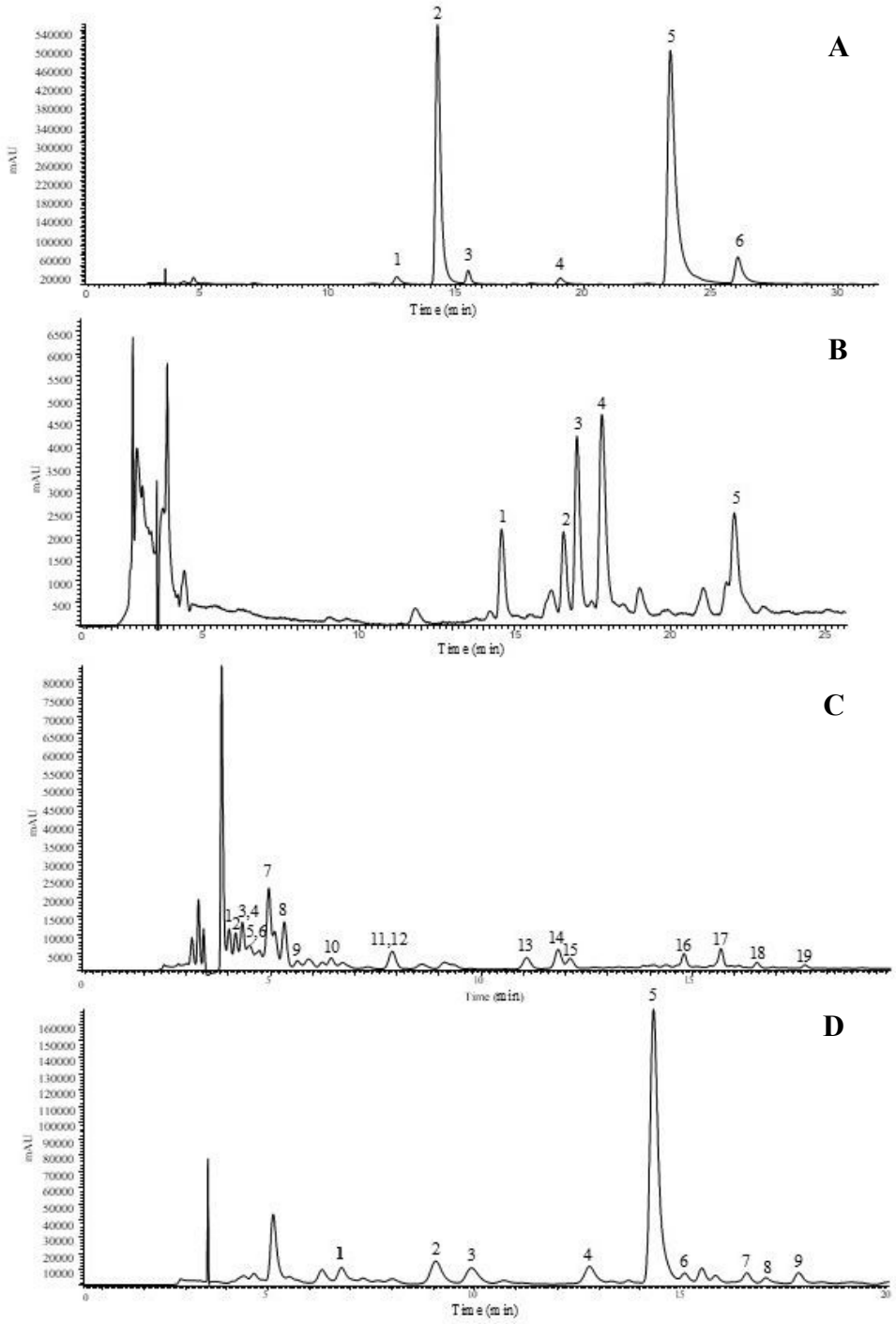

Figure 1. Chromatographic representation of the phenolic compounds profile obtained by HPLC-DAD-ESI-MS/MS for the hydroethanolic extracts at 360 nm. **A** = onion peel extract, peak 1: Quercetin-*O*-hexoside-*O*-hexoside (isomer 1), peak 2: Quercetin-*O*-hexoside-*O*-hexoside (isomer 2), peak 3: Isorhamnetin-*O*-hexosyl-hexoside, peak 4: Quercetin-*O*-hexoside (isomer 1), peak 5: Quercetin-*O*-hexoside (isomer 2),

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peak 6: Isorhamnetin-*O*-hexoside; **B** = zucchini extract, peak 1: Quercetin-*O*-dirhaminoside-hexoside, peak 2: Kaempferol-*O*-dirhaminoside-hexoside, peak 3: Isorhamnetin-*O*-rutinoside-rhaminoside, peak 4: rutin, peak 5: Isorhamnetin-*O*-rutinoside; **C** = kale extract, peak 1: Kaempferol 3-hydroxyferuloyl sophoroside-7-glucoside, peak 2: Kaempferol 3-*O*-caffeoyl sophoroside-7-glucoside, peak 3: Quercetin 3-*O*-p-coumaroyl sophoroside, peak 4: Quercetin 3-*O*-sinapoyl sophoroside-7-glucoside, peak 5: Kaempferol 3-*O*-sinapoyl-caffeoyl-sophoroside 7-*O*-glucoside, peak 6: Quercetin 3-*O*-sophoroside derivative, peak 7: Kaempferol 3-*O*-sinapoyl-caffeoyl-glucoside 7-*O*-glucoside, peak 8: Kaempferol 3-*O*-feruloyl sophoroside-7-glucoside, peak 9: Kaempferol 3-*O*-p-coumaroyl sophoroside-7-glucoside, peak 10: Kaempferol 3-*O*-coumaroyl caffeoyl-sophoroside, peak 11: Kaempferol 3-*O*-coumaroyl synapoyl sophoroside, peak 12: Kaempferol 3-*O*-feruloyl coumaroyl sophorotriose, peak 13: Kaempferol 3-*O*-p-coumaroyl sophoroside, peak 14: Kaempferol rutinoside, peak 15: Kaempferol-3 (7-*O*-glucosyl)galactoside, peak 16: Quercetin 3-sinapoyl sophoroside-7-glucoside, peak 17: Kaempferol disinapoyl sophorotriose-7-glucoside, peak 18: Kaempferol sophorotrioside derivative, peak 19: Kaempferol synapoyl sophorotrioside derivative; **D** = MIX extract, peak 1: Kaempferol-3-*O*-caffeoyl-sophoroside-7-glucoside, peak 2: Kaempferol-3-*O*-sinapoyl-sophoroside-7-glucoside, peak 3: Kaempferol-3-*O*-feruloyl-sophoroside-7-glucoside, peak 4: Kaempferol-3-*O*-p-coumaroyl diglucoside, peak 5: Quercetin-*O*-hexosyl-hexoside (isomer 1), peak 6: Quercetin-*O*-hexosyl-hexoside (isomer 2), peak 7: Kaempferol-*O*-dihexoside, peak 8: Isorhamnetin-*O*-rutinoside-rhaminoside, peak 9: rutin.

3.1.3. **Kale extract (CG).** CG revealed a more complex phenolic profile, constituted mainly of acylated and glycosylated flavonoids. It revealed the presence of 19 flavonoids, twelve kaempferol glycosides were acylated with *p*-coumaric, caffeic, ferulic, and sinapic acids, and three quercetin glycosides were acylated with *p*-coumaric and sinapic acids, derived from hydroxycinnamic acids. Finally, four compounds are non-acylated kaempferol and quercetin glycosides. These compounds are characteristic of the *Brassica* family and have been widely reported in the literature, as highlighted in studies by Lin and Harnly[46], Lin et al.[47], Li et al. [48], and Bianchi et al.[9]

Compounds **1** ([M-H]$^-$ *m/z* 963), **2** ([M-H]$^-$ *m/z* 933), **5** ([M-H]$^-$ *m/z* 1139), **7** ([M-H]$^-$ *m/z* 977), **8** ([M-H]$^-$ *m/z* 947), and **9** ([M-H]$^-$ *m/z* 917) exhibited a common initial fragmentation path corresponding to the loss of 162 Da ([M-H-162]$^-$). This loss is characteristic of a hexose moiety at position 7 of the aglycone and typical of flavonoid 3-*O*(acyl)glycoside-7-*O*-hexoside.[49] In such acylated flavonoids, acylation predominantly occurs on the sugar moiety attached at position 3 of the aglycone and is generally detected after the cleavage of the sugar at position 7.[50] Previous works [49, 51-53] have shown that, in the *Brassica* family, acylation is commonly associated with sophoroside units, consisting of two glucose molecules linked through a 1→2 interglycosidic linkage. This disaccharide can be identified by characteristic fragment ions such as [M-H-324]$^-$, [M-H-180]$^-$, [M-H-162]$^-$, and [M-H-120]$^-$. Additionally, the presence of fragment ions at *m/z* 284–285 suggests kaempferol as the aglycone. Based on the combined fragmentation patterns and UV–Vis data (λ_{max}), these compounds were tentatively assigned as kaempferol derivatives, namely kaempferol-3-hydroxyferuloyl-sophoroside-7-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-glucoside, kaempferol-3-*O*-sinapoyl-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-caffeoyl-glucoside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-glucoside, and kaempferol-3-*O*-*p*-coumaroyl-sophoroside-7-glucoside, in agreement with previously reported data for *Brassica* species.[46, 48]

Compounds **3** ([M-H]$^-$ *m/z* 771), **4** ([M-H]$^-$ *m/z* 993), **6** ([M-H]$^-$ *m/z* 993), and **16** ([M-H]$^-$ *m/z* 1361) presented fragment ions of higher intensity in MS^2 at *m/z* 301, characteristic of the deprotonated quercetin aglycone. In particular, compound **3** presented a [M-H-146-324]$^-$ fragmentation pathway, with the initial loss of 146 Da suggesting a fragment characteristic of a *p*-coumaric acid molecule [50] and the subsequent loss of 324 Da, a sophoroside. The formation of a fragment at *m/z* 446 ([M-H-180]$^-$) supports this interpretation.[53] Based on these data, compound **3** was tentatively identified as quercetin-3-*O*-*p*-coumaroyl-sophoroside.[46]

Finally, the other compounds showed fragmentation patterns similar to those mentioned above, with differentiation in the acylation or non-acylation group, whose provisional names are presented in **Table 1**.

CG showed a total phenolic content of 5.83 ± 0.04 mg/g extract. Regarding the identified compounds, compounds **7** and **8** were the most abundant, with concentrations of 0.680 ± 0.004 and 0.760 ± 0.001 mg/g extract, respectively, while compound **19** was the least abundant (0.130 ± 0.002 mg/g extract). Compounds **3** and **4** (0.3300 ± 0.0001 mg/g extract), **5** and **6** (0.360 ± 0.001 mg/g extract), and **11** and **12** (0.400 ± 0.003 mg/g extract) co-eluted under the analytical conditions. As a result, their concentration are presented as the combined values of each pair.

3.1.4. **Mixture of onion peel, zucchini, and kale extract (MIX).** Based on the individual phenolic profiles, residues of onion peel, zucchini, and kale were combined in equal proportions to further explore their bioactive potential and reuse. The resulting hydroethanolic extract was subsequently analysed for phenolic compound identification and quantification. This approach was designed considering two main aspects. First, these by-products are often discarded together in industrial settings, making it relevant to evaluate the bioactivity of their combined extraction. Second, onion peel is known for its high phenolic content, whereas zucchini and kale typically present lower concentrations. Therefore, combining these residues not only reflects real disposal practices but also represents a valorisation strategy that enhances the bioactive potential of less abundant matrices, supporting their sustainable reuse and application as functional ingredients.

Thus, the phenolic profile obtained for the vegetable mixture showed a total of eight tentatively identified compounds, already mentioned in individual samples. Regarding the concentration of individual phenolic compounds, quercetin-*O*-hexoside-hexoside (isomer **1**) was the majority with 2.440 ± 0.001 mg/g extract, being the second most abundant present in CB. However, the concentration of this compound in CB was 8.2500 ± 0.0003 mg/g extract.

Furthermore, the total concentration of phenolic compounds in MIX (7.15 ± 0.04 mg/g extract) did not exceed the sum of the individual extracts, suggesting the occurrence of an antagonistic

effect during the extraction process. This effect should be interpreted in terms of extraction efficiency rather than intrinsic bioactivity, as phenolic compound recovery is strongly influenced by solvent composition and compound polarity.[54, 55] In mixed systems, phenolic compounds with different polarities may compete for solubilisation in the hydroethanolic medium, leading to selective extraction rather than additive recovery.[55-57] In addition, the presence of complex plant matrices, particularly polysaccharides and proteins from zucchini and kale, may promote non-covalent interactions (e.g., hydrogen bonding or hydrophobic interactions) with phenolic compounds, reducing their extractability or stability.[58]

These findings highlight the complexity of the extraction systems of multiple compounds and suggest that combining plant residues does not necessarily result in additive recovery of bioactive compounds.[59, 60] Further studies focusing on mechanistic approaches, such as fractionation, model systems, or controlled co-extraction experiments, would be valuable to better elucidate the interactions governing these antagonistic effects.

3.2. Bioactivity evaluation of hydroethanolic extracts

3.2.1. **Antioxidant activity assessment**. The antioxidant activity of the hydroethanolic extracts of CT, CG, CB, and MIX is shown in **Fig. 2**, and the results were expressed as EC_{50} (µg/mL) (concentration required to achieve 50% antioxidant efficacy).

CB showed the lowest EC_{50} values of 10.4 ± 0.3 µg/mL for TBARS and 9.7 ± 0.3 µg/mL for DPPH, both statistically different ($p < 0.05$) from the other samples analyzed, including the positive control potassium metabisulfite. CG also showed relevant antioxidant activity with EC_{50} concentrations of 261.2 ± 8.2 µg/mL for TBARS and 101.00 ± 3.7 µg/mL for DPPH. On the other hand, CT presented the highest EC_{50} concentrations in the two assays analyzed (817.3 ± 31.2 µg/mL - TBARS and 762.7 ± 17.7 µg/mL - DPPH), indicating a significantly lower antioxidant capacity than the others.

These results are supported by the literature, where authors presented similar DPPH results for a hydroethanolic extract of yellow onion peel, with an EC_{50} of 12.5 ± 0.4 µg/mL. On the other hand, Nile et al.[62] reported an EC_{50} of 60.5 µg/mL for the hydroethanolic extract of yellow onion peel, which represents a higher concentration to achieve 50% efficacy, but is considered an excellent result. Radünz et al.[63] also reported excellent antioxidant activity in the DPPH method of kale extract, with 89.2% inhibition at a concentration of 200 mg/mL of the radical. The same was reported by Armesto et al.[64], with a radical inhibition ranging from 75.10 to 91.32% of methanolic kale extract. In the case of CT, Hamissou et al.[65] reported that zucchini peel displayed low DPPH radical scavenging capacity, being only 12.19% as effective as ascorbic acid. Saavedra et al.[66] showed a DPPH radical inhibition capacity of 52.2 ± 0.7 µmol Trolox/g for pumpkin peels belonging to the same *Cucurbita* family. Furthermore, Leichtweis et al.[43] presented higher EC_{50} values for different parts of pumpkins of the same family, with the seeds showing the best antioxidant activity in the TBARS assay. These results show that factors such as chemical composition, plant matrix, and extraction conditions, as well as cultivar, geographic region, climate, and many other factors, can significantly affect the antioxidant capacity of by-products.[64]

The results confirm better antioxidant performance for CB, which can be correlated with the high presence of phenolic compounds [38], since it was the extract that presented the highest concentration of phenolic compounds. In particular, this effect may be related to the presence of quercetin, the main compound in the extract in its glycosylated form, and widely recognized in the literature for its high antioxidant capacity.[67, 68]

The antioxidant capacity observed for MIX is consistent with the antagonistic behaviour discussed in Section 3.1.4, where the combination of residues did not result in a synergistic effect but rather in a reduction of overall antioxidant efficiency. Nevertheless, the EC_{50} values presented (309 ± 13 µg/mL - TBARS and 164.6 ± 2.6 µg/mL -DPPH) indicate a relevant antioxidant activity, with TBARS values statistically similar ($p > 0.05$) to those presented by CG. Results such as these highlight the complexity of interactions among multiple component systems, where combining different phenolic profiles does not necessarily increase antioxidant functionality.[60] Similar antagonistic effects have been reported in mixtures of flavonoids and plant extracts, where certain combinations exhibit lower antioxidant activity than individual components, highlighting the influence of the interactions between compounds in complex systems.[69, 70]

This behaviour may be explained by interactions among compounds that affect radical scavenging mechanisms, as well as by dilution effects, in which the contribution of highly active compounds (such as those from onion peel) is reduced when combined with matrices of lower antioxidant potential, such as zucchini and kale.

Consequently, onion peel appears to be the primary contributor to the antioxidant activity of the mixture, while the inclusion of other matrices may limit its overall effectiveness. Although the combination of residues represents a promising strategy for sustainable valorisation, optimisation of mixture composition or extraction conditions may be required to minimise antagonistic effects and maximise functional performance.

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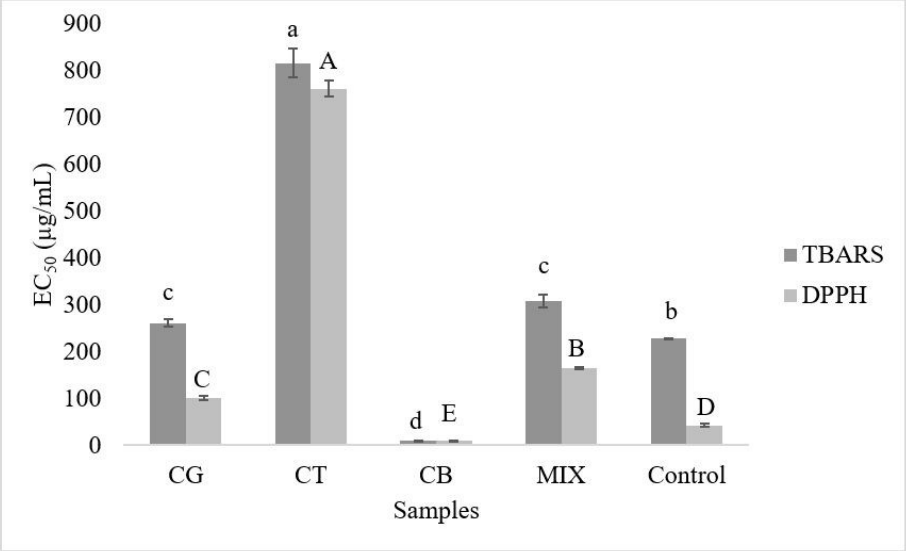

Figure 2. Antioxidant activity of the hydroethanolic extracts from vegetable by-products. CT – hydroethanolic extract of zucchini; CG – hydroethanolic extract of kale; CB – hydroethanolic extract of onion peel; MIX – hydroethanolic extract of the mixture of onion peel, zucchini, and kale; Control – potassium metabisulfite. ANOVA analysis – For each analysis (TBARS – lowercase letters; DPPH – capital letters), different letters mean significant differences among the samples according to Tukey's HSD test ($p > 0.05$).

3.2.2. Antidiabetic activity assessment. The antidiabetic activity of the hydroethanolic extracts was evaluated using α-glucosidase enzyme inhibition tests, and the results are presented in **Table 2.**

All samples exhibited inhibitory activity against the enzyme *α*-glucosidase, though with varying potencies. CB showed the strongest inhibitory effect, with an IC_{50} value of 0.176 ± 0.008 mg/mL, being statistically similar ($p > 0.05$) to both the MIX (0.30 ± 0.02 mg/mL) and the positive control (0.33 ± 0.01 mg/mL). In contrast, CG and CT presented significantly lower inhibitory activity, with IC_{50} values of 4.5 ± 0.3 mg/mL and 3.9 ± 0.2 mg/mL, respectively ($p < 0.05$), indicating a reduced capacity to inhibit *α*-glucosidase when compared to CB, MIX, and the control.

These results indicate that CB has the potential to modulate the activity of this enzyme, which can be attributed to the presence of quercetin, known for its antidiabetic effects.[68] However, in onion peel extracts, not only quercetin is responsible for the *α*-glucosidase inhibition activity, but also the presence of other bioactive fractions. For instance, Kim et al.[71] reported a correlation between the enzyme inhibition capacity and the presence of additional compounds beyond quercetin. In this study, the hydroethanolic extract of onion peel showed the ability to inhibit *α*-glucosidase with an IC_{50} of 1.27 mg/mL. Silva et al.[72] also reported that onion peel extract exhibited a greater capacity to inhibit α-glucosidase than pure quercetin, suggesting that additional compounds present in the extract contribute to the overall inhibitory activity. This behaviour supports the contribution of matrix components and potential interactions among phenolic compounds, which may enhance or modulate the observed biological effects.

MIX showed potential to inhibit the enzyme *α*-glucosidase with an IC_{50} of 381.3 ± 27.9 µg/mL, although with significantly lower efficacy compared to CB and the positive control ($p < 0.05$). This reduced activity is consistent with the antagonistic behavior discussed previously and may be attributed to dilution effects or interactions among compounds, which limit the bioactivity of key phenolic constituents. Nevertheless, considering that CT and CG individually exhibited weaker α-glucosidase inhibitory activity, their combination with onion peel results in an extract with improved functionality relative to these matrices alone.

These findings suggest that, despite a reduction in activity compared to the most potent extract, the mixture represents a compromise between bioactivity and the valorization of less active by-products, supporting their potential application as functional ingredients.

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Table 2. α-glucosidase inhibition of the hydroethanolic extracts (mean ± standard deviation).

Samples	α – glucosidase inhibition - IC_{50} values(mg/mL)
CG	4.5 ± 0.3[c]
CT	3.9 ± 0.2[b]
CB	0.176 ± 0.008[a]
MIX	0.3 ± 0.02[a]
Control	0.33 ± 0.01[a]

CT – hydroethanolic extract of zucchini; CG – hydroethanolic extract of kale; CB – hydroethanolic extract of onion peel; MIX – hydroethanolic extract of the mixture of onion peel, zucchini, and kale; Control – acarbose. ANOVA analysis – In the column, different letters mean significant differences among the samples according to Tukey's HSD test ($p > 0.05$).

3.2.3. **Antimicrobial activity assessment.** The antimicrobial and antifungal activity of the hydroethanolic extracts were evaluated against a panel of bacterial and fungal strains of food importance, to assess the potential of these extracts as preservative agents. The results obtained are presented in **Table 3.**

CB and CG presented the best results of antibacterial activity, with MIC and MBC ranging from 0.25 to 2 mg/mL for the bacteria tested. However, CT and MIX also presented good antimicrobial capacity with MIC and MBC ranging from 0.25 to 4 mg/mL. *Bacillus cereus* was the most susceptible bacteria for all the extracts tested, since spore-forming bacteria, such as this one, are more susceptible to inhibition by the presence of phenolic compounds.[73] These results were similar to those presented by the positive control E211 and showed better activity when compared with the positive control E224.

The antifungal activity data showed greater specificity of action among the extracts. CT and MIX did not show efficacy against the tested strains up to the concentration of 8 mg/mL. On the other hand, CB and CG exhibited relevant antifungal activity, with MIC and MFC lower than or equal to the positive control E211 for all strains, except *Aspergillus versicolor* (CB) and *Aspergillus niger* (CG).

Table 3. Antibacterial and antifungal activity of hydroethanolic extracts tested (mg/mL)

Antibacterial activity							
Samples	**mg/mL**	***Staphylococcus aureus***	***Bacillus cereus***	***Listeria monocytogenes***	***Escherichia coli***	***Salmonella Typhimurium***	***Enterobacter cloacae***
CT	MIC	2	0.25	1	1	1	1
	MBC	4	0.5	2	2	2	2
CG	MIC	1	0.25	0.5	0.5	0.5	0.5
	MBC	2	0.5	1	1	1	1
CB	MIC	0.5	0.25	0.5	1	0.5	1
	MBC	1	0.5	1	2	1	2
MIX	MIC	1	0.25	1	1	2	2
	MBC	2	0.5	2	2	4	4
E211	MIC	4.0	0.5	1.0	1.0	1.0	2.0
	MBC	4.0	0.5	2.0	2.0	2.0	4.0
E224	MIC	1.0	2.0	0.5	0.5	1.0	0.5
	MBC	1.0	4.0	1.0	1.0	1.0	0.5
Antifungal activity							

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		Aspergillus fumigatus	*Aspergillus niger*	*Aspergillus versicolor*	*Penicillium funiculosum*	*Penicillium verrucosum var. cyclopium*	*Trichoderma viride*
CT	MIC	>8	>8	>8	>8	>8	>8
	MFC	>8	>8	>8	>8	>8	>8
CG	MIC	1	2	1	1	1	1
	MFC	2	4	2	2	2	2
CB	MIC	1	1	2	0.5	0.5	1
	MFC	2	2	4	1	1	2
MIX	MIC	>8	>8	>8	>8	>8	>8
	MFC	>8	>8	>8	>8	>8	>8
E211	MIC	1.0	1.0	2.0	1.0	2.0	1.0
	MFC	2.0	2.0	2.0	2.0	4.0	2.0
E224	MIC	1.0	1.0	1.0	0.5	1.0	0.5
	MFC	1.0	1.0	1.0	0.5	1.0	0.5

CT – hydroethanolic extract of zucchini; CG – hydroethanolic extract of kale; CB – hydroethanolic extract of onion peel; MIX – hydroethanolic extract of mixture of onion peel, zucchini, and kale; E211 - Sodium benzoate; E224 - potassium metabisulphite; MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; and MFC – minimum fungicide concentration.

3.3. Proximate composition and chemical characterization of solid residue resulting from phenolic compound extraction

Analysing the residues from the hydroethanolic extraction process of the vegetable by-products (RCG, RCB, RCT, and RMIX) is essential for understanding their potential and how we can use them more sustainably. While the focus of the extraction was on phenolic compounds, the residues generated during this process also have great value. They can be used as sources of biopolymers, such as fibers and proteins, which have huge potential for applications in various industries, including the food industry. By characterizing these residues, it gains a more complete view of their nutritional and chemical composition, uncovering the value of by-products that are often discarded without being utilized. With this, the solid residues were characterized according to their proximate composition and some chemical parameters. The results are presented in **Table 4**.

Regarding the proximate composition of the residues, the fiber content was more prevalent in all the samples analyzed. RCB stood out with the highest TDF, IDF, and SDF content with 69.54 ± 1.22, 54.61 ± 0.53, and 14.45 ± 0.37 g/100 g dw, significantly ($p < 0.05$) higher than the other samples. This result is justified by the fact that the main component of yellow onion peel is fiber among all the macromolecules present.[74]

The high TDF content observed in this study suggests a potential use of these residues as food ingredients that could enrich food formulations. However, among all the characteristics necessary for an ingredient to be considered a good source of dietary fiber, the balance between SDF and IDF is of great importance as it reflects the functional balance of digestive health. A ratio of 1:2 between SDF and IDF is considered acceptable.[75] In the case of the present study, the samples analyzed did not have this ratio (RCG: 1:10, RCT: 1:3, RCB: 1:4, RMIX: 1:5), but it is an expected result since most plant matrices have a higher IDF content than SDF.[76] Nevertheless, RCT and RCB presented ratios of 1:3 and 1:4, which are more similar to what is considered viable and can be used directly for food fortification. The ratio between SDF and IDF can be improved by physical, chemical, and biological methods, which can promote the conversion of IDF to SDF, increasing the possibility of use as a food ingredient.[76] In addition to the balance between the fractions, characteristics such as water retention, fermentation rate, knowledge of the fiber matrix, and even its structure are of great importance when considering good-quality fiber for fortifying a product.[77] Therefore, further studies are needed to confirm the potential of residues from the hydroethanolic extraction process of vegetable by-products as a source of dietary fiber.

The protein content was the second largest component detected in the extraction residues. RCT presented the highest contents, with 30.67 ± 0.49 g/100 g dw for crude protein and 27.43 ± 0.03 g/100 g dw for true protein, statistically different from the others ($p < 0.05$). The difference observed between the two methods (approximately 3.3 g/100g dw) highlights the presence of non-protein nitrogenous compounds in the sample, which interfere with crude protein analysis. These may include free amino acids, amines, alkaloids, nucleotides, or other bioactive compounds that contain nitrogen and that are quantified in the total nitrogen analysis but do not contribute to the structural protein fraction.[78] Therefore, the application of the true protein method, which includes a precipitation step, was essential to avoid overestimation and to obtain a more accurate representation of the bioavailable protein content.

A consistent trend was observed between the true protein content and total amino acid content across samples. RCT and RCG, which exhibited the highest true protein content (30.67 ± 0.49 and 23.34 ± 0.02 g/100 g dw), also presented the highest concentration of total amino acids (228.05 ± 11.30 and 196.20 ± 9.62 mg/g dw), reinforcing the integrity and nutritional relevance of their protein fractions. In contrast, RCB, with the lowest true protein (7.82 ± 0.02 g/100 g dw), also showed the lowest amino acid concentration (58.43 mg/g dw), suggesting a reduced contribution of its protein fraction

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to nutritional value. RMIX presented intermediate values of both true protein (18.49 g/100 g dw) and total amino acids (156.42 mg/g dw), reflecting the weighted contribution of each component. This suggests that mixing may help balance nutritional quality, especially by mitigating the lower protein content of RCB through the inclusion of RCT and RCG. Moreover, the essential amino acid content in RCT and RCG (98.55 ± 4.70 mg/g dw and 82.21 ± 3.90 mg/g dw) highlights their importance as primary contributors to the amino acid profile of the RMIX, which likely benefits from their presence

Aspartic and glutamic acids were the main amino acids in all samples analyzed, with no significant differences ($p > 0.05$) between RCT and RCG. However, RCB also showed arginine as one of the predominant amino acids. Asparagine and glutamine were not detected in any of the samples analyzed, since the acidic conditions promoted by the acid hydrolysis performed in this assay cause the conversion of these compounds into aspartic and glutamic acid. The nine essential amino acids (histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, methionine, and valine) were detected in all samples, with leucine and phenylalanine being the main amino acids in RCT and RCG and therefore in RMIX. With these results and the protein content of RCT and RCG, these residues become interesting sources of high nutritive value protein for plant-based diets. In particular, the phenylalanine content stands out, as it is essential for protein synthesis, thyroid hormone production, melanin production, and other metabolic functions.[79] Furthermore, leucine, together with isoleucine and valine, the branched-chain amino acids (BCAAs) present in the samples, play a fundamental role in muscle protein synthesis and energy metabolism.[80] Thus, these residues can be valorized by incorporation into food preparations and dietary supplements aimed at improving nutrition and physical performance.

Regarding the chemical characterization of the hydroethanolic extraction residues, the individual profiles of free sugars and organic acids were also determined. In the case of free sugars, low concentrations were observed for all samples, with the maximum observed for RCT at 3.02 ± 0.04 g/100 g dw, mainly due to its fructose and glucose content, which presented significantly higher values ($p < 0.05$). This low concentration of free sugars is probably due to partial solubilization during hydroethanolic extraction, since ethanol-water mixtures can extract polar molecules together with phenolic compounds.[81] According to previous studies, the total free sugar content in kale (*Brassica oleracea* L. var. Acephala) is about 4 g/100 g dw[82], while in onion peels it is about 20 g/100 g.[83] In the flesh of species of the *Cucurbita sp*. family, the values can vary between 19 and 38 g/100 g dw.[84] The low sugar content found in these samples suggests that these residues may be suitable for low glycemic index formulations while providing functional properties.

Regarding the organic acid content, a statistically significant variation ($p < 0.05$) was observed among the residues analyzed. The highest content was observed for RCT (16.36 ± 0.16 g/100 g dw), mainly due to its concentration of fumaric acid (10.99 ± 0.19 g/100 g dw), a food additive and acidulant used in the food industry, capable of acting in microbial control, pH, and flavour enhancement.[85] RCG had the highest statistically significant ($p < 0.05$) levels of citric acid (4.47 ± 0.18 g/100 g dw) and oxalic acid (2.90 ± 0.03 g/100 g dw). Succinic acid was only detected in RCB, which may contribute to potential metabolic and feed additive benefits.[86] As observed for the sugar content, the organic acid content of the analyzed residues is lower than that of the natural sample, since some of these compounds are also extracted during the hydroethanolic extraction process. Since some compounds are polar and others are soluble in water and alcohols, the use of the ethanol-water mixture facilitates their extraction.[87] Therefore, the organic acids detected in the residue may correspond to the fraction most strongly associated with the matrix, since the determination of this profile is performed after extraction with metaphosphoric acid.

Finally, the results indicate that these plant residues from the hydroethanolic extraction process have potential as sustainable sources of dietary fiber and protein with a low glycemic index. RCT and RCG appear to be potential protein supplements with an interesting amino acid profile, while RCB appears to be particularly rich in dietary fiber, reinforcing its potential for applications in the food industry. The combination of different residues, as in RMIX, can produce synergistic effects, optimizing nutritional and functional properties, but it did not surpass the residue of a single plant material. Future studies should explore its applicability in food formulations, focusing on digestibility, bioavailability, and potential health benefits.

Table 4. Chemical characterization and nutritional value-proximate composition of the residues of hydroethanolic extraction of the samples analyzed (mean ± standard deviation).

	RCG	RCT	RCB	RMIX
Centesimal composition (g/100 g dw)				
Total fats	3.34 ± 0.09 ^{b}	2.62 ± 0.11 ^{c}	2.39 ± 0.08 ^{d}	3.62 ± 0.06 ^{a}
Ash	11.57 ± 0.04 ^{a}	9.37 ± 0.09 ^{c}	8.48 ± 0.02 ^{d}	10.07 ± 0.11 ^{b}
Crude protein	26.90 ± 0.15 ^{b}	30.67 ± 0.49 ^{a}	7.87 ± 0.16 ^{d}	18.71 ± 0.69 ^{c}
True protein	23.34 ± 0.02 ^{b}	27.43 ± 0.03 ^{a}	7.82 ± 0.02 ^{d}	18.49 ± 1.01 ^{c}
Total dietary fibers (TDF)	49.07 ± 1.20 ^{c}	41.85 ± 1.79 ^{d}	69.54 ± 1.22 ^{a}	53.64 ± 2.00 ^{b}
Insoluble dietary fibers (IDF)	46.64 ± 0.27 ^{b}	34.03 ± 0.08 ^{c}	54.61 ± 0.53 ^{a}	47.08 ± 0.43 ^{b}
Soluble dietary fibers (SDF)	4.50 ± 0.09 ^{d}	10.35 ± 0.47 ^{b}	14.45 ± 0.37 ^{a}	8.69 ± 0.26 ^{c}

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Amino acids (mg/g dw)				
Aspartic acid	24.40 ± 1.16 [a]	24.21 ± 1.18 [a]	7.70 ± 0.35 [c]	18.23 ± 1.02 [b]
Glutamic acid	25.98 ± 1.25 [a]	27.80 ± 1.39 [a]	8.09 ± 0.37 [c]	19.50 ± 1.09 [b]
Asparagine	n.d.	n.d.	n.d.	n.d.
Serine	10.14 ± 0.52 [b]	12.66 ± 0.65 [a]	3.45 ± 0.16 [d]	8.28 ± 0.45 [c]
Glutamine	n.d.	n.d.	n.d.	n.d.
*Histidine	4.99 ± 0.28 [b]	5.68 ± 0.23 [a]	1.02 ± 0.04 [d]	4.09 ± 0.17 [c]
Glycine	10.47 ± 0.46 [b]	12.25 ± 0.59 [a]	2.94 ± 0.08 [d]	8.90 ± 0.41 [c]
*Threonine	8.71 ± 0.44 [a]	8.90 ± 0.39 [a]	2.55 ± 0.09 [c]	6.48 ± 0.34 [b]
Arginine	13.67 ± 0.71 [b]	17.33 ± 1.01 [a]	8.02 ± 0.40 [c]	12.14 ± 0.66 [b]
Alanine	11.10 ± 0.54 [b]	13.53 ± 0.72 [a]	3.62 ± 0.17 [d]	9.40 ± 0.51 [c]
Tyrosine	5.82 ± 0.31 [b]	7.03 ± 0.47 [a]	1.65 ± 0.09 [d]	4.40 ± 0.26 [c]
*Valine	11.02 ± 0.55 [b]	13.98 ± 0.74 [a]	3.05 ± 0.13 [d]	9.38 ± 0.52 [c]
*Methionine	1.98 ± 0.23 [b]	2.57 ± 0.11 [a]	0.32 ± 0.02 [d]	1.08 ± 0.10 [c]
*Tryptophan#	1.78 ± 0.17 [a]	0.94 ± 0.08 [b]	0.41 ± 0.02 [c]	0.92 ± 0.08 [b]
*Phenylalanine	13.32 ± 0.73 [b]	15.66 ± 0.92 [a]	3.24 ± 0.14 [d]	10.84 ± 0.60 [c]
*Isoleucine	7.89 ± 0.39 [b]	11.15 ± 0.55 [a]	2.39 ± 0.10 [c]	6.93 ± 0.38 [b]
*Leucine	21.71 ± 0.92 [b]	26.94 ± 1.34 [a]	6.09 ± 0.22 [d]	17.59 ± 0.92 [c]
*Lysine	10.81 ± 0.23 [b]	12.71 ± 0.49 [a]	1.62 ± 0.10 [d]	8.58 ± 0.22 [c]
Hydroxyproline	1.96 ± 0.09 [b]	2.90 ± 0.14 [a]	0.55 ± 0.02 [c]	1.79 ± 0.09 [b]
Proline	10.44 ± 0.83 [a]	11.80 ± 0.62 [a]	1.70 ± 0.09 [c]	8.28 ± 0.39 [b]
∑ Total amino acids	196.20 ± 9.62 [b]	228.05 ± 11.30 [a]	58.43 ± 2.23 [d]	156.82 ± 8.14 [c]
*∑ Essential amino acids	82.21 ± 3.90 [b]	98.55 ± 4.70 [a]	20.71 ± 0.67 [d]	65.88 ± 3.27 [c]
Organic acids (g/100 g dw)				
Oxalic acid	2.88 ± 0.03 [a]	1.33 ± 0.05 [b]	0.46 ± 0.01 [d]	1.00 ± 0.03 [c]
Malic acid	2.78 ± 0.11 [b]	3.40 ± 0.07 [a]	1.17 ± 0.02 [d]	2.37 ± 0.05 [c]
Shikimic acid	n.d.	tr	tr	n.d.
Citric acid	4.47 ± 0.18 [a]	0.64 ± 0.03 [d]	0.91 ± 0.01 [c]	2.22 ± 0.02 [b]
Succinic acid	n.d.	n.d.	1.92 ± 0.00	n.d.
Fumaric acid	2.60 ± 0.07 [b]	10.99 ± 0.19 [a]	tr	0.63 ± 0.02 [c]
Total organic acids	12.73 ± 0.26 [b]	16.36 ± 0.17 [a]	4.46 ± 0.02 [d]	6.22 ± 0.12 [c]
Sugars (g/100 g dw)				
Fructose	0.42 ± 0.01 [c]	1.55 ± 0.04 [a]	0.18 ± 0.01 [d]	0.81 ± 0.01 [b]
Glucose	0.22 ± 0.01 [d]	1.07 ± 0.01 [a]	0.38 ± 0.01 [c]	0.60 ± 0.01 [b]
Sucrose	n.d.	0.21 ± 0.01 [a]	n.d.	0.07 ± 0.01 [b]
Trehalose	n.d.	0.21 ± 0.01 [a]	n.d.	0.16 ± 0.03 [b]
Total sugars	0.64 ± 0.02 [c]	3.02 ± 0.04 [a]	0.56 ± 0.02 [c]	1.64 ± 0.04 [b]

n.d. – no detected; # - determined by basic hydrolysis; * - essential amino acids; RCT – residue of zucchini extraction; RCG – residue of kale extraction; RCB – residue of onion peel extraction; RMIX – residue of the mixture of onion peel, courgette, and kale extraction; tr – traces. ANOVA analysis – In each line, different letters mean significant differences among the samples according to Tukey's HSD test for comparison of three or more means and Student's t-test for comparison of two means ($p > 0.05$).

Conclusions

This study demonstrated the potential of hydroethanolic extracts and the residues from onion, cabbage, and zucchini peels as sources of phenolic compounds, dietary

fiber, and protein. The extracts exhibited significant antioxidant activity, with CB showing the highest phenolic content and strongest radical scavenging capacity. The α-glucosidase inhibition assay confirmed CB as a promising antidiabetic agent, comparable to acarbose, a positive control. At the same time, MIX showed moderate but non-additive inhibition in the combination of residues. Antimicrobial assays showed that CB and CG had strong antibacterial and antifungal properties, supporting their application as natural food preservatives. In the characterization of residues from hydroethanolic extraction, RCB stood out as a source of dietary fiber. RCT and RCG stood out as potential sources of protein, with a favorable amino acid profile, indicating a possible use as protein supplements. In addition, the low sugar content and the presence of some organic acids support their valorisation for use in functional food formulations.

This study not only demonstrated the potential of hydroethanolic extracts and residues from onion, zucchini, and kale by-products as sources of phenolic compounds, dietary fiber, and protein, but also highlighted the novel approach of simultaneously valorising both phenolic compounds and the extraction solid residue. While most research tends to focus on the phenolic compounds, this study highlights the often overlooked value of the extraction residues. By characterizing them, we uncovered important nutritional components such as fiber and protein that can be used to develop functional foods and supplements. This dual approach not only reduces waste but also opens up new possibilities for more sustainable practices in the food industry.

In general, this work has highlighted the feasibility of valorising plant by-products through hydroethanolic extraction, promoting the identification and quantification of molecules that can be valorised through their application in food fortification and the development of functional ingredients with associated biological properties. Future research should focus on the evaluation of bioavailability, digestibility, and technological applications to optimize their use in the food industry, as well as the optimization of methods that promote greater recovery capacity of these molecules.

Author contributions

Tatiane C. G. Oliveira: Writing- Original draft, Investigation, Formal analysis, Data curation. **Tayse F. F. Da Silveira:** Writing – original draft, Investigation, Formal analysis**. Dejan Stojković**: Methodology, Investigation, Formal analysis. **Javier Cano-Lou**: Methodology, Investigation, Formal analysis**. Gema Casado-Hidalgo**: Methodology, Investigation, Formal analysis**. Susana Machado**: Methodology, Investigation, Formal analysis**. Liliana Espírito Santo**: Methodology, Investigation, Formal analysis. **M. Beatriz P.P. Oliveira:** Writing-Review & editing, Supervision. **Eliana Pereira**: Writing- review & editing, Supervision, Methodology, Investigation, and Conceptualization. **Lillian Barros**: Writing- review & editing, Supervision, Resources, Methodology, Investigation, and Conceptualization.

Conflicts of 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.

Data availability

Data will be made available on request.

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References

1. R. Bhat, em *Valorization of Agri-Food Wastes and By-Products*, ed. R. Bhat, Academic Press, 2021, Chapter 1, pp. 1-27.
2. FAO, *Food wastage footprint: impacts on natural resources: Summary report*, Food & Agriculture Organization of the UN (FAO), 2013.
3. C. V. Berenguer, C. Andrade, J. A. M. Pereira, R. Perestrelo and J. S. Câmara, Current challenges in the sustainable valorisation of agri-food wastes: A review, *Processes*, 2023, **11**, 20.
4. M. Beuving, W. C. McNabb and N. W. Smith, Global nutrient content embedded in food losses and waste: Identifying the sources and magnitude along the food supply chain, *Sustainable Production and Consumption*, 2024, **51**, 519-531.
5. A. R. Soares Mateus, A. Pena and A. Sanches-Silva, Unveiling the potential of bioactive compounds in vegetable and fruit by-products: Exploring phytochemical properties, health benefits, and industrial opportunities,

Current Opinion in Green and Sustainable Chemistry, 2024, **48**, 100938.
6. FAOSTAT, Food Balances (2010-), 2022.
7. A. Bains, K. Sridhar, B. N. Singh, R. C. Kuhad, P. Chawla and M. Sharma, Valorization of onion peel waste: From trash to treasure, *Chemosphere*, 2023, **343**, 140178.
8. M. Kumar, M. D. Barbhai, M. Hasan, S. Punia, S. Dhumal, Radha, N. Rais, D. Chandran, R. Pandiselvam, A. Kothakota, M. Tomar, V. Satankar, M. Senapathy, T. Anitha, A. Dey, A. A. S. Sayed, F. M. Gadallah, R. Amarowicz and M. Mekhemar, Onion (Allium cepa L.) peels: A review on bioactive compounds and biomedical activities, *Biomedicine & Pharmacotherapy*, 2022, **146**, 112498.
9. G. Bianchi, V. Picchi, A. Tava, F. Doria, P. G. Walley, L. Dever, M. C. di Bella, D. Arena, H. Ben Ammar, R. Lo Scalzo and F. Branca, Insights into the phytochemical composition of selected genotypes of organic kale (Brassica oleracea L. var. acephala), *Journal of Food Composition and Analysis*, 2024, **125**, 105721.
10. P. K. Mukherjee, S. Singha, A. Kar, J. Chanda, S. Banerjee, B. Dasgupta, P. K. Haldar and N. Sharma, Therapeutic importance of Cucurbitaceae: A medicinally important family, *Journal of Ethnopharmacology*, 2022, **282**, 114599.
11. D. Šamec, B. Urlić and B. Salopek-Sondi, Kale (Brassica oleracea var. acephala) as a superfood: Review of the scientific evidence behind the statement, *Critical Reviews in Food Science and Nutrition*, 2019, **59**, 2411-2422.
12. S. T, I. S, R. A and J. A, Antimicrobial and antioxidant properties of the acetone extracts of the leaves of Lagenaria siceraria and Cucurbita pepo, *Food Chemistry Advances*, 2023, **3**, 100556.
13. M. Paesa, D. P. Nogueira, G. Velderrain-Rodríguez, I. Esparza, N. Jiménez-Moreno, G. Mendoza, J. Osada, O. Martin-Belloso, M. J. Rodríguez-Yoldi and C. Ancín-Azpilicueta, Valorization of onion waste by obtaining extracts rich in phenolic compounds and feasibility of its therapeutic use on colon cancer, *Antioxidants*, 2022, **11**, 733.
14. L. G. Santos and V. G. Martins, Recovery of phenolic compounds from purple onion peel using bio-based solvents: Thermal degradation kinetics and color stability of anthocyanins, *Journal of Food Processing and Preservation*, 2022, **46**, e17161.
15. S. L. Rodríguez García and V. Raghavan, Microwave-assisted extraction of phenolic compounds from broccoli (Brassica oleracea) stems, leaves, and florets: Optimization, characterization, and comparison with maceration extraction, *Recent Progress in Nutrition*, 2022, **02**, 011.
16. M. Râpă, R. N. Darie-Niță and G. Coman, Valorization of fruit and vegetable waste into sustainable and value-added materials, *Waste*, 2024, **2**, 258-278.
17. B. N. Silva, V. Cadavez, C. Caleja, E. Pereira, R. C. Calhelha, M. Añibarro-Ortega, T. Finimundy, M. Kostić, M. Soković, J. A. Teixeira, L. Barros and U. Gonzales-Barron, Phytochemical composition and bioactive potential of Melissa officinalis L., Salvia officinalis L. and Mentha spicata L. extracts, *Foods*, 2023, **12**, 947.
18. S. M. F. Bessada, J. C. M. Barreira, L. Barros, I. C. F. R. Ferreira and M. B. P. P. Oliveira, Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb.f.: An underexploited and highly disseminated species, *Industrial Crops and Products*, 2016, **89**, 45-51.
19. L. Barros, A. M. Carvalho, J. S. Morais and I. C. F. R. Ferreira, Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties, *Food Chemistry*, 2010, **120**, 247-254.
20. M. Soković and L. J. L. D. van Griensven, Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus, *European Journal of Plant Pathology*, 2006, **116**, 211-224.
21. M. Soković, J. Glamočlija, P. D. Marin, D. Brkić and L. J. L. D. v. Griensven, Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model, *Molecules*, 2010, **15**, 7532-7546.
22. J. Cano-Lou, A. Millán-Laleona, R. Candrea, F. Les, A. Pina, G. Caprioli and V. López, Apple peels as an edible source of phenolic bioactive compounds with antidiabetic and antiglycation properties, *Food & Function*, 2025, **16**, 2947-2958.
23. AOAC, AOAC Official Methods of Analysis, AOAC International, 2016.
24. B. Melgar, E. Pereira, M. B. P. P. Oliveira, E. M. Garcia-Castello, A. D. Rodriguez-Lopez, M. Sokovic, L. Barros and I. C. F. R. Ferreira, Extensive profiling of three varieties of Opuntia spp. fruit for innovative food ingredients, *Food Research International*, 2017, **101**, 259-265.
25. S. Machado, A. S. G. Costa, F. Pimentel B, M. B. P. P. Oliveira and R. C. Alves, A study on the protein fraction of coffee silverskin: Protein/non-protein nitrogen and free and total amino acid profiles, *Food Chemistry*, 2020, **326**, 126940.
26. L. Barros, C. Pereira and I. C. F. R. Ferreira, Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection, *Food Analytical Methods*, 2013, **6**, 309-316.
27. L. Barros, E. Pereira, R. C. Calhelha, M. Dueñas, A. M. Carvalho, C. Santos-Buelga and I. C. F. R. Ferreira, Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L., *Journal of Functional Foods*, 2013, **5**, 1732-1740.
28. M. M. Elattar, H. M. Hammoda, D. A. Ghareeb, M. F. El-Hosseny, M. G. Seadawy, I. Celık, R. S. Darwish and H. M. Dawood, An integrated strategy combining UPLC-MS/MS, chemometrics, molecular docking, and molecular dynamics simulation for metabolic profiling of onion (Allium cepa L.) cultivars and unravelling potential anti-COVID-19 metabolites, *South African Journal of Botany*, 2023, **162**, 885-900.
29. H. Ullah, E. Sommella, C. Santarcangelo, D. D'Avino, A. Rossi, M. Dacrema, A. D. Minno, G. Di Matteo, L. Mannina, P. Campiglia, P. Magni and M. Daglia, Hydroethanolic extract of Prunus domestica L.: Metabolite profiling and in vitro modulation of molecular mechanisms associated to cardiometabolic diseases, *Nutrients*, 2022, **14**, 340.
30. M. Søltoft, J. H. Christensen, J. Nielsen and P. Knuthsen, Pressurised liquid extraction of flavonoids in onions. Method development and validation, *Talanta*, 2009, **80**, 269-278.
31. C. Moliner, S. Núñez, G. Cásedas, M. S. Valero, M. I. Dias, L. Barros, V. López and C. Gómez-Rincón, Flowers of Allium

cepa L. as nutraceuticals: Phenolic composition and anti-obesity and antioxidant effects in Caenorhabditis elegans, *Antioxidants*, 2023, **12**, 720.

32. R. M. V. Goh, K. H. Ee, A. Pua, Y. Huang, S. Q. Liu, B. Lassabliere and B. Yu, Neutral loss scan in complement with high-resolution MS/MS: Combination of detection methods for flavonoid and limonoid glycosides analysis, *Journal of Mass Spectrometry*, 2022, **57**, e4810.
33. J. I. Park, M. J. Kim, K. H. Lee, S. H. Oh, Y. H. Kang and H. Kim, Determination of flavonoid glycoside isomers using vision transformer and tandem mass spectrometry, *Plants,* 2024, **13**, 3401.
34. L. Campone, R. Celano, A. Lisa Piccinelli, I. Pagano, S. Carabetta, R. D. Sanzo, M. Russo, E. Ibañez, A. Cifuentes and L. Rastrelli, Response surface methodology to optimize supercritical carbon dioxide/co-solvent extraction of brown onion skin by-product as source of nutraceutical compounds, *Food Chemistry*, 2018, **269**, 495-502.
35. R. M. Pérez-Gregorio, M. S. García-Falcón, J. Simal-Gándara, A. S. Rodrigues and D. P. F. Almeida, Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest, *Journal of Food Composition and Analysis*, 2010, **23**, 592-598.
36. A. V. González-de-Peredo, M. Vázquez-Espinosa, E. Espada-Bellido, C. Carrera, M. Ferreiro-González, G. F. Barbero and M. Palma, Flavonol composition and antioxidant activity of onions (Allium cepa L.) based on the development of new analytical ultrasound-assisted extraction methods, *Antioxidants*, 2021, **10**, 273.
37. R. Metrani, J. Singh, P. Acharya, G. K. Jayaprakasha and B. S. Patil, Comparative metabolomics profiling of polyphenols, nutrients, and antioxidant activities of two red onion (Allium cepa L.) cultivars, *Plants*, 2020, **9**, 1077.
38. Ž. Fredotović, J. Puizina, M. Nazlić, A. Maravić, I. Ljubenkov, B. Soldo, E. Vuko and D. Bajić, Phytochemical characterization and screening of antioxidant, antimicrobial, and antiproliferative properties of Allium × cornutum Clementi and two varieties of Allium cepa L. peel extracts, *Plants*, 2021, **10**, 832.
39. C. C. Nweze, W. Tseaa and I. P. Ekpe, Anti-inflammatory properties of quercetin: A review, *Journal of Drug Delivery and Therapeutics*, 2022, **12**, 205-210.
40. V. Vukics and A. Guttman, Structural characterization of flavonoid glycosides by multi-stage mass spectrometry, *Mass Spectrometry Reviews*, 2008, **29**, 1-16.
41. F. M. Mashitoa, T. Shoko, J. L. Shai, R. M. Slabbert and D. Sivakumar, Changes in phenolic metabolites and biological activities of pumpkin leaves (Cucurbita moschata Duchesne ex Poir.) during blanching, *Frontiers in Nutrition*, 2021, **8**.
42. I. Iswaldi, A. M. Gómez-Caravaca, J. Lozano-Sánchez, D. Arráez-Román, A. Segura-Carretero and A. Fernández-Gutiérrez, Profiling of phenolic and other polar compounds in zucchini (Cucurbita pepo L.) by reverse-phase high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, *Food Research International*, 2013, **50**, 77-84.
43. M. G. Leichtweis, A. K. Molina, T. C. S. Pires, M. I. Dias, R. Calhelha, K. Bachari, B. E. C. Ziani, M. B. P. P. Oliveira, C. Pereira and L. Barros, Biological activity of pumpkin byproducts: Antimicrobial and antioxidant properties, *Molecules*, 2022, **27**, 8366.
44. L. Barros, M. Dueñas, I. C. F. R. Ferreira, A. Maria Carvalho and C. Santos-Buelga, Use of HPLC-DAD-ESI/MS to profile phenolic compounds in edible wild greens from Portugal, *Food Chemistry*, 2011, **127**, 169-173.
45. S. Piccolella, A. Bianco, G. Crescente, A. Santillo, G. Chieffi Baccari and S. Pacifico, Recovering Cucurbita pepo cv. 'Lungo Fiorentino' wastes: UHPLC-HRMS/MS metabolic profile, the basis for establishing their nutra- and cosmeceutical valorisation, *Molecules*, 2019, **24**, 1479.
46. L.-Z. Lin and J. M. Harnly, Phenolic component profiles of mustard greens, Yu Choy, and 15 other Brassica vegetables, *Journal of Agricultural and Food Chemistry*, 2010, **58**, 6850-6857.
47. L. Z. Lin, J. Sun, P. Chen and J. Harnly, UHPLC-PDA-ESI/HRMS/MS(n) analysis of anthocyanins, flavonol glycosides, and hydroxycinnamic acid derivatives in red mustard greens (Brassica juncea Coss variety), *Journal of Agricultural and Food Chemistry*, 2011, **59**, 12059-12072.
48. Z. Li, H. W. Lee, X. Liang, D. Liang, Q. Wang, D. Huang and C. N. Ong, Profiling of phenolic compounds and antioxidant activity of 12 cruciferous vegetables, *Molecules*, 2018, **23**, 1139.
49. S. Schmidt, M. Zietz, M. Schreiner, S. Rohn, L. W. Kroh and A. Krumbein, Identification of complex, naturally occurring flavonoid glycosides in kale (Brassica oleracea var. sabellica) by high-performance liquid chromatography diode-array detection/electrospray ionization multi-stage mass spectrometry, *Rapid Communications in Mass Spectrometry*, 2010, **24**, 2009-2022.
50. P. Velasco, M. Francisco, D. A. Moreno, F. Ferreres, C. García-Viguera and M. E. Cartea, Phytochemical fingerprinting of vegetable Brassica oleracea and Brassica napus by simultaneous identification of glucosinolates and phenolics, *Phytochemical Analysis*, 2011, **22**, 144-152.
51. Y. Wang, M. A. Berhow, M. Black and E. H. Jeffery, A comparison of the absorption and metabolism of the major quercetin in brassica, quercetin-3-O-sophoroside, to that of quercetin aglycone, in rats, *Food Chemistry*, 2020, **311**, 125880.
52. M. E. Cartea, M. Francisco, P. Soengas and P. Velasco, Phenolic compounds in Brassica vegetables, *Molecules*, 2011, **16**, 251-280.
53. H. Olsen, K. Aaby and G. I. A. Borge, Characterization and quantification of flavonoids and hydroxycinnamic acids in curly kale (Brassica oleracea L. Convar. acephala Var. sabellica) by HPLC-DAD-ESI-MSn, *Journal of Agricultural and Food Chemistry*, 2009, **57**, 2816-2825.
54. L. G. M. da Silva, C. A. R. da Costa, G. A. Batista, K. A. Amorim, D. J. M. de Abreu, L. A. S. Pio, E. V. d. B. Vilas Boas and E. E. N. Carvalho, Synergy between solvent polarity and composition for efficient extraction of bioactive compounds from pitaya, *Food Materials Research*, 2025, **5**, e012.
55. O. R. Alara, N. H. Abdurahman and C. I. Ukaegbu, Extraction of phenolic compounds: A review, *Current Research in Food Science*, 2021, **4**, 200-214.
56. J. Dai and R. J. Mumper, Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties, *Molecules*, 2010, **15**, 7313-7352.
57. F. Chemat, M. A. Vian and G. Cravotto, Green extraction of natural products: concept and principles, *International Journal of Molecular Sciences*, 2012, **13**, 8615-8627.

58. F. Shahidi and K. D. Athiyappan, Polyphenol-polysaccharide interactions: molecular mechanisms and potential applications in food systems - a comprehensive review, *Food Production, Processing and Nutrition*, 2025, **7**, 42.
59. L. K. Caesar and N. B. Cech, Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2, *Natural Product Reports*, 2019, **36**, 869-888.
60. X. Chen, H. Li, B. Zhang and Z. Deng, The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations, *Critical Reviews in Food Science and Nutrition*, 2022, **62**, 5658-5677.
61. T.-H.-A. Phan, T.-P. Nguyen and T.-T.-A. Tran, Extracting, evaluating biological activities of phenolic compounds from yellow onion peels (Allium cepa L.) and their applicability for fish preservation, *IOP Conference Series: Earth and Environmental Science*, 2021, **947**, 012042.
62. A. Nile, S. H. Nile, D. H. Kim, Y. S. Keum, P. G. Seok and K. Sharma, Valorization of onion solid waste and their flavonols for assessment of cytotoxicity, enzyme inhibitory and antioxidant activities, *Food and Chemical Toxicology*, 2018, **119**, 281-289.
63. M. Radünz, T. M. Camargo, C. d. O. Raphaelli, A. L. Radünz, E. Á. Gandra and E. d. R. Zavareze, Chemical composition, antimicrobial and antioxidant activities of broccoli, kale, and cauliflower extracts, *Plant Foods for Human Nutrition*, 2024, **79**, 700-706.
64. J. Armesto, L. Gómez-Limia, J. Carballo and S. Martínez, Effects of different cooking methods on the antioxidant capacity and flavonoid, organic acid and mineral contents of Galega Kale (Brassica oleracea var. acephala cv. Galega), *International Journal of Food Sciences and Nutrition*, 2019, **70**, 136-149.
65. M. Hamissou, A. C. Smith, R. E. Carter Jr and J. K. Triplett II, Antioxidative properties of bitter gourd (Momordica charantia) and zucchini (Cucurbita pepo), *Emirates Journal of Food and Agriculture*, 2013, **25**.
66. M. J. Saavedra, A. Aires, C. Dias, J. A. Almeida, M. C. B. M. De Vasconcelos, P. Santos and E. A. Rosa, Evaluation of the potential of squash pumpkin by-products (seeds and shell) as sources of antioxidant and bioactive compounds, *Journal of Food Science and Technology*, 2015, **52**, 1008-1015.
67. N. Joković, J. Matejić, J. Zvezdanović, Z. Stojanović-Radić, N. Stanković, T. Mihajilov-Krstev and N. Bernstein, Onion peel as a potential source of antioxidants and antimicrobial agents, *Agronomy*, 2024, **14**, 453.
68. R. Celano, T. Docimo, A. L. Piccinelli, P. Gazzerro, M. Tucci, R. Di Sanzo, S. Carabetta, L. Campone, M. Russo and L. Rastrelli, Onion peel: Turning a food waste into a resource, *Antioxidants*, 2021, **10**, 304.
69. M. Hidalgo, C. Sánchez-Moreno and S. de Pascual-Teresa, Flavonoid–flavonoid interaction and its effect on their antioxidant activity, *Food Chemistry*, 2010, **121**, 691-696.
70. L. Jakobek, M. Seruga and P. Krivak, The influence of interactions among phenolic compounds on the antiradical activity of chokeberries (Aronia melanocarpa), *International Journal of Food Sciences and Nutrition*, 2011, **62**, 345-352.
71. S.-H. Kim, S.-H. Jo, Y.-I. Kwon and J.-K. Hwang, Effects of onion (Allium cepa L.) extract administration on intestinal α-glucosidases activities and spikes in postprandial blood glucose levels in SD rats model, *International Journal of Molecular Sciences*, 2011, **12**, 3757-3769.
72. M. Gois Ruivo da Silva, M. Skrt, D. Komes, N. Poklar Ulrih and L. Pogačnik, Enhanced yield of bioactivities from onion (Allium cepa L.) skin and their antioxidant and anti-α-amylase activities, *International Journal of Molecular Sciences*, 2020, **21**, 2909.
73. G. J. E. Nychas and C. C. Tassou, in *Encyclopedia of Food Microbiology (Second Edition)*, eds. C. A. Batt and M. L. Tortorello, Academic Press, Oxford, 2014, Preservatives, pp. 113-118.
74. N. A. Sagar, Y. Kumar, R. Singh, C. Nickhil, D. Kumar, P. Sharma, H. Om Pandey, S. Bhoj and A. Tarafdar, Onion waste based-biorefinery for sustainable generation of value-added products, *Bioresource Technology*, 2022, **362**, 127870.
75. N. O'Shea, E. K. Arendt and E. Gallagher, Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products, *Innovative Food Science & Emerging Technologies*, 2012, **16**, 1-10.
76. S. Iqbal, Ö. Tirpanalan-Staben and K. Franke, Modification of dietary fibers to valorize the by-products of cereal, fruit and vegetable industry—A review on treatment methods, *Plants*, 2022, **11**, 3466.
77. C. Opperman, M. Majzoobi, A. Farahnaky, R. Shah, T. T. H. Van, V. Ratanpaul, E. W. Blanch, C. Brennan and R. Eri, Beyond soluble and insoluble: A comprehensive framework for classifying dietary fibre's health effects, *Food Research International*, 2025, 115843.
78. F. Mariotti, D. Tomé and P. P. Mirand, Converting nitrogen into protein—Beyond 6.25 and Jones' factors, *Critical Reviews in Food Science and Nutrition*, 2008, **48**, 177-184.
79. M. Kohlmeier, in *Nutrient Metabolism*, ed. M. Kohlmeier, Academic Press, London, 2003, Phenylalanine, pp. 314-321.
80. T. Ikeda and N. Morotomi, in *Treatments, Nutraceuticals, Supplements, and Herbal Medicine in Neurological Disorders*, eds. C. R. Martin, V. B. Patel and V. R. Preedy, Academic Press, 2023, Chapter 48, pp. 929-944.
81. A. Plaskova and J. Mlcek, New insights of the application of water or ethanol-water plant extract rich in active compounds in food, *Frontiers in Nutrition*, 2023, **10**.
82. F. A. Ayaz, R. H. Glew, M. Millson, H. S. Huang, L. T. Chuang, C. Sanz and S. Hayırlıoglu-Ayaz, Nutrient contents of kale (Brassica oleraceae L. var. acephala DC.), *Food Chemistry*, 2006, **96**, 572-579.
83. L. Jaime, E. Mollá, A. Fernández, M. A. Martín-Cabrejas, F. J. López-Andréu and R. M. Esteban, Structural carbohydrate differences and potential source of dietary fiber of onion (Allium cepa L.) tissues, *Journal of Agricultural and Food Chemistry*, 2002, **50**, 122-128.
84. M. G. Leichtweis, A. K. Molina, M. I. Dias, R. C. Calhelha, T. C. S. P. Pires, O. Pavli, M. B. P. P. Oliveira, S. A. Petropoulos, L. Barros and C. Pereira, Variability in chemical profile and bioactivities of the flesh of Greek pumpkin landraces, *Horticulturae*, 2023, **9**, 1232.
85. R. K. Das, S. K. Brar and M. Verma, in *Platform Chemical Biorefinery*, eds. S. Kaur Brar, S. Jyoti Sarma and K. Pakshirajan, Elsevier, Amsterdam, 2016, Chapter 8, pp. 133-157.

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86. S. Zhou, M. Zhang, L. Zhu, X. Zhao, J. Chen, W. Chen and C. Chang, Hydrolysis of lignocellulose to succinic acid: a review of treatment methods and succinic acid applications, *Biotechnology for Biofuels and Bioproducts*, 2023, **16**, 1.
87. V. S. Kislik, in *Solvent Extraction*, ed. V. S. Kislik, Elsevier, Amsterdam, 2012, Chapter 2, pp. 69-111.