INSTITUTO POLITÉCNICO DE BRAGANÇA
Escola Superior Agrária

Valorisation of quince (*Cydonia oblonga* Mill.) peel as a source of nutrients and bioactive polyphenols

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Dissertation presented to Escola Superior Agrária de Bragança
to obtain the Master Degree in Biotechnological Engineering

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Bragança
2021

This work was financially supported by European Regional Development Fund (ERDF)-Interreg España-Portugal programme through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P, and by ERDF through the Regional Operational Program North 2020, within the scope of Project GreenHealth – Digital strategies in biological assets to improve well-being and promote green health, Norte-01-0145-FEDER-000042.

ACKNOWLEDGEMENTS

Throughout the writing of this dissertation I have received a great deal of support and assistance.

I would first like to thank my supervisor, Dr José Pinela whose expertise was invaluable in formulating the research questions and methodology. Your insightful feedback pushed me to sharpen my thinking and brought my work to a better level. Thank you for sharing the expertise and providing important advice that made this project possible.

I would like to thank Dr Lillian Barros to give me this opportunity to work in her team.

I also want to thank Mikel Añibarro Ortega for his assistance and help in the laboratory.

To my parents and brother for their constant support and their confidence in me. without their encouragement this work could not have been carried out successfully.

Last but not least. For my other half, my soulmate Yasmine, the girl whose words cannot describe enough how I feel about her.

I will never forget the difficult moments we spent together, these moments surely were easier to handle because you were there. I won't also forget the sparks of happiness that we experienced together, our runaways, our nocturnal confessions, and our deep, intelligent discussions.

To the one I trust blindly, who I deeply relate to, to the one who understands me and forgives my mistakes, to the one who never stopped taking care of me.

Thank you for the quality time you brought to my life, I'm proud to have those precious moments with you.

ABSTRACT

Given the current and future needs of the human population and the finite resources our planet can provide, it is necessary to move from the current unsustainable food system to a circular and resource-efficient paradigm. Since the industrial production of quince-based foods generates a large amount of fruit by-products, this study was carried out to characterize the nutritional and chemical composition and bioactive properties of quince peel, following a "zero waste" approach. The proximate composition was estimated following official methods of food analysis, minerals were analysed by atomic absorption spectroscopy, free sugars and organic acids were analysed by high-performance liquid chromatography (HPLC) coupled to refractive index and photodiode array detectors, respectively, and fatty acids were identified by gas chromatography with flame ionization detection. Quince peel extracts obtained by dynamic hydroethanolic maceration and hot water extraction were investigated for their composition in phenolic compounds, by HPLC coupled to mass spectrometry, and antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities, using *in vitro* assays involving oxidizable cell substrates, foodborne microorganisms, lipopolysaccharide-stimulated macrophages, and tumour and non-tumour cell lines, respectively. The residues resulting from the extractions were also studied for fibre content. The peel dry powder was particularly rich in fibre, fructose, malic acid, and potassium. The hydroethanolic and aqueous extracts 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 haemolysis, and also showed better antimicrobial effects against foodborne pathogens, which agreed with the higher levels of flavan-3-os. The extracts were better than control food additives against some of the tested fungi and bacteria. On the other hand, no ability to inhibit nitric oxide production or toxicity to tumour and non-tumour cell lines was observed. Furthermore, the solid residues remaining after extraction were characterized as having 35–37 g/100 g of fibre. Overall, it was demonstrated that quince peel can be upcycled into fibre-rich and bioactive ingredients to endow the value chain with natural food fortifiers and preservatives.

Keywords: by-products valorisation, nutritional composition, phenolic compounds, flavan-3-ols, antioxidant activity, antimicrobial activity.

RESUMO

Dadas as necessidades atuais e futuras da população humana e os recursos finitos que o nosso planeta pode fornecer, é necessário passar do atual sistema alimentar insustentável para um paradigma circular e eficiente em termos de recursos. Uma vez que a produção industrial de alimentos à base de marmelo gera uma grande quantidade de subprodutos, este estudo teve com o objetivo de caracterizar a composição nutricional e química e as propriedades bioativas da casca de marmelo, seguindo uma abordagem de "desperdício zero". A composição centesimal foi estimada seguindo métodos oficiais de análise de alimentos, minerais foram analisados por espectroscopia de absorção atómica, açúcares livres e ácidos orgânicos por cromatografia líquida de alta eficiência (HPLC) acoplada a detetores de índice de refração e fotodíodos, respetivamente, e ácidos gordos por cromatografia gasosa com deteção por ionização de chama. Extratos obtidos por maceração hidroetanólica e extração com água quente foram estudados quanto à sua composição em compostos fenólicos, por HPLC acoplada a espectrometria de massa, e atividade antioxidante, anti-inflamatória, antimicrobiana e citotóxica, utilizando ensaios *in vitro* envolvendo substratos celulares oxidáveis, microrganismos de origem alimentar, macrófagos estimulados com lipopolissacarídeos e linhas de células tumorais e não tumorais, respetivamente. Os resíduos resultantes das extrações também foram estudados quanto ao teor de fibra. O pó seco da casca foi particularmente rico em fibra, frutose, ácido málico e potássio. Os extratos hidroetanólico e aquoso renderam 4,70 e 4,27 mg/g de polifenóis, respetivamente, com predomínio de flavan-3-ois. O extrato hidroetanólico foi o mais eficaz em inibir a peroxidação lipídica e a hemólise oxidativa, e também apresentou melhores efeitos antimicrobianos contra os microrganismos de origem alimentar, o que concordou com os maiores teores de flavan-3-ois. Os extratos foram melhores do que os aditivos alimentares controle contra alguns dos fungos e bactérias testadas. Por outro lado, não foi observada capacidade de inibir a produção de óxido nítrico ou toxicidade para as linhas celulares tumorais e não tumorais. Além disso, os resíduos resultantes da extração apresentaram 35–37 g/100 g de fibra. Desta forma, foi demonstrado que a casca do marmelo pode ser transformada em ingredientes ricos em fibra e bioativos para dotar a cadeia de valor com fortificantes e conservantes naturais.

Palavras-chave: valorização de subprodutos, composição nutricional, compostos fenólicos, flavan-3-ois, atividade antioxidante, atividade antimicrobiana.

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ABBREVIATIONS

2,2'-Azobis(2-amidinopropane) dihydrochloride	**AAPH**
Atomic absorption spectroscopy	**AAS**
Gastric adenocarcinoma cell line	**AGS**
Association of official analytical chemists	**AOAC**
Caucasian colorectal adenocarcinoma cell line	**CaCo-2**
Diode array detector	**DAD**
Dry weight	**dw**
Concentration providing 50% of nitric oxide production inhibition	**EC_{50}**
Electrospray ionization	**ESI**
Fatty acid methyl esters	**FAME**
Flame ionization detector	**FID**
Fresh weight	**fw**
Gas chromatography	**GC**
High-performance liquid chromatography	**HPLC**
Concentration inhibiting 50% of TBARS or oxidative haemolysis	**IC_{50}**
p-Iodonitrotetrazolium violet	**INT**
Lipopolysaccharide	**LPS**
Minimum bactericidal concentration	**MBC**
Breast adenocarcinoma cell line	**MCF-7**
Minimum fungicidal concentration	**MFC**
Minimum inhibitory concentration	**MIC**
Mass spectrometry	**MS**
Monounsaturated fatty acids	**MUFA**
Non-small cell lung cancer cell line	**NCI-H460**
Oxidative haemolysis inhibition assay	**OxHLIA**
Erythrocyte population that remained intact	***P***
Photodiode array detector	**PAD**
Phosphate buffered saline	**PBS**
Porcine liver primary cell culture	**PLP2**
Portuguese food information portal	**PortFIR**
Polyunsaturated fatty acids	**PUFA**
Saturated fatty acids	**SFA**
Sulforhodamine B	**SRB**
Thiobarbituric acid reactive substances	**TBARS**
United States Department of Agriculture	**USDA**
Normal African Green Monkey kidney epithelial cells	**Vero cells**

1. INTRODUCTION

Given the current and future needs of the human population and the finite resources that our planet can provide, it is necessary to move from the current environmentally unsustainable agri-food system to a healthy, circular and resource-efficient paradigm. 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*" (FAO, 2021). This statement underlies 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 sustainability-oriented innovation practice by contributing to waste reduction and more efficient use of bioresources, which has been called valorisation or upcycling (Aschemann-Witzel & Stangherlin, 2021).

Quince is the golden yellow pome fruit of *Cydonia oblonga* Mill., a deciduous small tree native to the Trans-Caucasia and north of Iran and which has spread to west and east Asia, Europe, and America (Abdollahi, 2019). In 2019, the world production of this fruit reached 666,589 tons in 93,699 ha of harvested area, with Turkey and China together contributing about 41% of the world total (FAOSTAT, 2021). This fruit has an intense aroma, flavour, and acidity, but most varieties are too hard and sour to be eaten raw, so it is cooked or processed into other food products such as jam, jelly, and quince pudding or marmalade (a traditional sweet originating from the Iberian Peninsula where it is known as *marmelada* (PT) and *carne de membrillo* or *dulce de membrillo* (ES)). For industrial production of marmalade, large quantities of raw materials are needed, especially quince and sugar (Decreto-Lei n.º 97/84). 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 for marmalade production.

Attempts to promote the upcycling of quince processing by-products (solid or liquid) have been very limited. Trigueros et al. (2011) studied quince scalding 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. Quince scalding water was also investigated by Shinomiya et al. (2009) for its antiallergic effects on type I allergic symptoms. A crude extract was prepared and when orally administrated to mice for 63 days, a significant decrease in the development of atopic dermatitis-like skin lesions was observed. Quince juice pomace, another nutrient-rich industrial by-product, was upcycled as a sustainable source of pectin (Brown et al., 2014) and antimicrobial ingredients for candies (Lele et al., 2018). Regarding quince peel, it has been described as containing hydroxycinnamic (caffeoylquinic) acids and flavonols (quercetin and kaempferol glycoside derivatives), which are also found in the fruit pulp (Essafi-Benkhadir et al., 2012; Magalhães et al., 2009; Stojanović et al., 2017). According to some studies, both quince peel and pulp extracts display antioxidant activity, including antihemolytic effects (Magalhães et al., 2009; Stojanović et al., 2017). The peel extract may actually be more effective in scavenging free radicals and inhibiting some microorganisms than the pulp (Fattouch et al., 2007). Antioxidant and antiproliferative activities of phytochemicals isolated from quince peel were investigated by Alesiani et al. (2010). The highest antioxidant activity was attributed to quercetin and its 3-*O*-rutinoside, followed by chlorogenic and neochlorogenic acids. Antiproliferative effects were attributed to triterpenoids, but quercetin-3-*O*-rutinoside also exhibited strong effects on murine B16-F1 melanoma cells. Anti-inflammatory effects were also previously attributed to a quince peel polyphenolic extract (Essafi-Benkhadir et al., 2012). All these studies highlight the bioactive potential of quince peel. However, the nutritional composition of this by-product remains unknown and more studies are needed to prove its suitability to be entirely reinserted in the value cycle as natural ingredients with nutritional and functional or bioactive value.

This work was carried out 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 contributed to the upcycling of quince peel into valuable natural ingredients (including food preservatives and fortifiers) following a “zero waste” approach. Although this work will not solve the major problems that have been marking today's society, it is a path towards sustainable food systems that can be extended to other plant by-products of the agri-food sector.

2. STATE OF THE ART

2.1. Quince

Quince is the fruit of the deciduous shrub or subtree in the Rosaceae family (which also includes apple, pear, and other fruits), *Cydonia oblonga* Mill. (syn. *Cydonia vulgaris* Pers.), and the only member of the genus *Cydonia*. This species can grow up to 6 m high and reach 4 m wide and produces a bright golden yellow pome fruit with a strong and long-lasting aroma when ripe (**Figure 1**). The fruit is commonly 70–120 mm long and 60–90 mm across, resembling an apple but differing by having many seeds in each carpel (Kafkas et al., 2018; UTAD, 2021). In the past, the quince symbolized fortune, fertility, and love, and was used as a medicinal plant by the Greeks. The Romans also consumed quinces, preserving them in honey.

Figure 1. Quince fruit and leaves. Source: https://jb.utad.pt/especie/Cydonia_oblonga

2.2. Production and consumption data

Quince is originally from the Caucasus region and has gradually spread to Central Europe and Mediterranean countries, having the potential to thrive in a variety of climates and can be successfully grown in latitudes as far north as Scotland (Abdollahi, 2019). Although its old reputation has been lost, quince continues to be grown all over the world. As shown **Figure 2**, the world production of quinces has increased in the last 10 years, reaching 666,589 tonnes in 2019 in an harvested area of 93,699 ha (FAOSTAT, 2021). For Portugal, FAOSTAT has no production data since 2017, but for this year, the quince production reach 5492 tonnes (**Figure 2**), representing less than 1% of the world total. In turn, Turkey and China together contributed to approximately 41% of the world total. Uzbekistan, Iran, Morocco, and Azerbaijan are other countries with a large production (FAOSTAT, 2021), which occurs mainly in backyards, but also in commercial orchards.

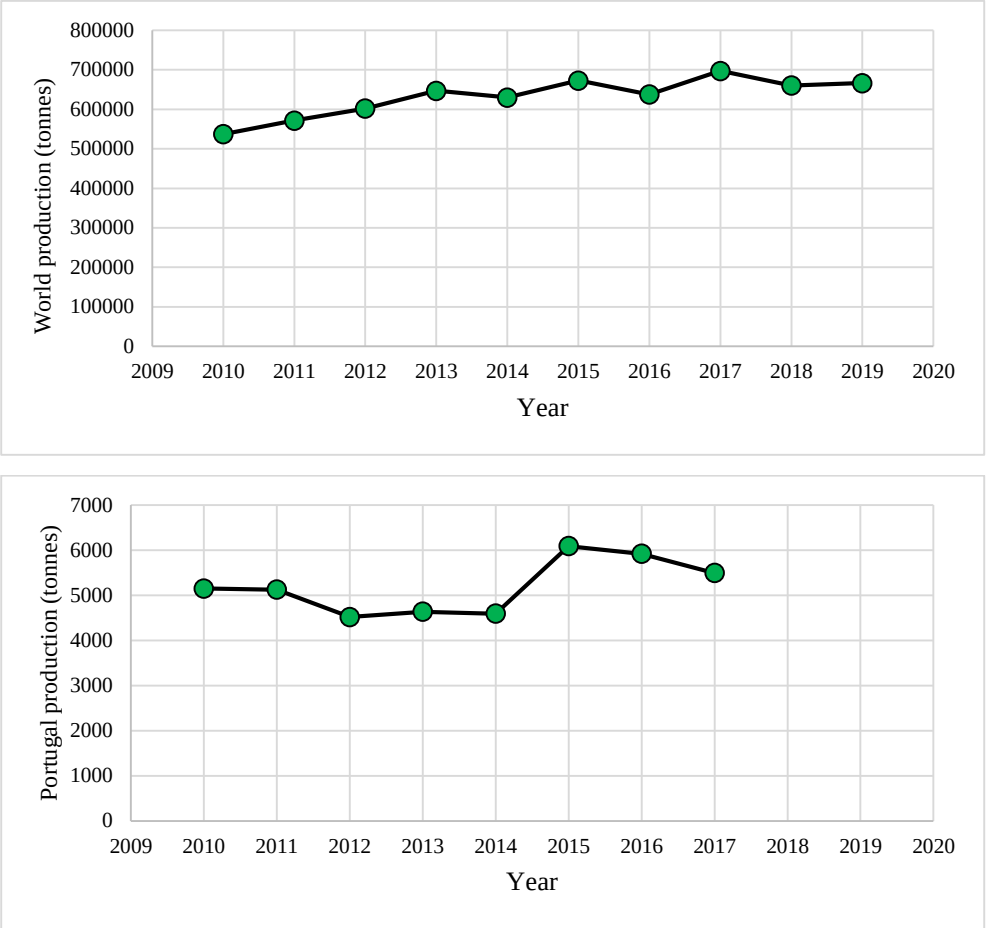


Figure 2. Data on quinces production in the world and in Portugal since 2010. FAOSTAT (2021).

Quince is not appreciated fresh because of the hard flesh, bitterness, and astringency, but when ripe it is highly demanded for processing marmalade, jams, jelly, and cakes, among other food products. In the northeast of Portugal, it was traditionally roasted in the fireplace and eaten with sugar.

2.3. Industrial processing and by-products valorisation

According to the Portuguese Legislation (Decreto-Lei n.º 97/84 de 28 de Março), marmalade is the product resulting from a homogeneous and consistent mixture, obtained exclusively from cooking the quince mesocarp with sugars. However, some additives are also added in industrially produced marmalades, including preservatives (such as benzoic and sorbic acids), antioxidants (such as ascorbic acid), and acidity regulators (such as citric and tartaric acids), among others. Considering the production volume of marmalade, large quantities of raw materials are needed, especially quince and sugar. Sometimes, other fruit pulps are also used. During the production of this Portuguese regional sweet, gelation depends on the type and concentration of pectin, the pH, and the amount of sugar.

It occurs when pectin is precipitated by the addition of sugar, altering the existing balance between pectin and water. Marmalade is high consumed in Portugal, but has ceased to be handcrafted and, when produced industrially, is sometimes of lower quality. Quince pectin is also of particular importance as a food additive.

Figure 3 illustrates the initial steps of the industrial production of the marmalade, some of which generate by-products. Initially, the quince is received and selected by visual examination for obtaining a high quality and uniform raw material, then it is washed with chlorinated water, cut to remove the fruit peel and damaged parts (an operation that can be performed manually or mechanically and that generates a solid by-product). After an antioxidant treatment with an ascorbic acid solution (resulting a liquid by-product), the fruits are scalded with water at 90 ºC to remove the air from the tissues, inactivate enzymes responsible for browning, and reduce the microbial load. Then, these are ground to obtain quince pulp, which is refined by centrifugation to eliminate the seeds. The pulp obtained after these steps follows the process line for the marmalade production.

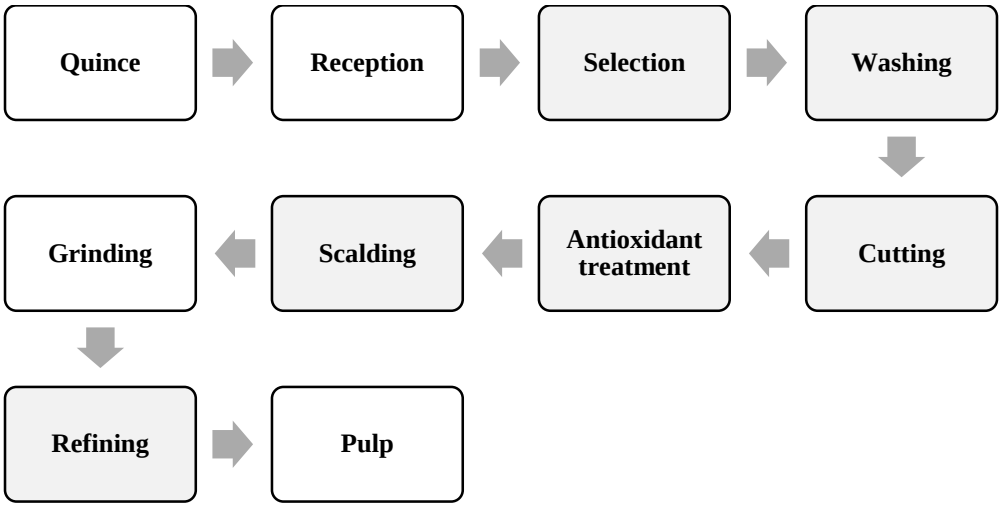


Figure 3. Flowchart of the initial steps of the industrial production of marmalade. All steps with the shaded box generate a solid or liquid by-product.

The reuse of quince processing by-products by the industrial sector has been very limited. However, some products have been developed from quince scalding water, which is empirically known to be effective for treating sore throats and to relieve coughs. A previous study proposed the use of quince scalding water as a functional food ingredient due to its content in antioxidant polyphenols, organic acids, and sugars (Trigueros et al., 2011). The authors incorporated this ingredient in set style yogurts and evaluated their quality during 28 days of refrigerated storage. This new formulation induced colour

changes and reduced the yogurt sensory scores due to its acidic nature. The nutrient-rich water had an inhibitory effect against lactic acid bacteria, probably due to its high polyphenols content, which increased the pH and lowed the lactic acid content of the enriched yogurts, compared to the control ones. Thus, the rheological and textural properties were also affected, originating soft yogurts of higher deformability and lower elastic behaviour and viscosity. During cold storage of the enriched yogurts, the pH decreased, the gel structure was reinforced, the lactobacilli population decreased, and mould and yeast population increased. Based on these unpromising results, the authors proposed further studies to extract the bioactive compounds from this liquid by-product for their application as bio-based food ingredients or health promoting agents.

In a different study, Shinomiya et al. (2009) proposed the use of quince scalding water for its antiallergic effects on type I allergic symptoms. A crude extract was prepared and tested *in vivo* and *in vitro*. When orally administrated to mice for 63 days, a significant decrease in the development of atopic dermatitis-like skin lesions was observed. The concentration of IgE antibodies in the serum was reduced in a dose-dependent manner, and the release of β-hexosaminidase from rat basophilic leukaemia RBL-2H3 cells was inhibited after a 24-h treatment. Additionally, the extract fraction of less than 3 kDa was able to reduce the mRNA expression of the high-affinity IgE receptor (FcεRI) γ subunit, which has been associated to an immediate hypersensitive reaction against environmental allergens and an elevated level of IgE antibodies produced against the allergens. Based on these results, quince scalding water could be exploited as a functional food ingredient.

Despite the high-added value of the quince scalding water, it should be noted that the direct application of this heat-treated by-product is nowadays limited by regulations.

The potential of quince juice pomace (a nutrient-rich by-product) for being used as ingredients in chewing candies was evaluated by Lele et al. (2018), and was interesting to note the antimicrobial affects against *Salmonella enterica*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus cereus*, and methicillin-resistant *Staphylococcus aureus* (MRSA), among other microorganism.

The valorisation of quince pomace as a sustainable source of pectin was investigated by Brown et al. (2014), who optimized alternative methods for pectin extraction comprising steps of pomace pre-treatment, drying, acid extraction, and pectin extract concentration. The pH was the extraction variable that induced major effects, and also interacted to other variables. Additionally, low pressure superheated steam drying and ultrafiltration were also important process steps for maximizing the response.

2.4. Compositional characteristics of quince

2.4.1. Nutritional composition

The quince edible part (~79%) is a rich source of macronutrients, minerals, and vitamins importance for consumer's health and well-being. **Tables 1** and **2** present the quince composition according to the USDA (USDA, 2019) and PortFIR (INSA, 2018) Nutrient Databases. Despite the observed compositional differences, it is possible to conclude that quince is rich in water and total carbohydrates, followed by dietary fibre. It is a low protein and fat food. The lipid fraction is constituted mostly by unsaturated fatty acids, with predominance of linoleic acid (C18:2), an essential fatty acid that must be obtained from the diet, and oleic acid (C18:1). In addition to C18:2 and C18:1, Szychowski et al. (2014) reported palmitic (C16:0), stearic (C18:0), and arachidic (C20:0) acids as abundant lipophilic constituents of the quince seeds. The energy value of the quince edible part is 53–57 kcal/100 g.

Table 1. Proximate composition, energy, and lipid fraction composition of quince (per 100 g fw).

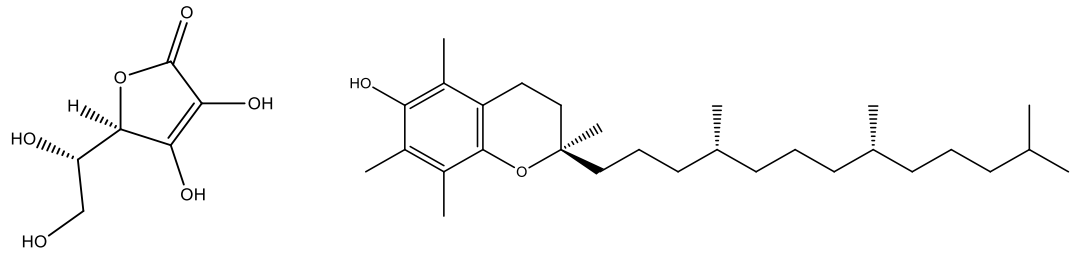
	USDA (2019)	**INSA (2018)**
Water (g)	83.8	83.6
Carbohydrates (g)	15.3*	9.3
Sucrose	-	0.3
Ash (g)	0.4	0.35
Protein (g)	0.4	0.3
Dietary fibre (g)	1.9	6
Fat (g)	0.1	0.2
Saturated fatty acids	0.01	0
Monounsaturated fatty acids	0.036	0.1
Polyunsaturated fatty acids	0.05	0.1
Energy (kcal)	57	53

*Total carbohydrates calculated by difference (including dietary fibre).

As shown in **Table 2**, potassium is the most abundant mineral in quince, followed by potassium and calcium. The minerals content, given as ashes in **Table 1**, is obtained by mineralization (incineration of the organic matter) at high temperatures (> 450 ºC) (Pereira et al., 2011). Vitamin C (ascorbic acid) and α-tocopherol are the vitamins described in higher amounts (**Figure 4**), but many others can be detected in quince.

Table 2. Minerals and vitamins composition of quince (per 100 g fw).

	USDA (2019)	INSA (2018)
Ca (mg)	11	14
Fe (mg)	0.7	0.1
Mg (mg)	8	7
P (mg)	17	14
K (mg)	197	200
Na (mg)	4	4
Zn (mg)	0.04	0.1
Cu (mg)	0.13	-
α-Tocopherol (mg)	-	0.55
Vitamin C (mg)	15	14
Thiamine (mg)	0.02	0.02
Riboflavin (mg)	0.03	0.04
Niacin (mg)	0.2	0.1
Vitamin B6 (mg)	0.04	0.04
Folates (µg)	3	3
Vitamin A (µg)	2	5



A B

Figure 4. Chemical structures of (A) ascorbic acid and (B) α-tocopherol.

Organic acids are also important quince constituents. Silva et al. (2002) indented citric, ascorbic, malic, quinic, shikimic, and fumaric acids in quince peel and pulp samples, with total contents ranging from 7 to 14 g/Kg in both samples. The chemical structure of some of these organic compounds is presented in **Figure 5**. In some cases, the amount of organic acids was higher in pulps than in the corresponding peels, while in other cases the differences were very small. Furthermore, the sum of both malic and quinic acids accounted for 96–99% of the total acid content in pulps and 93–98% in peels. All other organic acids were present in small amounts, with the exception of citric acid. In turn, Szychowski et al. (2014) reported a profile consisted constituted by phytic, malic, quinic, citric, and tartaric acids. The major acids in ripe quinces were phytic and malic

acids, with much lower contents of quinic, citric, and tartaric acids. However, phytic acid is a non-nutrient than can reduce the bioavailability of certain nutrients, such as minerals.

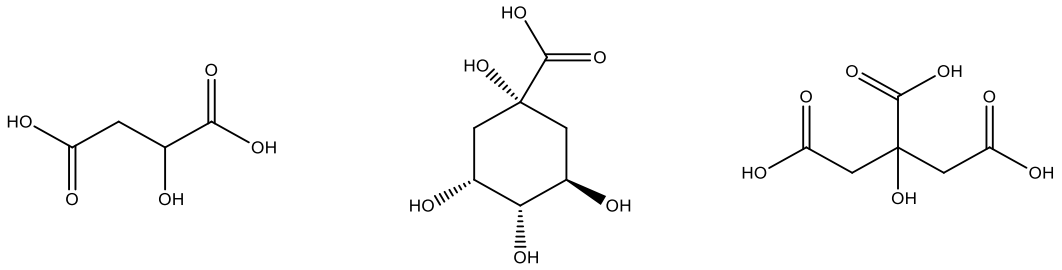


Figure 5. Chemical structures of (A) malic acid, (B) quinic acid, and (C) citric acid.

As referred above, quince is rich in pectin, a structural acidic heteropolysaccharide found in the primary and middle lamella and cell walls of terrestrial plants, which affects the gelation process during marmalade manufacture. Its main component is galacturonic acid, a sugar acid derived from galactose. Forni et al. (1994) investigated the galacturonic acid content and the neutral sugar composition of a quince pectin extract and reported a high galacturonic acid content (~78%) and a degree of methoxylation of ~59% corresponding to a medium-high methoxyl pectin.

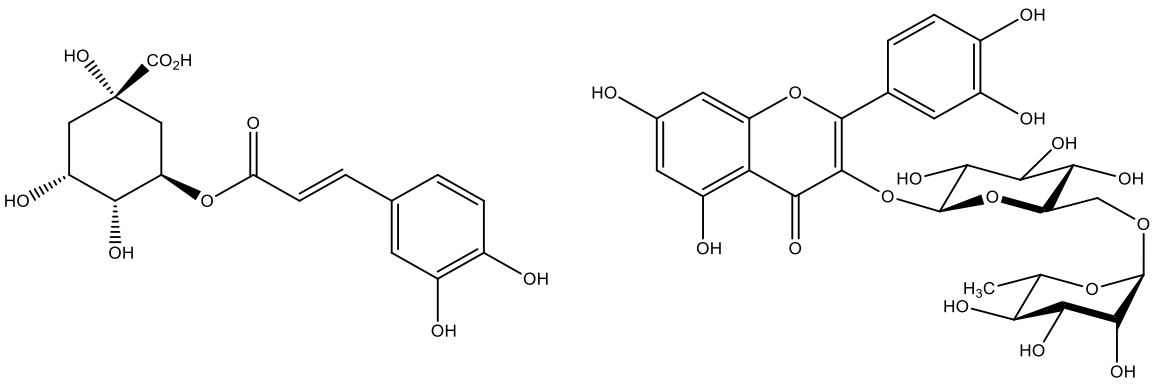
2.4.2. Polyphenolic composition

Quince is source of many compounds with health-promoting effects such as polyphenols. These can be estimated spectrophotometrically based on colorimetric assays (expressed as total content) or identified by high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS^n, **Figure 6** shows the system available at CIMO laboratories. However, other advanced analytical techniques are also available such as nuclear magnetic resonance spectroscopy.

Essafi-Benkhadir et al. (2012) reported hydroxycinnamic acids, mainly chlorogenic acid (13%), and quercetin-3-*O*-rutinoside (rutin) as the major polyphenol (36%) in quince peel extract (**Figure 7**). Flavonols were detected as mixture of different aglycone and glycosylated quercetin and kaempferol. Flavanols were essentially catechins and procyanidins. In the study by Magalhães et al. (2009), quince pulp extract presented a profile composed by 3-*O*- and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicaffeoylquinic acid, and quercetin-3-*O*-rutinoside (rutin). Caffeoylquinic acids were the major phenolic compounds (99%) and the most abundant was 5-*O*-caffeoylquinic acid (57%). The

authors also characterized the peel extract, where they detected the same compounds found in pulp and also quercetin-3-*O*-galactoside, kaempferol-3-*O*-glycoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, two quercetin glycosides acylated with *p*-coumaric acid and a kaempferol glycoside acylated with *p*-coumaric acid. 3-*O*- and 5-*O*-caffeoylquinic acid and quercetin-3-*O*-rutinoside were the main phenolic compounds. In turn, *C*-glycosil flavones were the major phenolic compounds (85%) detected in seed extract and the most abundant was stellarin-2 (18%). Stojanović et al. (2017) detected six hydroxycinnamic acids in quince peel and pulp methanol and acetone extracts, namely 3-*O*-caffeoylquinic acid, 4-*p*-coumaroylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, a *p*-coumaroylquinic acid derivative, and 3,5-dicaffeoylquinic acid. Among these, the most abundant was 5-*O*-caffeoylquinic acid with 259–481 mg/kg fw in peel and 97–217 mg/kg in pulp. Flavonols were also detected, namely quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, and a quercetin derivative.

Figure 6. HPLC-DAD-ESI/MS^n system available at CIMO laboratories.



A B

Figure 7. Chemical structures of (A) chlorogenic acid and (B) quercetin-3-*O*-rutinoside.

2.4.3. Other chemical constituents

As previously mentioned, quince has a strong and long-lasting aroma due to the presence of aromatic compounds, which can be extracted from the pulp and mostly from the peel. Tateo and Bononi (2010) identified more than 40 volatile compounds in quince. Of the esters of higher organic acids, ethyl hexanoate and ethyl octanoate were those detected in higher quantity. Additionally, while ethyl octanoate notoriously increased with ripening, the two sesquiterpenes, α-bergamotene and α-farnesene, were decreased.

Oleanane triterpenes, such as oleananic acid hederagenic acid, and triterpenes characterised by an ursane skeleton, including uvaol and it acyl derivative, ursanaldehyde, ursolic acid and it 3-linoleoyl derivative, 2α-hydroxy ursolic acid, pomolic acid, and annurcoic acid were also already identified in quince (Alesiani et al., 2010).

2.5. Bioactive properties of quince

Quince has been recognized as a dietary source of health-promoting compounds with antioxidant, anti-inflammatory, antiproliferative, antimicrobial, and antiulcerative effects. The *in vitro* evaluation of these biological activities is a common way of accessing the food functionality, which can be conferred by the different bioactive nutrients (including ascorbic acid, tocopherols, and minerals) and non-nutrients (such as polyphenols) referred above, which, after absorption by the human body, offer protection against the oxidative damage and various degenerative diseases (Carocho & Ferreira, 2013a, 2013b).

Magalhães et al. (2009) studied the phenolic profile and antioxidant activity of quince. First, quince pulp, peel, and seeds were used to prepare methanolic extracts. Phenolic compounds were characterized by HPLC/UV and the antioxidant activity was evaluated by the ability to quench the DPPH radical and to inhibit the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative haemolysis of human erythrocytes. The main phenolic compounds were 5-*O*-caffeoylquinic acid for pulp and peel (57% and 29%, respectively) and stellarin-2 for seed (18%). The total phenolics content was higher in the peel extract, followed by the pulp extract. However, both pulp and peel extract showed similar DPPH radical scavenging activities. Under the oxidative action of the AAPH free radical generator, the two extracts showed significant protection of the erythrocyte membrane from haemolysis. These results highlighted the potential of application for these extracts as novel food and nutraceutical ingredients.

The antioxidant activity of quince peel and pulp extracts prepared with 60–80% methanol or acetone was tested by Stojanović et al. (2017) using the DPPH, ABTS, FRAP, and reducing power assays. According to the *in vitro* results, the activity of both peel and pulp extracts were ordered as follows: 80% acetone > 60% acetone > 80% methanol > 60% methanol, thus highlighting 80% acetone as suitable solvent for extracting antioxidants.

Alesiani et al. (2010) investigated by antioxidant and antiproliferative activities of quince peel phytochemicals. Fifty-nine secondary metabolites were isolated from the peels, characterised by nuclear magnetic resonance (NMR) and mass spectrometry (MS), and tested for their antioxidant activities by measuring their capacity to scavenge DPPH and anion superoxide radicals, and to reduced Mo(VI) to Mo(V). The antiproliferative activity of the most abundant compounds was also tested by the MTT bioassay on murine B16-F1 melanoma cells. The highest antioxidant capacity was attributed to the flavonol quercetin and its 3-*O*-rutinoside. Quercetin reduced the DPPH radical by 57% and the anion superoxide radical by 80%. In turn, chlorogenic and neochlorogenic acids determined an average DPPH reduction of 35% and were able to scavenge O_2^- by 53% and 52%, respectively. Among triterpenes, ω-hydroxylinoleate was able to reduce the DPPH radical by 16% and the anion superoxide radical by 23%. Additionally, the detected carotenoid derivatives appeared to be strong radical-scavengers, with activities comparable to that of the positive standards α-tocopherol and ascorbic acid. A significant antiproliferative activity was attributed to triterpenoids, with ursolic acid as the most active compound. Quercetin and quercetin-3-*O*-rutinoside also exhibited strong effects on B16-F1 cells.

In another study, Essafi-Benkhadir et al. (2012) found that a quince peel polyphenolic extract modulates lipopolysaccharide (LPS)-induced inflammation in human THP-1-derived macrophages through the inhibition of nuclear factor-kappaB (NF-κB), p38 mitogen-activated protein kinase (MAPK), and Akt kinase, which are major cellular effectors of inflammation. These effects were related to the major phenolics compounds detected in the extract, namely catechin, rutin, hyperin (quercetin-3-*O*-galactoside), procyanidin, chlorogenic acids, and kaempferol and quercetin derivatives.

The antioxidant activity and cytotoxicity of two quince extracts were also evaluated by Pacifico et al. (2012). While a lipophilic wax extract had interesting antilipoperoxidant effects but was ineffective as radical scavenger, an aqueous fermented extract presented a marked antioxidant activity and cytotoxicity towards HepG2, A549, and HeLa cells.

Regarding antimicrobial effects, Fattouch et al. (2007) showed that quince peel extracts inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* more effectively than pulp extracts, when tested by the disk and well diffusion techniques. This effect was related to chlorogenic acid and its possible synergism with other constituents of the aqueous acetone extracts. A total phenolic content ranging from 105–157 mg/100 g of fresh weight was quantified in the quince peel, while the pulp contained from 37–47 mg/100 g of fresh weight. Rutin (quercetin 3-*O*-rutinoside) was the main one in the peel (36%), while chlorogenic acid (5-*O*-caffeoylquinic acid) was the most abundant phenolic compound in the pulp (37%), when analysed by HPLC-DAD-ESI/MS^n. The peel extract also showed a stronger DPPH radical scavenging activity than the pulp extract.

3. OBJECTIVES

This Master dissertation was carried out with the MAIN OBJECTIVE of valorising the quince peel as a source of nutrients and functional/bioactive compounds. Therefore, the following SPECIFIC OBJECTIVES were followed:

- Quince samples collection and preparation;
- Determination of the proximate composition of quince peel by official methods of food analysis;
- Analysis of mineral elements, free sugars, organic acids, and fatty acids by spectroscopic and chromatographic techniques;
- Preparation of quince peel extracts by solid-liquid extraction methods;
- Characterization of the phenolic profile by HPLC-DAD-ESI/MS^n;
- Evaluation of *in vitro* bioactive properties, namely antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities;
- Data analysis and elaboration of the dissertation.

Overall, it was intended to contributed to a better bioresource-use efficiency and circularity in the agri-food sector following a “zero waste” approach, and provide novel natural ingredients for food application containing dietary fibre, phenolic compounds, and other high value-added molecules.

4. MATERIAL AND METHODS

4.1. Preparation of the plant material

Quince peels were supplied by local producers from Bragança, civil parish of Calvelhe, Portugal, in October 2020. The whole quinces are shown in **Figure 8**. The peel sample (~1 kg) was frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA), reduced to a fine powder with a domestic grinder, and homogenized to obtain a representative sample that was kept at −20 °C until analysis.

Figure 8. Quinces produced in region of Bragança.

4.2. Standards and reagents

HPLC-grade acetonitrile (99.9%), n-hexane (95%), and ethyl acetate (99.8%) were purchased from Fisher Scientific (Lisbon, Portugal). Caesium buffer and lanthanum solution were acquired from Thermo Fisher Scientific Co. (Waltham, MS, USA). Acetic acid, ascorbic acid, ellipticine, ferrous sulfate, formic acid, metaphosphoric acid, phosphate buffered saline (PBS), sulforhodamine B (SRB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), trypan blue, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), the fatty acids methyl ester (FAME) reference standard 47885-U, and standards of sugars ((D(-)-fructose, D(+)-sucrose, D(+)-glucose, D(+)-trehalose and D(+)-raffinose pentahydrate) and organic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin-ethylenediaminetetraacetic acid (EDTA), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), RPMI-1640 and DMEM media were purchased from Hyclone (Logan, UT, USA). Dimethylsulfoxide (DMSO) was acquired from Merck (KGaA, Germany). Mueller-Hinton broth (MHB) and *p*-iodonitrotetrazolium

chloride (INT) were acquired from the Institute of Immunology and Virology (Mittelhäusern, Switzerland) and Biochemica (Panreac, Barcelona, Spain), respectively. Ethanol and other reagents were purchased from common sources. Water was treated in a Milli-Q water purification system (Millipore, model A10, Billerica, MA, USA).

4.3. Compositional analysis of quince peel

4.3.1. Proximate composition and energy

The quince peel sample was analysed for moisture, protein, fat, and ash contents following the procedures of the Association of Official Analytical Chemists (AOAC) (AOAC International, 2016). These determinations were performed in triplicate.

The moisture content was determined comparing the sample weight before and after drying in oven at 105 ºC until constant weight, using the following equation:

$$misture = \frac{m1 - m2}{m1} \times 100 \qquad \text{Eq. (1)}$$

where *m1* corresponds to the weight of the fresh sample and *m2* corresponds the weight of the dried sample. The result was presented in g per 100 g of fresh weight (fw).

The crude protein content was estimated by the macro-Kjeldahl method (AOAC 978.04), which allows the determination of the crude protein content based on the nitrogen content of the sample. In this analysis, ~1 g of quince peel sample was placed in digestion tubes containing 15 mL of sulphuric acid and 2 selenium tablets to catalyse the reaction, and then it was digested for approximately 2 h at 410 °C in the digestion block (Bloc Digest 12, JP Selecta, Barcelona) (**Figure 9**). After cooling, distilled water was added to each tube to reduce acidity and distillation and titration processes were made in an automatic equipment (Pro-Nitro-A, JP Selecta, Barcelona). The protein content was calculated by multiplying the obtained nitrogen content by the conversion factor of 6.25. The result was presented in g per 100 g of dw and also fresh weight (fw) by conversion.


A

B

C

Figure 9. Protein analysis: (A) digester and (B) digested sample quince peel, and (C) automatic distillation and titration unit.

The crude fat content was determined by subjecting the quince peel sample (~3 g) to Soxhlet extraction with petroleum ether for approximately 8 h (AOAC 920.85). The solvent containing the solubilized fat was eliminated and the fat content was determined using the following equation:

$$fat = \frac{m1 - m2}{m1} \times 100 \qquad \text{Eq. (2)}$$

where *m*1 corresponds to the initial weight of sample and *m*2 corresponds to the weight of crude fat obtained. The result was presented in g per 100 g of dw and also fw by conversion.

The ash content was determined by incineration of ~500 mg of quince peel sample in a muffle furnace (Lenton Thermal designs; ECF12/22) at 550 ± 15 °C for about 12 h, using crucibles previously calcined and weighed (AOAC 923.03). After that, the crucibles containing the ashes were cooled in a desiccator and weighed. The ash content was calculated using the following equation:

$$ash = \frac{m1 - m2}{m1} \times 100 \qquad \text{Eq. (3)}$$

where *m1* corresponds to the initial weight of sample and *m2* corresponds to the weight of ash obtained. The result was presented in g per 100 g of dw and also fw by conversion.

The dietary fibre content was determined by an enzymatic-gravimetric method (AOAC International, 2016). In brief, the quince peel sample was treated with alpha-amylase, protease, and amyloglucosidase. The residue from the digests was dried at 100 ºC, and ash and protein contents were determined in the residue. The result was obtained applying the following equation and presented as g per 100 g of dw and also fw by conversion.

dietary fibre = (g residue) – (g ash + g protein) Eq. (4)

The carbohydrate content was estimated by difference using the following equation and the result was presented in g per 100 g of dw and also fw by conversion.

$$carbs = 100 - (\text{g moisture} + \text{ g protein} + \text{g fat} + \text{g ash} + \text{g dietary fibre}) \qquad \text{Eq. (5)}$$

The energy value was calculated according to the Regulation (EC) No. 1169/2011 of the European Parliament and of the Council (European Union, 2011), as follows:

$$energy = 4 \times (\text{g protein} + \text{g carbohydrates}) + 9 \times (\text{g fat}) + 2 \times (\text{g dietary fiber})$$

4.3.2. Mineral elements

Mineral elements were analysed by atomic absorption spectroscopy (AAS) using a Perkin Elmer PinAAcle 900T Spectrometer (Waltham, MA, USA). The analysis included potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu). For the determination, ~0.25 g of quince peel sample was weighed and digested with 10 mL of nitric acid, using heat and microwaves (1600 W). The microwave used the following temperature ramp rate: up to 180 °C for 15 min and then held for 15 min. After cooling down, the obtained solutions were diluted up to 50 mL with deionized water and analysed by AAS, with prior treatment for specific elements. For the determination of K and Na, the sample was diluted in a cesium chloride solution (1 g/L); for Ca and Mg, the sample was diluted in a lanthanum chloride solution (1 g/L); for Mn and Cu, a magnesium nitrate solution was used as a matrix modifier; and Fe and Zn were directly analysed. Phosphorus (P) was analysed by a colorimetric method (Walinga et al., 1995). The determination of elements was achieved by comparing the absorbance responses to pure analytical solutions. The results were expressed in mg per 100 g of dw and also fw by conversion.

4.3.3. Free sugars

Approximately 1 g of quince peel sample was weighed and mixed with 1 mL of melezitose as internal standard (25 mg/mL) and 40 mL of 80% ethanol. The extraction was performed in a water bath at 80 ºC with shaking for 90 min. The mixture was then centrifuged (K24OR refrigerated centrifuge, Centurion, West Sussex, United Kingdom) for 10 min at 3500 rpm and filtered through Whatman paper no. 4. After concentration of the filtrate under reduced pressure (Büchi R-210 rotary evaporator, Flawil, Switzerland) in a round-bottom flask to eliminate the ethanol, the aqueous fraction was then subjected to a washing step performed three times with 10 mL of ethyl ether to remove the remaining lipids (**Figure 10**). The sample was then concentrated at 40 ºC to remove the residual ethyl ether, redissolved in distilled water to a final volume of 5 mL, and filtered through a 0.2 μm syringe filter into a vial for further injection.

The chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system (Knauer, Smartline 1000 system, Berlin, Germany) coupled to a refractive index (RI) detector (Knauer Smartline 2300) as previously described by the Barros, Pereira, Calhelha, Dueñas, et al. (2013). The separation was made on a 100-5 NH2 Eurospher column (4.6 × 250 mm, 5 μm, Knauer) using

acetonitrile/deionized water, 70:30 (*v/v*) as mobile phase, at a flow rate of 1 mL/min in isocratic mode. The identification of free sugars was made by comparing the relative retention times of the sample peaks with those of the standards and the quantification was performed by internal normalization of the peak chromatographic area, using the melezitose peak as a standard. The results were expressed in g per 100 g of dw and also fw by conversion.

Figure 10. Delipidification step performed with ethyl ether for sugars analysis.

4.3.4. Organic acids

Approximately 1 g of quince peel sample was weighed into a glass beaker protected from light (wrapped in aluminium foil) and stirred with 25 mL of metaphosphoric acid (4.5%, *v/v*) for 20 min at room temperature. The mixture was then filtered, first through Whatman paper no. 4 and then through a 0.2 μm nylon syringe filter into an amber glass vial for further injection.

The chromatographic analysis was performed by ultra-fast liquid chromatography coupled to photodiode array detection (UFLC-PDA; Shimadzu Coperation, Kyoto, Japan) as previously described by Barros, Pereira, Calhelha, Dueñas, et al. (2013). The separation was made on a C18 reverse phase column (250 mm × 4.6 mm, 5 μm, Phenomenex), thermostated at 35 ºC. The PDA detector was programmed for the preferred wavelengths of 215 nm and 245 nm (for ascorbic acid). Elution was made in isocratic mode with sulphuric acid (3.6 mM). The identification of organic acids was achieved by comparing the retention times and UV-Vis spectra with those of commercial standards. Quantification was performed using seven-level calibration curves constructed with the commercial standards of oxalic ($y = 1\times10^{7}x + 231891$; $R^2 = 0.9999$; LOD = 6.3 μg/mL; LOQ = 20.8 μg/mL), quinic ($y = 671557x + 14583$; $R^2 = 0.9998$; LOD = 11.3

µg/mL; LOQ = 37.6 µg/mL), and malic (y = 950041*x* + 6255.6; R^2 = 0.9999; 15.9 µg/mL; LOQ = 52.9 µg/mL) acids. The results were expressed in g per 100 g of dw and also fw by conversion.

4.3.5. Fatty acids

Fatty acids were analysed by gas chromatography with flame ionization detection (GC-FID). The crude fat obtained after Soxhlet extraction was methylated with 5 mL of methanol/sulphuric acid 95%/toluene 2:1:1 (*v/v/v*) for 12 h in a water bath at 50 ºC with shaking (160 rpm). Then, 3 mL of deionised water were added to obtain phase separation; the fatty acids methyl esters (FAME) were mixed with 3 mL of diethyl ether in a vortex and the upper phase was recovered and dehydrated with sodium sulphate anhydrous. The sample was then filtered through a 0.2 µm nylon syringe filter into a glass vial.

The fatty acid profile was characterized in a DANI model Gas Chromatography 1000 instrument. Separation was made on a Zebron–Kame column (30 m × 0.25 mm; 0.20 µm; Phenomenex, Lisbon, Portugal) operating under the following oven temperature program: initial temperature of 100 ºC, held for 2 min, increase at 10 ºC/min to 140 ºC, followed by a 3 ºC/min ramp to 190 ºC, a 30 °C/min ramp to 260 °C and held for 2 min. Hydrogen was used as carrier gas at the flow-rate of 1.1 mL/min, measured at 100 ºC. The injection split ratio was 1:50, with injector and detector temperatures being set at 250 ºC and 260 ºC, respectively. The identification of fatty acid was achieved by comparing the relative retention times of the sample FAME peaks those of the standard, using the Clarity DataApex 4.0 Software (Prague, Czech Republic). The results were expressed as the relative percentage of each fatty acid. The conversion factor of 0.8 stablished by Greenfield and Southgate (2003) for fruits and vegetables was used for calculation and expression of the fatty acids content in 100 g of quince peel (dw and fw by conversion), considering the fat weight obtained by the Soxhlet extraction.

4.4. Preparation of quince peel extracts

Hydroethanolic and aqueous extracts were prepared by maceration of quince peel. For hydroethanolic extraction, 1 g of sample was mixed with 30 mL of ethanol/water (80:20, *v/v*) and stirred at ~150 rpm for 1 h at room temperature. The mixture was filtered and the residue was re-extracted under the same conditions with 30 mL more of solvent. The combined extracts were concentrated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and the aqueous phase was lyophilized. For aqueous

extraction, 1 g of sample 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 fibre content by the enzymatic-gravimetric method referred above.

4.5. Characterization of phenolic compounds

For analysis of phenolic compounds, the quince peel extracts (~10 mg) were re-dissolved in ethanol/water (20:80, *v/v*) to a final concentration of 10 mg/mL and filtered through 0.2 μm nylon syringe filters into glass vials for further injection.

The chromatographic analysis was performed in a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a quaternary pump, an automatic injector thermostated at 5 ºC, a degasser, and a column compartment with thermostat at 35 ºC (**Figure 6**). A methodology previously described by Bessada et al. (2016) was followed. The compounds detection was carried out with a diode array detector (DAD) at the wavelengths of 280 nm, 330 nm, and 370 nm. Chromatographic separation was made on a Waters Spherisorb S3 ODS-2 C18 reverse-phase column (4.6 × 150 mm, 3 μm; Milford, USA) using (A) formic acid/water (0.1%) and (B) acetonitrile as mobile phase. The elution gradient was isocratic: 10% to 15% B up to 5 min, 15-20% B up to 5 min, 20-25% B for 10 min, 25-35% B for 10 min, 35-50% B for 10 min, and rebalancing the column for 10 min, at a flow rate of 0.5 mL/min.

The HPLC system was connected to an Ion Trap Linear LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. The carrier gas was nitrogen (50 psi). The system worked with a spray voltage of 5 kV, at an initial temperature of 325 ºC and capillary voltage of -20 V. The voltage of the tube lens offset was maintained at -66 V. The spectra were recorded in negative ion mode between 100 and 1500 *m/z*. The collision energy used was 35 (arbitrary units). Data were collected and processed using Xcalibur (Thermo Finnigan, San Jose, CA, USA).

For the identification of phenolic compounds, the data obtained (retention times and UV-Vis spectra and mass spectra) were compared with data available in the literature and, when available, with commercial standards. For quantitative analysis, a calibration curve was constructed ($R^2 \geq 0.999$) for the available phenolic standards, namely chlorogenic acid (y = 168823x – 161172, LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL), *p*-coumaric acid (y = 301950x + 6966.7, LOD=0.68 μg/mL and LOQ=1.61 μg/mL), catechin (y = 84950x – 23200, LOD = 0.17 μg/mL; LOQ = 0.68 μg/mL), and quercetin-3-*O*-glucoside (y =

34843x – 160173, LOD = 0.21 µg/mL; LOQ = 0.71 µg/mL). The results were expressed as mg per g of extract, thus same compounds were expressed in mg of equivalents of its basic constituent or similar compound.

4.6. Evaluation of bioactive properties

4.6.1. Antioxidant activity

Two cell-based *in vitro* assays were performed to evaluate the antioxidant activity of the quince peel extracts through oxidative haemolysis and thiobarbituric acid reactive substances (TBARS) formation inhibition using sheep erythrocytes and porcine brain cells as oxidizable substrates, respectively.

4.6.1.1. Oxidative haemolysis inhibition assay (OxHLIA)

Sheep blood samples were collected from healthy animals and centrifuged at 1000*g* for 5 min at 10 ºC. Plasma and buffy coats were discarded and the erythrocytes were first washed once with NaCl (150 mM) and three times with phosphate-buffered saline (PBS, pH 7.4) (Evans et al., 2013). The erythrocyte pellet was resuspended in PBS at 2.8% (*v/v*). Using a flat bottom 48-well microplate, 200 µL of erythrocyte solution was mixed with 400 µL of either: i) PBS solution (control); ii) extract solutions (0.125–4 mg/mL in PBS); iii) water for complete haemolysis; and iv) the positive control trolox (3.91–125 µg/mL). After pre-incubation at 37 ºC for 10 min with shaking, 200 µL of AAPH (160 mM in PBS) was added and the optical density was measured at 690 nm. The microplate was incubated again under the same conditions and the optical density was measured every 10 min at the same wavelength until complete haemolysis (Lockowandt et al., 2019).

The percentage of the erythrocyte population that remained intact (*P*) was calculated as follows:

$$P\ (\%) = (S_t - CH_0 / S_0 - CH_0) \times 100 \qquad \text{Eq. (6)}$$

where S_t and S_0 correspond to the optical density of the sample at *t* and 0 min, respectively, and CH_0 is the optical density of the complete haemolysis at 0 min.

The results were expressed as delayed time of haemolysis (Δt), which was calculated as follows:

$$\Delta t\ (\text{min}) = Ht_{50}\ (\text{sample}) - Ht_{50}\ (\text{control}) \qquad \text{Eq. (7)}$$

where Ht_{50} is the 50% haemolytic time (min) graphically obtained from the haemolysis curve of each antioxidant sample concentration.

The Δt values were then correlated to the extract concentrations and, from the correlation obtained for each extract, the inhibition concentration (IC_{50} value, µg/mL) able to promote a Δt haemolysis delay of 60 min, 120 min, and 180 min were calculated.

4.6.1.2. Thiobarbituric acid reactive substances (TBARS) formation inhibition capacity

A brain cell suspension was prepared by vigorously mixing pig (*Sus scrofa*) brain tissue with Tris-HCl buffer (20 mM, pH 7.4) at a ratio of 1:2 (*w/v*) and then centrifuging at 3500 rpm for 10 min at 10 ºC to collect the supernatant. In 2 mL eppendorfs, 200 µL of extract solution (0.04–24 mg/mL in PBS) or trolox (3.125–100 µg/mL, the positive control) was mixed with 100 µL of ascorbic acid (0.1 mM), 100 µL of iron sulphate (10 mM), and 100 µL of the brain cell solution. After incubation at 37.5 ºC for 1 h, the reaction was stopped by adding 500 µL of trichloroacetic acid (28%, *w/v*). Then, 380 µL of thiobarbituric acid (TBA; 2%, *w/v*) was added and the mixture was heated at 80 ºC for 20 min to promote formation of pink coloured complexes between TBA and malondialdehyde (MDA) that results from the lipid peroxidation of the polyunsaturated fatty acids of the brain cell membranes. The mixture was centrifuged at 3500 rpm for 5 min and the colour intensity of the MDA-TBA complex in the supernatant was measured at a wavelength of 532 nm (spectrophotometer AnalytikJena 200, Jena, Germany) (Lockowandt et al., 2019).

The percentage of lipid peroxidation inhibition was calculated using the equation:

$$Inhibition\ (\%) = \frac{\mathrm{A} - \mathrm{B}}{\mathrm{A}} \times 100 \qquad \text{Eq. (8)}$$

where A and B refer to the absorbance of the control and the extract solution, respectively.

The extract concentration (µg/mL) able to provide 50% lipid peroxidation inhibition (IC_{50} value) was calculated from the graph of percentage TBARS inhibition as a function of the extract concentration.

4.6.2. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the extract ability to inhibit the nitric oxide production by lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells (obtained from Leibniz Institute DSMZ – German Collection of Microorganisms

and Cell Cultures). Both quince peel extracts were dissolved in water at a concentration of 10 mg/mL and successively diluted to 0.16 mg/mL. The nitrite concentration in the cell culture medium was determined using a Griess Reagent System kit (Promega Corporation, Madison, WI, USA), by measuring the absorbance at 540 nm (microplate reader ELX800 Biotek, Bio-Tek Instruments, Inc., Winooski, VT, EUA) (Barros et al., 2013). The corticosteroid dexamethasone (50 mM) was used as a positive control. The percentage of nitric oxide production was calculated for each extract concentration, considering the negative control (samples without LPS), and the results were expressed as the concentration (μg/mL) providing 50% of nitric oxide production inhibition (EC_{50}).

4.6.3. 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 Mycological laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Serbia.

Minimum inhibitory concentrations (MIC), defined as the lowest extract concentration (mg/mL) that inhibits the visible microbial growth (at the binocular microscope), were determined by the serial microdilution method and the rapid *p*-iodonitrotetrazolium violet (INT) colorimetric assay as previously described (Soković et al., 2010; Soković & van Griensven, 2006). Minimal bactericidal (MBC) and fungicidal (MFC) concentrations were determined by measuring the lowest concentration that yielded no growth; thus, MBC and MFC were the lowest concentrations (mg/mL) required to kill the original inoculum. The food additives sodium benzoate (E211) and potassium metabisulfite (E224), the antibiotics streptomycin and ampicillin, and the azole antifungals ketoconazole and bifonazole were used as positive controls, while 30% ethanol was the negative control.

4.6.4. Cytotoxic activity against tumour and non-tumour cells

The extract capacity to inhibit the cell growth was screened against human tumour cell lines, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), AGS (gastric adenocarcinoma), and CaCo-2 (colorectal adenocarcinoma) cells, and non-tumour cell lines, namely normal African Green Monkey kidney epithelial Vero cells and porcine liver primary PLP2 cells. The sulforhodamine B (SRB) assay was followed as previously described (Vaz et al., 2010). Briefly, exponentially growing cells were seeded in 96-well plates and exposed to different extract concentrations. After 24 h of exposure, cells were fixed by adding 25 mL of cold 50% (*w/v*) trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. Plates were then washed with deionized water and dried, followed by addition of 50 mL of SRB solution (0.1%, *w/v*, in 1% acetic acid) and the plates were incubated for 30 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. The extract concentration (μg/mL) causing 50% of cell growth inhibition **(**GI_{50}**)** was calculated and used to express the results.

4.7. Statistical analysis

Three independent samples were used and analysed in triplicate. The results were expressed as mean ± standard deviation (except for antimicrobial activity). A two-tailed paired Student's t-test was applied for assessing differences between two samples. For antioxidant activity, differences among samples were assessed using one-way analysis of variance (ANOVA). The fulfilment of the ANOVA requirements was tested by means of the Shapiro Wilk's and the Levene's tests. The dependent variables were compared using Tukey's HSD or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified (p-value > 0.05) or not (p-value < 0.05), respectively. A Pearson's correlation was also run to assess correlations between phenolic compounds and antioxidant activity. All statistical tests were performed at a 5% significance level in SPSS Statistics (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

5. RESULTS AND DISCUSSION

5.1. 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.

5.1.1. Proximate composition and energy value

The proximate composition of quince peel is presented in **Table 3**. The moisture, fat, protein, ash, dietary fibre, and carbohydrate contents are presented. 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) (INSA, 2018; Rasheed et al., 2018; USDA, 2019). The dry quince peel powder contained mostly carbohydrates (76 g/100 g), followed by dietary fibre (20.2 g/100 g). Ash (total minerals), protein, and fat contents were detected in smaller amounts. Although there are no previous reports on the proximate 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) (Hegedűs et al., 2013; Rasheed et al., 2018; USDA, 2019), and dietary fibre (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 (Hegedűs et al., 2013; INSA, 2018; Rasheed et al., 2018; USDA, 2019) encompassing the result of the peel sample obtained in this study.

Table 3. Proximate composition and energy value of quince peel.

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

As shown in **Table 3**, the quince peel has a low energy value and carbohydrates are by far the main contributors (85.6%), followed by dietary fibre (11.4%) and finally protein and fat with a similar contribution (1.5%). Steel, according to the INSA (2018) and USDA (2019) Nutrient Databases, the energy value of the pulp (53 and 57 kcal/100 g fw) is lower than that of the peel (90 kcal/100 g fw), probably due to the higher moisture content.

5.1.2. Composition in mineral elements

The quince peel composition in macrominerals and trace elements is presented in **Table 4**. Potassium (K) was the most abundant mineral element detected in the studied sample. It is the main intracellular cation in the human body and required for the normal cellular function (Otten et al., 2006). 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 in the maintenance of 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 (Otten et al., 2006). P is also an essential elements required for a wide range of processes, including bone mineralization and ATP synthesis (Serna & Bergwitz, 2020).

Table 4. Mineral elements composition of quince peel.

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

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 fw) (Rop et

al., 2011; Sharma & Joshi, 2011). With the exception of Ca, the levels of macrominerals detected in quince peel are among those described in the literature for the fruit pulp.

Among the analysed trace elements, iron (Fe) represented a considerable fraction (**Table 4**). This element is a component of several proteins, such as haemoglobin, which is present in circulating erythrocytes and is involved in the oxygen transport (Otten et al., 2006). 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 (Otten et al., 2006). Among trace elements, manganese (Mn) was the one detected in lowest concentrations. The dietary intake of this element is essential for the formation of bone and for specific reactions related to amino acid, cholesterol, and carbohydrate metabolism (Otten et al., 2006). 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 (USDA, 2019).

5.1.3. Composition in free sugars and organic acids

The free sugars profile of quince peel is shown in **Figure 11** and the quantified contents are presented in **Table 5**.

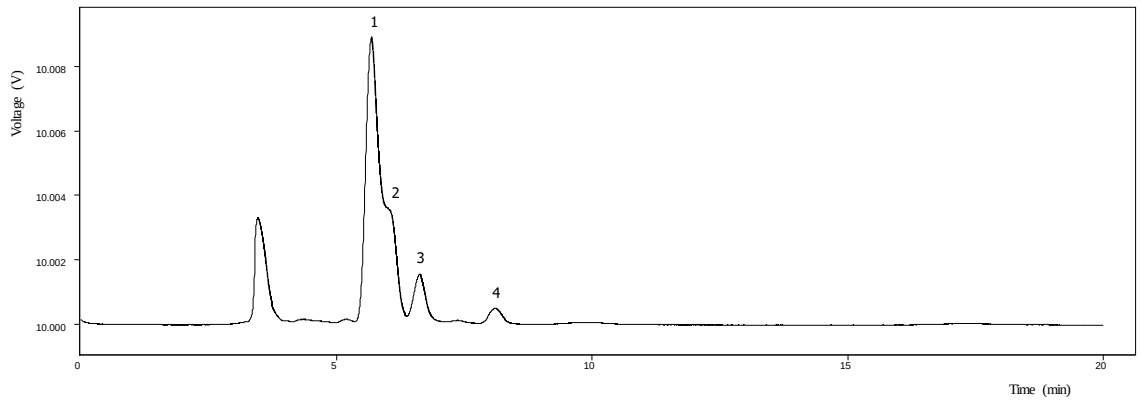


Figure 11. HPLC profile of free sugars detected in quince peel. 1- fructose; 2- glucose; 3- sucrose; 4- melezitose (internal standard).

Three free sugars were detected and fructose corresponded to about 74% of the total sugars detected, followed by glucose and the disaccharide sucrose (**Table 5**). To the best of the author' knowledge, this is the first report describing the free sugar composition of quince peel. Despite this, quince pulp is well characterized for its composition in sugars and other carbohydrates. Szychowski et al. (2014) detected fructose (0.42–0.58 g/100 g fw) as the major sugar in the pulp of Spanish quince, flowed 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, totalizing 0.57–0.79 g/100 g fw of these water-soluble compounds. Fructose (5.31–10.89%) and glucose (4.08–5.61%), and lower amounts of sucrose (1.51–241%) and maltose (0.31–0.42%), were also reported in quince juice (Rodríguez-Guisado et al., 2009). These results show that the sugars that predominate in the pulp also predominate in the peel of this fruit, despite notable quantitative differences.

Table 5. Free sugars composition of quince peel.

	Content per 100 g	
	Fresh weight	**Dry weight**
Fructose (g)	8.6±0.5	34±2
Glucose (g)	2.11±0.09	8.3±0.4
Sucrose (g)	1.02±0.03	4.0±0.1
Total sugars (g)	11.8±0.6	46±2

The quince peel composition in organic acids is shown in **Table 6**. Oxalic, quinic, and malic acids (**Figure 5**) 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. (2009), 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. (2002), 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.

Table 6. Organic acids composition of quince peel.

	Content per 100 g	
	Fresh weight	**Dry weight**
Oxalic acid (g)	0.35±0.01	1.38±0.05
Quinic acid (g)	1.00±0.02	3.95±0.09
Malic acid (g)	1.82±0.05	7.2±0.2
Total organic acids (g)	3.2±0.06	12.5±0.2

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 sourness and astringency, 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.

5.1.4. Composition in fatty acids

The 21 fatty acids detected in the quince peel are listed in **Table 7** and the GC-FID profile is shown in **Figure 13**. Palmitic (C16:0), stearic (C18:0), and oleic (C18:1*n*-9) acids were the major fatty acids detected (**Figure 12**), corresponding to 156 mg, 49.1 mg, and 48.1 mg per 100 g portion of dry quince peel, respectively. With lower contents came myristic (C14:0), arachidonic (C20:4*n*-6), and linoleic (C18:2*n*-6) acids. Saturated fatty acids (SFA) corresponded to 68% of the total fatty acids detected (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. 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 7**).

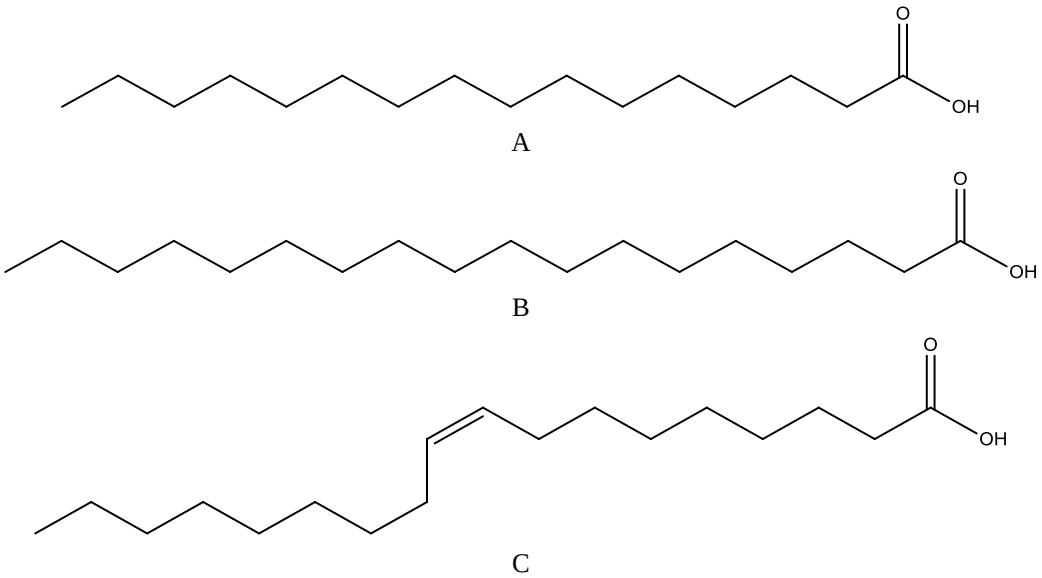


Figure 12. Chemical structures of (A) palmitic acid (C16:0), (B) stearic acid (C18:0), and (C) oleic acid (C18:1*n*-9).

Table 7. Fatty acids composition of quince peel. The relative percentage and content of each fatty acid and the contents of saturated and mono- and polyunsaturated fatty acids are presented.

	Relative percentage (%)	Content (mg/100 g)*	
		Fresh weight	Dry weight
C8:0	1.24±0.02	1.44±0.02	5.67±0.09
C10:0	2.14±0.03	2.50±0.04	9.8±0.1
C11:0	0.72±0.01	0.84±0.01	3.31±0.05
C12:0	3.15±0.05	3.67±0.06	14.5±0.2
C13:0	0.299±0.06	0.348±0.007	1.37±0.03
C14:0	8.4±0.2	9.8±0.2	38.7±0.8
C15:0	1.24±0.2	1.44±0.03	5.7±0.1
C16:0	34.0±0.8	40±1	156±4
C16:1	0.71±0.01	0.82±0.01	3.25±0.05
C17:0	1.28±0.02	1.49±0.02	5.87±0.07
C18:0	10.7±0.1	12.5±0.2	49.1±0.7
C18:1*n*-9	10.5±0.1	12.2±0.2	48.1±0.6
C18:2*n*-6	5.55±0.08	6.5±0.1	25.5±0.4
C18:3*n*-6	0.274±0.004	0.319±0.005	1.26±0.02
C18:3*n*-3	1.44±0.01	1.68±0.02	6.63±0.07
C20:0	0.96±0.01	1.12±0.01	4.41±0.05
C20:4*n*-6	6.92±0.07	8.07±0.089	31.8±0.3
C20:3*n*-3	3.27±0.04	3.81±0.04	15.0±0.2
C22:1	2.31±0.03	2.69±0.04	10.6±0.1
C24:0	3.89±0.04	4.53±0.05	17.9±0.2
C24:1	1.04±0.02	1.21±0.02	4.8±0.1
SFA	68.0±0.7	79.3±0.9	312±4
MUFA	14.5±0.1	16.9±0.2	66.7±0.7
PUFA	17.5±0.2	20.3±0.2	80.2±0.8
PUFA/SFA		0.257±0.001	
PUFA *n*-3/*n*-6		0.370±0.003	

Caprylic acid (C8:0), capric acid (C10:0), undecylic acid (C11:0), lauric acid (C12:0), tridecylic acid (C13:0), myristic acid (C14:0), pentadecylic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1*n*-9), linoleic acid (C18:2*n*-6), α-linolenic acid (C18:3*n*-3), arachidic acid (C20:0), arachidonic acid (C20:4*n*-6), *cis*-11,14,17-eicosatrienoic acid (C20:3*n*-3), erucic acid (C22:1), lignoceric acid (C24:0), nervonic acid (C24:1). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
*Contents determined based on the conversion factor (0.8) stablished by Greenfield and Southgate (2003).

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 over 0.45 and lower than 4.0, respectively (Mapiye et al., 2011). As presented in **Table 7**, 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 C18:1*n*-9 and C18:2*n*-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 (USDA, 2019). The two predominant in the pulp are also the most abundant in the lipid fraction of the seed, corresponding to 90% of the total fatty acids (Szychowski et al., 2014). Thus, PUFA (53–56%) and MUFA (34–37%) predominate over SFA (9–10%), being a healthier fatty acid profile than that characterized for quince peel.

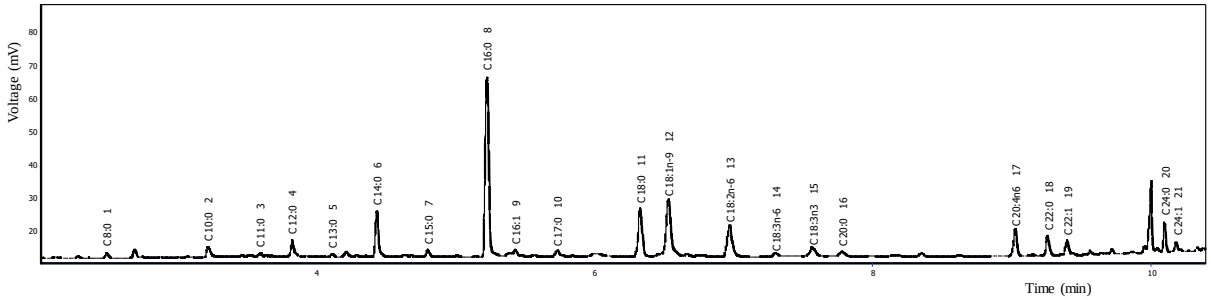


Figure 13. Profile of fatty acids in quince peel. 1: Caprylic acid (C8:0), 2: capric acid (C10:0), undecylic acid (C11:0), lauric acid (C12:0), tridecylic acid (C13:0), myristic acid (C14:0), pentadecylic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1*n*-9), linoleic acid (C18:2*n*-6), α-linolenic acid (C18:3*n*-3), arachidic acid (C20:0), arachidonic acid (C20:4*n*-6), *cis*-11,14,17-eicosatrienoic acid (C20:3*n*-3), erucic acid (C22:1), lignoceric acid (C24:0), nervonic acid (C24:1).

5.2. Extraction yield and fibre content of the extraction residues

The quince peel powder was subjected to solid-liquid extractions by dynamic maceration using ethanol/water (80:20, *v/v*) and aqueous heat extraction, which yielded 53±2% (*w/w*) and 54±1% (*w/w*) of extracted solids, respectively, values that did not differ statistically (p-value > 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 fibre 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-value = 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 contributed to better bioresource-use efficiency and circularity

through the supply of both phenolic extracts and novel fibre-rich natural ingredients that can be used in food fortification.

Similar methodological approaches were previously followed with date seeds. Al-Farsi and Lee (2008) measured 57.9 g/100 g of total dietary fibre in the seeds, and 83.5 and 82.2 g/100 g in the residues from water and acetone extractions, respectively. The higher fibre content in the aqueous residue was justified by the better efficiency of this solvent for the recovery of phenolic compounds and other constituents, such as proteins and soluble sugars, which agrees with the results of the present study. Later, Afifi et al. (Afifi et al., 2017) obtained a solid residue containing 79.4% crude fibre, resulting from a 3 h extraction with 25% ethanol at 55 °C; despite this, the yield of the residue was low, thus having a greater purity. These conditions also promote the recovery of total phenolics and flavonoids.

5.3. Composition in phenolic compounds

Phenolic compounds are secondary metabolites widely found in fruits and vegetables, representing 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 (Cory et al., 2018; Santos-Buelga, 2021). **Figure 14** and **Table 8** show the HPLC phenolic profile of the quince peel extracts and the chromatographic data (retention time, wavelengths of maximum absorption in the visible region (λ_{max}), and deprotonated ion) 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 flavonoid glycosides (quercetin-*O*-deoxyhexoside-hexoside and kaempferol-*O*-deoxyhexoside-hexoside), which were previously described in this species (Karar et al., 2014; Szychowski et al., 2018; Wojdyło et al., 2014; Zapata et al., 2019). Therefore, the compounds' identity was assigned by comparing the chromatographic data in **Table 8** with those in the literature.

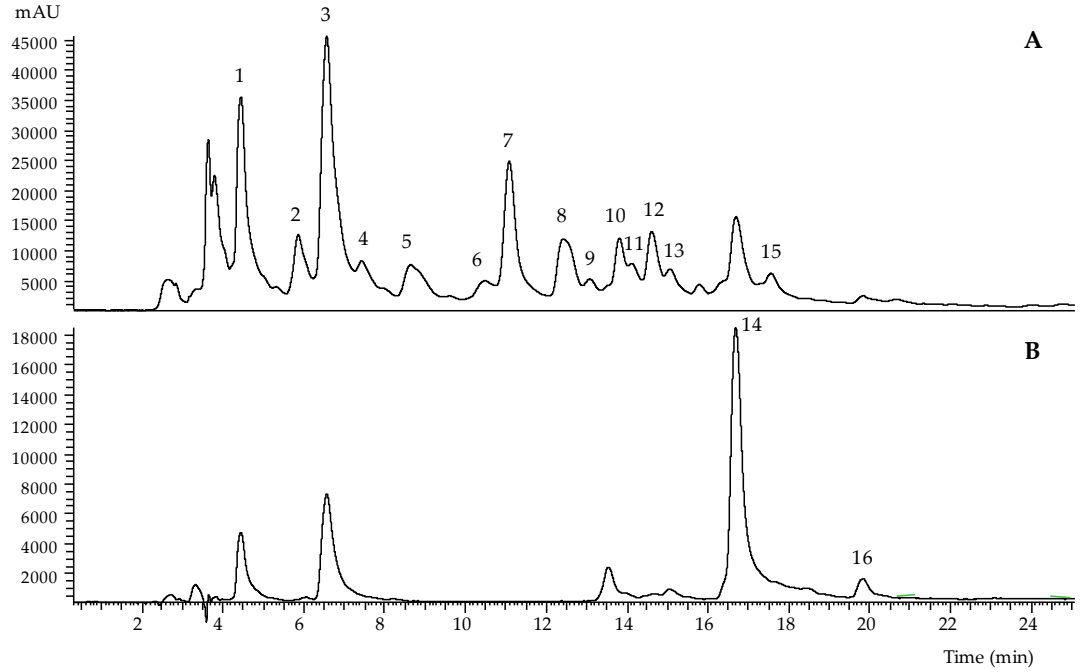


Figure 14. HPLC phenolic profile of the quince peel hydroethanolic extract recorded at (A) 280 nm and (B) 370 nm. Peak identification is shown in **Table 8**.

The results in **Table 8** show that the extraction method affected the quantitative phenolic profile. Flavan-3-ols were the major compounds in quince peel, corresponding to approximately 56.64% and 47.78% of the 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*-caffeoylquinic (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 (0.427–0.490 and 0.403–0.435 mg/g extract, respectively). These results demonstrated that the dynamic hydroethanolic maceration is preferable for obtaining higher amounts of flavan-3-ols, whereas the hot water extraction can be more indicated to recover phenolic acids and flavonols; the high temperature may have caused the degradation of the flavan-3-ols.

Table 8. Content of the phenolic compound identified in the quince peel extracts. The retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), and mass spectral data are presented.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	Tentative identification	Content (mg/g extract)		Statistics*
					Hydroethanolic	Aqueous	*p*-Value
1 [A]	4.43	325	353	3-*O*-Caffeoylquinic acid	0.372±0.004	0.399±0.008	0.02
2 [B]	5.85	292	337	3-*O*-*p*-Coumaroylquinic acid	0.087±0.004	0.082±0.002	0.045
3 [A]	6.56	326	353	*cis*-5-*O*-Caffeoylquinic acid	0.498±0.005	0.63±0.02	<0.001
4 [A]	7.43	323	353	*trans*-5-*O*-Caffeoylquinic acid	0.192±0.007	0.183±0.006	0.088
5 [C]	8.63	275	289	(+)-Catechin	0.218±0.003	0.182±0.002	<0.001
6 [B]	10.47	311	337	5-*O*-*p*-Coumaroylquinic acid	0.028±0.002	0.037±0.002	0.001
7 [C]	11.08	276	865	β-Type (epi)catechin trimer	0.76±0.02	0.557±0.006	<0.001
8 [C]	12.52	272	1153	β-Type (epi)catechin tetramer	0.330±0.008	0.226±0.007	<0.001
9 [C]	13.83	298	577	β-Type (epi)catechin dimer	0.3±0.02	0.233±0.002	0.001
10 [C]	14.16	280	1153	β-Type (epi)catechin tetramer	0.192±0.009	0.152±0.002	<0.001
11 [C]	14.57	271	865	β-Type (epi)catechin trimer	0.385±0.005	0.302±0.005	<0.001
12 [C]	15.10	275	865	β-Type (epi)catechin trimer	0.185±0.007	0.146±0.006	0.001
13 [C]	15.76	275	865	β-Type (epi)catechin trimer	0.128±0.002	0.126±0.004	0.235
14 [D]	16.70	355	609	Quercetin-*O*-deoxyhexoside-hexoside	0.427±0.001	0.490±0.002	<0.001
15 [C]	17.52	268	863	Procyanidin with A-type linkage	0.171±0.007	0.120±0.002	<0.001
16 [D]	19.86	357	593	Kaempferol-*O*-deoxyhexoside-hexoside	0.435±0.004	0.403±0.003	<0.001
				Total phenolic acids	1.18±0.02	1.33±0.03	<0.001
				Total flavan-3-ols	2.662±0.005	2.04±0.01	<0.001
				Total flavonols	0.862±0.003	0.893±0.005	<0.001
				Total phenolic compounds	4.70±0.02	4.27±0.03	<0.001

Standards used in quantification: [A] chlorogenic acid; [B] *p*-coumaric acid; [C] catechin; and [D] quercetin-3-*O*-glucoside. *Significant differences (*p*-value < 0.05) between samples were assessed by a Student's t-test.

Previous studies describe a similar phenolic composition for quince. Karar et al. (2014), Szychowski et al. (2018) and Wojdyło et al. (2014) 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, 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 colour change processes. Stojanović et al. (2017) described 5-*O*-caffeoylquinic 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.

5.4. Bioactive properties of quince peel extracts

To evaluate the bioactive properties of quince peel, hydroethanolic and aqueous extracts were prepared by dynamic maceration and hot water extraction, respectively.

5.4.1. Antioxidant activity

The antioxidant activity of the quince peel extracts was evaluated by the capacity to inhibit the formation of TBARS and to protect erythrocytes from oxidative haemolysis; the results are shown in **Figure 15**. The lower the IC_{50} values, the higher the antioxidant capacity. For the TBARS assay, the IC_{50} value correspond to the extract concentration providing 50% of lipid peroxidation inhibition; while, in OxHLIA, it corresponds to the concentration required to protect 50% of the erythrocyte population from the haemolytic action caused by the used oxidizing agent, AAPH, for a certain period of time.

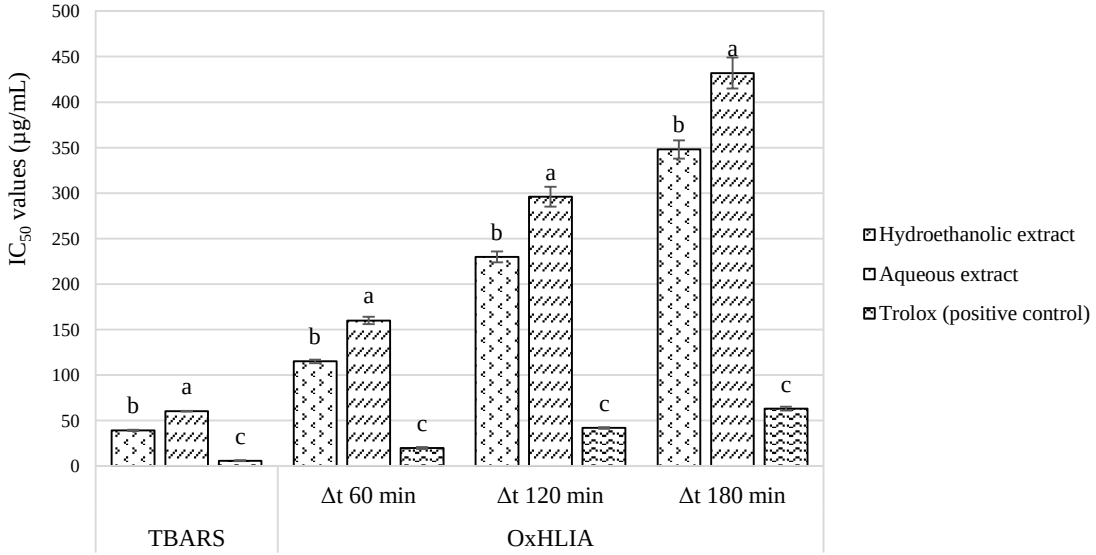


Figure 15. Antioxidant activity of the quince peel extracts evaluated by the TBARS formation inhibition and OxHLIA assays. For each antioxidant activity assay and Δ*t* in OxHLIA, different letter (a–c) indicate statistically significant differences (*p*-value < 0.001) between samples.

As presented in **Figure 15**, the hydroethanolic extract presented lower IC_{50} values than the aqueous extract in both *in vitro* assays, a result that can be justified by the higher content of flavan-3-ols, which were found strongly correlated with the antioxidant activity (**Table 9**). It should be noted that trolox, which had higher activity (lower IC_{50} values), is a pure antioxidant compound, while plant extracts are complex mixtures of different phytoconstituents, some of which do not exhibit bioactivity. The TBARS assay provides information on the extracts ability to inhibit the formation of highly reactive compounds,

such as malondialdehyde (MDA), which is generated from the decomposition of lipid peroxidation products. The MDA monitoring was achieved by adding thiobarbituric acid (TBA) to the reaction mixture, heating at 80 °C for 20 min, and subsequently measuring the absorbance of the formed pink coloured MDA-TBA complex.

For OxHLIA, the Δt values (min) resulting from the half haemolysis time (Ht_{50} values) obtained from the haemolytic curves (**Figure 16A**) of each extract concentration minus the Ht_{50} value of the PBS control were correlated to the respective extract concentrations to obtained the IC_{50} values (µg/mL), which were calculated for time periods of 60 min, 120 min, and 180 min. As mentioned above, the hydroethanolic extract was more effective in protecting the erythrocytes, which is also translated by the higher slope value obtained for the correlation between Δt values and extract concentrations shown in **Figure 16B**. In this assays, the erythrocytes were subjected to the haemolytic action of hydrophilic and lipophilic radicals (Zou et al., 2001). The hydrophilic radicals resulted from the thermal decomposition of AAPH, which is a peroxyl radical initiator that attacks the erythrocyte membranes and eventually causes haemolysis. In turn, the lipophilic radicals were generated as a consequence of this initial attack a through lipid peroxidation phenomenon.

The antioxidant activity of quince peel extracts has been evaluated by different methodologies. Magalhães et al. (2009) observed that quince peel and pulp extracts protect the erythrocyte membrane from haemolysis induced by AAPH in a concentration- and time-dependent manner, indicating that endogenous antioxidants can efficiently quench free radicals to protect the membranes against haemolysis. The antioxidant activity of quince peel methanolic extracts was found correlated with total phenolics and manly with caffeoylquinic acids. The phenolic compounds were thus reported to be able to quench the chain propagating peroxyl radicals, thus inhibiting haemolysis.

Alesiani et al. (2010) tested the radical-scavenging activity against DPPH and anion superoxide radicals and 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 9**).

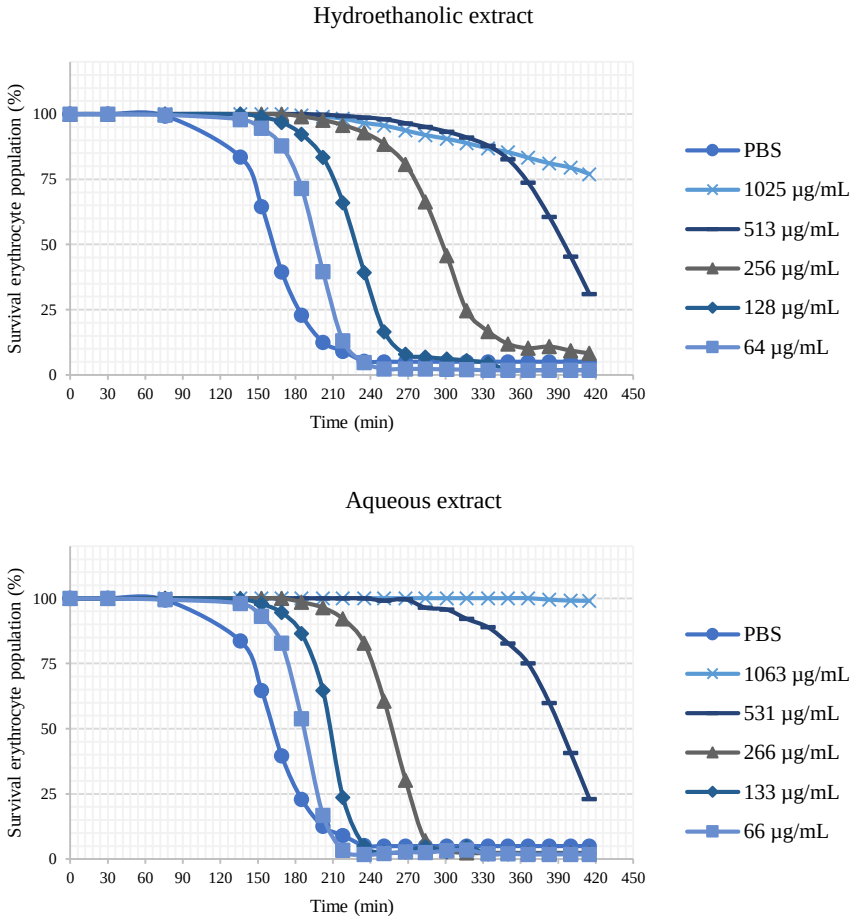
Table 9. Pearson's correlation coefficients (*R*) of phenolic compounds and antioxidant activity in quince peel extracts.

Phenolic compounds		**TBARS assay**	**OxHLIA assay**		
			Δ*t* 60 min	**Δ*t* 120 min**	**Δ*t* 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*-deoxyhexoside-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*-deoxyhexoside-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 flavonoids	*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

*Significant at $p \leq 0.05$. **Significant at $p \leq 0.01$.

≥ 0.9 Very strong correlation 0.7–0.9 Strong correlation 0.5–0.7 Moderate correlation

A: Hemolytic curves obtained with increasing extract concentrations*



B: Correlation between Δ*t* values and extract concentrations*

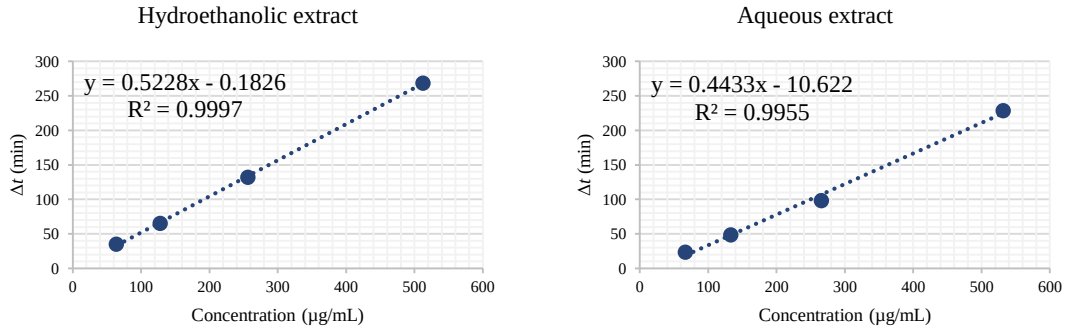


Figure 16. Kinetic curves of the survival erythrocyte population obtained for the quince peel extracts at different concentrations. The correlations between Δ*t* values and extract concentrations used to calculate the IC_{50} values (µg/mL) able to promote Δ*t* haemolysis delays of 60 min, 120 min, and 180 min are presented for each extract. *The well dilution factor was considered.

In another study, Szychowski et al. (2014) measured the total antioxidant activity of hydrophilic and lipophilic fractions obtained from quince peel and pulp using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the peroxidase enzyme, and its oxidant

substrate (hydrogen peroxide), in which ABTS•$^{+}$ radicals are generated and monitored at 730 nm. The peel antioxidant activity was higher than that of the pulp. Additionally, the activity of the hydrophilic fractions was higher in both pulp and peel in two quince clones, while the activity of the lipophilic faction was higher in four of the studied quince clones. Fattouch et al. (2007) and Silva et al. (2005) 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 hydrophilic chemical constituents contributing to the antioxidant activity of the pulp, these compounds were found weakly correlated to this activity in the quince peel (Szychowski et al., 2014).

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

5.4.2. Antimicrobial activity

The antimicrobial activity of the quince peel extracts was tested against pathogenic and foodborne bacterial and fungal strains and the results are presented in **Table 10** and **Table 11**, respectively. As positive controls, two food additives (sodium benzoate (E211) and potassium metabisulfite (E224)) and two antibiotics (streptomycin and ampicillin) were used. As shown in **Table 10**, 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 of the many common staphylococcal bacteria, and *Enterobacter cloacae* (lower MBC). It is also interesting to note that both quince peel extracts were more effective 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.

Table 10. Antibacterial activity of the quince peel extracts and positive controls (sodium benzoate (E211), potassium metabisulfite (E224), streptomycin, ampicillin, ketoconazole, and bifonazole). The results are presented as minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (mg/mL).

	Quince peel extracts				**Positive controls**							
	Hydroethanolic		**Aqueous**		**E211**		**E224**		**Streptomycin**		**Ampicillin**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus cereus	1.50	3.00	1.50	3.00	0.50	0.50	2.00	4.00	0.10	0.20	0.25	0.40
Staphylococcus aureus	1.50	3.00	2.00	3.00	4.00	4.00	1.00	1.00	0.04	0.10	0.25	0.45
Listeria monocytogenes	1.50	6.00	1.50	6.00	1.00	2.00	0.50	1.00	0.20	0.30	0.40	0.50
Escherichia coli	1.50	3.00	1.50	3.00	1.00	2.00	0.50	1.00	0.20	0.30	0.40	0.50
Enterobacter cloacae	1.50	3.00	1.50	6.00	2.00	4.00	0.50	0.50	0.20	0.30	0.25	0.50
Salmonella typhimurium	1.50	3.00	3.00	6.00	1.00	2.00	1.00	1.00	0.20	0.30	0.75	1.20

Table 11. Antifungal activity of the quince peel extracts and positive controls (sodium benzoate (E211), potassium metabisulfite (E224), streptomycin, ampicillin, ketoconazole, and bifonazole). The results are presented as minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (mg/mL).

	Quince peel extracts				**Positive controls**							
	Hydroethanolic		**Aqueous**		**E211**		**E224**		**Ketoconazole**		**Bifonazole**	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus fumigatus	0.75	1.50	0.75	1.50	1.00	2.00	1.00	1.00	0.25	0.50	0.15	0.20
Aspergillus ochraceus	0.75	1.50	0.75	1.50	1.00	2.00	1.00	1.00	0.20	0.50	0.10	0.20
Aspergillus niger	1.50	3.00	1.50	3.00	1.00	2.00	1.00	1.00	0.20	0.50	0.15	0.20
Trichoderma viride	0.50	0.75	0.50	0.75	1.00	2.00	0.50	0.50	2.50	3.50	0.20	0.25
Penicillium funiculosum	0.75	1.50	1.50	3.00	1.00	2.00	0.50	0.50	0.20	0.50	0.20	0.25
Penicillium verrucosum var. *cyclopium*	0.75	1.50	1.50	3.00	1.00	2.00	0.50	0.50	0.20	0.30	0.10	0.20

Regarding the antifungal activity results (**Table 11**), 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) (Taniwaki et al., 2018), 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. (2007) 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 microbial infections, in addition to antibiotics, given the apparent trend toward the appearance of 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.

Stojkovic et al. (2018) 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 *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli*), 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.

5.4.3. Anti-inflammatory and cytotoxic activities

In this study, the anti-inflammatory activity of the quince peel extracts was assessed through its capacity to inhibit the production of nitric oxide by lipopolysaccharide (LPS)-

stimulated RAW 264.7 macrophage cells. As presented in **Table 12**, 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. (2012) showed that aqueous acetone extracts can induced 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 (NF-κB), p38MAPK and Akt. This difference in bioactivity may be due to the different origin of the plant material, the extraction method, and the different *in vitro* assay.

Table 12. Anti-inflammatory activity of the quince peel extracts assessed trough NO production inhibition capacity. The results are presented as EC_{50} values (μg/mL).

	Quince peel extracts		**Positive control**
	Hydroethanolic	**Aqueous**	**Dexamethasone**
RAW 246.7 cells	>400	>400	6.3±0.4

The cytotoxicity of the quince peel extracts against tumour (breast carcinoma – MCF-7, lung carcinoma – NCI-H460, gastric adenocarcinoma – AGS, and colorectal adenocarcinoma – CaCo-2 cells) and non-tumour (normal African green monkey kidney epithelial cells – Vero) cell lines are shown in **Table 13**. As observed for the anti-inflammatory activity, the extracts also did not show cytotoxicity to the tested cell lines (up to 400 μg/mL). These results are in agreement with those of Essafi-Benkhadir et al. (2012), who did not observe toxicity for THP-1-derived macrophages of quince peel aqueous acetone extract at concentrations ranging from 20 to 1000 μg/mL. On the other hand, Alesiani et al. (2010) recorded a strong cytotoxic effect on murine B16-F1 melanoma cells for the triterpenoid annurcoic acid and the polyphenolic compounds quercetin and rutin.

Despite the ineffectiveness of our quince peel extracts in inhibiting the growth of the tested tumour cells, the non-tumour cell line was also not affected; and this result is somewhat promising since it is necessary to guarantee consumer safety when developing new ingredients and food products from plant by-products such as quince peel.

Table 13. Cytotoxic activity of the quince peel extracts to tumour and non-tumour cell lines. The results are presented as GI_{50} values (μg/mL).

	Quince peel extracts		Positive control
	Hydroethanolic	**Aqueous**	**Ellipticine**
Tumour cell lines			
MCF-7 cells	>400	>400	1.02±0.02
NCI-H460 cells	>400	>400	1.01±0.01
AGS cells	>400	>400	1.23±0.03
CaCo-2 cells	>400	>400	1.21±0.02
Non-tumour cell line			
PLP2	>400	>400	3.2±0.7
Vero cells	>400	>400	1.41±0.06

6. CONCLUSIONS

6.1. Concluding remarks

This valorisation study allowed the identification of valuable functional compounds in quince peel, among which fibre, fructose, malic and quinic acids, and potassium, and some fatty acids stand out. On the other hand, while the solid residues remaining from the extraction were characterized as interesting sources of dietary fibre, 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.

6.2. Future studies

Future studies will be of interest to optimize the extraction of target compounds, namely malic acid, fructose, phenolic compounds, and dietary fibre, using an experimental design coupled to response surface methodology. It will also be interesting to investigate the profile in volatile compounds and terpenoids of the quince peel, and the biological activity of phenolic fraction and isolated compounds. This future-oriented approach could promote the large-scale exploitation of quince peel and be extended to other agri-food by-products and industrial waste in order to identify valuable molecules and their potential application.

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