Valorization of quince peel into functional food ingredients: A path towards “zero waste” and sustainable food systems

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G R A P H I C A L  A B S T R A C T

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Quince (Cydonia oblonga Mill.) is an astringent fruit widely processed into marmalade and other sweets through processes that discard the peel as a by-product. Therefore, this study was performed to characterize the quince peel composition in nutrients and phytochemicals and evaluate its in vitro biological activity, following a “zero waste” approach. The quince peel dry powder was particularly rich in fiber (20.2 g/100 g), fructose (34 g/100 g), malic acid (7.2 g/100 g), and potassium (692 mg/100 g). Extracts prepared by dynamic hydroethanolic maceration and hot water extraction yielded 4.70 and 4.27 mg/g of phenolic compounds, respectively, with a prevalence of flavan-3-ols. The hydroethanolic extract was the most effective in inhibiting lipid peroxidation and oxidative hemolysis, and also presented better antimicrobial effects against foodborne pathogens, which agreed with the highest flavan-3-ol contents. The extracts were better than control synthetic food additives against some tested fungal and bacterial strains. On the other hand, no ability to inhibit nitric oxide production or toxicity to the tumor and non-tumor cell lines was observed. Furthermore, the solid residues remaining after extraction contained 35–37 g/100 g of fiber. Overall, quince peel can be upcycled into fiber-rich and bioactive ingredients to endow the value chain with natural food fortifiers, preservatives, and health promoters.

A R T I C L E  I N F O

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1. Introduction

Nowadays, to meet the current and future needs of the human population and food security issues worldwide, it is necessary to implement strategies that allow transforming the current linear and environmentally unsustainable agri-food chain into a circular and more efficient production and consumption system. According to Qu Dongyu, Director-General of the Food and Agriculture Organization of the United Nations (FAO), “agri-food systems lie at the heart of sustainable development” and, even before the world population reached an expected 10 billion in 2050, “food systems are already exceeding planetary boundaries for key resources” [1]. This statement underlines the urgent need for a major social and technological change at various levels and among all stakeholders involved in the production and consumption chain. In this sense, the reinsertion of agri-food by-products in the value cycle emerges as an important but challenging sustainability-oriented strategy by contributing to biowaste reduction and more efficient use of bioresources, which has been called valorization or upcycling [2].

Quince is the bright golden-yellow pomaceous fruit of Cydonia oblonga Miller (Rosaceae), a small tree native to northern Iran and Trans-Caucasia that has spread to other regions including Europe and America [3]. In 2020, the world production of quince reached around 696,861 tons and China and Turkey were the main producers, holding approximately 43% of the world total [4]. This fruit has an intense and pleasant aroma, but as most varieties are too hard, sour, and astringent to be eaten raw, it is cooked and mostly processed into sweet food products such as jelly, jam, and quince pudding or marmalade (a traditional sweet from the Iberian Peninsula where it is known as “marmelada”, in Portugal, and “dulce de membrillo”, in Spain). For industrial production of marmalade, large quantities of raw materials are needed, especially quince and sugar [5]. The production steps involve the quince reception and selection, washing with chlorinated water, and cutting to remove the peel and damaged parts (generating a solid by-product). After an antioxidant treatment with ascorbic acid solution, the fruit mesocarp is scalded to inactivate enzymes and reduce the microbial load, and then it is ground to obtain the pulp that is used to produce the marmalade.

Attempts to promote the upcycling of quince processing by-products (solid or liquid) have been very limited. Trigueros et al. [6] studied quince scaling water as a functional food ingredient due to its content in antioxidant polyphenols, organic acids, and sugars. However, the low-fat yogurts formulated with this ingredient had reduced sensory scores due to their acidic nature, and affected rheological and textural properties. The authors then proposed further studies to extract bioactive compounds instead of directly incorporating this liquid by-product. Quince scaling water was also investigated by Shinomiya et al. [7] for its antiallergic effects on type I allergic symptoms. When a crude extract was orally administrated to mice for 63 d, a significant reduction in IgE antibodies and inhibition of β-hexosaminidase release from rat basophilic leukemia RBL-2H3 cells. Although regulations limit the direct use of quince scaling water, its recycling will contribute to industrial eco-efficiency.

Quince juice pomace, another nutrient-rich industrial by-product, was already upcycled as a sustainable source of pectin [8] and antimicrobial ingredients for chewing candies [9]. Regarding quince peel, it has been described as containing hydroxyxynamic acids (caffeoylquinic acids) and flavonols (quercetin and kaempferol glycoside derivatives), which were also found in the fruit pulp [10, 11, 12]. According to some studies [10, 12], both quince peel and pulp extracts display antioxidant activity, including antihemolytic effects. The peel extract may actually be more effective in scavenging free radicals and inhibiting some microorganisms than the pulp [13]. Antioxidant and antiproliferative effects of compounds isolated from quince peel were investigated by Alesiani et al. [14]. The highest antioxidant activity was attributed to quercetin and its 3-O-rutinoside, followed by chlorogenic and neochlorogenic acids. Significant antiproliferative activity was attributed to triterpenoids, but quercetin and quercetin-3-O-rutinoside also strongly affected murine B16-F1 melanoma cells. Anti-inflammatory effects have also been attributed to quince peel extracts [11]. All these studies highlight the bioactive potential of quince peel. However, to the best of the authors’ knowledge, the nutritional composition of this by-product remains unknown and more studies are needed to prove its suitability to be entirely reinserted in the food value cycle as natural ingredients with nutritional and functional or bioactive value.

This work was performed to characterize the nutritional and chemical composition of quince peel, and evaluate its antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities in vitro. The impact of the extraction method on the phenolic profile and bioactivity was also investigated. In this way, it was intended to contribute to the upcycling of quince peel into valuable natural ingredients (including food preservatives and fortifiers) following a “zero waste” approach.

2. Materials and methods

2.1. Chemicals and standards

High-performance liquid chromatography (HPLC)-grade acetonitrile (99.9%) was acquired from Fisher Scientific (Lisbon, Portugal), Cesium chloride and lanthanum chloride were acquired from Thermo Fisher Scientific (Waltham, MA, USA). Phosphate buffered saline (PBS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, thio-barbituric acid (TBA), trichloroacetic acid, 2,2’-azobis(2-methylpropionamidine) dihydrochloride (AAPH), streptomycin, epithelium, sulfonhodamine B, lipopolysaccharide (LPS), dexamethasone, the fatty acids methyl ester (FAME) standard (Sigma-Aldrich, USA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The phenolic compound standards were purchased from Extrasynthèse (Genay, France). Ampicillin, bifenazole, ketoconazole, and p-iodonitrotetrazolium violet were acquired from Panfarma (Belgrade, Serbia), Srbolek (Belgrade, Serbia), Zorkapharma (Sabac, Serbia), and Biochemica (Panrea, Barcelona, Spain), respectively. Further chemicals were supplied by common sources.

2.2. Plant material

Quince peels were supplied by local farmers from Bragança, the civil parish of Calvelhe, Portugal, in October 2020. The peel sample was freeze-dried (FreeZone 4.5, Labconco, Kansas City, MO, USA), ground with a domestic grinder until passing through a 20-mesh sieve, and homogenized by mixing to obtain a representative sample that was maintained at −20 °C until use.

2.3. Compositional analysis of quince peel

2.3.1. Centesimal composition and energy

Quince peel was analyzed for moisture, protein, fat, ash, and dietary fiber contents following official food analytical methods [15]: the protein was determined by multiplying the quantified nitrogen by the conversion factor 6.25 (AOAC 920.152); the fat obtained by Soxhlet was determined gravimetrically after solvent removal (AOAC 920.85); ash was obtained by incineration at about 550 °C (AOAC 900.26); and dietary fiber was obtained by an enzymatic-gravimetric method (AOAC 985.29). Carbohydrates correspond to the remaining fraction. The results were presented as g/100 g of fresh weight (fw) and dry weight (dw).

The energy value (kcal/100 g of fw and dw) was calculated using the following conversion factors: 9 kcal/g for fat, 4 kcal/g for proteins and carbohydrates, and 2 kcal/g for fiber [16].

2.3.2. Mineral elements

Mineral elements were analyzed by atomic absorption spectroscopy (AAS) using a Perkin Elmer PinAAcle 900T Spectrometer (Waltham, MA, USA). The analysis included potassium, sodium, calcium, magnesium, carbon, and hydrogen.
manganese, zinc, iron, and copper. The quince peel powder (~0.25 g) was heat and microwave (1600 W) digested with 10 mL of nitric acid. The microwave used the following temperature ramp rate: up to 180 °C for 15 min and then held for 15 min. Before analysis, the obtained solutions were diluted up to 50 mL with deionized water and subjected to the following treatments: sample dilution in a cesium chloride solution (1 g/L) or in a lanthanum chloride solution (1 g/L) for analysis of K and Na and Ca and Mg, respectively; matrix modification with a magnesium nitrate solution for Mn and Cu; and Fe and Zn were directly analyzed. Phosphorus (P) was analyzed by a colorimetric method [17]. The elements in the sample (mg/100 g of dw and fw) were determined by comparing the signal with standard solutions.

2.3.3. Soluble sugars

Soluble sugars were analyzed in a HPLC system coupled to a refractometer (RI) detector, following analytical procedures previously described by the authors [18]. The extraction was performed with 80% ethanol at 80 °C for 90 min and melezitose (5 mg/mL) was used as internal standard. Separation was performed on a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 μm, Knauer), using acetonitrile/water 70:30 (v/v) as mobile phase. The detected compounds were identified by chromatographic comparison with standards and quantified (g/100 g of fw and dw) by the internal standard method.

2.3.4. Organic acids

Organic acids were analyzed in an ultra-fast liquid chromatography (UFLC) system (Shimadzu 20A series, Kyoto, Japan) coupled to a photodiode array detector, following analytical procedures previously described by the authors [19]. The extraction was performed with meta-phosphoric acid for 45 min. Separation was achieved in reverse phase on a C18 column (250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA). The compounds detected at 215 nm were identified and quantified (g/100 g of fw and dw) based on the peak areas and standard calibration curves.

2.3.5. Fatty acids

The crude fat obtained by Soxhlet was transesterified and the obtained fatty acid methyl ester (FAME) mixture was analyzed in a YOUNG IN Chromas 6500 Gas Chromatography System (Y Instrumenta, Anyang, Korea) with a flame ionization detector [18]. Separation was made on a Zebron™ ZB-FAME column (30 m × 0.25 mm, 0.20 μm). The detected fatty acids were identified by comparison of the retention times of the FAME and standard peaks. In addition to the relative percentage of each fatty acid, the contents (mg/100 g of fw and dw) were further estimated based on the conversion factor (0.8) established by Greenfield and Southgate [20].

2.4. Preparation of quince peel extracts

Ethanol is an extraction solvent authorized in the industry for the production of foodstuffs and food ingredients and does not leave residues or derivatives in the product after removal [21]. In turn, water is a good choice for extraction due to its safety, accessibility, and low cost. Therefore, hydroethanolic and aqueous extracts were prepared by maceration of quince peel. For hydroethanolic extraction, 1 g of plant material was mixed with 30 mL of ethanol/water (80:20, v/v) and stirred at ~150 rpm for 60 min at room temperature. The mixture was filtered and the residue was re-extracted under the same conditions. After that, the ethanol was removed under reduced pressure and the water by sublimation. For aqueous extraction, 1 g of plant material was mixed with 30 mL of water, boiled for 5 min, and the obtained filtrate was lyophilized. Both dry extracts were stored in a desiccator until analysis, while the solid residues remaining after filtration were lyophilized and characterized for dietary fiber content by the enzymatic-gravimetric method referred above.

2.5. Analysis of phenolic compounds

The extracts (10 mg) were dissolved in 2 mL of methanol/water 20:80 (v/v), filtered through 0.22 μm syringe filters, and analyzed in an ultra-performance liquid chromatography (UPLC) system equipped with a diode array detector (at 280 and 370 nm) and a LTQ XL linear ion trap mass spectrometer with an electrospray ionization source (HPLC-DAD-ESI/MS). Separation was performed in a Waters Spherisorb S3 ODS-2 C18 column (4.6 mm × 150 mm, 3 μm; Waters, Milford, MA, USA). The equipment and chromatographic conditions were previously described by the authors, as well as the identification and quantification procedures [22]. The detected compounds were quantified (mg/g of extract) using calibration curves (R² ≥ 0.999) constructed with chlorogenic acid (γ = 168,823x – 161,172, limit of detection (LOD) = 0.20 μg/mL, limit of quantification (LOQ) = 0.68 μg/mL), p-coumaric acid (γ = 301,950x + 6966.7, LOD = 0.68 μg/mL and LOQ = 1.61 μg/mL), catechin (γ = 84,950x – 23,200, LOD = 0.17 μg/mL, LOQ = 0.68 μg/mL), and quercetin-3-O-glucoside (γ = 34,843x – 16,0173, LOD = 0.21 μg/mL, LOQ = 0.71 μg/mL); thus, same compounds were expressed in mg of equivalents of its similar compound or basic structure.

2.6. Evaluation of bioactive properties

2.6.1. Antioxidant activity

The antioxidant activity of quince peel extracts was measured in vitro by means of oxidative hemolysis inhibition (OxHLIA) and thiorbarbituric acid reactive substances (TBARS) formation inhibition assays previously described by the authors [23, 24]. Sheep red blood cell and porcine brain tissue solutions were used in the OxHLIA and TBARS assays. Trolox (3.125–250 μg/mL) was used as a positive control. The results were expressed as half-maximal inhibitory concentration (IC₅₀) values (μg/mL). Since the OxHLIA is a kinetic assay, the IC₅₀ values were calculated for Δ of 60 min, 120 min, and 180 min.

2.6.2. Anti-inflammatory activity

The anti-inflammatory activity was assessed by the ability of quince peel extracts to inhibit the production of nitric oxide by the lipopoly saccharide (LPS)-treated murine macrophage cell line (RAW 264.7), determined by the quantification of nitrite in the medium by the colorimetric Griess assay as previously described [24]. Dexamethasone and LPS-free medium were used as positive and negative controls, respectively. The results were given as half-maximal effective concentration (EC₅₀) values (μg/mL).

2.6.3. Antimicrobial activity

The extracts redissolved in 30% ethanol were screened against the Gram-positive bacteria Bacillus cereus (human isolate), Staphylococcus aureus (ATCC 11632), and Listeria monocytogenes (NCTC 7973), the Gram-negative bacteria Escherichia coli (ATCC 35210), Enterobacter cloacae (human isolate), and Salmonella Typhimurium (ATCC 13311), and the micromycetes Aspergillus fumigatus (ATCC 1022), Aspergillus versicolor (ATCC 11730), Aspergillus niger (ATCC 6275), Trichoderma viride (IAM 5061), Penicillium funiculosum (ATCC 36839), and Penicillium verrucosum var. cyclopium (food isolate). The microorganisms were obtained from the Institute for Biological Research “Siniša Stanković”-National Institute of Republic of Serbia, University of Belgrade, Serbia. Minimum inhibitory concentrations (MIC) were determined by the serial microdilution method and the p-iodonitrotetrazolium violet (INT) colorimetric assay and corresponded to the extract concentration (μg/mL) that inhibited the visible microbial growth [25, 26]. Minimal bactericidal (MBC) and fungicidal (MFC) concentrations were measured as the lowest extract concentrations (μg/mL) needed to kill the original inoculum. The food additives potassium metabisulfite (E224) and sodium benzoate (E211), the antibiotics streptomycin and ampicillin, and the
azole antifungals ketoconazole and bifonazole were used as positive controls, while 30% ethanol was the negative control.

2.6.4. Cytotoxic activity against tumor and primary cell lines

The toxicity of quince peel extracts was tested on five human tumor cell lines (acquired from Leibniz Institute DSMZ), namely: MM127 (metastatic melanoma), MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), Hela (cervical carcinoma), and HepG2 (hepatocellular carcinoma). The extracts were also tested on PLP2 cells (porcine liver primary culture). The sulforhodamine B assay was followed as previously described by the authors [27, 28], using ellipticine as a positive control. The results were given as growth inhibition 50% (GI50) values (μg/mL).

2.7. Statistical analysis

Three independent samples were analyzed and measured in triplicate. The results were expressed as mean ± standard deviation (SD) (except for antimicrobial activity); the SD was rounded to one significant figure, which dictated the decimal place of the uncertain digit of the mean value. Statistical differences (p < 0.05) between two samples were assessed by Student’s t-test, while for the antioxidant activity results, a one-way analysis of variance was applied and the dependent variables were compared by Tukey’s HSD test. Furthermore, a Pearson’s correlation was performed to assess correlations between phenolic compounds and antioxidant activity. All tests were performed using SPSS Statistics (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

3. Results and discussion

3.1. Nutritional and chemical composition of quince peel

Quince peel is an industrial by-product that can be upcycled inside the food chain as a source of nutrients and functional compounds. Therefore, its nutritional and chemical composition was determined and the results are described below and compared with those available in the literature for quince peel, pulp, or seeds.

3.1.1. Centesimal composition and energy value

The centesimal composition of quince peel is presented in Table 1, including the moisture, fat, protein, ash, dietary fiber, and carbohydrate contents. The water content corresponded to 75.6 g/100 g fw, a content lower than that previously reported for the fruit pulp (~84 g/100 g) [29, 30, 31]. The dry quince peel powder contained primarily carbohydrates (76 g/100 g), followed by dietary fiber (20.2 g/100 g). Dietary fiber comprises a wide variety of carbohydrates that are not hydrolyzed or absorbed by the human small intestine and whose daily intake has been correlated with health benefits, particularly in gastrointestinal tract function [32]. According to European regulations [33], food can use the nutrition claim “source of fiber” if it contains at least 3 g/100 g and can claim “high fiber” if it contains ≥6 g/100 g or more. In this sense, the dry quince peel powder could be labelled as “high fiber content”. Ash (total minerals), protein, and fat contents were detected in smaller amounts. Although there are no previous reports on the centesimal composition of quince peel (as far as we know), the fruit pulp has been described as containing less carbohydrates (9.1–15.3 g/100 g fw) [29, 30, 34], and dietary fiber (1.65–6 g/100 g fw), ash (0.4–0.62 g/100 g fw), protein (0.3–0.49 g/100 g fw), and fat (0.1–0.24 g/100 g fw) contents [29, 30, 31, 34] encompassing the result of the peel sample obtained in this study.

As shown in Table 1, the quince peel has a low energy value and carbohydrates are by far the main contributors (85.6%), followed by dietary fiber (11.4%) and finally protein and fat with a similar contribution (1.5%). Steel, according to the INSA [31] and USDA [30] Nutrient Databases, the energy value of the quince pulp (53 and 57 kcal/100 g fw, respectively) is lower than that of the peel (90 kcal/100 g fw), probably due to the higher moisture content.

3.1.2. Composition in mineral elements

The quince peel composition in macrominerals and trace elements is presented in Table 2. Potassium (K) was the most abundant mineral element detected in the studied by-product. It is the main intracellular cation in the human body and required for the normal cellular function [35]. With lower contents came magnesium (Mg), phosphorus (P), calcium (Ca), and sodium (Na). Mg is involved in many enzymatic processes in the human body, as well as in bone health and maintaining intracellular levels of K and Ca. Mg and manly Ca play a key role in the bone health. In fact, more than 99% of total body Ca is found in the bones and teeth [35]. P is also an essential element required for a wide range of processes, including bone mineralization and ATP synthesis [36]. To the best of the author’s knowledge, this is the first study describing the mineral composition of quince peel. The pulp, on the other hand, has been described as containing high levels of K (108–252 mg/100 g fw), followed by P (12–26 mg/100 g fw), Ca (7.2–19 mg/100 g fw), Mg (4.24–11.1 mg/100 g fw), and Na (0.82–8.03 mg/100 g fw) [37, 38]. With the exception of P and Ca, the levels of macrominerals detected in the quince peel are among those described in the literature for the pulp of this fruit.

Among the analyzed trace elements, iron (Fe) represented a considerable fraction (Table 2). This element is a component of several proteins, such as hemoglobin, which is present in circulating red blood cells and is involved in the oxygen transport [35]. Lower levels of zinc (Zn) and copper (Cu) were quantified. In the human body, while Zn is essential for proper growth and development, given its catalytic, structural, and regulatory functions, Cu functions as a component of several metalloenzymes [35]. Among trace elements, manganese (Mn) was the one

### Table 1. Centesimal composition and energy value of quince peel.

| Component          | Content (g/100 g) | Fresh weight | Dry weight |
|--------------------|------------------|--------------|------------|
| Moisture           | 74.6 ± 0.2       |              |            |
| Ash                | 0.519 ± 0.006    | 2.04 ± 0.02  |            |
| Proteins           | 0.34 ± 0.01      | 1.34 ± 0.04  |            |
| Crude fat          | 0.146 ± 0.009    | 0.57 ± 0.04  |            |
| Dietary fiber      | 5.13 ± 0.06      | 20.2 ± 0.2   |            |
| Carbohydrates      | 19.2 ± 0.3       | 76 ± 1       |            |
| **Energy value (kcal) and its distribution by nutrient** |                  |              |
| Energy value       | 90 ± 1           | 354 ± 5      |            |
| Crude fat          | 1.31 ± 0.08      | 5.2 ± 0.3    |            |
| Proteins           | 1.36 ± 0.04      | 5.4 ± 0.2    |            |
| Dietary fiber      | 10.3 ± 0.2       | 40.4 ± 0.6   |            |
| Carbohydrates      | 77 ± 1           | 303 ± 5      |            |

The results are presented as mean ± standard deviation.

### Table 2. Mineral elements composition of quince peel.

| Element             | Content (mg/100 g) | Fresh weight | Dry weight |
|---------------------|--------------------|--------------|------------|
| Potassium, K        | 176 ± 1            | 692 ± 4      |            |
| Sodium, Na          | 1.7 ± 0.2          | 6.7 ± 0.8    |            |
| Calcium, Ca         | 2.93 ± 0.01        | 11.6 ± 0.1   |            |
| Magnesium, Mg       | 9.99 ± 0.02        | 39.4 ± 0.4   |            |
| Phosphorus, P       | 8.2 ± 0.5          | 32 ± 2       |            |
| Manganese, Mn       | 0.031 ± 0.002      | 0.123 ± 0.008|            |
| Zinc, Zn            | 0.227 ± 0.007      | 0.89 ± 0.03  |            |
| Iron, Fe            | 0.88 ± 0.02        | 3.48 ± 0.06  |            |
| Copper, Cu          | 0.181 ± 0.001      | 0.71 ± 0.01  |            |

The results are presented as mean ± standard deviation.
detected in lowest concentrations. The dietary intake of this element is essential for the formation of bones and for specific reactions related to the metabolism of amino acids, carbohydrates, and cholesterol [35]. According to the USDA Nutrient Database, 100 g portions of raw quince pulp contain 0.7 mg of Fe, 0.13 mg of Cu, and 0.04 mg of Zn [30].

### 3.1.3. Composition in soluble sugars and organic acids

The soluble sugars composition of quince peel is shown in Table 3. Three free sugars were detected and fructose accounted for about 74% of total sugars, followed by glucose and the disaccharide sucrose. To the best of the authors' knowledge, this is the first report describing the soluble sugar composition of quince peel. Despite this, quince pulp is well characterized for its composition in sugars and other carbohydrates. Szychowski et al. [39] detected fructose (0.42–0.58 g/100 g fw) as the major sugar in the pulp of Spanish quince, followed by glucose (0.08–0.11 g/100 g fw), sorbitol (0.04–0.10 g/100 g fw), and traces of sucrose and maltose, totaling 0.57–0.79 g/100 g fw of these water-soluble compounds. Fructose (5.31–10.8%) and glucose (4.08–5.61%), and lower amounts of sucrose (1.51–2.41%) and maltose (0.31–0.42%), were also reported in quince juice [40]. These results show that the sugars that predominate in the pulp also predominate in the fruit peel, despite notable quantitative differences.

The quince peel composition in organic acids is also shown in Table 3. Oxalic, quinic, and malic acids were identified and the latter corresponded to 57.6% of the total acids. This result is in line with the previous report by Rodríguez-Guisado et al. [40], who describe malic acid as the main organic acid (0.78%) in quince pulp juice. The authors described tartaric acid as the second most abundant (0.22%) organic acid, but did not report quinic acid in their samples. The prevalence of malic and quinic acids in quince peel and pulp was also observed by Silva et al. [41], who additionally reported citric, ascorbic, shikimic, and fumaric acids. A total organic acid content ranging from 0.7 to 1.4 g/100 g was quantified in both pulp and peel samples and, in general, the pulp tended to contain more organic acids than the peel.

Quince has been exploited for industrial purposes (such as production of marmalade and other sweets) since it has a relatively low sugar content and high astringency and sourness, sensory properties that make the raw fruit have a low consumer acceptance. This study shows that quince fruit can be a low-cost source of malic acid, a dicarboxylic acid used in the food industry as a food additive, and also fructose, the sweetest of all naturally occurring carbohydrates.

### 3.1.4. Composition in fatty acids

The 21 fatty acids detected in quince peel are listed in Table 4. Palmitic (C16:0), stearic (C18:0), and oleic (C18:1n-9) acids were the major fatty acids detected, corresponding to 156 mg, 49.1 mg, and 48.1 mg per 100 g portion of dry quince peel, respectively, followed by myristic (C14:0), arachidonic (C20:4n-6), and linoleic (C18:2n-6) acids. Saturated fatty acids (SFA) corresponded to 68% of the total fatty acids (given the high contents of C16:0, C18:0, and C14:0), corresponding to have 312 mg and 79.3 mg per 100 g portions of dry and fresh quince peel, respectively.

#### Table 3. Soluble sugars and organic acids composition of quince peel.

| Content (g/100 g) | Fresh weight | Dry weight |
|-------------------|--------------|------------|
| Fructose          | 8.6 ± 0.5    | 34 ± 2     |
| Glucose           | 2.11 ± 0.09  | 8.3 ± 0.4  |
| Sucrose           | 1.02 ± 0.03  | 4.0 ± 0.1  |
| Total sugars      | 11.8 ± 0.6   | 46 ± 2     |
| Oxalic acid       | 0.35 ± 0.01  | 1.38 ± 0.05|
| Quinic acid       | 1.00 ± 0.02  | 3.95 ± 0.09|
| Malic acid        | 1.82 ± 0.05  | 7.2 ± 0.2  |
| Total organic acids| 3.17 ± 0.06  | 12.5 ± 0.2 |

The results are presented as mean ± standard deviation.

#### Table 4. Fatty acids composition of quince peel. The relative percentage and content of each fatty acid and its classes are presented.

| Fatty acid | Relative percentage (%) | Content (mg/100 g) |
|------------|-------------------------|--------------------|
| Fresh weight | Dry weight       |                    |
| C8:0       | 1.24 ± 0.02           | 1.44 ± 0.02        |
| C10:0      | 2.14 ± 0.03           | 2.50 ± 0.04        |
| C11:0      | 0.72 ± 0.01           | 0.84 ± 0.01        |
| C12:0      | 3.15 ± 0.05           | 3.67 ± 0.06        |
| C13:0      | 0.299 ± 0.06          | 0.348 ± 0.007      |
| C14:0      | 8.4 ± 0.2             | 9.8 ± 0.2          |
| C15:0      | 1.24 ± 0.2            | 1.44 ± 0.03        |
| C16:0      | 34.0 ± 0.8            | 40 ± 1             |
| C16:1      | 0.71 ± 0.01           | 0.82 ± 0.01        |
| C17:0      | 1.28 ± 0.02           | 1.49 ± 0.02        |
| C18:1n-9   | 10.7 ± 0.1            | 12.5 ± 0.2         |
| C18:2n-6   | 5.55 ± 0.08           | 6.5 ± 0.1          |
| C18:3n-6   | 0.274 ± 0.004         | 0.319 ± 0.005      |
| C18:3n-3   | 1.44 ± 0.01           | 1.68 ± 0.02        |
| C20:0      | 0.96 ± 0.01           | 1.12 ± 0.01        |
| C20:4n-6   | 6.92 ± 0.07           | 8.07 ± 0.089       |
| C20:3n-3   | 3.27 ± 0.04           | 3.81 ± 0.04        |
| C22:1      | 2.31 ± 0.03           | 2.69 ± 0.04        |
| C24:0      | 3.89 ± 0.04           | 4.53 ± 0.05        |
| C24:1      | 1.04 ± 0.02           | 1.21 ± 0.02        |
| SFA        | 68.0 ± 0.7            | 79.3 ± 0.9         |
| MUFAs      | 14.5 ± 0.1            | 16.9 ± 0.2         |
| PUFA       | 17.5 ± 0.2            | 20.3 ± 0.2         |
| PUFA/SFA   | 0.257 ± 0.001         | 0.370 ± 0.003      |
| PUFA n-3/n-6| 0.370 ± 0.003         |                    |

The results are presented as mean ± standard deviation. Caprylic acid (C8:0), capric acid (C10:0), undecyl acid (C11:0), lauric acid (C12:0), tridecyl acid (C13:0), myristic acid (C14:0), pentadecyl acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6), e- linoleic acid (C18:3n-3), arachidic acid (C20:0), arachidonic acid (C20:4n-6), cis-11,14,17-eicosatrienic acid (C20:3n-3), erucic acid (C22:1), lignoceric acid (C24:0), nervonic acid (C24:1). SFA: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Contents estimated based on the conversion factor (0.8) established by Greenfield and Southgate [28].

Unsaturated fatty acids, on the other hand, comprised 32% of the fatty acid fraction, with a slight predominance of polyunsaturated fatty acids (PUFA) over monounsaturated ones (MUFA) (Table 4).

The PUFA/SFA and PUFA n-3/n-6 ratios have been used as indicators of the nutritional quality and healthiness of foodstuff, which should be >0.45 and <4, respectively [42]. As shown in Table 4, the by-product under analysis does not meet the requirement for the PUFA/SFA ratio, but meets the second quality criterion. As there is no data on the fatty acid profile of the quince peel, the results are compared with those of the pulp and seeds. The USDA Nutrient Database describes C18:1n-9 and C18:2n-6 as the most abundant MUFA and PUFA of the raw fruit pulp, respectively. Lower levels of the SFA C16:0 and C18:0 are also presented [30]. The two predominant fatty acids in the pulp are also the most abundant in the lipid fraction of the seed, corresponding to 90% of the total fatty acids [39]. Thus, PUFA (53–56%) and MUFA (34–37%) predominate over SFA (9–10%), being a healthier fatty acid profile than that herein characterized for quince peel.

#### 3.2. Extraction yield and fiber content of the extraction residues

The quince peel sample was subjected to solid-liquid extractions by dynamic maceration using ethanol/water (80:20, v/v) and hot water
extraction, which yielded 53 ± 2% (w/w) and 54 ± 1% (w/w) of extracted solids, respectively, values that did not differ statistically (p > 0.05). In order to investigate the impact of the extraction method, the extracts were characterized and compared for their composition in phenolic compounds and in vitro bioactive properties. On the other hand, although approximately half of the plant material was lost during the extraction, our results showed that these extraction residues contain high total dietary fiber contents, ranging from 35 ± 1 g/100 g (with hydroethanolic extraction) to 37 ± 1 g/100 g (with aqueous extraction), but not differing statistically (p = 0.147); the remaining fraction is likely made up mostly sugars and other carbohydrates. Hence, a “zero waste” approach can be followed in the quince processing industry to contribute to better bioresource-use efficiency and circularity through the supply of both phenolic extracts and novel fiber-rich natural ingredients that can be used in food fortification.

Similar methodological approaches were previously followed with date seeds. Al-Farsi and Lee [43] measured 57.9 g/100 g of total dietary fiber (91% insoluble) in the seeds, and 83.5 and 82.2 g/100 g in the residues from water and 50% acetone extractions, respectively. These higher levels of total fiber in the extraction residues were justified by the extraction of phenolic compounds and other constituents, such as proteins and soluble sugars, with the solvents used, which agrees with the results of the present study. The authors also observed an increase in the insoluble fraction (cellulose and hemicellulose), while most of the soluble fiber (pectins, inulin, and gums) was extracted together with the phenolic compounds, thus decreasing in the solid residues. Later, Afifi et al. [44] obtained a solid residue containing 79.4% crude fiber, resulting from a 3 h extraction with 25% ethanol at 55 °C; although the residue yield was relatively low, it was of high purity. These conditions also promote the recovery of total phenolics and flavonoids. It is also worth noting that, in the present work, the use of 80% ethanol in the extraction may have solubilized the soluble fiber.

### 3.3. Phenolic profile of quince peel extracts

Phenolic compounds represent the largest group of antioxidants present in the human diet. Epidemiological studies and meta-analyses have shown that long-term consumption of diets rich in polyphenols offers protection against the development of inflammatory and neurodegenerative diseases and various types of cancer [45, 46]. Figure 1 and Table 5 show a representative chromatogram of the phenolic profile of the quince peel extracts and the chromatographic data used in the tentative identification of the detected compounds, respectively. Sixteen compounds were tentatively identified, including five phenolic acids (caffeoylquinic acids), nine flavan-3-ols ((-)catechin, β-type (epi)catechin dimers, trimers, and tetramers, and a procyanidin with A-type linkage), and two flavonol glycosides (quercetin-O-deoxyhexoside-hexoside and kaempferol-O-deoxyhexoside-hexoside), which were previously described in this species [47, 48, 49, 50]. Therefore, the compounds’ identity was assigned by comparing the chromatographic data in Table 5 with those in the literature.

As shown in Table 5, the extraction method quantitatively affected the phenolic profile. Flavan-3-ols were the most predominant compounds in quince peel, corresponding to about 56.64% and 47.78% of the total phenolic compounds quantified in the hydroethanolic and aqueous extracts, respectively. The proanthocyanidin β-type (epi)catechin trimer (compound 7) was detected in large amount (0.557–0.76 mg/g extract). Phenolic acids ranked second with cis-5-O-cafeoylquinic (compound 3) among the predominant compounds (0.498–0.63 mg/g extract) and, contrary to what was verified for flavan-3-ols, they predominated in the aqueous extract. Furthermore, although only 2 flavonols were detected, these glycosides of quercetin (compound 14) and kaempferol (compound 16) had a notable individual numerical expression (0.427–0.435 mg/g extract, respectively). These results showed that hydroethanolic maceration was preferable to obtain larger amounts of flavan-3-ols, while hot water extraction was more suitable for extracting phenolic acids and flavonoids; the high temperature may have led the degradation of flavan-3-ols.

Previous studies describe a similar phenolic composition for quince. Karar et al. [47], Szychowski et al. [48] and Wojdylo et al. [49] also identified flavan-3-ols (polymeric procyanidins) as the predominant class of polyphenols, followed by hydroxycinnamic acids, and then flavonols. According to these studies, the content of polymeric procyanidins can be correlated to the quince astringency and bitterness. In turn,
Table 5. Content of the phenolic compound identified in the quince peel extracts. The retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), and deprotonated ion are presented.

| Peak | Rt (min) | λmax (nm) | [M-H] (m/z) | Tentative identification | Content (mg/g extract) | Statistics* |
|------|----------|-----------|-------------|--------------------------|------------------------|-------------|
|      |          |           |             |                          | Hydroethanolic | Aqueous | p-Value |
| 1A   | 4.43     | 325       | 353         | 3-O-Caffeoylquinic acid   | 0.372 ± 0.004        | 0.399 ± 0.008 | 0.02   |
| 2B   | 5.85     | 292       | 337         | 3-O-p-Coumaroylquinic acid | 0.087 ± 0.004        | 0.082 ± 0.002 | 0.045 |
| 3A   | 6.56     | 326       | 353         | cinn-5-O-Caffeoylquinic acid | 0.498 ± 0.005        | 0.63 ± 0.02  | <0.001 |
| 4A   | 7.43     | 323       | 353         | trans-5-O-Caffeoylquinic acid | 0.192 ± 0.007        | 0.183 ± 0.006 | 0.088 |
| 5C   | 8.63     | 275       | 289         | (-)-Catechin             | 0.218 ± 0.003        | 0.182 ± 0.002 | <0.001 |
| 6A   | 10.47    | 311       | 337         | 5-O-Coumaroylquinic acid | 0.028 ± 0.002        | 0.037 ± 0.002 | 0.001 |
| 9A   | 11.08    | 276       | 865         | β-Type (epi)catechin tetramer | 0.330 ± 0.008        | 0.226 ± 0.007 | <0.001 |
| 8A   | 12.52    | 272       | 1153        | β-Type (epi)catechin trimer | 0.3 ± 0.02           | 0.233 ± 0.002 | 0.001 |
| 9B   | 13.83    | 298       | 577         | β-Type (epi)catechin dimer | 0.192 ± 0.009        | 0.152 ± 0.002 | <0.001 |
| 10A  | 14.16    | 280       | 1153        | β-Type (epi)catechin trimer | 0.385 ± 0.005        | 0.302 ± 0.005 | <0.001 |
| 11C  | 14.57    | 271       | 865         | β-Type (epi)catechin trimmer | 0.185 ± 0.007        | 0.146 ± 0.006 | 0.001 |
| 12C  | 15.10    | 275       | 865         | β-Type (epi)catechin trimmer | 0.128 ± 0.002        | 0.126 ± 0.004 | 0.235 |
| 13C  | 15.76    | 275       | 865         | β-Type (epi)catechin trimmer | 0.427 ± 0.001        | 0.490 ± 0.002 | <0.001 |
| 14D  | 16.70    | 355       | 609         | Quercetin-O-deoxyhexosyl-hexoside | 0.427 ± 0.001        | 0.171 ± 0.007 | <0.001 |
| 15C  | 17.52    | 268       | 863         | Procyanidin with A-type linkage | 0.503 ± 0.004        | 0.493 ± 0.003 | <0.001 |
| 16D  | 19.86    | 357       | 593         | Kaempferol-O-deoxyhexosyl-hexoside | 1.18 ± 0.02          | 1.33 ± 0.03  | <0.001 |
|      |          |           |             | Total phenolic acids      | 2.662 ± 0.005        | 2.04 ± 0.01  | <0.001 |
|      |          |           |             | Total flavan-3-ols        | 0.862 ± 0.003        | 0.893 ± 0.005 | <0.001 |
|      |          |           |             | Total flavonoids          | 4.70 ± 0.02          | 4.27 ± 0.03  | <0.001 |

The results are presented as mean ± standard deviation. Standards used in quantification: A chlorogenic acid; β-coumaric acid; C catechin; and quercetin-3-O-glucoside.

Significant differences (p < 0.05) between samples were assessed by a Student’s t-test.

Caffeoylquinic acids can be important when manufacturing quince-based products, since these phenolic acids are substrates of the catecholase activity of polyphenol oxidase and, therefore, can influence oxidation and color change processes. Stojanović et al. [10] described 5-O-cafeoylquinic acid as the most abundant hydroxycinnamic acid in quince, with a higher content in the peel than in the pulp. Higher levels of total phenolics and flavonoids were also quantified in peel extracts.

3.4. Bioactive properties of quince peel extracts

3.4.1. Antioxidant activity

The antioxidant activity of the quince peel extracts was evaluated by the ability to inhibit the formation of TBARS and to protect red blood cell membranes from oxidative lysis; the results are shown in Figure 2. The lower the IC50 values, the more antioxidant the extract. For the TBARS assay, the IC50 value correspond to the extract dosage providing 50% of lipid peroxidation inhibition; while in OxHLIA it corresponds to the dosage necessary to protect 50% of the red blood cells from lysis caused by AAPH-derived free radicals for a certain period of time.

As shown in Figure 2, the hydroethanolic extract yielded lower IC50 values than the aqueous extract in both assays (thus higher bioactivity), which can be related to the higher levels of flavan-3-ols, since these extract constituents were strongly correlated with the antioxidant activity (Table 6). Trolox had higher activity (lower IC50 values) than the extract, while this is a pure antioxidant, quince peel extracts are complex mixtures of several constituents with or without bioactive properties. The TBARS assay provides indications on the extracts ability to inhibit the formation of highly reactive compounds, such as...
malondialdehyde (MDA) that results from the degradation of lipid peroxidation products. The MDA monitoring was achieved by adding thiorbarbituric acid (TBA) to the reaction mixture, heating at 80 °C for 20 min, and subsequently measuring the absorbance of the formed pink colored MDA-TBA adducts.

Table 6. Pearson’s correlation coefficients (R) of phenolic compounds with antioxidant activity in quince peel extracts.

| Phenolic compounds                  | TBARS assay | OxHLIA assay |
|-------------------------------------|-------------|--------------|
|                                     | Δt 60 min   | Δt 120 min   | Δt 180 min   |
| 3-O-Caffeoylquinic acid             | R 0.961**   | 0.936**      | 0.907*       | 0.891*       |
|                                     | Sig. 0.002  | 0.006        | 0.012        | 0.017        |
| 3-O-p-Coumaroylquinic acid         | R -0.826*   | -0.846*      | -0.859*      | -0.863*      |
|                                     | Sig. 0.043  | 0.034        | 0.028        | 0.027        |
| cis-5-O-Caffeoylquinic acid         | R 0.992**   | 0.975**      | 0.954**      | 0.941**      |
|                                     | Sig. 0.000  | 0.001        | 0.003        | 0.005        |
| trans-5-O-Caffeoylquinic acid       | R -0.752    | -0.783       | -0.804       | -0.813*      |
|                                     | Sig. 0.085  | 0.065        | 0.054        | 0.049        |
| (+)-Catechin                        | R -0.995**  | -0.989**     | -0.974**     | -0.964**     |
|                                     | Sig. 0.000  | 0.000        | 0.001        | 0.002        |
| 5-O-p-Coumaroylquinic acid          | R 0.975**   | 0.976**      | 0.964**      | 0.956**      |
|                                     | Sig. 0.001  | 0.001        | 0.002        | 0.003        |
| β-Type (epi)catechin trimer         | R -0.997**  | -0.989**     | -0.975**     | -0.965**     |
|                                     | Sig. 0.000  | 0.000        | 0.001        | 0.002        |
| β-Type (epi)catechin tetramer       | R -0.997**  | -0.986**     | -0.970**     | -0.959**     |
|                                     | Sig. 0.000  | 0.000        | 0.001        | 0.002        |
| β-Type (epi)catechin dimer          | R -0.977**  | -0.963**     | -0.942**     | -0.928**     |
|                                     | Sig. 0.001  | 0.002        | 0.005        | 0.008        |
| β-Type (epi)catechin tetramer       | R -0.979**  | -0.966**     | -0.944**     | -0.931**     |
|                                     | Sig. 0.001  | 0.002        | 0.005        | 0.007        |
| β-Type (epi)catechin trimer         | R -0.997**  | -0.985**     | -0.967**     | -0.955**     |
|                                     | Sig. 0.000  | 0.000        | 0.002        | 0.003        |
| β-Type (epi)catechin trimer         | R -0.981**  | -0.984**     | -0.979**     | -0.974**     |
|                                     | Sig. 0.001  | 0.000        | 0.001        | 0.001        |
| β-Type (epi)catechin trimer         | R -0.574    | -0.625       | -0.658       | -0.673       |
|                                     | Sig. 0.233  | 0.185        | 0.155        | 0.143        |
| Quercetin-O-deoxyhexosyl-hexoside   | R 0.999**   | 0.990**      | 0.975**      | 0.964**      |
|                                     | Sig. 0.000  | 0.000        | 0.001        | 0.002        |
| Procyanidin with A-type linkage     | R -0.991**  | -0.983**     | -0.967**     | -0.956**     |
|                                     | Sig. 0.000  | 0.000        | 0.002        | 0.003        |
| Kaempferol-O-deoxyhexosyl-hexoside  | R -0.993**  | -0.992**     | -0.983**     | -0.975**     |
|                                     | Sig. 0.000  | 0.000        | 0.000        | 0.001        |
| Total phenolic acids                | R 0.976**   | 0.954**      | 0.929**      | 0.913*       |
|                                     | Sig. 0.001  | 0.003        | 0.007        | 0.011        |
| Total flavan-3-ols                  | R -0.999**  | -0.991**     | -0.975**     | -0.964**     |
|                                     | Sig. 0.000  | 0.000        | 0.001        | 0.002        |
| Total flavonols                     | R 0.990**   | 0.973**      | 0.951**      | 0.937**      |
|                                     | Sig. 0.000  | 0.001        | 0.004        | 0.006        |
| Total phenolic compounds            | R -0.997**  | -0.994**     | -0.982**     | -0.974**     |
|                                     | Sig. 0.000  | 0.000        | 0.000        | 0.001        |

Very strong correlation: ≥ 0.9; Strong correlation: 0.7–0.9; Moderate correlation: 0.5–0.7.
*Significant at p ≤ 0.05.
**Significant at p ≤ 0.01.

For OxHLIA, the Δt resulting from the Ht50 values of each hemolytic curve represented in Figure 3A were correlated with the corresponding extract dosage in order to determine the IC50 values, which were presented for Δt of 60 min, 120 min, and 180 min. As mentioned above, the hydroethanolic extract was more effective in protecting the red blood
cells, which is also translated by the higher slope value obtained for the correlation between Δt values and extract dosages shown Figure 3B. In this assay, the red blood cells were exposed to the hemolytic action of both hydrophilic and lipophilic radicals [51]. While the former resulted directly from the AAPH decomposition, the latter resulted from lipid peroxidation initiated by the first oxidative reaction.

Figure 3. Kinetic curves of the survival red blood cell population obtained for the quince peel extracts at different concentrations. The correlations between Δt values and extract concentrations used to calculate the IC50 values (μg/mL) able to promote Δt hemolysis delays of 60 min, 120 min, and 180 min are presented for each extract. *The well dilution factor was considered.
The antioxidant activity of quince peel extracts has been measured by different methodologies. Maghalhães et al. [12] observed that quince peel and pulp extracts protect the red blood cell membrane from hemolysis induced by AAPH in a concentration and time-dependent manner, indicating that endogenous antioxidants can efficiently quench free radicals to protect the membranes against hemolysis. The antioxidant activity of quince peel methanolic extracts was found correlated with total phenolics and mainly with caffeoylquinic acids. The phenolic compounds were thus reported to be able to quench the chain propagating peroxyl radicals, thus inhibiting hemolysis.

Alesiani et al. [14] tested the radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and anion superoxide radicals and the ability to induce the Mo(VI) reduction of secondary metabolites isolated from quince peel. The flavonol quercetin and its 3-O-rutinoside showed a strong capacity to scavenge free radicals. The quinic acid derivatives, chlorogenic and neochlorogenic acids led to an average DPPH radical reduction and were able to scavenge anion superoxide radicals by half. In our study, two of the detected caffeoylquinic acids also correlated moderately with the antioxidant affects (Table 6).

In another study, Szychowski et al. [39] measured the total antioxidant activity of hydrophilic and lipophilic fractions obtained from quince peel and pulp by the ABTS+ radical scavenging assay and observed greater activity for the peel than for the pulp. Furthermore, the activity of the hydrophilic fractions was higher in both pulp and peel in two quince clones, while the activity of the lipophilic fraction was higher in four of the studied quince clones. Fattouch et al. [13] and Silva et al. [52] also attributed greater antioxidant activity to peel extracts than to pulp extracts, which was related to qualitative and quantitative differences in the phenolic profiles. Moreover, while phenolic compounds were the main constituents contributing to the antioxidant activity of the quince pulp, these compounds were weakly correlated with the activity of the peel [39].

All these results highlight the antioxidant properties of quince peel, which may represent an accessible source of antioxidants with potential applications in various fields, such as food preservation in which free radicals are involved.

3.4.2. Antimicrobial activity

The antimicrobial activity of the quince peel extracts was tested against pathogenic and foodborne bacterial and fungal strains and the results are shown in Table 7. As positive controls, two food additives (E211 and E224), two antibiotics (streptomycin and ampicillin) and two antymycotics (ketoconazole and bifenazole) were used. As shown in Table 7, the hydroethanolic extract was most effective against the tested bacteria than the aqueous extract, particularly against Salmonella Typhimurium (lower MIC and MBC), one of the leading causes of inflammatory gastroenteritis in humans, Staphylococcus aureus (lower MIC), the most dangerous of all the many common staphylococcal bacteria, and Enterobacter cloacae (lower MIC). It is also interesting to note that both quince peel extracts were more bioactive against Staphylococcus aureus and Bacillus cereus than the synthetic food additives E211 and E224, respectively. The hydroethanolic extract was also more effective than E211 against Enterobacter cloacae. For the positive control antibiotics, the concentrations required to inhibit and kill the tested microorganisms were considerably lower. Overall, these results highlight the suitability of quince peel extracts for controlling the growth of some food poisoning and food spoilage microorganisms.

Regarding the antifungal activity results (Table 7), the tested microorganisms showed similar degrees of susceptibility, except for Penicillium funiculosum and Penicillium verrucosum var. cyclopium, which were more sensitive to the hydroethanolic extract. Compared to the E211 additive, the extracts were more effective in inhibiting and killing Aspergillus fumigatus, Aspergillus versicolor (known to produce sterigmatocystin, one of the most abundant food-contaminating mycotoxins) [53], and Trichoderma viride. For these fungi, the MIC obtained with E224 were also greater than or equal to those of the peel extracts. The antifungal ketoconazole was also found to be less effective than the extracts against Trichoderma viride. Therefore, it was interesting to highlight the antifungal capacity of the natural extracts under analysis, which were as effective or better than molecules currently used in industry as food preservatives.

In a previous study, Fattouch et al. [13] attributed greater antimicrobial activity to aqueous acetone extracts from quince peel than from pulp. The reported activity was higher against Staphylococcus aureus and Pseudomonas aeruginosa, and moderate against Escherichia coli and Candida albicans. No activity against Salmonella sp. and Aspergillus niger was observed. Among the detected and tested phenolic compounds, chlorogenic acid was the strongest microbial inhibitor against Pseudomonas aeruginosa and Escherichia coli. The authors also reported that this phenolic acid appeared to act synergistically with other compounds in the extract. These results suggested that quince peel extracts may be useful for the treatment of some microbial infections, given the apparent trend toward the appearance of more resistant strains. It will be important to understand how the bioactive compounds present in quince peel extracts work and inhibit the microbial growth in order to promote the development of functional foods or novel food ingredients.

### Table 7. Antibacterial and antifungal activity of the quince peel extracts and positive controls (sodium benzoate (E211), potassium metabisulphite (E224), streptomycin, ampicillin, ketoconazole, and bifenazole). The results are presented as minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (mg/mL).

| Antimicrobial Activity | Quince peel extracts | Positive controls |
|------------------------|----------------------|-------------------|
|                        | Hydroethanolic | Aqueous | E211 | E224 | Streptomycin | Ampicillin |
| Bacillus cereus          | MIC 1.50 | MBC 3.00 | 0.50 | 0.50 | 1.00 | 2.00 | 0.50 | 0.50 | 0.20 | 0.30 | 0.40 |
| Staphylococcus aureus    | MIC 1.50 | MBC 3.00 | 4.00 | 4.00 | 1.00 | 1.00 | 0.04 | 0.10 | 0.25 | 0.45 |
| Listeria monocytogenes   | MIC 1.50 | MBC 3.00 | 1.00 | 2.00 | 0.50 | 1.00 | 0.20 | 0.30 | 0.40 | 0.50 |
| Escherichia coli         | MIC 1.50 | MBC 3.00 | 1.00 | 2.00 | 0.50 | 1.00 | 0.20 | 0.30 | 0.40 | 0.50 |
| Enterobacter cloacae     | MIC 1.50 | MBC 3.00 | 2.00 | 4.00 | 0.50 | 0.50 | 0.20 | 0.30 | 0.25 | 0.50 |
| Salmonella Typhimurium   | MIC 1.50 | MBC 3.00 | 1.00 | 2.00 | 1.00 | 1.00 | 0.20 | 0.30 | 0.75 | 1.20 |
|                        |                   |       | MIC 0.25 | MFC 0.50 | MBC 1.00 | 0.25 | 0.50 | 0.15 | 0.20 |
|                        |                   |       | MIC 0.25 | MFC 0.50 | MBC 1.00 | 0.25 | 0.50 | 0.10 | 0.20 |
|                        |                   |       | MIC 0.25 | MFC 0.50 | MBC 1.00 | 0.20 | 0.50 | 0.15 | 0.20 |
|                        |                   |       | MIC 2.50 | MFC 3.50 | MBC 0.20 | 0.25 | 0.25 |
|                        |                   |       | MIC 2.00 | MFC 0.50 | MBC 0.20 | 0.10 | 0.20 |
|                        |                   |       | MIC 0.20 | MFC 0.30 | MBC 0.10 | 0.20 | 0.20 | 0.25 |

**Note:** MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration.
Stojkovic et al. [54] investigate the effects of quince peel and leaf ethanolic extracts on biofilm formation and verified its ability to disrupt already formed biofilms (including those of Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella Typhimurium), with a MIC in the range of 10–100 μg/mL for leaf extract and 5–60 μg/mL for peel extract. These results were comparable to or even higher than the reference controls.

3.4.3. Anti-inflammatory and cytotoxic activities

The anti-inflammatory activity of the quince peel extracts was assessed through its capacity to inhibit the nitric oxide production by LPS-stimulated RAW 264.7 macrophage cells. However, the tested extracts did not show activity at the tested concentrations (up to 400 μg/mL). Despite these results, other authors have already attributed anti-inflammatory effects to quince peel extracts. Essafi-Benkhadir et al. [11] showed that aqueous acetone extracts can induce the secretion of the pro-inflammatory cytokine TNF-α and the chemokine IL-8 by LPS-treated human THP-1-derived macrophages, and also increased the secretion of the anti-inflammatory cytokine IL-10. The extract also inhibited the LPS-mediated activation of cellular pro-inflammatory effectors, namely nuclear factor-kappa B, p38MAPK, and Akt. This difference in bioactivity may be due to the different origin and composition of the plant material, the extraction method and solvent, and the different in vitro assay performed by the authors.

The quince peel extracts were also tested against human breast (MCF-7), lung (NCI-1), and colorectal (CaCo-2) tumor cell lines and normal African green monkey kidney epithelial cells (Vero). As observed for the anti-inflammatory activity, the extracts (up to 400 μg/mL) also did not show cytotoxicity for these cell lines. These results are in agreement with those of Essafi-Benkhadir et al. [11], who reported no toxicity of quince peel aqueous acetone extract, at concentrations ranging from 20 to 1000 μg/mL, for THP-1-derived macrophages. On the other hand, Alesiani et al. [14] attributed a strong cytotoxic effect to phytoconstituents isolated from quince peel, namely rutin and quercetin and the triterpenoid annuarcoic acid, in murine B16-F1 melanoma cells.

4. Conclusions

This future-oriented study allowed the identification of commercially valuable functional compounds in quince peel, among which fiber, fructose, malic and quinic acids, and potassium stand out. On the other hand, while the solid residues remaining from the extraction were characterized as interesting sources of dietary fiber, the extracts presented mostly flavan-3-ols and also phenolic acids and flavonol glycosides. The hydroethanolic extract obtained by dynamic maceration showed better antioxidant and antimicrobial activities than the extract obtained with boiling water, which agreed with the highest levels of flavan-3-ols. Although these extraction methods use different solvents, processing times, and temperatures, the hydroethanolic extraction was more suitable due to the higher contents of polyphenols and greater bioactivity. However, a cost-benefit analysis will be important to draw more conclusions about the sustainability of these methods. Overall, it was demonstrated that quince peel can be reinserted into the value cycle via upcycling into natural ingredients for incorporation into foodstuff as preservatives, fortifiers, or health promoters. Future studies are planned to characterize the individual fibers and to optimize the extraction of target compounds, which could promote the large-scale exploitation of this by-product by the industrial sector. Furthermore, this approach can be extended to other agri-food by-products in order to identify valuable molecules and their potential application.

Declarations

Author contribution statement
Souha Othman, Mikel Anibarro-Ortega, Filipa Mandim: Performed the experiments.

Maria Inês Dias, Ana Ćirić: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marina Soković, Lillian Barros: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Isabel C. F. R. Ferreira: Conceived and designed the experiments. José Pinela: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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