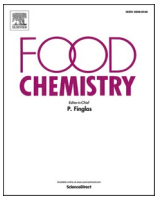
Food Chemistry 486 (2025) 144641

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



A biorefinery approach for the simultaneous obtention of essential oils, organic acids and polyphenols from citrus peels: Phytochemical characterization and bioactive potential

Esther Gómez-Mejía [a,b,c,*], Maria Inês Dias [a,b], Carla Pereira [a,b], Tânia C.S.P. Pires [a,b], Jesús Palá-Paúl [d], Noelia Rosales-Conrado [c], María Eugenia León-González [c], Ricardo Calhelha [a,b,*], Custódio Lobo Roriz [a,b]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain
[d] Department of Biodiversity Ecology and Evolution, Faculty of Biology, Complutense University of Madrid, 28040 Madrid, Spain

ARTICLE INFO

Keywords:
Citrus peels
Essential oils
Organic acids
Phenolic compounds
Bioactive properties
Multiproduct valorization

ABSTRACT

This investigation evaluates the valorization of citrus peels (lemon, tangerine, and orange) to recover both non-polar and polar fractions simultaneously. Citrus essential oils, abundant in limonene (74.4–33.7 %), exhibited great antioxidant activity (IC_{50} = 2.002 mg/mL) and *Campylobacter jejuni* halo inhibition (2.9 cm), particularly in tangerine and orange essential oils. The aqueous extracts were rich in quinic and malic acid (10–78.8 g/100 g), along with polyphenols (22.7–5.2 mg/g), such as diosmetin, luteolin, and eriodictyol glycosides. Tangerine's aqueous fraction showed the highest inhibition of oxidative hemolysis (IC_{50} = 102 μg/mL) and *Staphylococcus aureus* (MIC = 2.5 mg/mL). Whereas lemon was most effective against lipid peroxidation (IC_{50} = 1.33 mg/mL) and gastric adenocarcinoma proliferation (IG_{50} = 83 μg/mL). Principal component analysis correlated the in vitro bioactivities with each compound and citrus type, underscoring the potential of citrus peels as a cost-effective, sustainable source of value-added compounds with tailored commercial applications.

1. Introduction

Citrus fruits, classified under the *Rutaceae* family, represent one of the world's predominant crops, with global annual production exhibiting a consistent upward trend. According to the Foreign Agricultural Service of the United States Department of Agriculture (USDA), the production of tangerines, oranges, lemons, limes, and grapefruits has approached nearly 103 million tons as of July, 2024 (United States Department of Agriculture / Foreign Agricultural Service, 2024), highlighting the consumption and cultivation of tangerines, oranges and lemons in Western Europe (Mukhametzyanov et al., 2024; Vilas-Boas et al., 2023). However, this surge in production has led to a substantial volume of waste throughout the food supply chain, from initial production to final consumption (Soares et al., 2023). The consumption of citrus fruits results in up to 50–60 % of the fruit's total weight being discarded as waste, mostly as peels, but also as seeds, pomace, and membranes (Wedamulla et al., 2022). Addressing this challenge is critical, as conventional disposal methods such as landfilling, animal feeding utilization, incineration, or composting encounter inefficiencies due to the low pH, nitrogen content, and high humidity of citrus waste (Jensch et al., 2022; Wedamulla et al., 2022). These methods also pose environmental and economic challenges, including greenhouse gas emissions, extensive land use, and high resource expenditure (Gómez-Mejía et al., 2021; Soares et al., 2023).

Therefore, the search for sustainable waste management strategies, to promote a more sustainable food system, has gained magnitude in recent years, becoming a priority for the United Nations and the European Commission (Soares et al., 2023).

Among the most efficient alternatives in the citrus waste stream is valorization, which leverages citrus peel biomass as a renewable,

* Corresponding author at: Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain (Esther Gómez-Mejía); Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal (Ricardo Calhelha).
E-mail addresses: egomez03@ucm.es (E. Gómez-Mejía), calhelha@ipb.pt (R. Calhelha).

https://doi.org/10.1016/j.foodchem.2025.144641
Received 21 January 2025; Received in revised form 26 April 2025; Accepted 3 May 2025
Available online 6 May 2025

economical, and abundant source of value-added natural compounds with promising industrial and economic relevance (Wedamulla et al., 2022). This can be accomplished through the more comprehensive and promising biorefinery approach, which involves converting biomass into a diverse spectrum of marketable high-value-added products, including terpenoids, polyphenols, and organic acids, as well as energy (Arango-Manrique et al., 2024). This ensures the optimal biomass utilization, enhances economic viability, and reduces the carbon footprint (Espinosa et al., 2022; Jensch et al., 2022).

In this regard, Espinosa et al. (2022) sequentially recovered polyphenols and nanocellulose from orange peel, using a combination of ultrasound and high-pressure homogenization systems. Hilali et al. (2019) sequentially extracted essential oils, polyphenols, and pectin from orange peels using a hydrodistillation system followed by solid-liquid extraction in an acidic medium. Vilas-Boas et al. (2023) proposed a multi-step extraction of essential oils via steam hydrodistillation, hesperidin and narirutin via microwave hydrodiffusion and gravity and pectin and lignocellulosic biomass by hot acid extraction. Meanwhile, Chakroun et al. (2023) extracted non-polar (terpenoids, long-chain alkanes, phytosterols, and fatty acids) and polar compounds (phenolic compounds) from orange peel by two independent methods, Soxhlet, and maceration, respectively.

Nevertheless, previous proposals for sequential or separate-stage biorefineries face environmental and technological limitations. Particularly, multiple stages demand substantial investment in infrastructure and specialized equipment, markedly increasing capital and maintenance costs. Additionally, they entail prolonged processing times and heightened operational complexity, constraining profitability and industrial feasibility (Engelberth, 2020). Furthermore, using organic solvents such as methanol, ethanol, and acetone in certain steps undermines the sustainability and safety of the process (Gómez-Mejía et al., 2019). Consequently, scientific endeavors should prioritize enhancing the efficiency of aqueous extraction and developing safer, simpler, and industrially viable single-stage multiproduct extraction processes. As such, hydrodistillation, renowned for its efficiency, simplicity, and industrial applicability in recovering citrus essential oils, requires minimal instrumentation and utilizes water as a solvent (Arango-Manrique et al., 2024). Yet, a substantial proportion of the biomass, including the distillation water, is still regarded as waste, thereby foreclosing another valuable source of bioactive compounds enriched during the hydrodistillation heating. Therefore, repurposing distillation waters as functional extracts rather than discarding them would enable the simultaneous recovery of essential oils and citrus aqueous, enriched in polar compounds such as polyphenols and organic acids in a single step.

Additionally, the existing literature on citrus biorefinery exclusively focuses on oranges (Arango-Manrique et al., 2024; Chakroun et al., 2023; Espinosa et al., 2022; Hilali et al., 2019; Vilas-Boas et al., 2023), limiting the applicability and understanding of other citrus fruits, and have barely explored the bioactive potential of the extracts obtained, thereby restricting the insight into their potential applicability. To address this shortcoming, in-depth research is needed on the multiproduct valorization of various citrus peels in a single, simple procedure, combining chemical and bioactive evaluations.

Given the above, the main objective of this study is to evaluate the simultaneous multiproduct valorization of citrus peel wastes of lemon, tangerine, and orange peels, to obtain non-polar (essential oils) and polar (organic acids and phenolic compounds) extracts by hydrodistillation. In addition to the phytochemical profile characterization by chromatographic techniques (GC–MS, GC-FID, UPLC-PDA, and UPLC-ESI-DAD-MS), the in vitro antioxidant, antimicrobial, anti-inflammatory, and antiproliferative properties of both fractions have been investigated and correlated by multivariate analysis.

2. Material and methods

2.1. Citrus samples

Fruit peels from lemon (*Citrus* × *limon* (L.) Burm. fil.), orange (*Citrus* × *aurantium* L.) and tangerine (*Citrus reticulata* Blanco) have been used in this study. All samples, originating from Valencia (Spain), were purchased in bulk from Spanish suppliers. The peels were separated from the fruit using a stainless-steel knife, obtaining pieces approximately (1 × 4) cm in size. Afterwards, the peels were thoroughly cleaned with distilled water. Following the removal of excess moisture by blotting, the fresh peels were frozen at −20 °C in airtight, opaque containers for up to three months, until extraction of the bioactive compounds.

2.2. Isolation of bioactive compounds: Volatile and non-volatile compounds

Hydrodistillation was employed to simultaneously isolate essential oils and hydrophilic compounds from orange, lemon, and tangerine peels. Fig. 1 illustrates the extraction system used for the multiproduct valorization of citrus peels. Hydrodistillation was conducted following the recommendations of the Spanish Pharmacopoeia (Real Farmacopea Española, 3a Ed., 2005). A sample of 300–450 g of citrus peels was combined with distilled water at a 1:10 ratio in a Clevenger-type apparatus, equipped with an electric mantle heater, for 8 h. The resulting oils were dehydrated with anhydrous magnesium sulphate and stored in the dark at 4 °C until further analysis. The yield of essential oils extracted from the different samples was calculated in fresh weight as volume (mL) per 100 g of fresh peel weight (fw). Additionally, the aqueous extract, enriched with non-volatile compounds from the distillation water during the heating process, was collected by decantation and centrifugation, freeze-dried (FreeZone 4.5, Labconco, USA) and stored for later analysis.

2.3. Chemical characterization of bioactive compounds

2.3.1. Determination of volatile compounds of essential oils by GC–MS and GC-FID

Volatile compounds in citrus peel essential oils were determined using GC–MS and GC-FID, following the procedures developed elsewhere (Palá-Paúl et al., 2012; Usano-Alemany et al., 2016). The GC–MS analysis was carried out on an Agilent 6890 N Network gas chromatograph equipped with a fused DB5-MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness), coupled to an Agilent 5973 mass analyzer. The oven temperature started with an isotherm at 60 °C for 4 min, followed by a temperature gradient from 60 °C to 240 °C at 3 °C/min. Helium was used as carrier gas at a flow rate of 1.5 mL/min, and mass spectral data were recorded in scan mode at 70 eV, ranging from 41 *m/z* to 415 m/z. The injection volume was 0.1 μL of pure oil in splitless mode. Compounds were identified by their GC retention indices relative to *n*-alkanes and known compounds, and by comparing their full-scan mass spectra with known compounds, the spectrometer database library "WILEY.L." or published spectra (Adams, 2007; Joulain & König, 1998; Swigar & Silverstein, 1981).

Additionally, a semi-quantitative analysis was conducted using GC-FID on a Varian 3300 gas chromatograph equipped with a fused silica methyl silicone DB-5 column (50 m × 0.25 mm, 0.25 μm film thickness). The oven temperature was programmed to increase from 95 to 240 °C at 4 °C/min. Samples were injected at 250 °C in split mode (1:100), with nitrogen as the carrier gas at a flow rate of 1.5 mL/min. Detection was performed using a flame ionization detector (FID) (Palo Alto, California, USA) set at 300 °C. The relative contents of the volatile components, expressed as percentages, were determined by integrating the relative areas of the GC-FID peaks, using phenethyl acetate (0.8 mg/mL) as the internal standard.

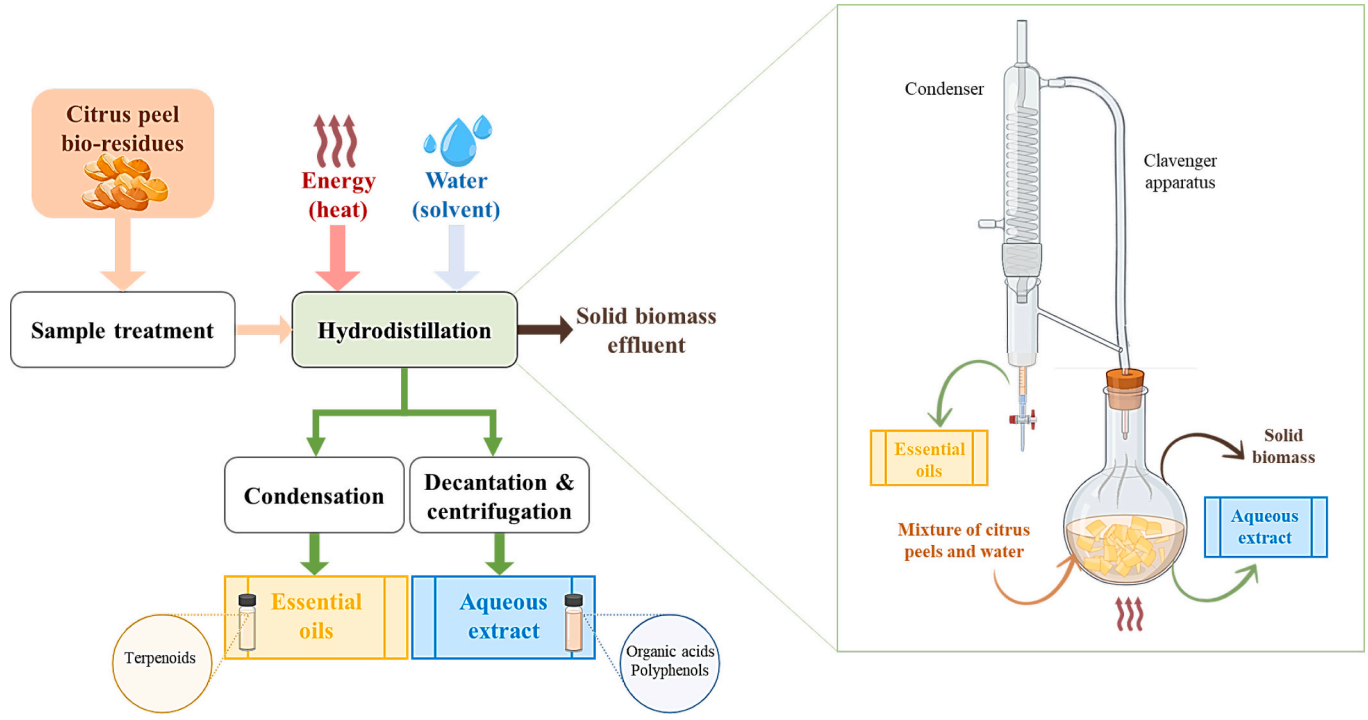


Fig. 1. Schematic flowchart of the multi-product valorization strategy of citrus peel bio-residues by hydrodistillation.

2.3.2. Determination of phenolic compounds by UPLC-ESI-DAD-MS

Phenolic profile characterization was carried out using a Dionex Ultimate 3000 UPLC liquid chromatograph (Thermo Scientific, San Jose, CA, USA) equipped with a diode-array detector (DAD) and an electrospray ionization source (ESI) operating in negative mode, in tandem with a linear ion trap (LTQ XL, Thermo Scientific, San Jose, CA, USA).

Chromatographic separation was achieved on a Spherisorb S3 ODS-2C18 column (3 μm, 4.6 mm × 150 mm, Waters, Mil-ford, MA, USA) maintained at 35 °C, according to the method previously described by (Bessada et al., 2016). The mobile phase gradient consisted of a mixture of 0.1 % (v) formic acid in water and acetonitrile a flow rate of 0.5 mL/min. The lyophilized aqueous extracts of the samples were reconstituted in water-methanol solution (20:80 *v/v*) at a concentration of 50 mg/mL and filtered through disposable 0.22 μm LC nylon filters.

On-line dual identification was performed using UV–Visible spectra collected at three distinct wavelengths (280, 330, and 370 nm) alongside mass fragmentation pattern. For quantification, seven-level external calibration curves were established for the phenolic standards. When specific compounds were commercially unavailable, quantification was performed using the most similar standard that could be obtained. Results were recorded and processed using the Xcalibur data system (Thermo Finnigan, San Jose, CA, USA), and then expressed as mg per gram of extract.

2.3.3. Determination of organic acids by UPLC-PDA

The determination of organic acids was carried out using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan), which was equipped with a DGU-20A degasser, a Nexera SIL-20A autosampler, a CTO-20AS column oven, and a SPDM20A photodiode array detector (PDA). Chromatographic separation was conducted on a SphereClone C18 column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA, USA), maintained at 35 °C. The elution was performed in isocratic elution mode using 3.6 mM sulfuric acid, and a flow rate of 0.80 mL/min (Barros et al., 2013). Samples extracts were prepared by dissolving the lyophilized extract in 4.5 % (*w/v*) HPO_3 at 40 mg/mL, and the solution was filtering through a 0.22-μm disposable LC nylon filter.

Organic acids were identified based on their adjusted retention time and peak purity at 215 and 245 nm, in comparison to commercial standards. Quantification was based on external calibration curves obtained from each analyte's standard (Sigma, St Louis, MIO, USA). Specifically, citric, fumaric, malic, oxalic, quinic, shikimic and succinic acids were measured at 215 nm, while ascorbic acid was measured at 245 nm. The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and expressed in g per 100 g of extract.

2.4. Evaluation of the bioactive properties of aqueous and volatile extracts

2.4.1. Antioxidant activity

DPPH assay: The antioxidant activity of the volatile essential oils extracted from citrus peels was evaluated on their ability to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals (Bessada et al., 2016). In brief, 30 μL of essential oil solutions, dissolved in methanol at concentrations ranging from 20 mg/mL to 0.34 mg/mL, were mixed with 270 μL of $6{\cdot}10^{-5}$ M DPPH methanolic solution (Alfa Aesar, Ward Hill, MA, USA). DPPH and trolox (Sigma-Aldrich, St. Louis, MIO, USA) served as negative and positive controls, respectively. After incubating in darkness for one hour, absorbance was measured at 515 nm using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA). Radical-scavenging activity was calculated as the percentage of DPPH decolorization at IC_{50} (mg/mL). The experiment was conducted in triplicate.

Thiobarbituric reactive substances assay (TBARS): This colorimetric method was used to evaluate the inhibition of lipid peroxidation by aqueous extract of the citrus peel (Lockowandt et al., 2019). In this procedure, 200 μL of porcine brain cell homogenates were incubated with 100 μL of aqueous extract (8.0–0.5 mg/mL), 100 μL of $FeSO_4$ (1.52 mg/L, Sigma Aldrich) and 100 μL of ascorbic acid (17.6 mg/L, Sigma Aldrich) at 37.5 °C for 1 h. The resulting mixture reacted with 380 μL of thiobarbituric acid (2 % (*w/v*) in water, Sigma-Aldrich). The absorbance of the complex was measured at 532 nm, and the results were expressed as IC_{50} values (mg/mL). Trolox was used as a positive control.

Oxidative hemolysis assay (OxHLIA): The inhibition of oxidative

hemolysis by aqueous extracts of citrus peel was conducted as described by Lockowandt et al. (2019). In this assay, 200 μL of a 2.8 % (*v*/v) of erythrocyte solution in Phosphate-Buffered Saline (PBS) was mixed with 400 μL of either extract in PBS (3500–70 μg/mL), PBS solution, or water. After pre-incubating the mixture at 37 °C for 10 min with agitation, 200 μL of 43.40 g/L 2–2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, Sigma-Aldrich, St. Louis, MIO, USA) in PBS was added. The optical density was measured at 690 nm every 15 min until full hemolysis, using a microplate reader. Trolox was used as a positive control. The assay was performed in duplicate, and the results were expressed as IC_{50} values (μg/mL) at Δt of 60 min.

2.4.2. Antimicrobial activity

Antibacterial activity of essential oils: The antibacterial activity of the essential oils was tested against three Gram-positive bacteria (*Bacillus subtilis*, *Listeria* spp. and *Staphylococcus aureus*) and four Gram-negative bacteria (*Campylobacter jejuni*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* spp.), provided by the Department of Genetic, Physiology and Microbiology of the Biology Faculty from the Complutense University of Madrid, using the agar disc diffusion method (Palá-Paúl et al., 2012). A bacterial suspension (0.5 McFarland scale concentration) was spread on Petri dishes with Mueller Hilton medium (MH, Sigma-Aldrich, St. Louis, USA) or Columbia agar with sheep blood (Thermo Fisher Scientific, Madrid, Spain) for *Listeria* and *C. jejuni*. Then, 10 μL of the pure essential oil were placed on sterilized filter paper discs (9 mm diameter) and incubated for 24 h at 35 °C or 42 °C in anaerobiosis for *C. jejuni*. Growth inhibition halos were quantified based on the inhibition zone in centimeters (including disc diameter). The assay was performed in duplicate.

Antibacterial activity of citrus aqueous extracts: The antibacterial activity of citrus aqueous extracts was evaluated using the microdilution method combined with *p*-iodonitrotetrazolium chloride staining (INT, Panreac Applichem, Barcelona, Spain), as previously described (Pires et al., 2018). Five Gram-negative (*Enterobacter cloacae* (ATCC49741), *E. coli* (ATCC25922), *P. aeruginosa* (ATCC9027), *S. enterica* (ATCC13076), *Yersinia enterocolitica* (ATCC8610)) and three Gram-positive bacteria (*B. cereus* (ATCC11778), *L. monocytogenes* (ATCC19111) and *S. aureus* (ATCC25923)), supplied by Frilabo (Porto, Portugal), were tested. The aqueous extracts were dissolved in autoclaved distilled water with 5 % (*v*/v) dimethyl sulfoxide (DMSO) at 20 mg/mL. Then, 100 μL of the extract was mixed with 90 μL of Tryptic Soy Broth (TSB) (Frilabo, Porto, Portugal) and 10 μL of bacteria suspension prepared in TSB medium at 1.5×10^6 CFU/mL, resulting in test solutions ranging from 0.312 mg/mL and 10 mg/mL. Non-inoculated TSB and the aqueous extract were prepared as negative controls. Ampicillin, streptomycin and methicillin antibiotics were used as positive controls. After 24-h incubation at 37 °C, 40 μL of 0.2 mg/mL INT was added, and the pink staining of viable microorganisms allowed visual screening of bacterial growth inhibition. Antibacterial results were expressed as minimum inhibitory concentrations (MIC; mg/mL) and/or minimum bactericidal concentrations (MBC; mg/mL) if 99.5 % suppression of the original inoculum was observed after an additional 24 h at 37 °C.

Antifungal activity of citrus aqueous extracts: The antifungal activity of the citrus aqueous extracts against *Aspergillus fumigatus* (ATCC204305) and *Aspergillus brasilensis* (ATCC16404), supplied by Frilabo (Porto, Portugal), was evaluated using the serial dilution method described by (Heleno et al., 2013). In this assay, 100 μL of the aqueous extract (0.312 mg/mL and 10 mg/mL, prior dissolved in water and DMSO 5 %), 90 μL of Malt Extract Broth (MEB, Liofilchem, Abruzzi, Italy) and 10 μL of fungal spores (1.0×10^6 in sterile saline) were incubated for 72 h at 25 °C. The lowest concentration without visible growth under binocular microscopy was estimated as the MIC. Conversely, the minimum fungicidal concentration (MFC) was defined as the minimal concentration required to suppress 99.5 % of the original inoculum after an additional 72 h at 25 °C. Two negative controls were prepared: one with non-inoculated MH and Tween, and another with the aqueous extract. A positive control with ketoconazole (Frilabo, Porto, Portugal) was also included. The assay was conducted twice.

2.4.3. Antiproliferative and anti-inflammatory activity

Cytotoxicity of citrus aqueous extracts: The cytotoxicity of citrus aqueous extracts was assessed using a slightly modified sulforhodamine B (SRB) colorimetric method (Barros et al., 2013). Four human tumor cell lines, provided by the Leibniz-Institute DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, were used: AGS (gastric adenocarcinoma), Caco-2 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung carcinoma). A non-tumor cell line, Vero (African green monkey kidney), was used to evaluate the extract's toxicity against healthy cells.

Briefly, 10 μL of citrus extracts at concentrations ranging from 400 to 6.25 μg/mL (dissolved in a 50:50 mixture of water and DMSO) were mixed with 190 μL of the cell lines (10,000 cell/mL or 19,000 cell/mL for Vero) and incubated for 72 h at 37 °C with 5 % CO_2. The adherent cells were attached with 100 μL of 10 % trichloroacetic acid, incubated for 60 min at 4 °C, and washed with deionized water. Then, 100 μL of 0.057 % (*w*/*v*) SRB was added, and the excess was removed with 1 % acetic acid solution. The adhered SRB was resolved with 200 μL of 1.21 g/L Tris, and the absorbance was read at 490 nm. Positive and negative controls, ellipticine (Sigma-Aldrich, St Louis, MO, USA) and cell solution without sample, respectively, were prepared. Results were expressed as the concentration of extract needed to inhibit 50 % of cell proliferation (GI_{50}, μg/mL). The assay was performed in duplicate.

Anti-inflammatory activity of citrus aqueous extracts: The anti-inflammatory potential of citrus aqueous extracts was evaluated using the method described by Sobral et al. (2016). The RAW 264.7 mouse macrophage cell line (European Collection of Authenticated Cell Cultures) was seeded at 1.5×10^5 cell/well with 6.25–400 μg/mL citrus extracts and 30 μL of 1 mg/mL lipopolysaccharide (LPS). After 24-h incubation, NO levels were quantified using the Griess reagent system kit (Promega, Madison, WI, USA). Results were presented as the concentration of extracts causing 50 % inhibition of NO production (IC_{50}, μg/mL). Dexamethasone (Sigma-Aldrich, Saint Louis, MO, USA) served as positive control, and samples without LPS (Sigma-Aldrich, Saint Louis, MO, USA) acted as negative controls.

2.5. Statistical analysis

The results of chemical composition and bioactivity, along with their corresponding confidence level, were expressed as mean ± standard deviation, when applicable. Such data were explored and compared, with a level of confidence of 95 %, based on one-way Analysis of Variance (ANOVA), the Fisher's Least Significant Difference test (LSD), and Principal Component Analysis (PCA), all of them conducted with Statgraphics Centurion 19 (Statgraphics Technologies. Inc., Rockville, MD, USA) software package.

3. Results and discussion

3.1. Chemical characterization

3.1.1. Essential oil composition

Essential oils were extracted from citrus peels using a reliable hydrodistillation method, with comprehensive guidelines. This methodology provides high oil yields, increased purity, extended shelf life, elevated limonene content, and low phototoxicity compared to alternative cold pressing, which favors the eventual industrial application of the essential oils (Vilas-Boas et al., 2023).

The volatile components were identified according to their GC retention indices relative to *n*-alkanes and known compounds and by comparison of their full-scan mass spectra with known compounds. **Fig. S1** shows the GC–MS profiles of the volatile compounds in the essential oils extracted from lemon, orange, and tangerine peels. The

content of each volatile compound was determined based on its relative percentage to the internal standard phenethyl acetate. Table 1 summarized the volatile compounds identified in the essential oils of the analyzed citrus fruits, their retention indices and their percentage compositions, listed in order of elution on the DB-5 column.

A total of 122 compounds were identified, representing more than 95 % of the total oil of the samples analyzed, most of which were terpenoids. Monoterpene hydrocarbons were especially dominant in orange (84.9 %) and tangerine (73.4 %) oils, while this fraction was slightly lower in lemon oil (52.9 %). These were followed by oxygenated monoterpenes, which nearly completed the entire chemical profiling of the essential oils, with 41.3 %, 24.4 % and 13.3 % for lemon, tangerine and orange oils, respectively. Other fractions, such as sesquiterpene hydrocarbon, were present at levels lower than 5.8 % (Table 1).

Limonene was the main compound identified in all the samples (33.7–74.4 %) and the only one for orange and tangerine oils. Classified as Generally Recognized As Safe (GRAS) by the Food and Drug Administration, limonene's designation supports its expanding use across various sectors, including food preservation, antimicrobial packaging, flavoring, and biopesticides, highlighting the applicability of the obtained citrus essential oils (Vilas-Boas et al., 2023). In the essential oil from lemon peel, nerol (10.9 %) was the other principal component identified. Other representative constituents of this oil included γ-terpinene (7.4 %), geranial (7.2 %), α-terpineol (6.7 %), neral (6.2 %), and β-pinene (5.2 %). All these compounds were detected in orange and tangerine samples, but their percentage composition did not reach 5 %. Although all the compounds contributed to the oil's fragrance, only linalool (5.3 %) can represent tangerine oil. These results agree with previous reports (Aguilar-Hernández et al., 2020; Boukroufa et al., 2015; Chakroun et al., 2023; Hilali et al., 2019). The chemical composition of these species includes principal compounds with potential medical or cosmetic applications. The stability of these essential oil components, responsible for antimicrobial, antioxidant, and anti-inflammatory effects, supports their future use (Chakroun et al., 2023; Gupta et al., 2021).

The number of compounds identified is noteworthy, with extraction yields of 1.00 % for lemon, 0.74 % for orange, and 0.61 % for tangerine peels. Yet, these citrus peel samples have only been profiled without considering other factors such as variety, post-harvest changes, phenology, soil, or rootstock. Therefore, it would be interesting to further evaluate whether any of these aspects could affect the yield, composition, and biological activity of the multiproduct valorization of citrus peel residues.

3.1.2. Organic acid composition

One of the most abundant natural compounds in citrus peels is organic acids. These phytochemicals are widely used in the food industry as antimicrobial, flavoring, acidulants and pH-adjusting agents (Gómez-Mejía et al., 2021; Liew et al., 2018). Therefore, this study proposes the reuse of hydrodistillation waters as functional bioactive extracts, making it essential to determine the identity and content of these metabolites in this aqueous fraction. **Fig. S2** exemplifies the profile of organic acids present in the aqueous extracts of lemon peels.

Four organic acids were detected in all the samples studied, namely, malic, quinic, ascorbic and fumaric acid. Oxalic acid was only found in the aqueous extract of orange and tangerine peels, while citric acid was exclusively present in the aqueous extract of lemon peels (Table 2). In agreement, citric acid levels are significantly higher in lemon peels than in tangerine and orange peels (Shofinita et al., 2015). Additionally, degradation of this organic compound has been observed when extraction is prolonged (Fernandes et al., 2022). Thus, the lower expected contents in orange and tangerine peels, combined with the extended extraction time (6 h) and the temperatures reached during hydrodistillation, could have led to the complete breakdown of citric acid in these matrices (Table 2). Quinic and malic acids constituted more than 50 % of the total anions in the peel extract of the citrus fruits studied, with quinic acid ranging from 19.4 to 78.8 g/100 g DW. Previously, Rosa et al. (2023) identified this acid as the most representative organic acid detected in both the flavedo and albedo peel of *Citrus limon* var. *pompia* Camarda at a concentration of 22 μg/mL, and Liew et al. (2018) reported malic acid as the third main compound in *Citrus sinensis* peel extract, with concentrations up to 8 mg/g. This is significantly lower than the concentration reported in this study for orange peels (100 mg/g), highlighting the effectiveness and viability of utilizing distillation waters as a functional extract rich in value-added organic acids.

The most notable source of oxalic acid was orange peel extract, constituting 15 % of the composition of organic acids in this citrus matrix (Table 2). This acid has also been reported as predominant in bitter orange (*Citrus aurantium*) peel, although at lower concentrations (257 mg/100 g) than in the present study (Ersus & Cam, 2007), likely due to the different varieties of citrus fruit and the efficacy of the extraction method employed. Regarding ascorbic acid, also known as vitamin C, the aqueous extract of tangerine peel was the only one in which this compound could be quantified (Table 2). Although the determined concentration was modest (0.325 g/100 g), this is likely because ascorbic acid is severely degraded upon exposure to air, light, or heat (Guimarães et al., 2010). Nevertheless, vitamin C is a valuable natural compound, widely exploited as an antioxidant in the food and cosmetic industries, which underscores the potential interest of the aqueous tangerine peel as a source of ascorbic acid in these fields.

Overall, the significantly higher richness of the lemon peel extracts in total organic acids (176.9 ± 0.2 g/100 g DW) is remarkable, followed by the other two citrus extracts, both of which were statistically comparable (p-value >0.05) with a total concentration around 40 g/100 g DW. Therefore, all aqueous extracts of citrus peels can be considered natural resources enriched in quinic and malic acids, with great potential for obtaining citric, oxalic and ascorbic acids, depending on their matrix nature.

3.1.3. Phenolic profile

Table 3 presents the phenolic profile of the aqueous peel extracts of *C.* × *limon* (L.) Burm. fil., *C.* × *aurantium* L. and *C. reticulata* Blanco, focusing on the identification and quantitation of bioactive compounds using non-targeted UPLC-DAD-ESI-MS analysis. **Fig. S3** illustrates the phenolic profiles of the aqueous extracts from lemon, orange, and tangerine peels. In total, thirty-four compounds were determined, with thirty-two natural bioactives tentatively identified in citrus peels based on available literature (Buyukkurt et al., 2019; Cebadera-Miranda et al., 2019).

The chemical composition of the citrus peel extracts was qualitatively similar, with flavonoids, specifically flavonols, flavones and flavanones, predominating. Significant differences (p-value <0.05) were observed in the concentration of most phenolic compounds, depending on the type of citrus (Table 3).

Regarding flavonoids, flavones were the most prevalent phenolics in the aqueous citrus peel extracts, ranging from 44 % to 75 % of the total phenolic compounds, with notable presence of luteolin, apigenin, chrysoeriol, and diosmetin. Specifically, luteolin-C-hexoside accounted for up to 1.87 ± 0.06 mg/g in tangerine extract, while chrysoeriol 6,8-di-C-glucoside (stellarin-2) was found at 1.05 ± 0.06 mg/g and 2.1 ± 0.1 mg/g in orange and lemon peel extracts, respectively. Diosmetin-6,8-di-C-β-D-glucopyranoside was the second main polyphenol in lemon peel extract, with a concentration of 4.46 ± 0.08 mg/g. Other authors have also described the richness of luteolin and diosmetin sugar derivatives, other than their aglycone forms, in the studied citrus peels. For instance, Wang et al. (2016) reported that the luteolin-C-glucoside content was higher in *C. reticulata* and *C. limon* than in *C. sinensis* peel extract, as shown in Table 3. However, phenolic extracts obtained with methanol-water 70:30 (v/v) reported lower levels than in the present study (< 50 μg/g vs. 1.8 mg/g). It is noteworthy that flavone C-glycosides, including stellarin-2, have been reported to inhibit NO production in LPS-stimulated RAW macrophages at concentrations of 100 μg/mL

Table 1
Essential oil composition (%) of lemon (*C.* × *limon* (L.) Burm. fil.), orange (*C.* × *aurantium* L.) and tangerine (*C. reticulata* Blanco) peel bio-residues.

Compound	Compound type	IK	IK-L	Lemon peel	Orange peel	Tangerine peel
1. Hexanal	O	800	797	*t*	*t*	0.1
2. Heptanal	O	905	901	*t*	*t*	*t*
3. α-Thujene	MH	927	924	0.2	*t*	*t*
4. α-Pinene	MH	938	932	1.2	0.8	0.8
5. Camphene	MH	953	946	0.1	*t*	*t*
6. Sabinene	MH	972	969	1.0	0.7	1.3
7. β-Pinene	MH	981	974	**5.2**	0.2	0.2
8. 6-methyl-5-Hepten-2-one	O	989		*t*	*t*	*t*
9. Myrcene	MH	992	988	1.9	3.3	2.7
10. n-Octanal	O	1001	998	*t*	*t*	*t*
11. α-Phellandrene	MH	1006	1002	0.4	3.0	2.6
12. δ-3-Carene	MH	1008	1008	*t*	0.5	*t*
13. α-Terpinene	MH	1016	1014	0.5	0.3	0.5
14. *p*-Cymene	MH	1018	1020	*t*	*t*	*t*
15. *o*-Cymene	MH	1019	1022	*n.d.*	0.8	0.6
16. Limonene	**MH**	**1026**	**1024**	**33.7**	**74.4**	**63.0**
17. β-Phellandrene	MH	1029	1025	*t*	0.1	*n.d.*
18. (*Z*)-β-Ocimene	MH	1038	1032	0.1	*t*	0.4
19. γ-Terpinene	MH	1053	1054	**7.4**	0.4	0.8
20. *n*-Octanol	O	1068	1063	*t*	0.7	0.3
21. cis-Linalool oxide	MO	1073	1067	0.1	*n.d.*	*n.d.*
22. Terpinolene	MH	1085	1086	1.1	0.3	0.4
23. p-Cymenene	MH	1093	1089	*n.d.*	*t*	*t*
24. Linalool	MO	1095	1088	2.2	2.9	**5.3**
25. n-nonanal	O	1103	1100	*t*	*t*	*t*
26. 1,3,8-*p*-Menthatriene	MH	1110	1108	*t*	0.1	0.2
27. endo-Fenchol	MO	1116	1114	0.2	*t*	*t*
28. dehydro-Sabina ketone	MO	1123	1117	t	*n.d.*	*n.d.*
29. cis-ρ-Menth-2-en-1-ol	MO	1124	1118	0.2	0.2	0.5
30. trans-Rose oxide	MO	1127	1122	*t*	*n.d.*	*n.d.*
31. cis-Limonene oxide	MO	1135	1132	0.1	0.2	0.5
32. trans-limonene oxide	MO	1140	1137	*n.d.*	*n.d.*	*t*
33. trans-ρ-Menth-2-en-1-ol	MO	1141	1136	0.1	*t*	*t*
34. Camphor	MO	1144	1141	*t*	*n.d.*	*t*
35. cis-β-terpineol	MO	1146	1140	0.4	0.1	0.2
36. Camphene hydrate	MO	1147	1145	*t*	*n.d.*	*n.d.*
37. Citronellal	MO	1150	1148	0.2	0.2	0.2
38. Borneol	MO	1169	1165	0.6	*n.d.*	t
39. ρ-Mentha-1,5-dien-8-ol	MO	1170	1166	*t*	*t*	*t*
40. n-Nonanol	O	1171	1165	*n.d.*	*t*	*t*
41. Terpinen-4-ol	MO	1175	1174	3.4	1.3	2.5
42. (*E*)-Isocitral	MO	1181	1180	0.3	*t*	*t*
43. ρ-Cymen-8-ol	MO	1183	1182	*n.d.*	*n.d.*	*t*
44. Cryptone	O	1186	1185	*n.d.*	*n.d.*	*t*
45. α-Terpineol	MO	1188	1186	**6.7**	1.2	1.5
46. cis-Piperitol	MO	1197	1195	*n.d.*	*n.d.*	*t*
47. cis-Dihydro carvone	MO	1199	1191	*n.d.*	*n.d.*	*t*
48. Verbenone	MO	1205	1204	*n.d.*	0.1	*n.d.*
49. trans-Piperitol	MO	1210	1207	*t*	0.1	0.6
50. trans-Dihydro carvone	MO	1212	1200	*n.d.*	t	t
51. n-Decanal (1204)	MO	1213	1201	0.2	1.3	1.3
52. trans-Carveol	MO	1217	1215	*t*	0.5	1.1
53. cis-Carveol	MO	1229	1226	*n.d.*	*t*	*n.d.*
54. Citronellol	MO	1230	1223	0.6	1.4	2.4
55. Nerol	**MO**	**1231**	**1227**	**10.9**	1.4	*t*
56. Carvone	MO	1241	1239	*t*	*t*	3.7
57. Neral	MO	1244	1235	**6.2**	0.2	*t*
58. Piperitone	MO	1250	1249	*t*	*n.d.*	*t*
59. Geraniol	MO	1251	1249	1.5	2.1	0.2
60. Geranial	MO	1266	1264	**7.2**	*t*	4.4
61. cis-Verbenyl acetate	O	1282	1280	*t*	*t*	0.1
62. α-Terpinen-7-al	MO	1285	1283	0.1	*n.d.*	*t*
63. ρ-Cymen-7-ol	MO	1291	1289	*t*	*n.d.*	*n.d.*
64. cis-2-tert-butyl-Cyclohexanol acetate	O	1295	1293	0.1	*t*	*t*
65. 3′-methoxy-Acetophenone	O	1301	1298	*t*	*t*	0.2
66. Geranyl formate	O	1302	1298	*t*	*n.d.*	*n.d.*
67. Carvacrol	MO	1303	1298	*n.d.*	*n.d.*	*t*
68. trans-Piperitol acetate	O	1344	1343	*n.d.*	*t*	*t*
69. Citronellyl acetate	O	1355	1350	0.1	*n.d.*	*n.d.*
70. Z-β-Damascenone	O	1363	1361	*t*	*n.d.*	*n.d.*
71. Neryl acetate	O	1366	1359	1.0	*t*	*t*
72. α-Copaene	SH	1375	1374	*t*	*t*	*t*
73. trans-Myrtanol acetate	O	1384	1385	*t*	*n.d.*	*n.d.*
74. Geranyl acetate	O	1387	1379	0.6	*t*	*t*

(*continued on next page*)

Table 1 (*continued*)

Compound	Compound type	IK	IK-L	Lemon peel	Orange peel	Tangerine peel
75. β-Cubebene	SH	1389	1387	*n.d.*	*t*	*t*
76. β-Elemene	SH	1392	1389	0.0	*n.d.*	*t*
77. Methyl eugenol	O	1405	1403	*t*	*n.d.*	*t*
78. α-*cis*-Bergamotene (23,564)	SH	1415	1411	*t*	0.1	0.1
79. (*E*)-Caryophyllene	SH	1419	1417	0.5	0.1	*t*
80. β-Gurjunene (24,04)	SH	1433	1431	*t*	*t*	*t*
81. α-*trans*-Bergamotene	SH	1435	1432	0.6	*t*	*t*
82. α-Guaiene	SH	1439	1437	*t*	*t*	*n.d.*
83. α-Humulene	SH	1453	1452	0.1	*t*	*t*
84. Neryl Propionate	O	1454	1454	*t*	*t*	*n.d.*
85. (*E*)-β-Farnesene	SH	1455	1454	0.1	*t*	0.1
86. β-Santalene	SH	1456	1457	0.1	*n.d.*	*n.d.*
87. γ -Muurolene	SH	1480	1478	*t*	*t*	*t*
88. Germacrene D	SH	1485	1484	*t*	*t*	*t*
89. β-Selinene	SH	1490	1489	0.1	*t*	*t*
90. cis-β-Guaine	SH	1493	1492	*n.d.*	*t*	*n.d.*
91. Valencene	SH	1497	1496	0.7	0.6	0.1
92. Bicyclogermacrene	SH	1503	1500	*t*	*t*	*t*
93. α-Muurolene	SH	1504	1500	*t*	*t*	*t*
94. Z-α-Bisabolene	SH	1507	1506	0.1	*t*	*t*
95. β-Bisabolene	SH	1509	1505	1.0	*t*	0.1
96. β-Curcumene	SH	1515	1514	*t*	*t*	*t*
97. Butylated hydroxytoluene	O	1517	1514	*n.d.*	*n.d.*	*t*
98. 7-*epi*-α-Selinene	SH	1522	1520	0.1	0.1	*t*
99. δ-Cadinene	SH	1526	1522	*t*	*t*	0.1
100. (*E*)-γ-Bisabolene	SH	1530	1529	*t*	*n.d.*	*n.d.*
101. Elemol	SO	1549	1548	*t*	*t*	*t*
102. (*Z*)-Nerolidol	SO	1533	1531	*t*	*n.d.*	*n.d.*
103. (*Z*)-Isoelemicin	O	1570	1568	*t*	*n.d.*	*n.d.*
104. (*E*)-Nerolidol	SO	1571	1561	0.1	*t*	*t*
105. Spathulenol	SO	1579	1577	*t*	*t*	*t*
106. Globulol	SO	1591	1590	*t*	*n.d.*	*n.d.*
107. Viridiflorol	SO	1593	1592	*t*	*n.d.*	*n.d.*
108. Cubeban-11-ol	SO	1598	1595	*t*	*n.d.*	*n.d.*
109. Rosifoliol	SO	1602	1600	*t*	*n.d.*	*n.d.*
110. Ledol	SO	1607	1602	*t*	*n.d.*	*n.d.*
111. γ-Eudesmol	SO	1631	1630	*t*	*t*	*t*
112. Cubenol	SO	1646	1645	*t*	*t*	*t*
113. α-Cadinol	SO	1653	1652	*t*	*t*	*t*
114. Valerianol	SO	1657	1656	0.2	0.1	t
115. epi-β-Bisabolol	SO	1671	1670	0.1	*n.d.*	*n.d.*
116. epi-α-Bisabolol	SO	1684	1683	0.1	*n.d.*	*n.d.*
117. β-Sinensal	SO	1701	1699	*t*	0.1	0.2
118. (2*Z*,6*E*)-Farnesol	SO	1723	1722	*t*	*n.d.*	*n.d.*
119. (2*E*,6*E*)-Farnesal	SO	1743	1740	*t*	*t*	*t*
120. α-Sinensal	SO	1757	1755	*t*	*t*	0.7
121. Nootkatone	SO	1805	1806	*t*	*t*	*t*
122. β-Bisabolenol	SO	1788	1789	*t*	*n.d.*	*n.d.*
Total monoterpene hydrocarbons (MH)				52.9	84.9	73.4
Total oxygenated monoterpenes (OM)				41.3	13.3	24.4
Total sesquiternene hydrocarbons (SH)				3.4	0.9	0.6
Total oxygenated sesquiternene (OS)				0.6	0.2	1.0
Others (O)				1.9	0.7	0.7

MH = monoterpene hydrocarbon, OM = oxygenated monoterpene, SH = sesquiterpene hydrocarbon, SO = oxygenated sesquiterpene, O = Other; *t* = traces (< 0.1 %); *n.d.* = not detected; KI = linear retention index relative to *n*-alkanes on DB-5 column; KI-L = literature linear retention index on DB-5 column.

(Kim et al., 2016). Additionally, luteolin 6-C-glucoside and luteolin 6,8-di-C-glucoside are effective antioxidant agents against free radicals generated in vitro in both lipophilic and hydrophilic media (Materska, 2015; Nicolescu et al., 2024), illustrating their commercial interest.

On the other hand, flavanones, primarily reported as polymethoxylated-glycosidic derivatives of eriodyctiol and naringenin, constituted less than 15 % in tangerine and orange peels. Although, they were found in greater quantities in lemon peels (43 %). Lemon peel extracts yielded eriodictyol-O-deoxyhexosyl-hexoside as high as 8.1 ± 0.4 mg/g, being the main compounds reported in the present study, with significantly lower amounts (p-value <0.05) in the other citrus peel extracts (Table 3), in agreement with previous studies (Rosa et al., 2023; Wang et al., 2016).

Conversely, naringenin-O-deoxyhexosyl-hexoside was poorly recovered in lemon and orange peel extracts (<0.3 mg/g). The final subfamily of flavonoids identified in the aqueous citrus extracts were flavonols, comprising glycosylated forms of quercetin, limocitrol and limocitrin (Table 3). These were found in minor quantities in all samples (<1 mg/g), consistent with former findings, which indicated that glycosylated flavanols are generally scarce (Li et al., 2020; Rosa et al., 2023; Wang et al., 2016).

Nonetheless, Nicolescu et al. (2024) identified quercetin-O-dideoxyhexosyl-hexoside as one of the bioactive principles responsible for antioxidant anti-glucosidase activity, found at concentrations similar to those in these samples (0.48–0.69 mg/g). C-glycoside flavonoids possess highly polar chemical structures, even more so than their aglycones (Materska, 2015). Due to this, these compounds can be absorbed unchanged and undergo enterohepatic recirculation, as well as hydrolysis, reduction, and conjugation to form bioavailable glucuronides, favoring their applicability as nutraceutical ingredients and dietary supplements (Kim et al., 2016).

Citrus peels are also a good source of phenolic acids, although to a

Table 2
Organic acid content of lemon (*Citrus* × *limon* (L.) Burm. fil.), orange (*Citrus* × *aurantium* L.) and tangerine (*Citrus reticulata* Blanco) peel aqueous extract.

Organic acid, g/100 g extract	Lemon peel	Orange peel	Tangerine peel
Oxalic acid	*n.d.*	6.4 ± 0.7^{cA}	2.78 ± 0.06^{cB}
Quinic acid	78.8 ± 0.2^{aA}	19.4 ± 0.3^{aC}	24 ± 1^{aB}
Malic acid	39.2 ± 0.5^{cA}	10 ± 1^{bC}	16 ± 1^{bB}
Ascorbic acid	*t*	*t*	0.326 ± 0.001^{dA}
Citric acid	52.253 ± 0.002^{bA}	*n.d.*	*n.d.*
Fumaric acid	6.52 ± 0.07^{dA}	4.1 ± 0.2^{dB}	0.487 ± 0.005^{cdC}
Total	176.9 ± 0.2^{A}	40 ± 2^{B}	43 ± 2^{B}

Data expressed as mean ± standard deviation ($n = 3$); *t* = traces; *n.d.* = not detected; DW = dry weight. Standard calibration curves: oxalic acid ($y = 8{\cdot}10^6\,x + 3.3{\cdot}10^5$, $R^2 = 0.9912$; LOD = 12.55 μg/mL; LOQ = 41.82 μg/mL), quinic acid ($y = 6.9{\cdot}10^5\,x + 1.2{\cdot}10^4$, $R^2 = 0.9983$; LOD = 15.40 μg/mL; LOQ = 46.68 μg/mL), malic acid ($y = 9.4{\cdot}10^5\,x + 3.8{\cdot}10^4$, $R^2 = 0.9987$; LOD = 36 μg/mL; LOQ = 120 μg/mL), ascorbic ($y = 5.0{\cdot}10^7\,x + 4.5{\cdot}10^5$, $R^2 = 0.9813$; LOD = 21.24 μg/mL; LOQ = 64.37 μg/mL), citric ($y = 9.7{\cdot}10^5\,x + 1.2{\cdot}10^4$, $R^2 = 0.9974$; LOD = 0.11 μg/mL; LOQ = 0,34 μg/mL), fumaric acid ($y = 9.0{\cdot}10^7\,x + 1.0{\cdot}10^5$, $R^2 = 0.9986$; LOD = 0.08 μg/mL; LOQ = 0.26 μg/mL). Lowercase letters indicate significant differences (*p*-values <0.05) within the same citrus extract, while uppercase letters indicate significant differences (*p*-values <0.05) between different citrus extracts, according to ANOVA and Fisher's LSD test.

lesser extent than flavonoids (Table 3). Among these, hydroxycinnamic acids occur much more abundantly than hydroxybenzoic acids (Rosa et al., 2023), as evidenced by the exclusive identification of glycoside derivatives of hydroxycinnamic acids, namely coumaric and ferulic acids, as well as feruloylquinic acid in the analyzed extracts (Table 3). Ferulic, coumaric and chlorogenic acids are the most representative hydroxycinnamic acids in the *Citrus* species (Gómez-Mejía et al., 2019; Li et al., 2020; Rosa et al., 2023). This is evident in Table 3, with the concentration of these phenolics being less than 0.3 mg/g in all samples. It has been suggested that glucaric derivatives of hydroxycinnamics contribute to α-glucosidase and NO inhibitory activities, mainly attributed to the release of glucaric acid, thoroughly investigated for a wide range of therapeutic and commercial applications, including the treatment of diabetes (Abd Ghafar et al., 2020).

Additionally, diferuloylquinic acid, the only aglycone determined in this study, stands out in tangerine peel aqueous extract at 0.14–0.19 mg/g. Similarly, Safdar et al. (2017) reported that feruloylquinic acid was one of the most abundant acids in *C. reticulate* L. peels, with concentrations between 15 and 22 μg/g. These results are much lower than those obtained for the tangerine extracts considered in the present work (Table 3), again showing the suitability of the hydrodistillation valorization strategy adopted here.

In addition, four limonoid glucosides were identified: limonin, nomilin, nomicilin, and obacunone glucosides (Table 3). Among these, only nomilin glucoside was quantified at a low concentration (20 μg/g) in the aqueous lemon peel extract (Table 3). Similarly, Wang et al. (2016) reported higher levels of nomilin in lemon, with the lowest levels observed in orange.

Overall, aqueous lemon peel extracts were significantly richer in total phenolic compounds (22.7 ± 0.6 mg/g extract) compared to tangerine and orange peels (about 5.5 mg/g extract), with no significant differences between the latter two (*p*-value <0.05). This highlights the potential of lemon residues, primarily enriched with glycosylated flavones and flavanones, for industrial applications.

3.2. Bioactivities of volatile essential oils: Antioxidant and antibacterial activity

The volatile fraction from all citrus peel biowaste was studied in terms of its antioxidant and antibacterial activity to estimate their quality and potential industrial application of these essential oils in the agri-food sector. As shown in Fig. 2, the antioxidant activity of the three citrus essential significantly depended on the type of citrus (*p*-value <0.05). Tangerine peel essential oils exhibited the lowest IC_{50}, followed by lemon and orange peel essential oils (Fig. 2). Similarly, Lu et al. (2019) reported a higher antioxidant potential for *C. reticulata* Blanco compared to *C. sinensis* Osbeck and *C. sinensis Burm f. cv Eureka* essential oils. The low activity of orange essential oils could be due to their higher content of monoterpene hydrocarbons, especially limonene (74.4 %), which has been described as potentially prooxidant and ineffective against DPPH radicals (Lu et al., 2019).

On the other hand, tangerine essential oils had a larger limonene yield than lemon oils (Table 1). However, lemon oils contained higher amounts of other easily oxidizable and poorly antioxidant volatile compounds, such as α- and β-pinene, terpinen-4-ol, α-terpineol, and nerol (Lu et al., 2019). Overall, lemon essential oil showed a weaker antioxidant response than tangerine essential oils, highlighting the potential of tangerine volatile oils as an active ingredient in the food industry.

The antimicrobial activity of pure citrus essential oils was tested against well-known and widespread food pathogens. **Fig. S5** illustrates the most significant inhibitory effects of the essential oils against selected bacteria, and Table 4 summarizes the inhibition halos (cm). Orange peel essential oil showed a significantly lower potential against *B. subtilis*, *S. aureus*, *E. coli* and *Salmonella* spp., failing to inhibit the growth of the latter three microbes (Table 4). This could be due to the weak antimicrobial action of its main component, limonene, given its high volatility, easy oxidation, and low solubility in water (Ou et al., 2015). Lemon and tangerine essential oils were significantly more effective against all the microorganisms examined, except for *B. subtilis*, *Salmonella* spp. and *P. aeruginosa*, for which no significant differences were observed (Table 4). This may be linked to the abundance of lemon oils in (*E*)-isocitral (0.8 %), γ-terpinene (7.4 %), α-terpineol (6.4 %) and linalool (2.2 %), which are potent antimicrobials (Ou et al., 2015). Notably, a bacteriostatic effect was observed for *P. aeruginosa* with both lemon and tangerine essential oils, implying that these oils impede bacterial reproduction, leading to senescence and eventual death without progeny rather than completely inhibiting the bacteria.

Generally, Gram-negative bacteria are more efficient than Gram-positive bacteria in maintaining their membrane integrity, as seen in the 15-mm halo of tangerine oils for *B. subtilis* (Table 4). This is due to the peptidoglycan layer outside the outer membrane of Gram-negative bacteria with hydrophilic transmembrane channels that restrict the diffusion of hydrophobic compounds (Gupta et al., 2021). However, essential oils can also deplete the β-barrel protein, an essential outer membrane protein in lipopolysaccharide assembly, increasing bacterial cell membrane permeability and affecting the integrity of the Gram-negative bacteria (Gupta et al., 2021). This study demonstrated a high sensitivity of the Gram-negative bacterium *C. jejuni*, with inhibition halos ranging from 1.25 to 2.9 cm, particularly notable for orange and lemon oils (Table 4).

Moreover, orange peel essential oil was the only one to demonstrate antibacterial potential against *P. aeruginosa*. Similarly, a higher efficacy of orange volatile oils against Gram-negative bacteria like *Aeromonas jandaei* was noted (Chakroun et al., 2023). This variation in antibacterial effectiveness depends on the bacterial species, the fruit itself, and the oil's composition (Gupta et al., 2021). Overall, lemon and tangerine essential oils have shown the greatest promise as general antimicrobial additives or flavoring agents in food products or packaging. However, orange essential oils might be particularly beneficial for meat or dairy foods, which are the primary source of foodborne *C. jejuni* contamination.

Table 3
Retention time (Rt), wavelengths of maximum absorption (λ_{max}) and mass spectral data for the identification and quantification of phenolic compounds and limonoids in lemon (*Citrus* × *limon* (L.) Burm. fil.), orange (*Citrus* × *aurantium* L.) and tangerine (*Citrus reticulata* Blanco) peel aqueous extract.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2	Tentative identification	Lemon peel (mg/g extract)	Orange peel (mg/g extract)	Tangerine peel (mg/g extract)
1	4.93	305	355	337 (12), 209 (39), 191 (100), 173 (7), 147 (5)	Coumaroyl-glucarate or – galactate[1]	0.21 ± 0.01 ^{A}	0.17 ± 0.05 ^{A}	0.23 ± 0.01 ^{A}
2	5.01	309	365	321 (9), 303 (38), 263 (100), 221 (5), 143 (16), 125 (25)	Unknown + HMG * (cf. isomer 12)	*t*	*t*	*t*
3	5.16	321	385	340 (21), 191 (100), 173 (7), 147 (6)	Feruloyl-glucarate or – galactate[2]	*n.d.*	0.08 ± 0.03 ^{A}	*n.d.*
4	5.45	312	365	321 (9), 303 (38), 263 (100), 221 (5), 143 (16), 125 (25)	Unknown + HMG * (cf. isomer 12)	*t*	*t*	*t*
5	5.89	314	355	337 (12), 209 (39), 191 (100), 173 (7), 147 (5)	Coumaroyl-glucarate or – galactate[1]	0.15 ± 0.02 ^{A}	*n.d.*	0.14 ± 0.04 ^{A}
6	6.47	332	609	519(20),489(499,399(25),369(269	Luteolin-C-hexoside[3]	1.44 ± 0.06^{B}	1.3 ± 0.2^{B}	1.87 ± 0.06 ^{A}
7	6.94	324	385	340 (5),191(100),173 (5),147 (8)	Feruloyl-glucarate or – galactate[2]	*n.d.*	*n.d.*	0.18 ± 0.05 ^{A}
8	8.69	333	593	575 (8), 503 (49), 473 (100), 383 (31), 353 (40)	Apigenin-6-C-hexosyl-8-C-pentoside[4]	1.69 ± 0.08 ^{A}	0.54 ± 0.03^{B}	0.61 ± 0.01^{B}
9	9.76	276	357	195 (100), 151 (10)	Dihydro-feruloyl-O-glucoside (cf. isomer 10)[2]	0.106 ± 0.004^{B}	0.18 ± 0.04 ^{A}	*n.d.*
10	10.61	344	623	533(9),503(100),413(17)383(26)	Chrysoeriol 6,8-di-C-glucoside (stellarin-2) [3]	2.1 ± 0.1 ^{A}	1.05 ± 0.06^{C}	0.71 ± 0.05^{B}
11	11.17	330	663	501 (100), 357 (34), 195 (5)	Dihydro-feruloyl-glucoside-HMG-caffeoyl[2]	0.07 ± 0.02 ^{A}	0.07 ± 0.01 ^{A}	*n.d.*
12	11.8	346	623	533(7),503(100),413(13)383(31)	Diosmetin-6,8-di-C-*β*-D-glucopyranoside[3]	4.46 ± 0.08 ^{A}	0.15 ± 0.02^{C}	0.73 ± 0.05^{B}
13	12.26	335	649	605 (100), 543 (10), 443 (42), 209 (11)	Limonin 17β-D-glucopyranoside[5]	*t*	*n.d.*	*n.d.*
14	13.81	288/326	595	287 (100)	Eriodictyol-*O*-deoxyhexosyl-hexoside[6]	8.1 ± 0.4 ^{A}	0.45 ± 0.1^{B}	0.21 ± 0.07^{B}
15	14.97	326	561	367(94),193(100),191(10)173(12),134(25)	Diferuloylquinic acid[2]	*n.d.*	*n.d.*	0.15 ± 0.02 ^{A}
16	15.01	284/320	595	287 (100)	Eriodictyol-*O*-deoxyhexosyl-hexoside[6]	0.68 ± 0.02 ^{A}	0.30 ± 0.05^{B}	*n.d.*
17	15.51	286/322	595	287 (100)	Eriodictyol-*O*-deoxyhexosyl-hexoside[6]	0.55 ± 0.03 ^{A}	*n.d.*	*n.d.*
18	15.65	326	561	367(92),193(100),191(7)173(9),134(15)	Diferuloylquinic acid[2]	*n.d.*	*n.d.*	0.20 ± 0.04 ^{A}
19	16.63	349	609	301(100)	Quercetin-*O*-deoxyhexosyl-hexoside[7]	0.31 ± 0.05 ^{A}	0.29 ± 0.01 ^{A}	0.15 ± 0.01^{B}
20	16.97	344	593	285(100)	Luteolin-*O*-deoxyhexosyl-hexoside[7]	0.41 ± 0.01 ^{A}	0.180 ± 0.001^{B}	0.13 ± 0.01^{C}
21	17.74	270/340	579	271 (100)	Naringenin-*O*-deoxyhexosyl-hexoside[6]	0.30 ± 0.03 ^{A}	0.09 ± 0.01^{B}	*t*
22	19.61	349	609	301(100)	Quercetin-*O*-deoxyhexosyl-hexoside[7]	0.29 ± 0.01 ^{A}	0.141 ± 0.003^{B}	0.12 ± 0.01^{B}
23	20.01	348	609	301(100)	Quercetin-*O*-deoxyhexosyl-hexoside[7]	0.45 ± 0.03 ^{A}	0.29 ± 0.01^{B}	0.15 ± 0.02^{C}
24	20.39	278/341	843	783 (37), 753 (10), 723 (26), 681 (100), 619 (7), 579 (27), 537 (57), 375 (18), 360 (9), 317 (5)	Limocitrol-*O*-glucoside-HMG-O-glucose ester[5]	0.22 ± 0.05 ^{A}	*n.d.*	*n.d.*
25	21.04	279/332	693	633 (30), 565 (100), 507 (29), 395 (31)	Nomilin glucoside[5]	0.020 ± 0.002 ^{A}	*n.d.*	*t*
26	21.60	275/333	699	681 (5), 663 (10), 537 (5), 501 (100), 357 (25)	Dihydro-feruloyl-HMG-glucoside-caffeoyl-dihydrate[2] *	0.17 ± 0.01 ^{A}	*n.d.*	0.028 ± 0.005^{B}
27	22.25	276/343	711	651 (32), 607 (100)	Nomilinic acid glucoside[5]	*t*	*n.d.*	*t*
28	23.34	274/352	681	619 (20), 579 (74), 537 (100), 375 (34), 360 (20)	Limocitrol-*O*- glucoside -HMG[5]	0.93 ± 0.03 ^{A}	*t*	*t*
29	24.67	326	633	589 (22),427 (100),331 (35),289(32)	Obacunone glucoside[5]	*n.d.*	*t*	*t*
30	24.89	274/341	651	589 (23), 549 (49), 507 (100), 345 (32)	Limocitrin-*O*-glucoside-HMG[5] *	*t*	*n.d.*	*n.d.*
31	25.95	326	637	575(11),535(45),493(100),475(5),331(23)	8-Methoxyquercetin-3-*O*-[6″-HMG]-glucoside[7]	*n.d.*	*n.d.*	0.113 ± 0.001 ^{A}
32	27.97	275/343	795	651 (100), 549 (41), 507 (57), 345 (15)	Limocitrin-*O*-glucoside-di-HMG[5]	*t*	*n.d.*	*n.d.*
33	28.54	274/347	795	651 (100), 549 (41), 507 (57), 345 (15)	Limocitrin-*O*-glucoside-di-HMG isomer I[5]	*t*	*n.d.*	*n.d.*
34	29.97	267/347	825	723 (19), 681 (100), 619 (23), 579 (66), 537 (75), 375 (19)	Limocitrol-*O*-glucoside-di-HMG (cf. isomer 15)[5]	*t*	*n.d.*	*n.d.*
					Total phenolic acids	0.71 ± 0.03^{AB}	0.92 ± 0.02 ^{A}	0.5 ± 0.1^{B}
					Total flavonoids	22.0 ± 0.6 ^{A}	4.8 ± 0.1^{B}	4.7 ± 0.5^{B}
					Total phenolic compounds	22.7 ± 0.6 ^{A}	5.7 ± 0.2^{B}	5.2 ± 0.6^{B}

t = traces (compound detected but not quantifiable); *n.d.* = not detected; *HMG = 3-hydroxy-3-methylglutaryl glucoside. Standard calibration curves: [1]*p*-coumaric acid (y = 30,195× + 6966.7, R^2 = 0.9999, LOD = 0.68 μg/mL and LOQ = 1.61 μg/mL); [2]ferulic acid (y = 633,126× – 185,462, R^2 = 0.999, LOD = 0.20 μg/mL; 1.01 μg/mL); [3]luteolin-6-C-glucoside (y = 4087,1× + 72,589, R^2 = 0.9988, LOD = 0.32 μg/mL; 0.95 μg/mL); [4]apigenin-6-C-glucoside (y = 107,025× + 61,531, R^2 = 0.9989, LOD = 0.19 μg/mL; LOQ = 0.63 μg/mL); [5]hesperitin (y = 34,156× + 268,027, R^2 = 0.9993, LOD = 0.20 μg/mL; LOQ = 0.54 μg/mL); [6]naringenin (y = 184,433× + 78,903, R^2 = 0.9998, LOD = 0.17 μg/mL; LOQ = 0.81 μg/mL); and [7]quercetin-3-O-glucoside (y = 34,843×– 160,173, R^2 = 0.9998, LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL). Uppercase letters indicate significant differences (*p*-values <0.05) between different citrus extracts, according to ANOVA and Fisher's LSD test.

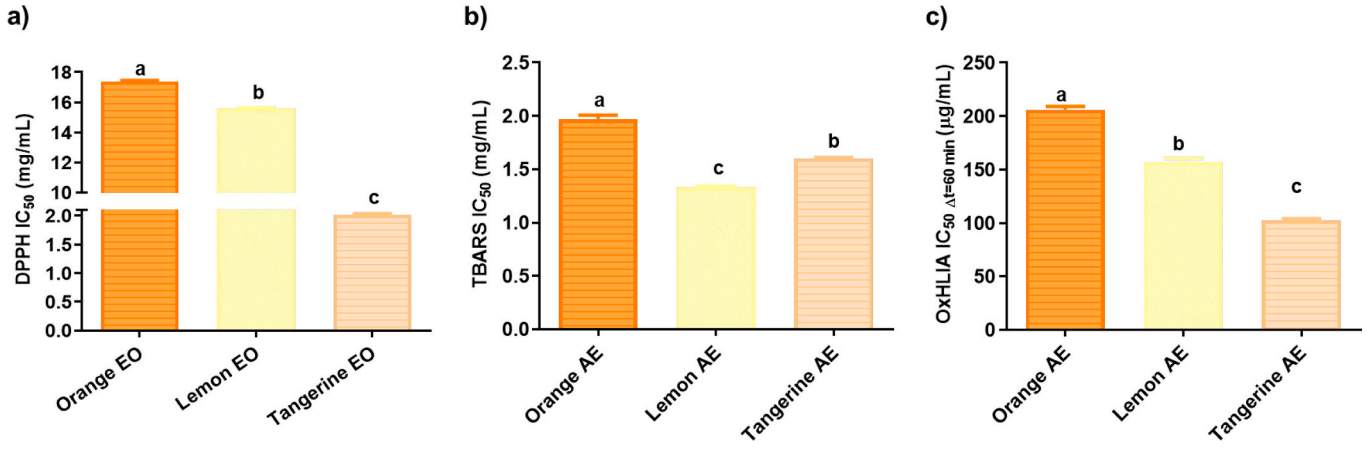


Fig. 2. Antioxidant activity, expressed as IC_{50}, of essential oils (EO) according to the DPPH assay (a) and of aqueous extracts (AE) according to the TBARS assay (b) and OxHLIA assay (c) of lemon (*C.* × *limon* (L.) Burm. fil.), orange (*C.* × *aurantium* L.) and tangerine (*C. reticulata* Blanco) peel bio-residues. Trolox IC_{50} values (μg/mL): 62 ± 3 (DPPH), 5.4 ± 0.3 (TBARS) and 21.8 ± 0.3 (OxHLIA). Data with different letters are significantly different at *p*-value <0.05, according to one-way ANOVA and Fisher's LSD test.

Table 4
Antibacterial activity of the essential oils from lemon (*Citrus* × *limon* (L.) Burm. fil.), orange (*Citrus* × *aurantium* L.) and tangerine (*Citrus reticulata* Blanco) peel bio-residues, expressed as diameter of inhibition zone (cm).

	Lemon peel	Orange peel	Tangerine peel
Gram-positive bacteria			
Bacillus subtilis	1.35 ± 0.07^{bA}	1.10 ± 0.01^{bB}	1.5 ± 0.2^{aA}
Listeria CET910	1.85 ± 0.07^{aA}	1.25 ± 0.07^{bB}	1.10 ± 0.01^{bB}
Staphylococcus aureus	1.95 ± 0.07^{aA}	*n.d.*	1.15 ± 0.07^{bB}
Gram-negative bacteria			
Campylobacter jejuni	2.1 ± 0.3^{aB}	2.9 ± 0.2^{aA}	1.25 ± 0.07^{abC}
Escherichia coli	1.9 ± 0.1^{aA}	*n.d.*	1.25 ± 0.07^{abB}
Pseudomonas aeruginosa	bacteriostatic	1.20 ± 0.01^{bA}	bacteriostatic
Salmonella spp.	1.4 ± 0.1^{bA}	*n.d.*	1.45 ± 0.07^{aA}

*The inhibition zone includes the 0.9 cm diameter of the disc. *n.d.* = not detected. Lowercase letters indicate significant differences (*p*-values <0.05) within the same essential oil, while uppercase letters indicate significant differences (*p*-values <0.05) between different oils for the same bacterium, according to ANOVA and Fisher's LSD test.

3.3. Bioactive properties of aqueous extracts

3.3.1. Antioxidant activity

The antioxidant activity of aqueous citrus peel extracts was evaluated using two different in vitro assays with biological relevance: lipid peroxidation and hemolysis in biological fluids and food models (Lockowandt et al., 2019). However, few studies have characterized the antioxidant properties of agri-food bio-residues using these assays, and even fewer have focused on the polar fraction of citrus peels. The hemolysis curves for aqueous extracts of lemon, tangerine, and orange peels at tested concentrations (3500–70 μg/mL), determined by the OxHLIA method, are graphically illustrated in **Fig. S4**.

As shown in Fig. 2, the aqueous orange peel extract exhibited the lowest antioxidant potential, whether inhibiting lipid peroxidation (IC_{50} = 1.97 mg/mL) or erythrocyte hemolysis (IC_{50} = 206 μg/mL). In contrast, aqueous extracts of lemon peels provided the highest antioxidant protection against lipid peroxidation (IC_{50} = 1.33 mg/mL), while tangerine extracts were the most effective according to OxHLIA (IC_{50} = 102 μg/mL). Taken as a whole, the antioxidant activity of citrus peel extracts can be mainly attributed to their phenolic composition (Rosa et al., 2023), although the minor secondary components, such as organic acids, also play a key role in achieving synergistic effects (Guimarães et al., 2010; Gülçin, 2012).

Guimarães et al. (2010) reported that the methanolic extract of *C. limon* peels showed a lower IC_{50} value for lipid peroxidation than that of *C. sinensis* peels, mainly due to the high flavonoid content in lemon peels. These findings align with the outcomes of this study, where the flavonoid levels reached up to 29 mg/g in lemon peel aqueous extracts, compared to 4.7 mg/g in other citrus extracts (Table 3). Conversely, the remarkable activity of aqueous tangerine extracts in quenching ROO• radicals, responsible for inducing hemoglobin oxidation, could be linked to their high hydroxycinnamic acid content (0.92 mg/g), which is 23 % higher than in lemon peels (Table 3). Ultimately, the concentrations required to achieve the IC_{50} parameter in both assays were significantly lower for the OxHLIA method (Fig. 2), indicating the greater potential of aqueous citrus peel extracts, mainly tangerine, as protective agents against this type of oxidation.

3.3.2. Antimicrobial activity

The potential of aqueous extracts from lemon, orange and tangerine peels against foodborne pathogens, including bacteria and fungi, was investigated. All citrus aqueous extracts inhibited the growth of the tested microorganisms, except for the Gram-negative bacteria *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* subsp. (MIC >10 mg/mL) (Table 5). This suggests that Gram-negative bacteria are more resistant to aqueous citrus peel extracts, possibly due to their bilayer membrane (Gupta et al., 2021). Additionally, none of the citrus aqueous extracts caused mortality of the microorganisms at the maximum tested concentrations (MBC and MFC > 10 mg/mL), merely preventing their outgrowth by altering the integrity of bacterial cell membranes or the metabolism and synthesis of DNA and RNA, among other mechanisms (Shehata et al., 2021; Zahr et al., 2023).

As shown in Table 5, similar MIC values were found for the three types of peel extracts against the examined microorganisms. *S. aureus*

Table 5
Antimicrobial, antiproliferative and anti-inflammatory activity of the aqueous extract from lemon (*Citrus* × *limon* (L.) Burm. fil.), orange (*Citrus* × *aurantium* L.) and tangerine (*Citrus reticulata* Blanco) peels.

		Lemon peel		Orange peel		Tangerine peel	
Antibacterial activity (mg/mL)		MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria	*Bacillus cereus*	5	> 10	10	> 10	>10	> 10
	Listeria monocytogenes	5	> 10	> 10	> 10	>10	> 10
	Staphylococcus aureus	5	> 10	5	> 10	2.5	> 10
Gram-negative bacteria	*Enterobacter Cloacae*	> 10	> 10	> 10	> 10	> 10	> 10
	Escherichia coli	> 10	> 10	> 10	> 10	> 10	> 10
	Pseudomonas aeruginosa	> 10	> 10	> 10	> 10	> 10	> 10
	Salmonella enterica	> 10	> 10	> 10	> 10	> 10	> 10
	Yersinia enterocolitica	5	> 10	5	> 10	10	> 10
Antifungal activity (mg/mL)		**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Fungi	*Aspergillus brasiliensis*	10	> 10	10	> 10	10	> 10
	Aspergillus fumigatus	10	> 10	5	> 10	5	> 10
Antiproliferative (GI_{50}; μg/mL) and anti-inflammatory activities (IC_{50}; μg/mL)							
AGS		83 ± 4	> 400	340 ± 17			
Caco-2		> 400	> 400	> 400			
MCF-7		> 400	286 ± 14	> 400			
NCI-H460		> 400	> 400	> 400			
Vero		> 400	> 400	> 400			
RAW 264.7		> 400	> 400	> 400			

n.t. = not treated; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; MFC = Minimum Fungicidal Concentration. Positive controls: Streptomycin (MIC = 0.007 mg/mL; MBC = 0.01 mg/mL); methicillin (MIC = 0.007 mg/mL; MBC = 0.007 mg/mL); ampicillin (MIC = 0.15 mg/mL; MBC = 0.631 mg/mL); ketoconazole (MIC = 0.125 mg/mL; MBC = 1 mg/mL). Ellipticine IC_{50} values (μg/mL): 1.23 ± 0.03 (AGS); 1.21 ± 0.02 (Caco-2); 1.02 ± 0.02 (MCF-7); 1.01 ± 0.01 (NCI-H460); 1.41 ± 0.06 (Vero); 6.3 ± 0.4 (RAW264.7).

was the most sensitive bacterium, with a MIC of 2.5 mg/mL in tangerine peel extract and 5 mg/mL in the other citrus extracts, followed by *Y. enterocolitica* with a MIC of 5 mg/mL for lemon and orange peels. Meanwhile, *B. cereus* and L. *monocytogenes* were inhibited only by lemon and, to a lesser extent, orange peel extracts (Table 5).

Regarding antifungal activity, tangerine and orange extracts exhibited higher efficiency (Table 5), primarily against *A. fumigatus*, where these citrus extracts achieved MIC values of 5 mg/mL. For inhibiting the growth of *Aspergillus brasiliensis*, all tested peel extracts achieved a MIC of 10 mg/mL. The inhibition of fungal growth by citrus phenolic extracts, mostly attributed to flavonoids along with phenolic acids, albeit to a lesser extent, involves several underlying mechanisms, including alteration of the plasma membrane, induction of mitochondrial dysfunction, and the efflux-mediated pumping system (Tan et al., 2022). Consequently, aqueous extracts of lemon and orange peel provide a broader efficacy spectrum against both types of microorganisms. Similarly, Shehata et al. (2021) reported greater effectiveness of ethanolic extracts of *C. sinensis* and *C. limon*, compared to *C. reticulate*, against *B. cereus*, *S. aureus*, *L. monocytogenes*, *Aspergillus parasiticus* and *Y. enterocolitica*, with inhibition halos between 23 and 9 mm (Shehata et al., 2021). Nevertheless, *C. reticulate* peel ethanolic extract proved to be significantly stronger against *S. aureus* than that of *C. limon* (14.1 mm vs. 10.6 mm) (Shehata et al., 2021), aligning with the present findings (Table 5). Therefore, tangerine peel extract may hold significant potential for preventing *S. aureus* contamination.

3.3.3. *Anti-proliferative and anti-inflammatory activity*

The antiproliferative and anti-inflammatory potential of citrus peel aqueous extracts was evaluated in vitro, with the results presented in Table 5. In this study, aqueous citrus extracts selectively inhibited AGS and MCF-7 cancer cell lines. The lemon peel extract demonstrated superior antiproliferative activity (p-value <0.05) against AGS cells, with an IG_{50} of 83 μg/mL, compared to 340 μg/mL for tangerine aqueous extract. Meanwhile, the orange peel extract exhibited an IG_{50} of 286 μg/mL against MCF-7 cells. Furthermore, no toxic effects were found against the primary non-tumorigenic Vero cell culture (Table 5), suggesting that these citrus extracts do not damage healthy cell lines and indicating their potential as nutraceutical agents.

Furthermore, the three citrus extracts tested showed no anti-inflammatory activity on RAW 264.7 macrophage cell line nor antiproliferative effects on Caco-2 and NCI-H460 cell lines at the maximum tested concentration (400 μg/mL). Other studies have investigated the biological potential of citrus waste as an antiproliferative agent. Camarda et al. (2007) reported that *C. sinensis* cv Sanguinello juice inhibited MCF-7 proliferation by 90.5 % at 10 % (*v*/v) concentration, whereas *C. clementina* juice did not significantly reduce the cell population, as observed in this study (Table 5). Additionally, Pagliara et al. (2019) found that AGS viability was reduced by 50 % when exposed to 20 μg/mL of lemon peel extract prepared by maceration with 80 % (v/v) ethanol. This antiproliferative effect was attributed to the reduction of interleukin-6-induced cell migration, primarily linked to the presence of hydroxybenzoic acids (Pagliara et al., 2019), whose derivatives are most abundant in lemon peel aqueous extract (Table 3).

3.4. *Multivariate analysis of bioactive properties and chemical profile of citrus aqueous extracts*

A Principal Component Analysis was conducted to establish a correlation between the phytochemical profile of the aqueous fraction of samples (chromatographic data) and their bioactivities (antioxidant, antibacterial, antifungal and antiproliferative activities). Only bioassay data that demonstrated activity in any of the samples were included. Meanwhile, compounds below the limit of quantification (LOQ) were excluded to ensure analytical robustness in the multivariate analysis. The PCA reduced thirty studied variables to two principal components (PCs), explaining 100 % of the total variance (64.8 % for PC1 and 35.2 % for PC2). The resulting two-dimensional graph, depicted in Fig. 3, revealed a distinct clustering of aqueous extracts from citrus peels, indicating variations in composition and bioactivity patterns according to the type of citrus fruit, with tangerine and orange peels showing higher similarity.

The aqueous orange peel extract was richest in oxalic acid and, to a lesser extent, dihydro-feruloyl-*O*-glucoside (cf. isomer 10). It demonstrated the weakest antioxidant and antiproliferative activity against the AGS cancer cell line while being the most effective against MCF-7. The in vitro bioactivities quantified as IC_{50} and GI_{50} values, i.e. antioxidant and anticarcinogenic activities, reveal a negative correlation with the citrus peel most strongly associated with them. Specifically, the opposite loading to these values denotes a higher efficacy against the respective activities (Fig. 3). This suggests a strong correlation between these compounds and MCF-7 cytotoxicity. The antiproliferative effect of oxalic acid could be due to the formation of oxalate crystals inside the cells that trigger their degradation (Radošević et al., 2018). Hence, the unique microenvironment of breast tissue and the metabolic pathways of the MCF-7 cell line may facilitate the carcinogenic effects of oxalic acid. For instance, Popović et al. (2023) observed a greater reduction in the viability of MCF-7 cells compared to colon adenocarcinoma cell lines Caco-2 and HT-29 in the presence of a choline chloride-oxalic acid NADES system.

On the other hand, tangerine peel extract was characterized by ascorbic acid, diferuloylquinic and feruloyl-glucarate/galactate acids, and the flavone luteolin-*C*-hexoside, contributing to a potent

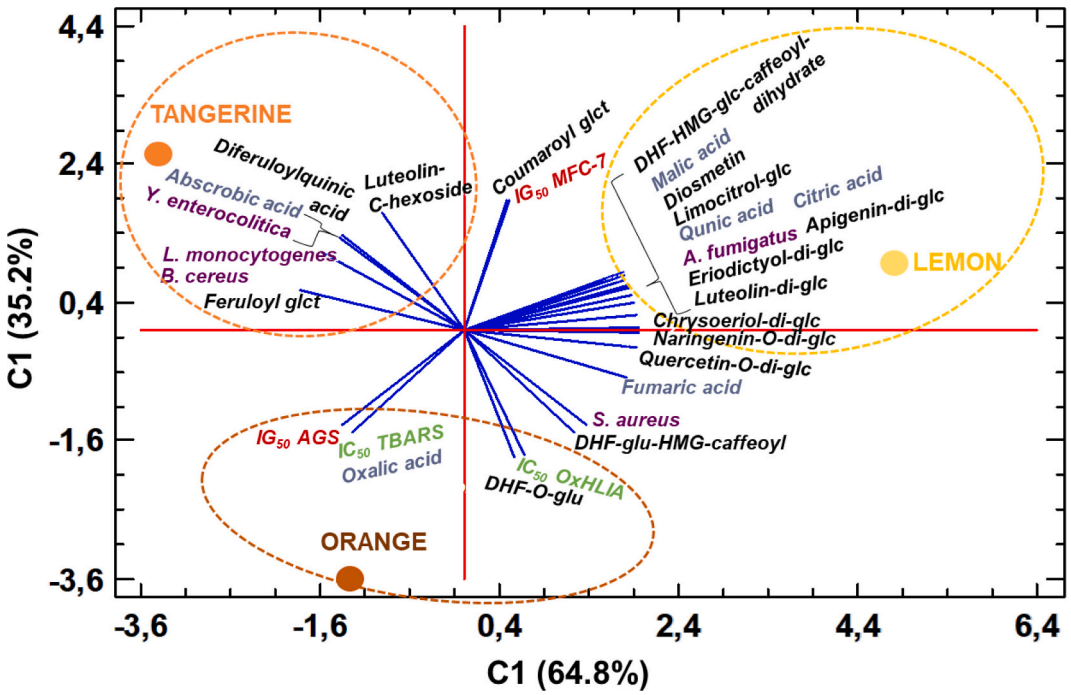


Fig. 3. PCA Principal Component Analysis bi-plot of the simultaneous evaluation of the loadings (chemical composition and bioactivities) and scores (citrus aqueous extracts) studied. *DHF-O-glu* = dihydro-feruloyl-O-glucoside (cf. isomer 10), *DHF-glu-HMG-caffeoyl* = dihydro-feruloyl-glucoside-HMG-caffeoyl, *feruloyl glct* = feruloyl-glucarate/galactate, *coumaroyl glct* = coumaroyl-glucarate/galactate, *quercetin-O-di-glc* = quercetin-O-deoxyhexosyl-hexoside, *naringenin-O-di-glc* = quercetin-O-deoxyhexosyl-hexoside, *chrysoeriol-di-glc* = chrysoeriol 6,8-di-C-glucoside (stellarin-2), *luteolin-di-glc* = luteolin-O-deoxyhexosyl-hexoside, *eriodictyol-di-glc* = eriodictyol-O-deoxyhexosyl-hexoside, *apigenin-di-glc* = apigenin-6-C-hexosyl-8-C-pentoside, *limocitrol-glc* = limocitrol-O-glc-HMG and limocitrol-O-glc-HMG-O-glucose ester, *DHF-HMG-glc-caffeoyl-dihydrate* = dihydro-feruloyl-HMG-glucoside-caffeoyl-dihydrate.

antihemolytic and antibacterial activity, particularly against *S. aureus* (loading opposite direction) (Fig. 3). This effect may stem from their ability to mitigate oxidative stress, especially by quenching singlet oxygen and preventing hemoglobin oxidation (Gülçin, 2012; Kylli et al., 2008). Furthermore, these acids induce acidification of microbial cytoplasm and interact with the anionic phospholipid of the bacterial cell walls, potentially disrupting *S. aureus* (Berton et al., 2020).

Regarding lemon peel extract, it was characterized by several glycoside flavonoids (diosmetin, limocitrol, apigenin, eriodictyol, chrysoeriol naringenin and quercetin), along with feruloyl-HMG-glucoside-caffeoyl-dihydrate hydroxycinnamic acid and organic acids (malic, quinic, and citric acids). This composition showed potent inhibition of lipid peroxidation, AGS cell line proliferation, and antibacterial activity against *Y. enterocolitica*, *L. monocytogenes* and *B. cereus* (opposite loadings). The synergy between flavonoids and organic acids, specifically citric acid's metal-chelating properties, enhances their antioxidant efficacy in lipid models (Guimarães et al., 2010; Gülçin, 2012). Additionally, quinic acid, naringin and quercetin have been described as potent antimicrobial agents through mechanisms such as increasing membrane permeability, reducing ATP production, and inhibiting DNA/RNA synthesis (Shehata et al., 2021).

Moreover, lemon peel extracts were enriched in polymethoxy and hydroxylated polymethoxyflavones, including naringenin, eriocitrin, and luteolin, as well as hydroxybenzoic acids like dihydro-feruloyl-HMG-glucoside-caffeoyl-dihydrate. These compounds have been highlighted for their anticancer potential, further reinforcing the bioactive significance of lemon peel phytochemicals (Pagliara et al., 2019; Zahr et al., 2023), which can be used for the development of dietary supplements for the nutraceutical and pharmaceutical industries.

4. Conclusions

This study has illustrated the feasibility of a simultaneous multi-component valorization of lemon, orange, and tangerine peels by a hydrodistillation system, contributing to the sustainable management of agri-food waste as proposed by the United Nations. Citrus peels have been proven to be a rich source of limonene (74.4–36.7 %), linalool (5.3–2.2 %), α-terpineol (6.7–1.2 %), and γ-terpinene (7.4–0.4 %), with a remarkable antiradical and antimicrobial properties against *C. jejuni* and *S. aureus*, particularly tangerine and lemon essentials oils. Additionally, the aqueous citrus extracts contained significant quantities of quinic and malic acids, with lemon peels also having a high citric acid content (52 g/100 g), as well as flavonoid glycosides, particularly in lemon peels, which proved to be the most cost-effective source. Furthermore, tangerine peel aqueous extract exhibited the strongest inhibition of oxidative hemolysis and bacteriostatic activity against *S. aureus*. Lemon aqueous extract primarily inhibited lipid peroxidation and AGS gastric cancer cell proliferation, while orange aqueous extract significantly inhibited the growth of MCF-7 breast cancer cells.

Additionally, the active organic acids and/or phenolic compounds contributing to these bioactivities have been elucidated through PCA. Thus, this research elucidates the abundance of high-value-added phytochemicals in citrus peels, showcasing their potential applications across various industrial sectors. It also advocates for the recovery of these valuable compounds through a sustainable, cost-effective, one-step extraction strategy, thereby promoting efficient and eco-friendly citrus waste management.

CRediT authorship contribution statement

Esther Gómez-Mejía: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Maria Inês Dias:** Writing – review & editing, Investigation. **Carla Pereira:** Writing – review & editing, Investigation. **Tânia C.S.P. Pires:** Writing – review & editing, Investigation. **Jesús Palá-Paúl:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization.

Noelia Rosales-Conrado: Writing – review & editing, Conceptualization. **María Eugenia León-González:** Writing – review & editing, Conceptualization. **Ricardo Calhelha:** Writing – review & editing, Visualization, Investigation. **Custódio Lobo Roriz:** Writing – review & editing, Visualization, Investigation.

Declaration of competing interest

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

Acknowledgements

The results are part of the grant PID2023-149424OB-I00, funded by MCIU/AEI/10.13039/501100011033 and ESF+. Moreover, the authors are grateful to the Complutense University [CT17/17-CT18/17, EB15/21], to the Foundation for Science and Technology (FCT), P.I. (FCT, Portugal) for the financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2021) and UIDB/50006/2020. National funding by FCT, P.I., through the institutional scientific employment program contract for M. I.D. (10.54499/CEECINST/00016/2018/CP1505/CT0004), C.P. (10.54499/CEECINST/00016/2018/CP1505/CT0010) and R.C.C. And to the European Regional Development Fund (ERDF) through the Competitiveness and Internationalization Operational Program for financial support to the project 100 % Figo (POCI-01-0247-FEDER-064977) and for C.L.R. post-Doc grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144641.

Data availability

Data will be made available on request.

References

Abd Ghafar, S. Z., Mediani, A., Maulidiani, M., Rudiyanto, R., Mohd Ghazali, H., Ramli, N. S., & Abas, F. (2020). Complementary NMR- and MS-based metabolomics approaches reveal the correlations of phytochemicals and biological activities in Phyllanthus acidus leaf extracts. *Food Research International, 136*, Article 109312. https://doi.org/10.1016/j.foodres.2020.109312

Adams, R. P. (2007). *Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy* (4th ed.). Allured Publishing Corporation: Carol Stream.

Aguilar-Hernández, M. G., Sánchez-Bravo, P., Hernández, F., Carbonell-Barrachina, Á. A., Pastor-Pérez, J. J., Legua, P., & Molina, L. (2020). Determination of the volatile profile of lemon Peel oils as affected by rootstock. *Foods, 9*(2). https://doi.org/10.3390/foods9020241

Arango-Manrique, S., Agudelo Patiño, T., Matallana Pérez, L. G., Ortiz-Sanchez, M., & Cardona Alzate, C. A. (2024). Conceptual design and economic optimization of different valorization routes for Orange Peel waste: The application of the biorefinery concept for an integral use of raw material. *Processes, 12*(10). https://doi.org/10.3390/pr12102298

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. *Journal of Functional Foods, 5*(4), 1732–1740. https://doi.org/10.1016/j.jff.2013.07.019

Berton, S. B. R., Cabral, M. R. P., de Jesus, G. A. M., Sarragiotto, M. H., Pilau, E. J., Martins, A. F., … Matsushita, M. (2020). Ultra-high-performance liquid chromatography supports a new reaction mechanism between free radicals and ferulic acid with antimicrobial and antioxidant activities. *Industrial Crops and Products, 154*, Article 112701. https://doi.org/10.1016/J.INDCROP.2020.112701

Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb. F.: An underexploited and highly disseminated species. *Industrial Crops and Products, 89*, 45–51. https://doi.org/10.1016/j.indcrop.2016.04.065

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., & Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry, 24*, 72–79. https://doi.org/10.1016/J.ULTSONCH.2014.11.015

Buyukkurt, O. K., Guclu, G., Kelebek, H., & Selli, S. (2019). Characterization of phenolic compounds in sweet lime (Citrus limetta) peel and freshly squeezed juices by LC-DAD-ESI-MS/MS and their antioxidant activity. *Journal of Food Measurement and Characterization, 13*(4), 3242–3249. https://doi.org/10.1007/S11694-019-00246-W/FIGURES/2

Camarda, L., Di Stefano, V., Del Bosco, S. F., & Schillaci, D. (2007). Antiproliferative activity of Citrus juices and HPLC evaluation of their flavonoid composition. *Fitoterapia, 78*(6), 426–429. https://doi.org/10.1016/J.FITOTE.2007.02.020

Cebadera-Miranda, L., Domínguez, L., Dias, M. I., Barros, L., Ferreira, I. C. F. R., Igual, M., … Cámara, M. (2019). Sanguinello and Tarocco (Citrus sinensis [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. *Food Chemistry, 270*, 395–402. https://doi.org/10.1016/J.FOODCHEM.2018.07.094

Chakroun, I., Bouraoui, Z., Ayachi, T., Hosni, K., Guerbèj, H., Snoussi, M., … Gharred, T. (2023). Phytochemical constituents and potential applications of Thomson navel orange (Citrus × aurantium var. sinensis L.) peel extracts: Antioxidant, antimicrobial and antiproliferative properties. *Industrial Crops and Products, 206*, Article 117597. https://doi.org/10.1016/J.INDCROP.2023.117597

Engelberth, A. S. (2020). Evaluating economic potential of food waste valorization: Onward to a diverse feedstock biorefinery. *Current Opinion in Green and Sustainable Chemistry, 26*, Article 100385. https://doi.org/10.1016/j.cogsc.2020.100385

Ersus, S., & Cam, M. (2007). Determination of organic acids, total phenolic content, and antioxidant capacity of sour Citrus aurantium fruits. *Chemistry of Natural Compounds, 43*(5), 500–501.

Espinosa, E., Rincón, E., Morcillo-Martín, R., Rabasco-Vílchez, L., & Rodríguez, A. (2022). Orange peel waste biorefinery in multi-component cascade approach: Polyphenolic compounds and nanocellulose for food packaging. *Industrial Crops and Products, 187*, Article 115413. https://doi.org/10.1016/J.INDCROP.2022.115413

Fernandes, F. A., Heleno, S. A., Pinela, J., Carocho, M., Prieto, M. A., Ferreira, I. C. F. R., & Barros, L. (2022). Recovery of citric acid from Citrus peels: Ultrasound-assisted extraction optimized by response surface methodology. *Chemosensors, 10*(7). https://doi.org/10.3390/chemosensors10070257

Gómez-Mejía, E., Roriz, C. L., Heleno, S. A., Calhelha, R., Dias, M. I., Pinela, J., … Barros, L. (2021). Valorisation of black mulberry and grape seeds: Chemical characterization and bioactive potential. *Food Chemistry, 337*. https://doi.org/10.1016/j.foodchem.2020.127998

Gómez-Mejía, E., Rosales-Conrado, N., León-González, M. E., & Madrid, Y. (2019). Citrus peels waste as a source of value-added compounds: Extraction and quantification of bioactive polyphenols. *Food Chemistry, 295*, 289–299. https://doi.org/10.1016/j.foodchem.2019.05.136

Guimarães, R., Barros, L., Barreira, J. C. M., Sousa, M. J., Carvalho, A. M., & Ferreira, I. C. F. R. (2010). Targeting excessive free radicals with peels and juices of citrus fruits: Grapefruit, lemon, lime and orange. *Food and Chemical Toxicology, 48*(1), 99–106. https://doi.org/10.1016/J.FCT.2009.09.022

Gülçin, I. (2012). Antioxidant activity of food constituents: An overview. In *, vol. 86*. *Archives of Toxicology* (pp. 345–391). https://doi.org/10.1007/s00204-011-0774-2. Issue 3.

Gupta, A., Jeyakumar, E., & Lawrence, R. (2021). Journey of limonene as an antimicrobial agent. *Journal of Pure and Applied Microbiology, 15*(3), 1094–1110. https://doi.org/10.22207/JPAM.15.3.01

Heleno, S. A., Ferreira, I. C. F. R., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., … Queiroz, M. J. R. P. (2013). Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology, 58*. https://doi.org/10.1016/j.fct.2013.04.025

Hilali, S., Fabiano-Tixier, A.-S., Ruiz, K., Hejjaj, A., Ait Nouh, F., Idlimam, A., … Chemat, F. (2019). Green extraction of essential oils, polyphenols, and Pectins from Orange Peel employing solar energy: Toward a zero-waste biorefinery. *ACS Sustainable Chemistry and Engineering, 7*(13), 11815–11822. https://doi.org/10.1021/acssuschemeng.9b02281

Jensch, C., Schmidt, A., & Strube, J. (2022). Versatile green processing for recovery of phenolic compounds from natural product extracts towards bioeconomy and Cascade utilization for waste valorization on the example of cocoa bean Shell (CBS). *Sustainability (Switzerland), 14*(5). https://doi.org/10.3390/su14053126

Joulain, D., & König, W. A. (1998). *The atlas of spectral data of Sesquiterpene hydrocarbons*. E.B.-Verlag.

Kim, M. K., Yun, K. J., Lim, D. H., Kim, J., & Jang, Y. P. (2016). Anti-inflammatory properties of flavone di-C-glycosides as active principles of Camellia mistletoe, Korthalsella japonica. *Biomolecules & Therapeutics, 24*(6), 630–637. https://doi.org/10.4062/biomolther.2016.019

Kylli, P., Nousiainen, P., Biely, P., Sipilä, J., Tenkanen, M., & Heinonen, M. (2008). Antioxidant potential of hydroxycinnamic acid glycoside esters. *Journal of Agricultural and Food Chemistry, 56*(12), 4797–4805. https://doi.org/10.1021/jf800317v

Li, W., Li, Y., Bi, J., Ji, Q., Zhao, X., Zheng, Q., Tan, S., & Gao, X. (2020). Effect of hot air drying on the polyphenol profile of Hongjv (Citrus reticulata Blanco, CV. Hongjv) peel: A multivariate analysis. *Journal of Food Biochemistry, 44*(5), Article e13174. https://doi.org/10.1111/JFBC.13174

Liew, S. S., Ho, W. Y., Yeap, S. K., & Bin Sharifudin, S. A. (2018). Phytochemical composition and in vitro antioxidant activities of Citrus sinensis peel extracts. *PeerJ, 2018*(8). https://doi.org/10.7717/peerj.5331

Lockowandt, L., Pinela, J., Roriz, C. L., Pereira, C., Abreu, R. M. V., Calhelha, R. C., … Ferreira, I. C. F. R. (2019). Chemical features and bioactivities of cornflower (Centaurea cyanus L.) capitula: The blue flowers and the unexplored non-edible part.

Industrial Crops and Products, 128, 496–503. https://doi.org/10.1016/j.indcrop.2018.11.059

Lu, Q., Huang, N., Peng, Y., Zhu, C., & Pan, S. (2019). Peel oils from three Citrus species: Volatile constituents, antioxidant activities and related contributions of individual components. *Journal of Food Science and Technology, 56*(10), 4492–4502. https://doi.org/10.1007/s13197-019-03937-w

Materska, M. (2015). Flavone C-glycosides from Capsicum annuum L.: Relationships between antioxidant activity and lipophilicity. *European Food Research and Technology, 240*(3), 549–557. https://doi.org/10.1007/s00217-014-2353-2

Mukhametzyanov, R. R., Brusenko, S. V., Khezhev, A. M., Kelemetov, E. M., & Kirillova, S. S. (2024). *Changing the global production and trade of Citrus fruits* (pp. 19–24). https://doi.org/10.1007/978-3-031-51272-8_4

Nicolescu, A., Babotă, M., Aranda Cañada, E., Inês Dias, M., Añibarro-Ortega, M., Cornea-Cipcigan, M., … Crișan, G. (2024). Association of enzymatic and optimized ultrasound-assisted aqueous extraction of flavonoid glycosides from dried Hippophae rhamnoides L. (Sea buckthorn) berries. *Ultrasonics Sonochemistry, 108*, Article 106955. https://doi.org/10.1016/j.ultsonch.2024.106955

Ou, M. C., Liu, Y. H., Sun, Y. W., & Chan, C. F. (2015). The composition, antioxidant and antibacterial activities of cold-pressed and distilled essential oils of Citrus paradisi and Citrus grandis (L.) Osbeck. *Evidence-based Complementary and Alternative Medicine*. https://doi.org/10.1155/2015/804091

Pagliara, V., Nasso, R., Di Donato, P., Finore, I., Poli, A., Masullo, M., & Arcone, R. (2019). Lemon peel polyphenol extract reduces interleukin-6-induced cell migration, invasiveness, and matrix metalloproteinase-9/2 expression in human gastric adenocarcinoma mkn-28 and ags cell lines. *Biomolecules, 9*(12). https://doi.org/10.3390/biom9120833

Palá-Paúl, J., Usano-Alemany, J., Granda, E., & Soria, A. C. (2012). Antifungal and antibacterial activity of the essential oil of Chamaecyparis Lawsoniana from Spain. *Natural Product Communications, 7*(10), 1383–1386. https://doi.org/10.1177/1934578X1200701036

Pires, T. C. S. P., Dias, M. I., Barros, L., Alves, M. J., Oliveira, M. B. P. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2018). Antioxidant and antimicrobial properties of dried Portuguese apple variety (Malus domestica Borkh. cv Bravo de Esmolfe). *Food Chemistry, 240*, 701–706. https://doi.org/10.1016/j.foodchem.2017.08.010

Popović, B. M., Gligorijević, N., Aranđelović, S., Macedo, A. C., Jurić, T., Uka, D., … Serra, A. T. (2023). Cytotoxicity profiling of choline chloride-based natural deep eutectic solvents. *RSC Advances, 13*(6), 3520–3527. https://doi.org/10.1039/D2RA07488E

Radošević, K., Čanak, I., Panić, M., Markov, K., Bubalo, M. C., Frece, J., … Redovniković, I. R. (2018). Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. *Environmental Science and Pollution Research, 25*(14), 14188–14196. https://doi.org/10.1007/s11356-018-1669-z

Real Farmacopea Española. (2005). 3ª Ed., Pub. L. No. SCO/3129/2005, ISBN: 84–340–1585-4 33271. https://www.boe.es/eli/es/o/2005/09/30/sco3129.

Rosa, A., Petretto, G. L., Maldini, M., Tirillini, Bruno, Chessa, M., … Sarais, G. (2023). Chemical characterization, antioxidant and cytotoxic activity of hydroalcoholic extract from the albedo and flavedo of Citrus Limon var. pompia Camarda. *Journal of Food Measurement and Characterization, 17*, 627–635. https://doi.org/10.1007/s11694-022-01659-w

Safdar, M. N., Kausar, T., Jabbar, S., Mumtaz, A., Ahad, K., & Saddozai, A. A. (2017). Extraction and quantification of polyphenols from kinnow (Citrus reticulate L.) peel using ultrasound and maceration techniques. *Journal of Food and Drug Analysis, 25*(3), 488–500. https://doi.org/10.1016/J.JFDA.2016.07.010

Shehata, M. G., Awad, T. S., Asker, D., El Sohaimy, S. A., Abd El-Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science, 4*, 326–335. https://doi.org/10.1016/J.CRFS.2021.05.001

Shofinita, D., Feng, S., & Langrish, T. A. G. (2015). Comparing yields from the extraction of different citrus peels and spray drying of the extracts. *Advanced Powder Technology, 26*(6), 1633–1638. https://doi.org/10.1016/j.apt.2015.09.007

Soares, C., Moreira, M. M., Ramos, S., Ramalhosa, M. J., Correia, M., Svarc-Gajić, J., … Barroso, M. F. (2023). A critical assessment of extraction methodologies for the valorization of agricultural wastes: Polyphenolic profile and bioactivity. *Processes, 11*(6), 1767. https://doi.org/10.3390/pr11061767

Sobral, F., Sampaio, A., Falcão, S., Queiroz, M. J. R. P., Calhelha, R. C., Vilas-Boas, M., & Ferreira, I. C. F. R. (2016). Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. *Food and Chemical Toxicology, 94*, 172–177. https://doi.org/10.1016/J.FCT.2016.06.008

Swigar, A. A., & Silverstein, R. M. (1981). *Monoterpenes*. Aldrich Chemical Co.

Tan, L. F., Yap, V. L., Rajagopal, M., Wiart, C., Selvaraja, M., Leong, M. Y., & Tan, P. L. (2022). Plant as an alternative source of antifungals against aspergillus infections: A review. *Plants, 11*(22), 3009. https://doi.org/10.3390/PLANTS11223009/S1

United States Department of Agriculture / Foreign Agricultural Service. (2024). Citrus: World Markets and Trade. https://fas.usda.gov/data/citrus-world-markets-and-trade-07252024.

Usano-Alemany, J., Palá-Paúl, J., & Herráiz-Peñalver, D. (2016). Essential oil yields and qualities of different clonal lines of Salvia lavandulifolia monitored in Spain over four years of cultivation. *Industrial Crops and Products, 80*, 251–261. https://doi.org/10.1016/J.INDCROP.2015.11.010

Vilas-Boas, A. A., Gómez-García, R., Campos, D. A., Correia, M., & Pintado, M. (2023). *Integrated biorefinery strategy for Orange juice by-products valorization: A sustainable protocol to obtain bioactive compounds* (pp. 113–124). https://doi.org/10.1007/978-1-0716-3303-8_8

Wang, S., Tu, H., Wan, J., Chen, W., Liu, X., Luo, J., Xu, J., & Zhang, H. (2016). Spatio-temporal distribution and natural variation of metabolites in citrus fruits. *Food Chemistry, 199*, 8–17. https://doi.org/10.1016/J.FOODCHEM.2015.11.113

Wedamulla, N. E., Fan, M., Choi, Y. J., & Kim, E. K. (2022). Citrus peel as a renewable bioresource: Transforming waste to food additives. *Journal of Functional Foods, 95*. https://doi.org/10.1016/j.jff.2022.105163

Zahr, S., Zahr, R., El Hajj, R., & Khalil, M. (2023). Phytochemistry and biological activities of Citrus sinensis and Citrus Limon: An update. *Journal of Herbal Medicine, 41*, Article 100737. https://doi.org/10.1016/J.HERMED.2023.100737