Chemical, nutritional and bioactive characterization of two varieties of pears with a view to their industrial enhancement

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List of abbreviations

A-549: Human lung carcinoma cell line

AAPH: 2,2'-azobis(2-methylpropionamidine) dihydrochloride

ABTS+: 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) cation

AGS: Gastric adenocarcinoma cell line

AOAC: Association of Official Analytical Chemists

ATCC: American type culture collection

CAA: Cellular antioxidant activity

CaCo: Colorectal adenocarcinoma cell line

CFU: Colony forming unit

DAD: Diode array detector

DCF: Fluorescent dichlorofluorescein

DCFH: 2',7'-dichlorohydrofluorescein

DMEM: Dulbecco's modified eagle medium

DMSO: Dimethyl sulfoxide

DPPH: 2-diphenyl-1-picrylhydrazyl

EC50: Extract concentration corresponding to 50% of antioxidant activity

ESI: Electrospray ionization

FAME: Fatty acid methyl ester

FAO: Food and Agriculture Organization

FBS: Fetal bovine serum

FID: Flame ionization detector

FRAP: Ferric reducing antioxidant power assay

GC: Gas chromatography

GC-FID: Gas chromatography with flame ionization detection

GI50: Sample concentration that inhibits 50% of cell growth

HBSS: Hank's saline solution

HCV-29T: Bladder cancer cell line

HPLC: High performance liquid chromatography

HPLC-RI: High performance liquid chromatography system coupled to a refractive index detector

IC50: Concentration of extract causing 50% inhibition of NO production

IDF: Insoluble dietary fiber

INT: Iodonitrotetrazolium choloride

LPS: Liposaccharide solution

MA: Malt agar

MBC: Minimum bactericidal concentration

MCF-7: Breast adenocarcinoma cell line

MDA: Malondialdehyde

MDA-TBA: Malondialdehyde- thiobarbituric acid complex

MFC: Minimum fungicidal concentration

MHB: Mueller-Hinton agar

MIC: Minimum inhibitory concentration

M-NFS-60: Mouse myelogenous leukemia cell

MS: Mass spectrometry

MUFA: Monounsaturated fatty acid

NCBI: National Center for Biotechnology Information

NCI-H460: Lung carcinoma cell line

NCTC: National collection of type cultures

NO: Nitric oxide

PUFA: Polyunsaturated fatty acids

RAW 264.7: Murine macrophage cell line

RI: Refractive index

SFA: Saturated fatty acids

SRB: Sulforodamine B

TBA: Thiobarbituric acid

TBARS: Thiobarbituric acid reactive substance assay

TCA: Trichloroacetic acid

TSB: Triptych soy broth

UFLC: Ultra-fast liquid chromatography

UFLC-DAD: Ultra-fast liquid chromatography coupled to a diode detector

VERO: African green monkey kidney cell line

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Abstract

Food waste and losses emerge as a currently worrying and urgent problem with significant environmental and economic impacts. The fruit and vegetable industry are considered the main producer of food waste. Seeds, pomace, peel and even the whole fruit when discarded by the producer, retailer, or consumer for various reasons, including non-compliance with commercial standards (size, color, shape, among others) are considered fruit bio-waste. However, this bio-waste is referred to as excellent sources of bioactive compounds of high interest, namely proteins, dietary fibers, vitamins, and phenolic compounds with antioxidant properties. In this context, many natural matrices have been highly explored by the scientific community as a source of nutrients with different properties related to human well-being and health.

Pyrus spp., commonly known as pear, belongs to the Rosacea family and is a popularly consumed and appreciated fruit throughout the world. The European pear (*Pyrus communis* L.) and the Asian pear (*Pyrus pyrifolia* (Burm.) Nak), are two species identified as the most commercialized in the world. In addition to being consumed fresh or processed, these species have been used since antiquity as a treatment for some diseases, due to their antitussive, anti-inflammatory, and diuretic activities.

However, the high consumption results in a very high amount of waste, representing negative consequences for the environment and causing great economic losses for the industry. Thus, the chemical, nutritional and bioactive characterization of this type of bio-waste is essential for the use of these raw materials and, consequently, their recovery.

In this sense, the present study aimed to carry out the nutritional and chemical characterization of two different varieties of pear bio-waste, *P. communis* and *P. pyrifolia*, supplied by a national company. Additionally, the bioactive potential of extracts obtained from different bio-waste was also studied. Moisture, ash, protein, fat, carbohydrate, and energy contents were determined following official AOAC analysis methodologies. For chemical characterization, quantification of free sugars was performed using an HPLC-RI system, organic acids by UFLC-DAD and fatty acids by GC-FID. Total phenols and total flavonoids were also determined according to colorimetric assay and different bioactivities (antioxidant, antimicrobial, anti-inflammatory, and cytotoxicity activities) were analyzed. through different *in vitro* assays.

The nutritional value revealed that carbohydrates were the predominant macroelement in the analyzed samples. Fructose was the major free sugar, malic acid was the major organic acid, while palmitic, oleic and linoleic acid were the predominant fatty acids. Moreover, the peels of the two pear varieties stand out as a promising source of bioactive compounds, showing a high antioxidant and antimicrobial results.

In short, this study aims to contribute to the valorization of these bio-waste as natural matrices on an industrial scale, by converting them into value-added products and at the same time contributing to the goal of zero waste.

Keywords: Bio-waste, Bioactive compounds, nutritional value, *Pyrus communis* L., *Pyrus pyrifolia* (Burm.) Nak.

Resumo

O desperdício e as perdas alimentares surgem como um problema atualmente preocupante e urgente com impactos ambientais e económicos significativos. A indústria hortofruticula é considerada a principal produtora de resíduos alimentares. Sementes, bagaço, casca e até mesmo o fruto inteiro quando descartado pelo produtor, retalhista ou consumidor por diversos motivos, incluindo a não conformidade com os padrões comerciais (tamanho, cor, forma, entre outros) são considerados bio-resíduos de frutos. No entanto, estes bio-resíduos são referenciados como excelentes fontes de compostos bioativos de elevado interesse, nomeadamente proteínas, fibras alimentares, vitaminas e compostos fenólicos com propriedades antioxidantes. Nesse contexto, muitas matrizes naturais têm vindo a ser altamente exploradas, pela comunidade científica, como fonte de nutrientes com diversas propriedades relacionadas com o bem-estar e a saúde humana.

Pyrus spp., comummente conhecida como pêra, pertence à família Rosacea e é uma fruta popularmente consumida e apreciada em todo o mundo. A pêra europeia (*Pyrus communis* L.) e a pêra asiática (*Pyrus pyrifolia* (Burm.) Nak), são duas espécies identificadas como as mais comercializadas no mundo. Além de serem consumidas *in natura* ou processadas, essas espécies são utilizadas desde a antiguidade como tratamento para algumas doenças, devido às suas atividades antitússica, anti-inflamatória e diurética.

No entanto, o elevado consumo resulta numa quantidade muito elevada de resíduos, representando consequências negativas para o meio ambiente e acarretando grandes perdas económicas para a indústria. Assim, a caracterização química, nutricional e bioativa deste tipo de bio-resíduos é essencial para o aproveitamento destas matérias-primas e, consequentemente, a sua valorização.

Neste sentido, o presente estudo pretendeu realizar a caracterização nutricional e química de duas variedades diferentes de bio-resíduos de pêra, *P. communis e P. pyrifolia*, fornecidos por uma empresa nacional. Adicionalmente, foi também estudado o potencial bioativo dos extratos obtidos a partir dos diferentes bio-residuos. Os teores de humidade, cinzas, proteínas, gorduras, hidratos de carbono e energia foram determinados seguindo as metodologias oficiais de análise AOAC. Para a caracterização química, a quantificação de açúcares livres foi realizada através de um sistema HPLC-RI, ácidos orgânicos por UFLC-DAD e ácidos gordos por GC-FID. Os fenóis e flavonoides totais foram, também, determinados de acordo com métodos colorimétricos e, as

diferentes bioatividades (atividades antioxidante, antimicrobiana, anti-inflamatória e citotoxicidade), foram analisadas através de diferentes ensaios *in vitro*.

O valor nutricional revelou que os hidratos de carbono foram o macroelemento predominante nas amostras analisadas. A frutose foi o principal açúcar livre, o ácido málico o principal ácido orgânico, enquanto os ácidos palmítico, oleico e linoleico foram os ácidos gordos predominantes. Além disso, as cascas de ambas as variedades de pêra destacam-se como uma fonte promissora de compostos bioativos, apresentando bons resultados nas atividades antioxidante e antimicrobiana.

Em suma, este estudo visa contribuir para a valorização destes bio-resíduos como matrizes naturais em escala industrial, pela sua conversão em produtos de valor agregado e ao mesmo tempo contribuir para a meta de desperdício zero.

Palavras-chave: Bio-resíduos, compostos bioativos, valor nutricional, *Pyrus communis* L., *Pyrus pyrifolia* (Burm.) Nak.

1. Introduction

1.1 Bio-waste in the agri-food industry

Agri-food industry residues, waste, and effluents are considerably produced on a large scale over the world (Agathos, 2011).

Food processing generates waste, and it has been estimated that one-third of the world's production, which represents 1.3 billion tons, is lost or wasted (FAO, 2011). In Europe, food industry processes lead to 100 Mt of food waste and by-products annually (Marić et al., 2018).

Bio-waste is defined in the landfill directive as biodegradable wastes from gardens, parks, food, household, and comparable waste from food processing plants, that can undergo an anaerobic or aerobic decomposition (Waldron, 2007; Kokkora, 2008).

Biological wastes from agri-food industry are a major source of worry for health and environmental departments. This type of waste is generated when the discarded food does not have the standard characteristics to be sold to the consumer or due to the disposal of pits, peels, among other elements in the processing of 2nd line products (Lee et al., 2020).

1.1.1 The problematic associated with the residues of the agri-food sector

Food loss and food waste can result at different stages of the food value-added chain, in some countries it mostly happens in the end, unlike other countries where it occurs at the beginning (Monzon-Santos, 2017). Those losses and wastes can also appear in farm stages including inadequate harvesting time, climatic conditions, inappropriate storage conditions, etc. Moreover, limited shelf life, necessity to respond to aesthetic standards in terms of colour, shape, and size, are the main causes of food waste at the retail stage (FAO, 2019).

Most of the countries consider that agri-food residues are pollutants with a high Biochemical Oxygen Demand causing a negative effect on the environment and consequently on the balance of the planet as well as the sustainability of the agri-food sector (Agathos, 2011).

According to the European Commission's roadmap, the value chain of food and drink in the European Union causes 17% of direct greenhouse gas emissions and 28% of material resource use (Papaj, 2016), principally with the emission of CO_2, major factor, in terms of quantity, in global warming (Monzon-Santos, 2017).

The Landfilling of bio-wastes contributes also to the degradation of the environment by producing polluting leachate and methane gas wish is one of the greenhouse gases that is also involved in global warming (Kokkora, 2008).

Furthermore, the cycle of food from production to consumption is known as a sector that requires a lot of energy and resources, and essentially the most polluting, through large quantities of pollutants released into the air, water, and soil (Papaj, 2016). According to Scherhaufer et al. (2018), the production phase contributes to approximately three quarters of the greenhouse gas emissions, in contrast to food waste treatment effects, which are not the major source of the environmental impact of food waste.

Thus, preventing food waste including reduction of the quantity of emitted methane and CO_2, as well as energy, water and other sources used during food processing will have an enormous potential to decrease the impacts of climate change and global warming (Smith et al., 2001).

Added to that, the blue water footprint, which is the water that has been sourced from surface or groundwater resources, of food wastage is about 250 km^3, which is a huge value and is equivalent to the annual water discharge of Volga River (Papaj, 2016). Moreover, residues of the agri-food sector can cause biodiversity loss, and it is stressful for the planetary limits in terms of climate change and Nitrogen and Phosphorus cycles (Aschemann-Witzel et al., 2015).

Therefore, uneaten food has significant social and economic costs. Global costs are calculated from the analysis of some parameters such as the atmosphere by calculating the costs of greenhouse gas emissions, water by estimating water scarcity, water pollution, and pesticides residues, soil by estimating the soil erosion, land occupation, and deforestation. Economic impacts are calculated through the value of products lost and wasted, and social impacts are calculated through livelihood and health damages (FAO, 2014).

According to the FAO final report, food wastage footprint, full-costs accounting, shows that in total 2,6 trillion USD is lost annually (Papaj, 2016). As indicated by the paper introduced by FUSIONS (Food Use for Social Innovation by Optimizing Waste Prevention Strategies), the expenses related to food waste for the 28 European countries are estimated to be approximately 143 billion Euros in 2012 (Stenmarck et al., 2016). **Figure 1** presents the costs related to food waste at each level of the food supply chain.

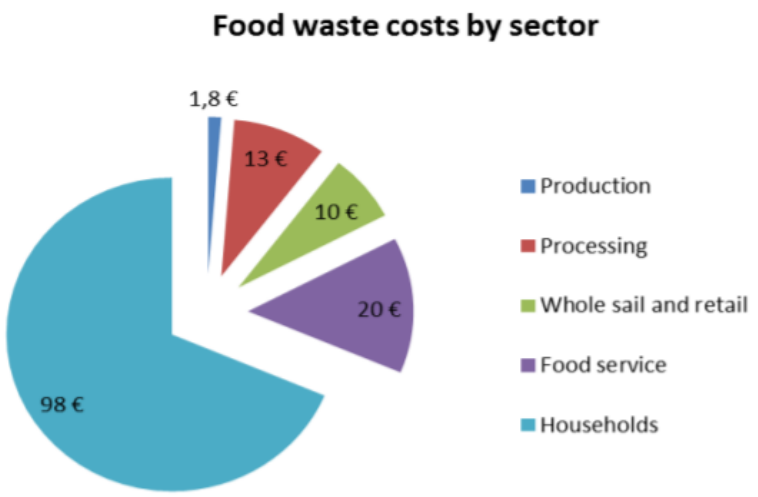

Figure 1 - Costs associated with food waste by sector in 2012 (values in billions of euros)

(Stenmarck et al., 2016)

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Therefore, it can be concluded that the economic impacts of food waste influence both producers and consumers. For the producer, it consists in the loss of resources like fossil fuel, processing or distribution, or other types of energy used, water, labor, and raw materials. In the case of the consumer, rejected food constitutes a cost and a loss of purchasing power (Monzon-Santos, 2017).

1.1.2 The particular case of fruit bio-wastes

Fruit wastes are one of the principal sources of municipal waste. Because of the intense usage and industrial processing of the edible parts of the fruit, bio-wastes of fruits such as fruit skins, seeds, rind, and pomace are generated in high quantities. Fruit wastes can also be fruits suitable for consumption but are abandoned by retailer or consumer (FAO, 2014; Deng et al., 2012).

In fact, there is a quite important rate of fruit that gets to the consumer not as the entire fruit, but as preparation from agri-food industry such as juices or pulps. Some of the technological processes consist into the separation of skin, seeds, and pomace, resulting in fruit bio-waste (Pascoalino et al., 2021).

The FAO has assessed that bio-wastes of fruit and vegetables are the most important compared to other food types and may reach up to 60% (Sagar et al., 2018).

Fruit wastes have become a serious environmental problem, and nowadays there are principally two techniques to deal with those wastes, which are incineration and landfill. However, mismanagement of landfilling will produce methane and Carbone dioxide and incineration will release many pollutants such as dioxins, furans, acid gases, and particulates. Therefore, it is a high priority to valorise fruit bio-waste. Indeed, the re-use of agri-food residues will limit the environmental impact and may be very profitable (Deng et al., 2012).

1.1.3 Extraction of bioactive compounds

Agri-food industry bio-waste are rich in bioactive compounds, which can be extracted using multiple techniques based on solvent extraction, supercritical fluid extraction, subcritical water extraction, use of enzymes, as well as ultrasound and microwave-assisted extraction (Kumar et al., 2017). Several factors such as the temperature, pressure, matrices, and solvent can affect the process of extraction (Hernández et al., 2009).

Soxhlet extraction, hydrodistillation and maceration are considered the main conventional techniques for extracting bioactive compounds from plant matrices (Sagar et al., 2018). The major disadvantage of these techniques is the long time they require and the fact that they are not considered ecofriendly, which has led to greater investment in the search for new innovative and ecologically more sustainable techniques (Garcia-Salas et al., 2010; Azmir et al., 2013).

Solvent extraction technique is a technique based on the reaction of raw material with organic solvents, that will extract soluble compounds of interest (Kumar et al., 2017). It is defined as a low-cost and easy methodology, but it uses big amounts of toxic solvents that should be evaporated or concentrated. Furthermore, bioactive compounds can undergo thermal degradation because of the high temperature of solvent and long-time extraction (Kumar et al., 2017).

In its turn, hydro-distillation is used to extract important oils and other bioactive compounds from different vegetal matrices (Sagar et al., 2018). This technique, although simple, has a major disadvantage when applied to thermolabile compounds since they can be degraded at higher temperatures (Vankar, 2004; Azmir et al., 2013).

Supercritical fluid extraction is an innovative technique for extracting bioactive compounds classified as green and ecofriendly since this technology uses supercritical carbon dioxide (SC-CO_2) instead of toxic organic solvents (Sihvonen et al., 1999; Kumar et al., 2017). Despite this

great ecological advantage and its low cost, this technique does not allow use with polar compounds (Abbas et al., 2008).

Subcritical water extraction is a very common extraction technique for extracting phenolic compounds from food matrices. The short extraction time and low cost are the main advantages pointed out to this ecological technique (Herrero et al., 2006). However, the high temperature used can cause thermal degradation of thermosensitive compounds (Zhang et al., 2020).

Bioactive compounds also can be extracted from food residues using enzymes. Antioxidant molecules are mainly extracted from plant tissues made up of polysaccharide such as cellulose, hemicellulose and pectins. Thus, the use of enzymes like cellulase, β-glucosidase, xylanase, β-gluconase and pectinase will help to release intracellular components (Moore et al., 2006; Singh et al., 2016). In this technique, it uses water as a solvent as an alternative to organic solvents, allowing it to be classified as a green technique with a high extraction rate. However, some disadvantages have been documented when using them, namely, the high cost of enzymes for large volumes of samples and the difficult viability on an industrial scale due to the behavior of enzymes under different conditions (Puri et al., 2012).

Ultrasound-assisted extraction is an easy and efficient green technique to extract bioactive compounds (Kumar et al., 2017). The main advantages of this technique include a short extraction time, reduced use of energy and solvents, and easy maintenance. However, for a maximum yield, ultrasound-assisted extraction requires optimization in the parameters such as the ultrasound frequency, nominal power of the device, geometry of the system, propagation cycle, input power, and amplitude of work (Azmir et al., 2013; Barba et al., 2015).

Finally, microwave-assisted extraction is an innovative extraction method that combines microwave energy and solvent extraction. This technique has an extraction rate using short extraction times, low amount of solvent and consequently low cost (Delazar et al., 2012). However, this technique has as main disadvantages the fact that the microwave efficiency can be low when the solvents or the target compound are volatile or non-polar and the high cost of the equipment (Wang & Weller, 2006; Zhang et al., 2011).

1.2 Bio-waste as a source of bioactive compounds

Agri-food industry bio-waste are a great source of bioactive as well as functional compounds, which can be used in food, nutraceutical, pharmaceutical and cosmetic formulations (Pascoalino et al., 2021). These wastes are rich in phenolic compounds, vitamins, carotenoids, polysaccharides, and many other bioactive compounds that are strictly associated with human well-being. In fact, these by-products are very useful in the food industry sector to develop functional food or food additives (Dueñas & García-Estévez, 2020).

1.2.1 Molecules of interest

Bioactive compounds extracted from fruits and vegetables bio-wastes or by-products are essentially polyphenols, tannins, flavonoids, vitamins (A and E), essential minerals, fatty acids, volatiles, and pigments (Ben-Othman et al., 2020). Researchers have shown that fruit bio-wastes and by products increases especially in the juice industry or after producing value-added products. In fact, peels, or skin of fruit, which are non-edible parts, are richer in bioactive components compared to edible parts like the pulp (Gorinstein et al., 2001a; Gorinstein et al., 2001b).

These compounds include a wide range of molecule of interests, following are examples of the most important biomolecules that can be extracted from fruit and vegetables bio-wastes:

- Dietary fiber: are the sum of non-starch polysaccharides, with a resistance to enzymatic digestion in small intestine, but have the potential to be fermented in large intestine. Dietary fibers are subdivided into two groups: soluble dietary fiber and insoluble dietary fiber (IDF) (Chen et al., 2018). IDF such as cellulose, hemicellulose and lignin, have the properties to reduce gastrointestinal transit period, prevent of colon cancer, diabetes, cardiovascular diseases and obesity (Wang et al., 2020). Some studies in the literature point to the presence of dietary fibers in the composition of bio-waste. For example, apple peel is richer in dietary fiber than the pulp, in addition to apple pomace which is a by-product from apple juice industry also contains an important amount dietary fiber (Sagar et al., 2018).
- Phenolic compounds: are one of the largest classes of bioactive compounds with numerous biological functions. The literature has highlighted fruits and vegetables as excellent sources of phenolic compounds; however, some studies have highlighted rind, peel and seeds with the highest content (Sagar et al., 2018). For example, orange, lemon, and grapes'

peel, and jackfruit, longans, avocados, and mangos' seeds have shown 15% higher phenolic concentrations than the fruit pulp (Gorinstein et al., 2001a; Soong & Barlow, 2004).

- Flavoring agents and aromas: such as flavors, ethanol, citric acid, lactic acid, among others (Zheng & Shetty, 1998). Consumers' preferences for natural and safe sources are increasing. Thus, the market of flavors, aromas and fragrances has grown. Vanillin, derived from vanillic acid is the major compound of vanilla flavor. As an example, in pineapple peel waste, a precursor for vanillic acid which is ferulic acid, was found. Other than vanillin, more aromas are found in vegetal by-products that could be added to many industrial products such as juices to improve their sensory quality (Sagar et al., 2018).
- Enzymes: such as amylases, cellulases, invertases, pectinases and other enzymes (Sagar et al., 2018). Fruit residues are used to produce amylase. In addition, cellulases are used in the process of extraction of phenolic compounds and the liberation of aroma-rich compound in the food sector. For example, when banana solid waste was mixed with a combination of bacteria (*Cellulomonas carte, Bacillus megaterium, Pseudomonas putida*, and *Pseudomonas fluorescence),* the quantity of cellulase has increased (Dabhi et al., 2014). Moreover, invertase is used in jam, candies, and confectionery products, as well as in pharmaceutical sector (Panda et al., 2016). And pectinase is commonly used in wine and fruit juice processes (Sagar et al., 2018).
- Organic acids: those molecules can be used in food, chemical and cosmetic industries, especially using the citric and lactic acids. Fermentation with yeasts, bacteria and molds produce citric acid (Sagar et al., 2018). Lactic acid is also important and has many applications in the food and non-food sectors. In food industry, it is used as preservative and acidulant (Rodríguez-Couto, 2008).
- Proteins: are very important to human health and well-being as they are a necessary component of many body molecules as well as their potential to form muscles (Sagar et al., 2018). Proteins are found in fruit and vegetables wastes in a high proportion, such as apple pomace, green pea peels, mango peel, orange peel, carrot pomace, among others (Sharma et al., 2016).

1.2.2 The particular case of phenolic compounds

Phenolic compounds contain an aromatic ring with one or more hydroxyl groups, comprise more than 8,000 compounds with different structures, and can be divided into 10 classes based on their chemical structure (Garcia-Salas et al., 2010).

Phenolic compounds are mainly the plant secondary metabolites that are closely linked to the sensory and nutritional quality of plant-based food. They are synthesized as a way of defense against pathogens (Karakaya, 2004). These compounds are also recognized for their excellent antioxidant and antimicrobial activities with potential use in different industrial areas (Chirinos et al., 2009).

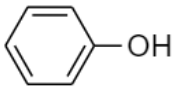
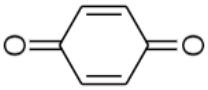
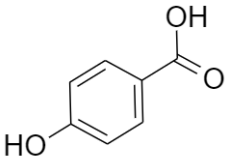
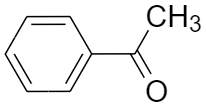
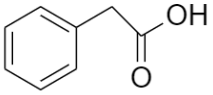
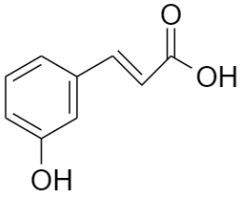
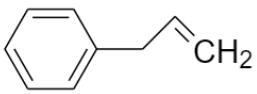
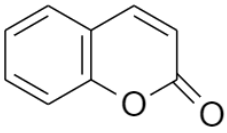
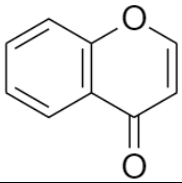
Phenolic compounds are a source of vitamins, protect enzymes, and avoid lipid peroxidation. They are natural antioxidant, preventing neurodegenerative and cardiovascular diseases and contribute to the prevention from oxidative stress. Food industries uses antioxidants to prolong food shelf life and thus protect them against lipid and vitamin oxidation (Pascoalino et al., 2021). Apple pomace including seeds, peels, core, stems, and soft tissues, is rich in polyphenols such as chlorogenic acid, epicatechin, catechin, quercetin, and hydroxycinnamates (Lee et al., 2020).

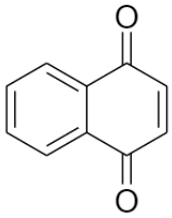
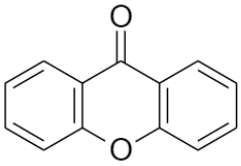
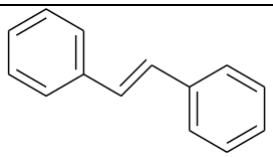
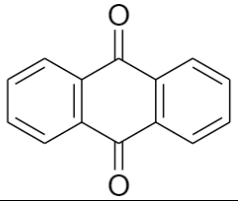
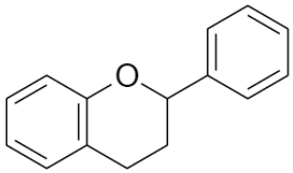
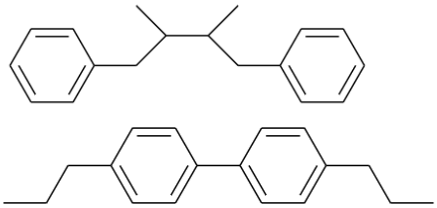
Phenolic compounds have shown an antimicrobial activity due to their capacity to inhibit the growth of a many microorganisms (Pascoalino et al., 2021).

In addition to their antioxidant and antimicrobial properties, phenolic compounds also have an anti-inflammatory activity, in fact, phenolic compounds have shown a great potential to treat inflammatory responses and disorders. (Pascoalino et al., 2021). As an example, phenolics contained in pear extracts have a key role in antioxidant and anti-inflammatory activities (Li et al., 2014). In a study conducted by Wang et al. (2015), pears have shown potent antioxidant capacity detected especially in peels through reducing power and DPPH (2-diphenyl-1-picrylhydrozyl) radical scavenging assay. Phenolic acids, vitamin C, flavonoids, tannins, and carotenoids were in direct correlation with antioxidant activity, as reported by researchers (Dimitrios, 2006).

As showed in **Table 1**, phenolic compounds include many families, essentially found in natural matrices in association with glycosides (mono- or polysaccharides) or functional derivatives such as esters or methyl esters. Furthermore, the principal sources of phenolic compounds are also presented.

Table 1 - Class, structures and source of some phenolic compounds (Garcia-Salas et al., 2010).

Carbon numbers	Class	Basic structure	Sources
C6	Simple phenols		
	Benzoquinones		
C6-C1	Hydroxybenzoic Acid		Cranberry, cereals
C6-C2	Acetophenones		Apple, apricot, banana, cauliflower
	Phenylaceticacid		
C6-C3	Hydroxycinnamic acid		Carrot, citrus, tomato, spinach, peaches, cereal, pears, eggplant
	Phenylpropene		
	Coumarins		Carrot, celery, citrus, parsley
	Chromones		

C6-C4	Naphthoquinones		Nuts
C6-C1-C6	Xanthones		Mango, Mangosteen
C6-C2-C6	Stilbenes		Grapes
	Anthraquinones		
C6-C3-C6	Flavonoids		Widely distributed
$(C6-C3)_2$	Lignans, neolignans		Sesame, rye, wheat, flax
$(C6-C1)_n$	Hydrosoluble tannins	Heterogeneous polymer composed of phenolic acids and simple sugars	Pomegranate, raspberry
$(C6-C3)_n$	Lignins	Highly crosslinked aromatic polymer	

Flavonoids are commonly found in the tissues of plants, and they contribute to the colour (red, orange, purple, yellow, and blue) of flowers, leaves and fruits (Khoddami et al., 2013). This family of biomolecules includes flavonols, flavanols, flavones, flavanones, isoflavones, and

anthocyanins (Pascoalino et al., 2021). Moreover, flavonoids have a protective role against many chronic diseases such as cancer, inflammation, and cardiovascular disorders. These components derive from, phenyalanine and tyrosine, two aromatic amino acids, and they are three-ringed structured (Routray & Orsat, 2012).

On the other side, phenolic acids are also one of the well-known classes of phenolic compounds that rarely appear in a free form but in the form of esters, glycosides or amides. Phenolic acids originate from hydroxycinnamic and hydroxybenzoic acid (Khoddami et al., 2013).

Additionally, phenolic components of the plant cell wall are a considerable class of phenolic compounds, they are hydrophobic and found in complexes with other components of cells. For example, according to some studies, polyphenol contained in pears have various structures and belongs to many classes such as flavonoids (flavan-3-ols) and flavonols, phenolic acids (hydroxycinnamic acids derived from caffeic acid and *p*-coumaric acid), and simple phenolics (arbutin) (Kolniak-Ostek, 2016b).

1.3 *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak

Pears belong to the family Rosaceae, subfamily Maloideae, and genus *Pyrus* (Li et al., 2016). Pears (*Pyrus* spp.) are a principal pome fruit that grows in temperate zones all over the world (Hancock & Lobos, 2008). Due to their geographical distribution, they include Oriental and Occidental varieties. Oriental pears are principally cultivated all over Eastern Asia (China, North and South Korea, and Japan), and include *Pyrus bretschnrideri* Reh, *Pyrus ussuriensis* Maxim, *Pyrus pyrifolia* Nakai and *Pyrus sinkiangensis* Yu, while major species of Occidental pears is the *Pyrus communis* species (Cui et al., 2005).

Fresh pear fruits are one of the most consumed fruits worldwide, it is also found in some food preparation such as drinks, candy, preserved fruit, and jam (Reiland & Salvin, 2015). Chinese pear (*Pyrus pyrifolia*) and European pear (*Pyrus communis* L.) are the world's two main commercial pear species (Hancock & Lobos, 2008).

1.3.1 Botanical characterization, origin and production

Pear is one of the most important temperate fruits species and comes in the fifth rank after banana, orange, apple and grape. Pear production is around 23.9 million metric tons with a harvest area of 1.61 million hm^2. China is the first producer (15.9 million t) with a planting area that

exceeds 1 million hm², followed by Italy, the United States, Argentina, Spain, Turkey, and South Africa (Li et al., 2016).

According to FAOSTAT (2020) European pear production is estimated at 2.35 million tons in with a harvested area of 107.080 ha in 2020. In Portugal "Pera Rocha do Oeste" is one of the most important Portuguese fruit products, and its production is in the Midwest region, with an area of approximately 11000 ha. It is also important in terms of economy and export (141.186 tons) (Pedro et al., 2020; Dias et al., 2021).

Pyrus communis L. **(Figure 2)** is a fruit native from Europe. The two well-known *Pyrus communis* cultivars in North America and Europe are 'Bartlett' (Williams or 'Williams Bon Chrétien') and 'Anjou', while 'Conference' cultivar is the most popular variety grown in European countries such as Italy, the United Kingdom among others (Li et al., 2016).

Figure 2- *Pyrus communis* L. fruits (http://perarocha.pt)

This fruit is well appreciated by consumers due to its good taste, nutritive properties, and low caloric level (Barroca et al., 2006). The taxonomic hierarchy of *Pyrus communis* L. is shown in the **Table 2** below.

Table 2 - Taxonomic hierarchy of *Pyrus communis* L. (NCBI, 2021).

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Rosales
Family	Rosaceae
Genus	***Pyrus***
Species	***Pyrus communis*** L.

Pyrus pyrifolia (Burm.) Nak **(Figure 3)** also called Asian, Apple, Chinese, Nashi or Sand pear, is a specie native to Eastern Asia, China, Japan and Korea.

Figure 3 - *Pyrus pyrifolia* (Burm.) Nak fruits (https://www.pepiniere-bretagne.fr/images/Photo_plantes/PYRUPYHO_2.jpg)

P. pyrifolia belongs to the family Rosaceae and genus *Pyrus* **(Table 3)**. It is grown over central and South China, East of Russia, Southern Japan, Korea, in the northern mountains of Vietnam, India and Thailand and lately it has been cultivated in USA, New Zeland, and Australia and in the warmer regions of Europe such as France and Italy. Sand pears fruit have a juicy, crispy, sweet flesh, they are eaten fresh, cooked or used in vinegar or soy-sauce. Generally, these pears are not used into jams or cooked in pies because of the high-water content, and, unlike the European pear, they have a crispy and grainy texture. Nashi pears have a unique taste and a large size, and thus, their price is high (Lim, 2012).

Table 3 - Taxonomic hierarchy of *Pyrus pyrifolia* (Burm.) Nak (NCBI, 2021).

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Rosales
Family	Rosaceae
Genus	***Pyrus***
Species	***Pyrus pyrifolia* (Burm.) Nak**

1.3.2 Nutritional and chemical characterization

Pears are considered as a major source of energy, minerals, vitamins, etc. with several nutritive properties (Li et al., 2016). The nutritional and chemical composition varies from a pear species to another. European pear (*Pyrus communis*) is richer in sugar and provide more calories than Asian pears (*Pyrus Pyrifolia*) which contains more water, less sugar and starch. Therefore, European pears are usually used as food ingredients whereas Asian pears are considered as healthy fruit with dietary properties (Li et al., 2016).

European pear's edible portion (100g) provides approximately 54 calories of food energy. Pear is composed of 85% of water, 14% carbohydrates, 2% fiber, 0,3% protein and 0,1% fat.

Ascorbic acid is one of the main vitamins that exists in pears, it presents 3mg (Kolniak-Ostek, 2016a).

Many researchers have focused on comparisons and analyses of pear's compounds such as sugars, vitamins, minerals, amino acids, and fatty acids (Tanrıöven & Ekşi, 2005). These components are very important regarding the qualitative evaluation of fruits, namely taste and colour (Colaric et al., 2005). Whereas organoleptic properties are evaluated throughout pear's chemicals such as total sugars, titrable acidity and soluble solids content (Teng & Liu, 1999).

Sugars influence the fruit quality and flavor. Fructose, glucose, sucrose and sorbitol are the principal saccharides of pear fruits, with fructose having a higher a value than glucose or saccharose (Yim & Nam, 2016).

Many amino acids in pears have been identified in pears, which are aspartic acid, glutamic acid, proline, threonine, valine, and phenylalanine. It has been shown also that aspartic acid and glutamic acid are the major amino acids in the major pear cultivar (Yim & Nam, 2016).

Malic, citric, quinic, shikimic acids are the major organic acids found in pears according to an investigation by Sha et al. (2011) where they analyzed the content of organic acids in the fruit of 40 cultivars of four major pear species including *Pyrus ussuriensis*, *Pyrus bretschneideri*, *Pyrus pyrifolia*, and *Pyrus communis*, to show that malic and citric acid are the two main organic acids in pears.

Minerals are important to human health with their multiple physiological effects. Pears contain macro minerals such as Calcium (Ca) that is important for bone structure as well as the control of salt balance, Magnesium (Mg) with its catalytic role in respiration, Potassium (K), which is the major mineral in pears. Additionally, microminerals such as Zinc (Zn), Iron (Fe), Copper (Cu), are found in many pears' cultivar (Yim & Nam, 2016).

Carotenoids are natural pigments in both fruit and vegetables, giving them the red colour. They have many health benefits as they are precursor to vitamin A. They also have antioxidant properties, participates in cell differentiation, stimulators of cell-cell communication and may improve immune system (Yim & Nam, 2016). Dias et al. (2009) have studied carotenoids in 10 varieties of five fruit species: orange, pear (*Pyrus communis* L. var Rocha), peach, apple, cherry. They concluded that fruits have a low content of carotenoids compared to vegetables, and that it could be variable with different species, varieties, time of harvest and geography.

Fibers are divided into two main groups, soluble fiber formed by pectin, mucilage, gums and hemicelluloses, and insoluble fibers that comprise cellulose, lignin, and a large range of hemicelluloses. Fruit and vegetables contain a high proportion of soluble dietary fibers, which may prevent many diseases and disorders such as cardiovascular diseases, diabetes, constipation and obesity (Li et al., 2016).

Numerous studies reported that the different part of pear trees such as pear peel, flower and other parts contain different types of phenolic compounds. Arbutin, chlorogenic acid, *p*-coumaroylquinic acid, *p*-coumaroylmalic acid, dicaffeoylquinic acids, vanillic acid derivatives, catechin, epicatechin, and many others were found in pears, with higher phenolic content in the pear skin than the flesh (Lin & Harnly, 2008).

1.3.3 Benefits and application

Pears are much appreciated all over the world for their tasty and juicy aspect. Generally, people eat it fresh or in processed products such as drinks, candy, preserved fruit, jams, fruit wine, puree and jellies (Li et al., 2016). For a long time, pears (*Pyrus spp.*) have been used as traditional medicine to treat coughing and for its anti-inflammatory, anti-tussive and diuretic activities (Li et al., 2014).

Phenolic compounds have important role in human health metabolism, they have antioxidant, antimutagenic, anticarcinogenic, antibacterial, antifungal potentials. Arbutin, chlorogenic acid and epicatechin are the major phenolic compounds in pear. Chlorogenic acid is a crucial antioxidative compound in pears, it has many health benefits such as preventing cancer and cardiovascular diseases, and it stimulates and enhances the immune system. Arbutin is also an important phenolic compound found in pears, it has an antibiotic role against the fire blight disease (Öztürk et al., 2015). Moreover, other compound found in pears such as fibers can be prebiotics (Hong et al., 2021).

According to recent studies, pears have shown an anti-diabetic effect, in fact, consuming both apple and pears reduced by 18% the risk of Type 2 *diabetes mellitus* (T2DM) (Velmururugan & Bhargava, 2013; Guo et al., 2017). According to Velmururugan and Bhargava (2013), ethyl acetate and ethanol extracts of pear (*Pyrus communis*), showed an important antihyperglycemic effect, stimulating the insulin secretion from pancreatic β-cells. They also have demonstrated the antihyperlipidemic effect of pears, since hyperlipidemia is frequent among diabetic patients, which

have higher risks of cardiovascular diseases. The hypolipidemic activity of pears was demonstrated through the reduction of some biochemical parameters such as total cholesterol, triglyceride, and low-density lipoprotein. Hence, the antihyperlipidemic effect of pears is associated with some components such as catechin, a phenolic compound found mainly in pears peels (Hong et al., 2021).

In addition, pears have an anti-inflammatory effect, through anthocyanin that reduces inflammation scores, including cytokines, oxidative stress and acute inflammation (Hong et al., 2021). In cosmetology, skin-whitening effect, anti-aging and anti-wrinkle effects have always been a challenge. In this purpose, arbutin, an abundant compound in pear fruit, is a skin-whitening compound (Cho et al., 2015).

Furthermore, bioactive potential of fruit bio-wastes and by-products is commonly used in food formulations to enhance functional, sensory and nutritional properties (Mahfoudhi et al., 2016). The application of bioactive compounds is either direct when applying these compounds to the food itself or indirect when applying bioactive compounds, for example, in active food packaging (Andrade et al., 2019).

2. Objectives

This study aimed to carry out the nutritional and chemical characterization of two pear varieties (*Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak), as well as to validate their bioactive properties, to promote their industrial valorization (**Figure 4**).

The specific objectives of this work are:

- Nutritional characterization (fat, ash, protein, carbohydrate and energetic value) of the pulp and skin of two pear varieties;
- Determination of chemical composition (namely total phenols and flavonoids, free sugars, fatty acids and organic acids) of all samples under study;
- Characterization of bioactive properties (antioxidant, antimicrobial, anti-inflammatory and cytotoxic activity) of the extract obtained from pulp and skin of two pear varieties.

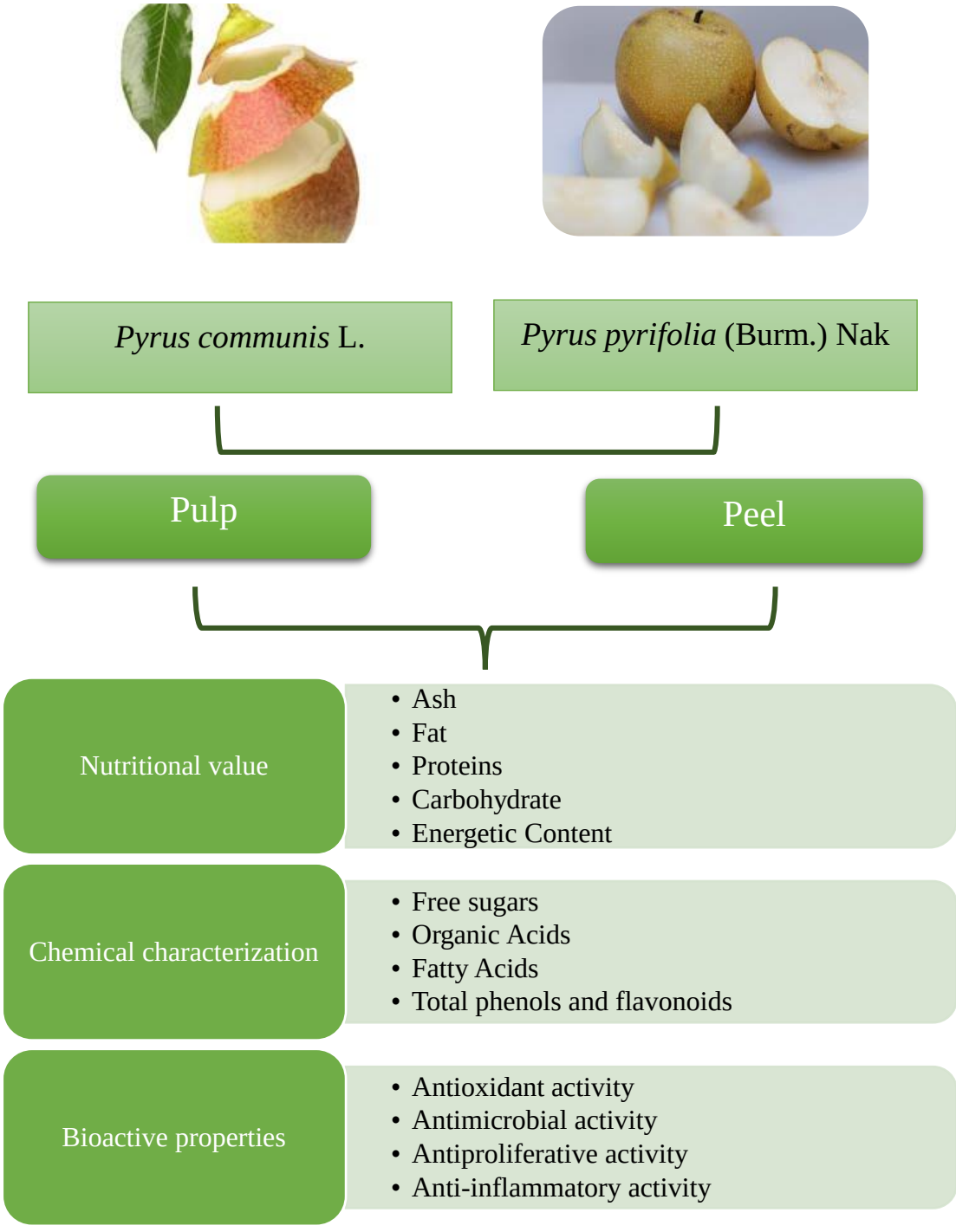


Figure 4 - Diagram of the main objectives and procedures of this study

3. Material and Methods

3.1 Standards and reagents

3.1.1 Nutritional and chemical characterization

HPLC-grade acetonitrile 99%, n-hexan 95%, etanol absolut (99,9%), and ethyl acetate 99,98% were acquired from Fisher Scientific (Lisbon, Portugal). The analytical grade methanol solvent was acquired from Paralab Company (Lisbon, Portugal). Analytical grade solvents ethyl ether was obtained from Lab-Scan (Lisbon, Portugal), toluene and sulfuric acid from Sigma-Aldrish (St. Louis, MO, EUA). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) , the sugar standards (D (-)-fructose, D(+)-sucrose, D(+)-glucose, D(+)-trehalose, D(+)-melezitosis and D(+)- raffinose pentahydrate), as well as the organic acid standards (L(+)-ascorbic acid; citric acid; malic acid; oxalic acid; succinic acid; fumaric acid and quinic acid) was purchased from Sigma-Aldrich (St. Louis, MO, EUA), as well as the other individual fatty acid isomers, nitric acid, formic acid. All other reagents were purchased from specialized retailers. Water treatment was performed in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

3.1.2 Bioactive characterization

Fetal bovine serum (FBS), l-glutamine, Hank's saline solution (HBSS), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), and Dulbecco's Modified Eagle Medium (DMEM) media were acquired from Hyclone (Logan, Utah, EUA). Acetic acid, ellipticin, sulforodamine B (SRB), trichloroacetic acid (TCA) and Tris were obtained from Sigma-Aldrich (St. Louis, MO, USA).

The Raw 264.7 mouse macrophage cell line were obtained from DMSMZ – Leibniz – Institut DSMZ -Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and cell culture), and Griess Reagent System Kit was obtained from Promega (Madison, WI, EUA).

Mueller-Hinton agar (MHB) was acquired from Biolab® (Hungary). The compound p-iodonitrotetrazolium chloride (INT) was purchased from Panreac Applichem (Barcelona, Spain). The antibiotics imipenem and vancomycin were obtained from Hikma pharmaceutical (Portugal SA) and Ampicillin from pharmaceutical Janssen (Belgium).

3.2 Sample preparation and acquisition

For this study the *Pyrus communis* L. (Rocha pear) and *Pyrus pyrifolia* (Burm.) Nak (Nashi pears) samples were acquired, in particular fruit bio-wastes resulting from the production chain (Campotec, Torres Vedras, Portugal). In order to understand in detail the characteristics and benefits of each part of the samples, the peels were finely separated from the pulp (**Figure 5**) and after frozen they were dehydrated by lyophilization (**Figure 6**) (FreeZone 4.5, Labconco, Kansas City, MO, USA). Then, the samples were crushed and the powder mixed to ensure the homogeneity of the samples and were stored in a cool, dry place, protected from light, for further analysis

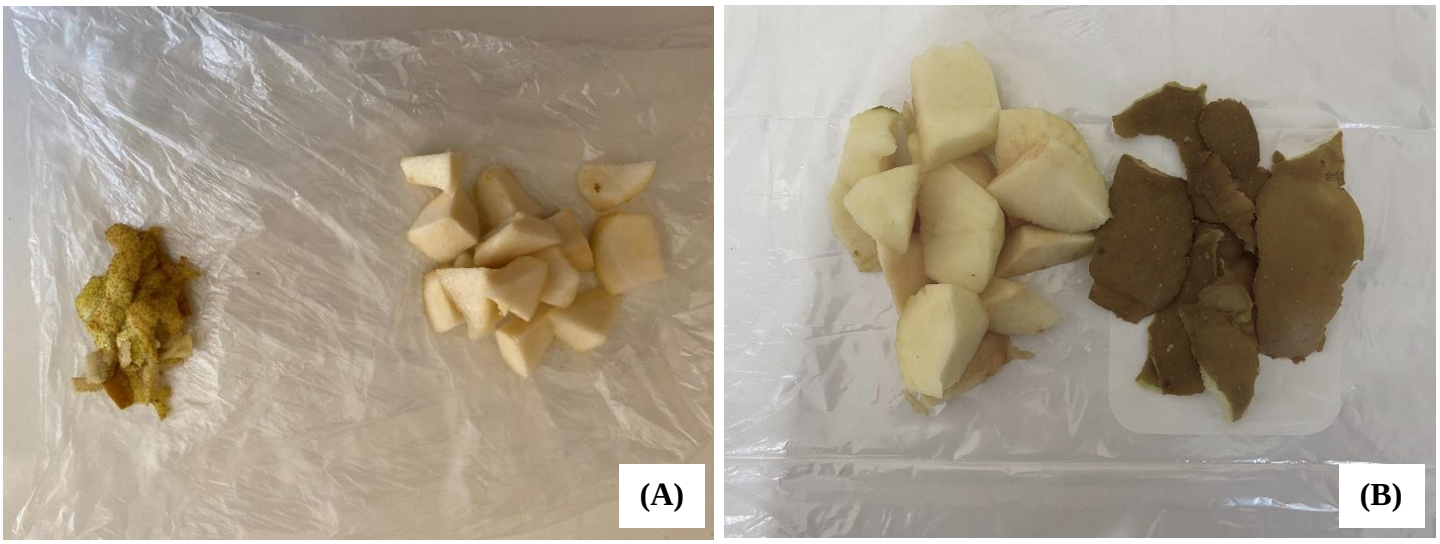


Figure 5 - Pulp and peel of the fruit of *Pyrus communis* L. (A) and *Pyrus pyrifolia* (Burm.) Nak (B)

Figure 6 - Lyophilized peel and pulp of the fruit of *Pyrus communis* L. (A; B) and *Pyrus pyrifolia* (Burm.) Nak (C; D)

3.3 Nutritional Characterization of the pulp and peel of *Pyrus Communis* L. and *Pyrus Pyrifolia* (Burm.) Nak biowastes

The nutritional evaluation of the pear biowastes (pulp and peel of the two varieties) was determined according to official food analysis methodologies (AOAC, 2016). In this way, the concentrations of protein, fat, carbohydrates, ash, moisture and also the total energy value were determined.

Lipids were determined by extracting an obtained known mass of the samples (3g) in a Soxhlet apparatus for which petroleum ether was used as the extraction solvent at a temperature of approximately 60 °C for 7 hours (**Figure 7**), then the solution was evaporated (AOAC 989.05).

Figure 7 - Soxhlet extraction of lipids

Total protein content (N×6.25) was calculated as nitrogen content by the Kjeldahl method (AOAC 991.02). To this end, concentrated sulfuric acid (H_2SO_4) was added to the sample (0.5 g), thus causing the digestion of organic matter (**Figure 8**) and the consequent formation of an inorganic salt, ammonium sulfate $(NH_4)_2SO_4$, in which nitrogen is retained. Then, the solution is made alkaline by adding sodium hydroxide (NaOH), which enhances the release of nitrogen in the form of ammonia, NH_3. The ammonia is then distilled and taken up in a solution of H_2SO_4 (0.1M). Finally, a titration is carried out with NaOH (0.1 M), using a red methyl indicator, according to (AOAC 991.02).

Figure 8 - Evaluation of protein content by Kjeldahl method

The ash content **(Figure 9)** was determined from the incineration of 250 mg of sample at a temperature of 550 ± 15°C during 12 hours (AOAC 935.42).

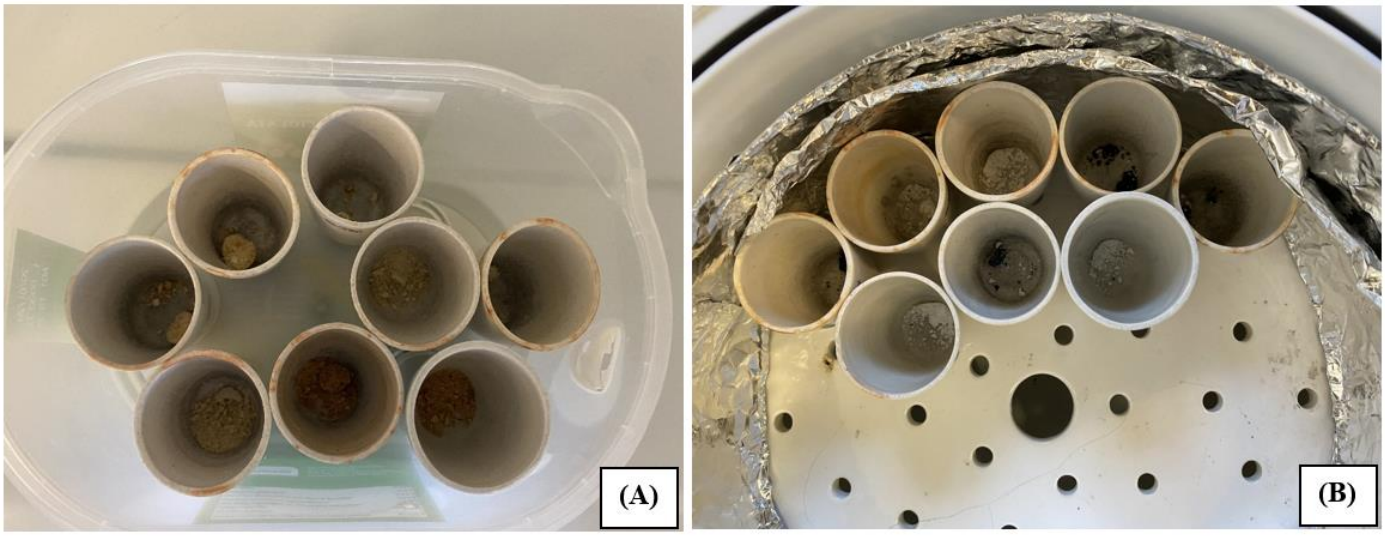


Figure 9 – Evaluation of ash content: before (A) and after (B) incineration

To determine the moisture present in the sample, a known mass of sample (1 g) was placed in the oven (100°C) until a constant mass was obtained.

Carbohydrates were calculated by difference according to **Equation 1**, and the energetic value was calculated as showed in **Equation 2**.

$$Carbohydrates = 100 - (g\ moisture + g\ proteins + g\ lipids + g\ ash)$$

Equation 1 - Calculation of carbohydrates

$$Energy\ (Kcal) = 4 \times (g\ proteins + g\ carbohydrates) + (9 \times g\ fats)$$

Equation 2 - Determination of total energy

3.4 Chemical composition of the pulp and peel of *Pyrus Communis* L. and *Pyrus Pyrifolia* (Burm.) Nak. biowastes

3.4.1 Free sugars

Free sugars were determined by high performance liquid chromatography system coupled to a refractive index detector (HPLC-RI) as previously described by Barros et al., (2013).

The sample (1 g) was enriched with melezitose (used as an internal standard, 25mg/mL) and extracted with 40 mL of ethanol (80/20, *v/v*) in an 80 °C bath (Julabo, SW22; Seelbach, Germany) for 1 hour and 30 minutes, with agitation every 15 minutes (**Figure 10**). Subsequently, the supernatant obtained was centrifuged (K24OR refrigerated centrifuge, Centurion, West Sussex, United Kingdom) at 15,000 g for 10 minutes and transferred to a glass flask to evaporate the ethanolic fraction, using a rotary evaporator (Büchi R -210, Flawil, Switzerland) (60°C, reduced pressure). The aqueous phase was washed 3 times with diethyl ether (10 ml), then the rest was evaporated. To the dry residue obtained, water was added to make up a final volume of 5 mL and 1.5 mL of it was filtered (nylon filters - 0.2 µm, Whatman) into a vial, for later analysis of the profile in sugars in the HPLC system.

The HPLC system is equipped with a pump (Knauer, Smartline 1000 System, Berlin, Germany), a degassing system (Smartline manager 5000), an autosampler (AS-2057 Jasco, Easton,

Maryland, USA) and a detector refractive index (Knauer Smartline 2300). Chromatographic separation was achieved through a Eurospher 100-5 NH_2 column (4.6 x 250 mm, 5 mm, Knauer), which operated at a temperature of 30°C (7971 R Grace).

Acetonitrile/deionized water (70:30; *v/v)* was used as mobile phase at a flow rate of 1 ml/min. The Clarity 2.4 Software (DataApex) was used to identify the compounds, from which the relative retention times of the sample peaks were compared with known standards. The results were obtained by the internal standard method and expressed in grams of compound per 100 g of fresh mass.

Figure 10 - Procedures for the free sugars profile evaluation

3.4.2 Organic acids

Organic acids were determined using high performance liquid chromatography coupled to a photodiode detector (UFLC-PDA), according to a procedure previously described by Barros et al., (2013). The extraction (**Figure 11**) was carried out in the dark by adding 25 mL of metaphosphoric acid (4.5%) to the sample (1.5 g), maintaining the temperature conditions of 25 ºC and constant agitation (150 rpm) for 45 minutes. After extraction, the mixture was filtered through filter paper (Whatman No. 4) and then through nylon filters (0.2 µm; Whatman) for analysis at UFLC (Shimadzu Corporation, Kyoto, Japan). Their separation was obtained through a C18 reversed-phase SphereClone column (Phenomenex, Torrance, California, USA) (5 µm, 250 mm, 4.6 mm i.d.) thermostated at 35 °C, eluted with sulfuric acid. (3.6 mM) at a flow rate of 0.8 ml/min.

The detection of organic acids was achieved using a DAD system, applying a wavelength of 215 nm (and 245 nm for ascorbic acid). The quantification of the compounds was performed by comparing the area of their recorded peaks, at the wavelengths, with the calibration curves obtained from the standards of the respective compound. The results were expressed in mg of compound per 100 g of fresh mass.

Figure 11 - Determination of organic acids

3.4.3 Fatty acids

Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID), as previously described by Pereira et al. (2012).

To the lipid extract previously obtained by Soxhlet extraction (**Figure 12**), 5 ml of a methanol/sulfuric acid/toluene solution were added, in a 2:1:1 (*v/v/v*) ratio, and the mixture remained in a bath (Julabo, SW22; Seelbach, Germany) at 50 °C (with 160 rpm agitation) for approximately 12 h. After removing the tubes from the bath and, to enhance the phase separation, deionized water (3 mL) was added to the mixture and, later, to recover the fatty acid methyl esters (FAME), diethyl ether (3 mL), both vertexing steps.

After separating the phases, the supernatant was transferred to a vial, in which anhydrous sodium sulfate was previously added, to dehydrate the supernatant. Finally, it was filtered through nylon filters (0.2 µm; Whatman) into a vial for further analysis in GC.

The fatty acid profile was obtained using a GC system (Model DANI GC 1000) equipped with a split/splitless injector, a flame ionization detector (FID, 260 °C) and a Zebron-Kame column (30 m x 0 .25 mm ID x 0.20 µm df; Phenomenex, Lisbon, Portugal). The temperature program applied was as follows: initial temperature of 100 °C for 2 min; progressive temperature increases: 10 °C/min to 140 °C; 3°C/min to 190°C; 30 °C/min to 260 °C which was held for 2 min. The carrier gas used was hydrogen with a flow rate of 1.1 ml/min, measured at 100°C. Split injection (1:50) was performed at 250 °C, with 1 µL of the sample being injected.

The identification of fatty acids was based on the relative retention times of FAME peaks from samples with known standards. For processing the results, the CSW 1.7 software (DataApex 1.7, Prague, Czech Republic) was used and these were expressed as a relative percentage (%) for each fatty acid detected.

Figure 12 - Evaluation of the fatty acid profile

3.4.4 Total phenols and total flavonoids

Extraction procedure. An extract was prepared from the dry fruit material (pulp and peel of the two pear varieties), previously dehydrated by lyophilization. The lyophilized sample (1 g) was subjected to maceration with an ethanol/water solution (80:20, *v/v*; 30 mL) at room temperature under constant agitation (150 rpm) during 1 hour. Subsequently, it was filtered through filter paper (Whatman N°4; Sigma-Aldrich, St. Louis, MO, EUA) and the process was repeated in the re-extracted with 30 mL of the same hydroethanolic solution. Finally, the alcoholic fraction of the obtained extracts was evaporated under vacuum (Büchi R-210, Flawil, Switzerland) and the aqueous fraction was lyophilized (47°C, 0.045 bar; FreeZone 4.5, Labconco, Kansas City, MO, EUA) to further analysis. A quantity of the obtained dry extract (10 mg) was subsequently re-dissolved in an EtOH/H_2O solution (20:80, *v/v*; 2 mL).

Total phenols were determined by the Folin-Ciocalteu assay according to Wolfe et al. (2003). The extract solution (5mg/mL; 0.5 mL) was mixed with Folin-Ciocalteu (2.5 mL, previously diluted with water 1:10, *v/v*) and sodium carbonate (75 g/L, 2 mL). The tubes were mixed using a vortex for 15 sec and allowed to stand for 30 min at 40 °C for color development. Absorbance was measured at 765 nm. Gallic acid was used to obtain a standard curve (9.4×10^{-3} – 0.15 mg/mL) and the results were expressed as mg gallic acid equivalent (GAE) per g of extract.

For flavonoids total quantification, the sample extract (5 mg/ml; 0.5 mL) was mixed with distilled water (2 mL) and $NaNO_2$ solution (5%, 0.15 mL). After 6 min, the $AlCl_3$ solution (10%, 0.15 mL) was added and left to stand for a further 6 minutes. Subsequently, NaOH solution (4%, 2 mL) and distilled water were added to a final volume of 5 mL. The mixture was properly stirred and allowed to stand for 15 min (Jia et al., 1999). The intensity of the pink color was evaluated by determining the absorbance at 510 nm. (+)-catechin was used to calculate the standard curve (1.5 x 10 -2 -1.0 mM) and the results were expressed as mg of (+)-catechin equivalents (EC) per gram of extract.

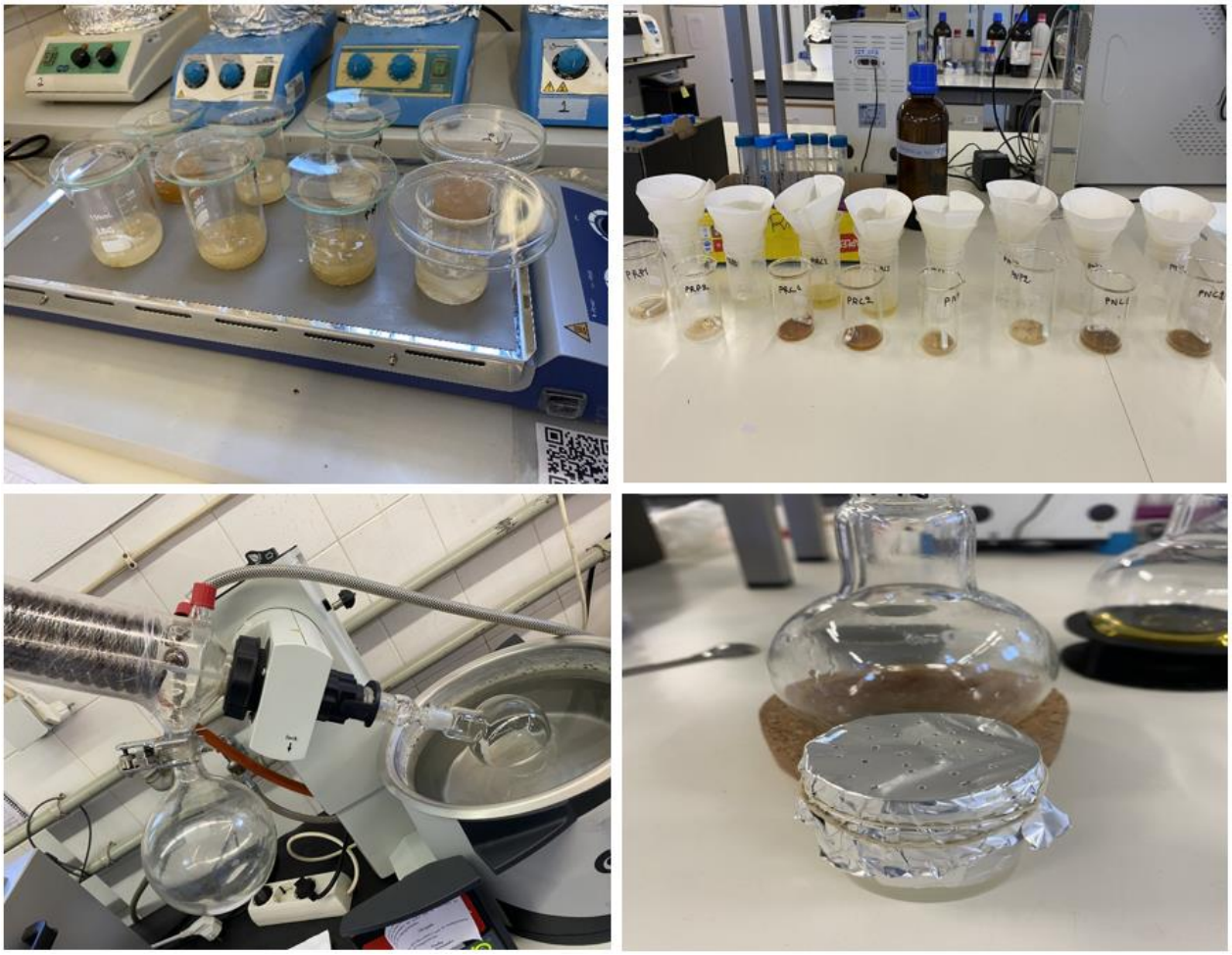


Figure 13 - Procedures used for the evaluation of the total phenols and flavonoids

3.5 Evaluation of bioactive potential of the extract obtained from pulp and peel of *Pyrus Communis* L. and *Pyrus Pyrifolia* (Burm.) Nak biowastes

For the evaluation of the antioxidant potential, the lyophilized extracts obtained in section 3.4.4 were re-dissolved in ethanol:water, 80:20 (*v/v*) to a concentration of 10 mg/mL. For the

antimicrobial activity, the lyophilized extracts were re-dissolved dimethyl sulfoxide (DMSO) (100 mg/mL), and for cytotoxic activity, hepatotoxicity and anti-inflammatory activity the extracts were re-dissolved in distilled water in a concentration of 8 mg/mL.

3.5.1 Antioxidant activity

3.5.1.1 TBARS

The thiobarbituric acid reactive substances (TBARS) assay is commonly used to determine lipid oxidation and antioxidant potential in food and physiological systems. The process of lipid oxidation consists of a free radical chain mechanism leading to many products mostly aldehyde. Malondialdehyde (MDA) is one of the most studied aldehydes, which reacts with thiobarbituric acid (TBA), giving pink-coloured compounds known as thiobarbituric acid reactive species (Ghani et al., 2017).

In TBARS assay, the substrate will be oxidized by adding a metal ion such as iron or copper. If any antioxidant fraction is added to the test solution, it will inhibit the oxidation process and if the chromogen (TBARS) formation is reduced, it indicates the antioxidant activity (Kumar et al., 2018).

In this assay (**Figure 14**), pig brains were obtained from official slaughtered animals. A portion of pig brain was weighed, in which twice of its mass of Tris-HCl buffer (20 mM, pH 7.4) was added and the solution was shaked. Then, it was centrifuged at 3500 g for 10 minutes.

In tests tubes, an aliquot (0.1 mL) of the supernatant was incubated using different concentration solutions (0.2 mL) with the addition of 0.1 mL of $FeSO_4$ (10 μM) and 0.1 mL ascorbic acid (0.1mM) at 37°C during 1 hour. The next step was to add 0.5 mL of trichloroacetic acid (28% , *w/v*) to stop the reaction , then 0.38 mL of thiobarbituric acid (TBA, 2%, *w/v*). The tubes were then placed in an 80°C bath for 20 minutes, centrifuged at 3000 g for 10 minutes in order to remove the precipitated protein. The supernatant contained the complex MDA-TBA which the colour intensity was measured by the absorbance at 532 nm. The inhibition ration (%) was calculated through the **Equation 3**. With A: is the absorbance of the control and B: the absorbance of the compound solution.

$$Inhibition\ ratio\ (\%) = \frac{A - B}{A} * 100$$

Equation 3- Determination of the inhibition ration in TBARS assay

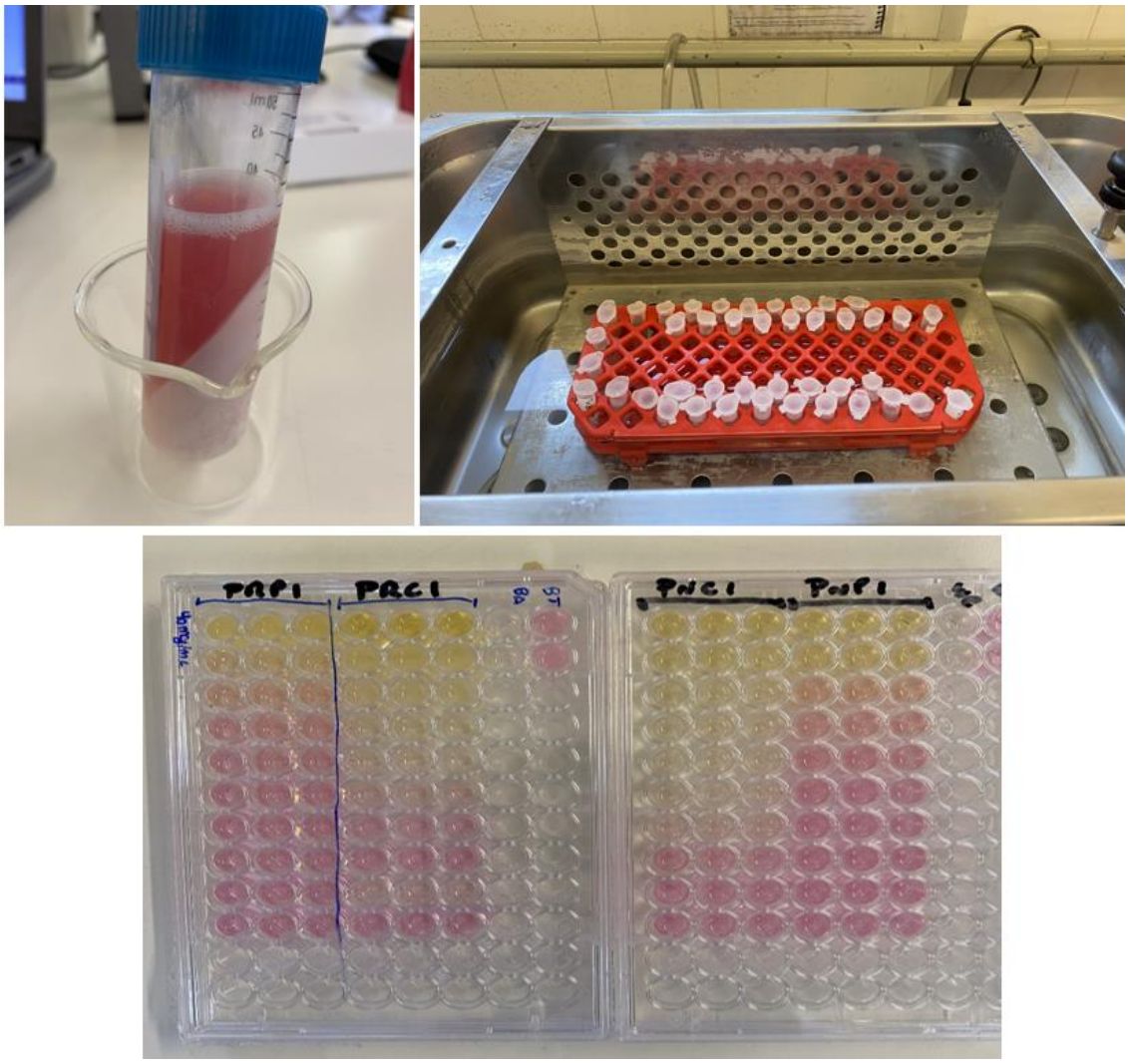


Figure 14 - Determination of antioxidant activity through TBARS assay

3.5.1.2 CAA

Cellular antioxidant activity (CAA) assay is used to quantify the antioxidant activity found in food extracts, phytochemicals or dietary products. This assay measures the ability of extracts to prevent the formation of fluorescent dichlorofluorescein (DCF), a product of the oxidation of dichlorofluorescin which is a probe that is trapped within cells (Wolfe & Liu, 2007).

To achieve this assay, the extracts were dissolved in H_2O to obtain a concentration of 8 mg/mL, from which successive dilutions were made with 2',7'-dichlorohydrofluorescein (DCFH)

prepared with ethanol and diluted with HBSS (50 μM), in order to obtain the concentrations to be tested (500 - 2000 μM). This procedure was followed according to Wolfe & Liu (2007).

RAW 264.7 (murine macrophage cells) was used as the cell line, which was incubated at 37°C, with a humidified atmosphere and 5% CO_2 and DMEM culture medium supplemented with L-glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL), fetal serum bovine (10%) and non-essential amino acids (2 mM).

In order to detach the murine macrophage cells, a cell scraper was used and the content was transferred to a falcon, and then centrifuged at 1200 rpm for 5 minutes. The culture medium was discarded and a new one was added in accordance with the size of the pellet obtained. Subsequently, a solution of 70,000 cells/mL was prepared, from which an aliquot (300 μL) was transferred to black microplates with clear-bottom (SPL Lifesciences) and maintained in an incubator during 48h. Once the incubation finished, the medium was discarded, the cells were washed using HBSS (2x, 100 μL), and treated with different concentrations of the extracts (200 μL; 32.5 - 2000 μM) and then incubated for 1 hour. After the incubation, the cells were washed with HBSS (2x, 100 μL) followed by the addition of a 2.2 2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) solution (100 μL; 600 μM). During 1 hour, the fluorescence was measured every 5 minutes (Biotek FLx800 microplate reader) at 485 nm excitation and 538 nm emission. Quercetin was used as a positive control, and dichlorohydroflurescein and DMEM culture medium were used as a negative control.

3.5.2 Antimicrobial activity

3.5.2.1 Antibacterial activity

The microorganisms used to evaluate the antibacterial activity 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.

The Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973), as well as the following Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311) and *Enterobacter cloacae* (clinical isolate) were used in order to determine potential antimicrobial activity of the samples.

The microdilution method was used in order to determine the minimum inhibitory concentration (MIC) as well as the minimum bactericidal concentration (MBC) of the extracts.

The bacterial cultures were adjusted to a concentration of 1×10^5 CFU/mL using the spectrophotometer, which corresponds to a bacterial suspension obtained in a spectrophotometer at 625 nm. The absence of contamination as well as the verification of the validity of the inoculum was checked through the growth of the inoculum dilutions in a solid medium. The wells contained 100 µL of triptych soy broth (TSB), in which the dilutions of the hydroethanolic extracts were pipetted, and then 10 µL of inoculum was added. The microplates were maintained in the incubator during 24 hours at 37°C. To determine the MIC (minimal inhibitory concentration), iodonitrotetrazolium choloride (INT) (40 µL, 0.2 mg/mL) was added, then it was incubated at 37°C for 30 minutes. The MIC was identified as the lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria comparing to the positive control.

The MICs obtained from the susceptibility test of various bacteria to the both pear's varieties extracts (peel and pulp) were determined through a colorimetric microbial viability assay based on the reduction of the INT colour in comparison with positive control for each bacterial strain.

Concerning the MBC (minimum bactericidal concentration), it was determined by sub-cultures in series, which consists of adding 10 µL from wells that did not change color in microplates containing 100 µL of TSB. The MBC was defined as the lowest concentrations that did not show growth once this subculture is achieved. The positive control used were streptomycin and ampicillin, while 5% dimethyl sulfoxide (DMSO) was used as a negative control. The results of MIC and MBC were expressed in mg/mL of *P. communis* and *P. pyrifolia* extracts.

3.5.2.2 Antifungal activity

For the evaluation of antifungal activity, six micromycetes were used: *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum var. cyclopium* (food isolate) and *Trichoderma viride* (IAM 5061). These microfungi were obtained from the Mycology Laboratory of the Department of Plant Physiology of the Biological Research Institute "Siniša Stanković" at the University of Belgrade in Serbia.

The micromycetes were carried on malt agar (MA), and the cultures were stored at 4°C and sub-cultured monthly. The spores of the fungi were washed from the surface of the agar plates with

0.85% sterile saline solution that contain 0.1 % (*v/v*) of Tween 80. The spore suspension was adjusted with a sterile saline solution to a concentration of approximately 1.0×10^5 CFU/mL in a final volume of 100 µL per well. Inocula were stored at 4°C for subsequent use. The dilution of the inocula were grown on solid MA in order to verify the absence of contamination and the inoculum validity. The successive dilution technique in 96-well microplates were used to determine MIC.

The sample solution was added to the malt medium with the fungal inoculum then the microplates were incubated at 28°C for 72 hours. The lowest concentrations with no visible growth (using a binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs) were obtained by serial sub-cultures of 2 µL in microplates that contain 100 µL per well of malt broth, and subsequently incubated at 28°C for 72 hours. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% of loss of the original inoculum. DMSO 5% was used as negative control, and bifonazole and ketoconazole were used as positive control.

The MIC and MFC results were expressed in mg/mL of *P. communis* L. and *P. pyrifolia* hydroethanolic extracts.

3.5.3 Anti-proliferative activity in tumour and non-tumour cell-lines

In order to evaluate the cytotoxic potential **(figure 15)** of the hydroethanolic extracts (80:20, *v/v*), the followig human tumor cell lines were used: AGS (gastric adenocarcinoma), CaCo (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), NCI-H460 (lung carcinoma). In addition, a non-tumor cell line were used: VERO (African green monkey kidney), to test the extracts toxicity. They were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL), except for the VERO cell line, which was maintained in DMEM medium supplemented with fetal bovine serum (10%), glutamine and antibiotics. The flasks containing the cultures were incubated at 37°C, with 5% CO_2, and the air was humidified. The cells were used if they had 70 to 80% confluence.

To obtain stock solutions with a concentration of 8 mg/mL, 8 mg of the extracts was dissolved in water (1 mL). From this solution was made successive dilutions in order to obtain different concentrations to be tested, starting from 0.125 to 8 mg/mL. 10 µL of each of the extract concentrations were incubated with 190 µL of the cell suspension of the cell line tested in a 96-well microplates during 72 hours. The temperature of incubation was set on 37°C and with 5% CO_2, in a humidified atmosphere and after verification of the cell adherance. All the cell lines were

tested at a concentration of 10,000 cells/well, in exception for VERO cells for which a 19,000 cells/well density was used. After the period of the incubation, cold trichloroacetic acid (TCA, 10%; 100 µL) was added to each well, and the microplates were incubated at 4°C for 1 hour. Next the plates were washed with water, and then, once dry, a solution of SRB (0.057%, *m/v*, 100 µL) was added and left for incubation at room temperature during 30 minutes. Subsequently, the plates were washed three times with acetic acid solution (1%, *v/v*) in order to remove non-adhered SRB, and then left to dry. The SRB was solubilized with Tris (10 mM, 200 µL). The absorbance was read at a 540 nm wavelength in a microplate reader (Bio-Tek Instruments, ELX800, Inc; Winooski, EUA). Ellipticin was used as a positive control. The results were expressed in GI_{50} values, which consists of the sample concentration that inhibits by 50% the cell growth, the units, were in µg/mL.

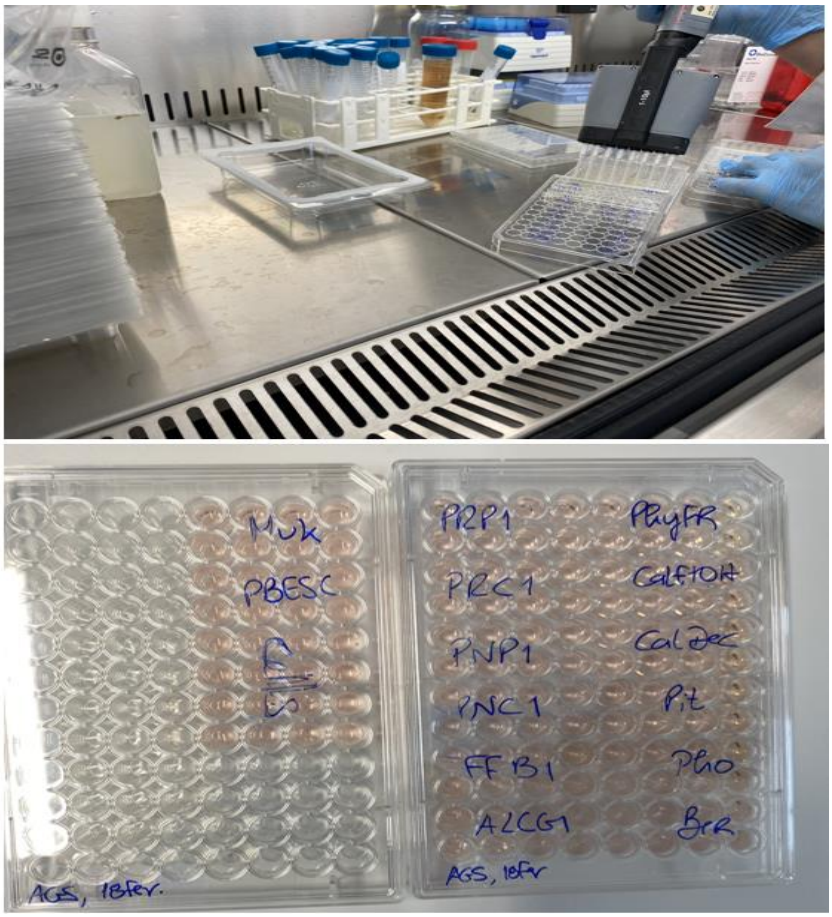


Figure 15 - Evaluation of cytotoxic activity

3.5.4 Anti-inflammatory activity

The evaluation of anti-inflammatory activity **(Figure 16)** was done according a procedure described by Taofiq et al. (2015). The macrophage cells RAW 264.7 were used, due to the ability to inhibit nitric oxide (NO) production.

The extracts were dissolved in H_2O, to obtain a concentration of 8 mg/mL. Successive dilutions were made from this solution in order to obtain the concentration to be tested (0.125 - 8 mg/mL).

The culture of the RAW 264.7 mouse macrophage was made in DMEM medium, supplemented with heat-inactivated (FBS) fetal serum (10%), glutamine and antibiotics, and maintained in an incubator at 37°C, with 5% CO_2, and under humidified air. In order to detach cells with active growth, a cell scraper was used. The cell density was fixed at 5×10^5 cells/mL, with a proportion of dead cells under 5% according to the Trypan blue exclusion test. An aliquot from the cells (300 µL) was then distributed in a 96-well plate. The microplate was then placed in an incubator for 24 hours, to grant adherence and multiplication of the cells. After the incubation period, the cells were treated with different concentrations of *P. communis* and *P. pyrifolia* extracts (15 µL, 0.125 - 8 mg/mL), and then an incubation for 1 hour was carried out. The cells were stimulated with liposaccharide solution (30 µL) LPS (1 mL/mL) then incubated for 24 hours. Negative control was performed without LPS and dexamethasone (50 mM) was used as positive one.

The quantification of the production of nitric oxide was determined using a Griess reagent system kit (nitrophenamide, ethylenediamine and nitrite solutions), as well as the nitrile calibration curve (100 mM sodium nitrite at 1.6 mM), which was prepared in a 96-well plate. The detection of nitric oxide produced was performed through the measure of absorbance at 540 nm (ELX800 Biotek microplate reader, Bio-Tek Instruments, Inc., Winooski, VT, USA), as well as the comparison with the standard calibration line. The results were determined via the graphical representation of the percentage of NO inhibition versus the concentration of the sample and were expressed as the concentration of extract causing 50% inhibition of NO production- IC_{50}.

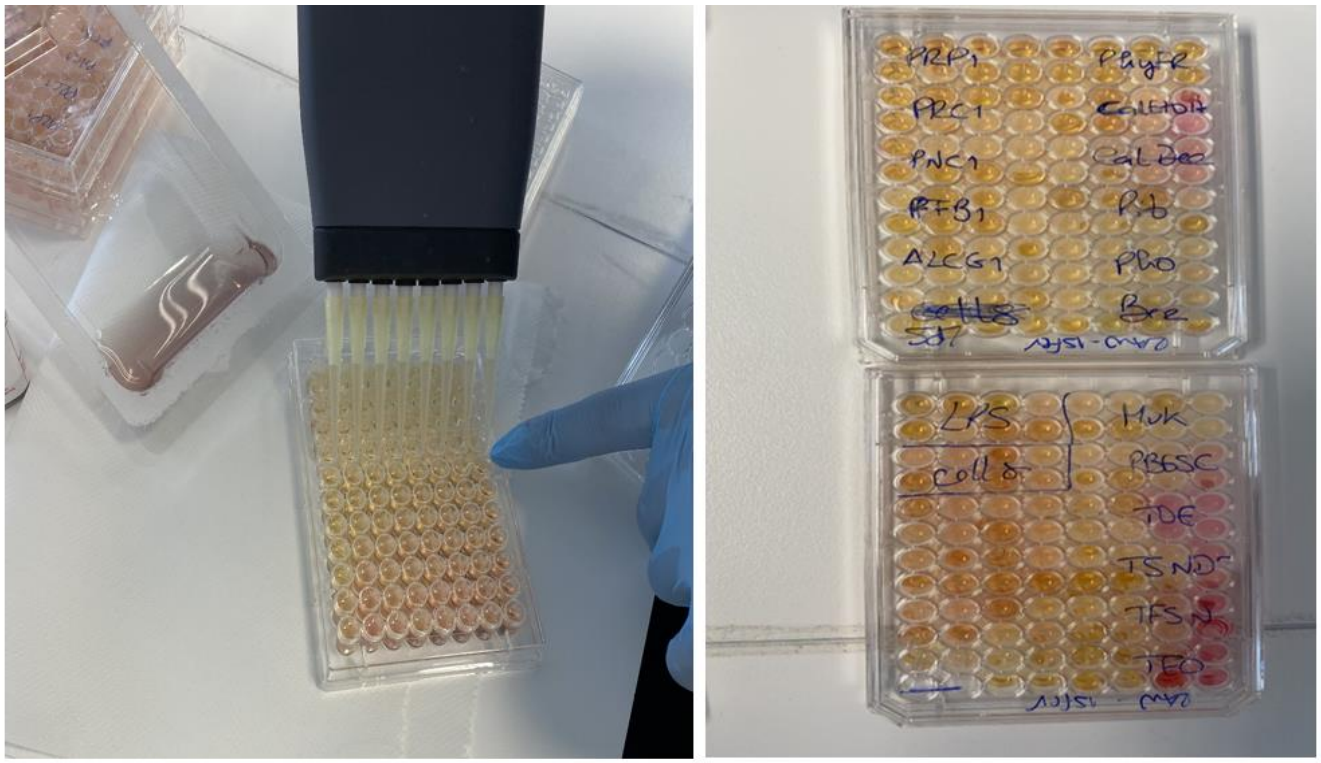


Figure 16 - Procedures for the evaluation of anti-inflammatory activity (Source: from the author)

3.6 Statistical analysis

The assays mentioned in this study were performed in triplicate and the results were expressed as mean ± standard deviation (SD). The statistical analysis of the data was performed to determine the significant differences between the different samples and was done through a one-way ANOVA analysis of variance, considering the different types of comparisons. In each table, was described which statistical test was applied. For this, we used the RStudio program, version 4.1.1 (R Core Team, 2021, Vienna, Austria) with the multcomp package, and then a *t-student* test was applied.

4. Results and Discussion

4.1 Nutritional value of the pulp and peel of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak biowastes

The results of the nutritional characterization of the pulp and peel of both pear's fruit varieties biowastes are present in **Table 4**.

Considering these results, carbohydrates were the highest macronutrients present in all samples (pulp and peel) of the two pears species. The pulp and peel of *P. communis* contained slightly higher amount of carbohydrates (14 ± 1 g/100 g fw for the pulp and 19.71 ± 0.01 g/100 g fw for the peel) when compared with *P. pyrifolia* (10.0 ± 0.2 g/100 g fw and 17.6 ± 0.3 g/100 g fw respectively in pulp and peels). However, in both species the peels contained higher values of carbohydrates. The remaining nutrients founded in the studied samples presented considerably lower concentrations. The protein content detected in *P. communis* and *P. pyrifolia* pulps were 0.299 ± 0.003 g/100 g fw and 0.60 ± 0.02 g/100 g fw, respectively; while in *P. communis* and *P. pyrifolia* peels were 0.390 ± 0.006 g/100 g fw and 0.57 ± 0.02 g/100 g fw, respectively. Also, the fat concentration showed values significantly low, especially in the pulp that contained 0.00516 ± 0.00002 g/100 g fw in *P. communis* and 0.0210 ± 0.0001 g/100 g fw in *P. pyrifolia*. Also in the lipids case, the peel of both species contained more concentration of this macronutrient. The ash composition was higher in pulp comparing to the peels, presenting values of 1.056 ± 0.003 g/100 g fw and 1.20 ± 0.03 g/100 g fw, respectively in *P. communis* and 0.14 ± 0.02 g/100 g fw and 0.36 ± 0.01 g/100 g fw, respectively in *P. pyrifolia*.

Concerning the moisture, the values have shown a high water content in both pulp and peel, with values of humidity ranging between 79.5 ± 0.1 % in *P. communis* pear's peel and 81.3 ± 0.2% in *P. pyrifolia* pear's peel, whereas pulps of both *P. communis* and *P. pyrifolia* had respectively 84.5 ± 0.5% and 87.3 ± 0.1% of water.

The energy level was higher in the peels, presenting value of 82.5 ± 0.1 Kcal/100g in *P. communis* peels and 75 ± 1 Kcal/100 g in *P. pyrifolia* peels, while the pulp of *P. communis* had an energetic value of 58 ± 2 Kcal/100 g and *P. pyrifolia* pear's pulp had 46 ± 1 Kcal/100 g.

So, after applying the statistical treatment, it was possible to verify that there are significant differences in the macronutrient content, when comparing the two pulps and the two skins of both pear varieties ($p < 0.05$).

Table 4 - Nutritional parameters of the pulp and peels of *Pyrus communis* and *Pyrus pyrifolia* (Burm.) Nak biowastes. The results are presented: mean ± sd.

	Pulp			**Peel**		
	P. communis	***P. pyrifolia***	***p-value***	***P. communis***	***P. pyrifolia***	***p-value***
Humidity (%)	84.5 ± 0.5	87.3 ± 0.1	<0.01	79.5 ± 0.1	81.3 ± 0.2	<0.01
Fat (g/100 g fw)	0.00516 ± 0.00002	0.0210 ± 0.0001	<0.01	0.24 ± 0.01	0.27 ± 0.01	<0.01
Proteins (g/100 g fw)	0.299 ± 0.003	0.60 ± 0.02	<0.01	0.390 ± 0.006	0.57 ± 0.02	<0.01
Ash (g/100 g fw)	1.056 ± 0.003	1.20 ± 0.03	<0.01	0.14 ± 0.02	0.36 ± 0.01	<0.01
Carbohydrates (g/100 g fw)	14 ± 1	10.0 ± 0.2	<0.01	19.71 ± 0.01	17.6 ± 0.3	<0.01
Energy (Kcal/100 g)	58 ± 2	46 ± 1	<0.01	82.5 ± 0.1	75 ± 1	<0.01
Energy (Kj/100 g)	242 ± 9	193 ± 3	<0.01	345 ± 1	314 ± 4	<0.01

fw- fresh weight

These results were in accordance with a study conducted by Hussain et al. (2015), that compared two pears (*Pyrus communis* L.) cultivars in terms of nutritional composition, which shows that pears have high carbohydrates and water content, low protein, fat and ash in their composition. In addition, according to Kolniak-Otstek (2016a), that have studied the chemical composition of different anatomical parts of pear (*Pyrus communis* L.), *P. communis* pears have an energetic value of 54 calories.

The previous studies were made on the entire fruit, and only few studies considered the pulp and peel characterization separately. This lack in the information in the literature led to the limitation in comparison with other studies.

Barroca et al. (2006) studied the chemical and microbiological characteristics of portuguese varieties of pears, and the results showed that the pulp of fresh pear has a low content of protein and ash with values ranging between 1.5% and 2.6% for the proteins for the four pear varieties studied (*Amêndoa, Amorim, Carapinheira Branca and S. Bartolomeu*), and the ash values were between 1.6% and 2.4%, which indicates that the samples had a low amount of mineral elements.

A recent study conducted by Lomba-Viana et al. (2022), focused on the valorization of clean label 'Rocha' pear (*Pyrus communis* L.) juice by-prodcucts. The results confirms that the puree and pomace of 'Rocha' pear (*Pyrus communis L.*), have high content of water (around 85 g per 100 g of fresh matter), low content of protein (1.40 and 1.46 g per 100 g of fresh matter, for puree and pomace respectively), a low total lipid level (0.04 g/100 g of fresh matter for the puree and 0.02 g/100 g of fresh matter for the pomace), and the ash values were 0.28 and 0.19 g per 100 g of fresh matter.

The differences observed when comparing the results of other authors with the present study are, certainly, due to the difference of the varieties of studied pears. In general, fruits have always low protein values and high sugars levels.

4.2 Chemical composition of the pulp and peel of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak biowastes

4.2.1 Free sugars, fatty acids and organic acids

The chemical composition's results (free sugars, fatty acids and organic acids) of the pulp and peel of *P. communis* and *P. pyrifolia* are presented in **Table 5**.

The free sugars profile have shown the predominance of fructose in pulp and peel of the both varieties. In fact the pulp has a higher content of fructose than the peel, with 35.6 ± 0.3 g/100 g fw in the pulp of *P. communis* and 27 ± 2 g/100 g fw in the pulp of *P. pyrifolia*; while in the peel the concentrations were 22 ± 1 g/100 g fw and 18.2 ± 0.4 g/100 g fw, respectively. Otherwise, glucose was detected at lower concentrations with values ranging between 8.11 ± 0.04 g/100 g fw (*P. communis* pulp) and 12 ± 0.7 g/100 g fw (*P. pyrifolia* pulp), while the peel had slightly less content (7.1 ± 0.4 g/100 g fw in *P. communis* and 7.4 ± 0.4 g/100 g fw in *P. pyrifolia*). According to statistical treatment presented in **Table 5**, glucose presented a similar result between the two pears' peel samples, since the *p*-value > 0.05. Sucrose was detected in the *P. communis* pulp with a value of 8.36 ± 0.04 g/100 g fw, but with lower concentrations in the remaining parts. Trehalose was the molecule identified at the lowest concentration in the pulp and peel of both pears' varieties, presenting no significant differences in the results (*p*-value > 0.05) for both pulp and peel. Considering the total sugars content, was higher in the pulp (52.3 ± 0.38 g/100 g fw, for *P. communis* and 41.8 ± 2.30 g/100 g fw, for *P. pyrifolia*) comparing to the peels (32 ± 1 g/100 g fw, for *P. communis* and 28 ± 1 g/100 g fw, for *P. pyrifolia*).

The quality of the fruit is considerably affected by the concentration of sugars, which affect the sweetness of the juice of the fruit, while the sugar content of the pulp gives information about the authenticity of fruit juices (Kolniak-Ostek, 2016a).

Wang et al. (2021) investigated the chemical composition of the pulp and peel of *Pyrus ussuriensis* cultivars. The total sugar content was higher in the pulp than in the peel, with values ranging from 54 to 101.9 mg/g fw in the peel and from 73.1 to 113.9 mg/g fw in the pulp. The authors also highlight fructose as the major free sugar, followed by glucose, sucrose and sorbitol. Those previous results are similar to the results of the present study.

According to other study, made by Kolniak-Ostek et al. (2016), the chemical composition and antioxidant capacity of different anatomical parts of pear (*Pyrus communis* L.) was studied and the results showed that fructose is the most abundant sugar in *Pyrus communis* L. especially in the pulp with a value of 227.46 g/kg of dry matter, followed by glucose, sorbitol and sucrose. The concentration of total sugars was most important in the pulp than in the peels, which is in accordance with our results.

Fatty acids were also evaluated in the present study, and the result are shown in **Table 5.** Nineteen fatty acids were identified in the different pear parts (pulp and peel) of the two varieties. Palmitic (C16:0), oleic (C18:1n9) and linoleic acids (C18:2n6c) were the most abundant fatty acids identified in pulps and peels. The palmitic acid presented values that ranging between 16.1 ± 0.4% (for *P. communis* pear's peel) and 32 ± 2% (for *P. communis* pear's pulp). On the other hand, oleic acid stood out with values of 51 ± 1% and 22.9 ± 0.1%, for samples of *P. communis* and *P. pyrifolia* peels, respectively. The third major fatty acid also showed very satisfactory values, ranging between 9.1 ± 0.01% in *P. communis* peels and 49 ± 1% in *P. pyrifolia* pulp. The remaining identified fatty acids were detected in much lower percentages (< 8%).

In general, the saturated fatty acids (SFA) showed values that ranging between 28.0 ± 0.4% and 59 ± 1%, emphasizing in greater quantity in the samples of *P. communis* pulp. Otherwise, the monosaturated fatty acid (MUFA) presented values between 6.6 ± 0.1% and 51 ± 1%, for *P. pyrifolia* pulp and *P. communis* peels, respectively. Finally, the polyunsaturated fatty acids (PUFA) revealed values that ranged from 20.7 ± 0.1% and 54 ± 1%, for *P. communis* peels and *P. pyrifolia* pulp, respectively.

Considering the statistical analyses, was visible a significant difference between pulp and peel for all the fatty acids (presented in **Table 5**), (p-value < 0.05), except for the palmitic acid when comparing the peels of both pears' varieties.

One of the predominant fatty acids in this study was the linoleic acid (C18:2n6), described as an essential for health promotion, growth and human development, and as humans cannot synthetize it, they should be obtained from exogenous sources (Le et al., 2013). Moreover, linoleic acid is an essential fatty acid that is the source of the whole ω−6 fatty acid family (Lai et al., 2015). Also, the consumption of oleic acid has shown beneficial effects on serum total lipid, triacylglycerol and glutathione, as well as protective effect against cardiovascular diseases and diabetes (Emekli-Alturfan et al., 2010).

According to a study conducted by Chen et al. (2007) with eight pear cultivars evaluating their chemical characterization, they have found that the most abundant fatty acids were linoleic and palmitic acid, constituting about 70-80% of the total fatty acids in the pears evaluated. For the palmitic acid, the values ranged between 22 ± 1% and 29 ± 1% which is quite close to our results. Linoleic acid percentages ranged between 59 ± 2% and 69 ± 1%, which is partially close to the results of the present study, especially in *P. pyrifolia* pears. This could be explained due to the fact that the pears of the above-mentioned study and *P. pyrifolia* pears belongs to the same geographical area and thus they may have similarities in environmental and genetic conditions which may affect the fatty acid profile (Sánchez-Salcedo et al., 2016). Chen et al. (2007), characterized chemically eight pear cultivars, and have also found low quantities of α-linolenic acid (C18:3n3) (between 1.13 ± 0.04% and 6.86 ± 0.03%), which agrees with the present result. This is of nutritional importance since diets high in meat, carbohydrates, fruit and vegetables are often low in ω-3 fatty acids (Chen et al., 2007).

PUFA are extremely important for human health, and particularly for the nervous system, since they play a major role in the memory, neurogenesis, learning, and emotions, they help to maintain the brain structure during embryonic and adult stages (Khan & He, 2017). In turn, MUFA help to maintain a healthy blood lipid profile, regulate blood pressure and regulate insulin sensitivity and glycemic control, however the intake of SFA must be reduced and replaced with unsaturated fats, in order to promote a good cardiovascular health (Gillingham et al., 2011).

Concerning the organic acids (**Table 5**), all the samples analysed presented in their composition six organic acids including oxalic, quinic, malic, shikimic, citric and fumaric acids.

Malic acid presented the highest values in both pear varieties, presenting respectively in the pulp of *P. communis* and *P. pyrifolia* 19.3 ± 0.2 mg/100 g fw and 15 ± 1 mg/100 g fw, while in the peels the values were 15.4 ± 0.2 mg/100 g fw and 8.6 ± 0.1 mg/100 g fw respectively, for *P. communis* and *P. pyrifolia* pears. Quinic acid was the next most important organic acid and presented the following values for *P. communis* pears: 2.1 ± 0.1 mg/100 g fw and 7.9 ± 0.3 mg/100 g fw respectively in pulp and peel. In *P. pyrifolia*, the values of quinic acid were 11.3 ± 0.1 mg/100 g fw and 2.6 ± 0.2 mg/100 g fw in pulp and peel respectively. Oxalic acid was the third most important organic acids in the samples, especially in the pulp, which contained slightly higher values than the peels, the values in peels were as following: 2.17 ± 0.03 mg/100 g fw in the pulp of *P. communis* and 2.96 ± 0.02 mg/100 g fw in the pulp of *P. pyrifolia.* While in the peels, oxalic acid represented values of 1.80 ± 0.01 mg/100 g fw in the *P. communi*s variety and 1.39 ± 0.03 mg/100 g fw in *P. pyrifolia* variety. The concentration of citric acid was important in the pulp of *P. pyrifolia* pears (8.7 ± 0.4 mg/100 g fw) and in the peels of *P. communis* pears (7.0 ± 0.4 mg/100 g fw), whereas in the pulp of *P. communis* and in the peels of *P. pyrifolia* the values of citric acid were much lower (0.96 ± 0.02 mg/100 g fw and 0.66 ± 0.03 mg/100 g fw respectively in the pulp of *P. communis* and peels of *P. pyrifolia*). Regarding shikimic acid, the concentrations were low and detected in the pulp with values of 0.20 ± 0.01 mg/100 g fw and 1.64 ± 0.04 mg/100 g fw respectively for *P. communis* and *P. pyrifolia* pear's varieties, while in the peels the values were as following 0.569 ± 0.004 mg/100 g fw and 0.129 ± 0.004 mg/100 g fw. Fumaric acid was identified as the lowest concentration, in the pulp the values ranged between 0.0042 ± 0.001 mg/100 g fw for *P. communis* and 0.083 ± 0.001 mg /100 g fw for *P. pyrifolia*, and for the peels, the concentrations were 0.061 ± 0.003 mg/100 g fw in the peels of *P. communis* L. and 0.028 ± 0.001 mg/100 g fw in the peels of *P. pyrifolia*.

The total concentration of organic acids in the pulp of *P. pyrifolia* pear presented the highest value (40 ± 1 mg/100 g fw), while in the pulp of *P. communis* pear it was 24.7 ± 0.3 mg/100 g fw. The total concentration in the peels of *P. communis* was higher (33 ± 1 mg/100 g fw) than in the peels of *P. pyrifolia* (13.5 ± 0.4 mg/100 g fw).

When comparing statistically the two pulps and the two peels of both varieties of pears, we can conclude that all the results presented a significant difference (p-value < 0.05).

Organic acids have a major role in the flavour of fruit and in nutrition. The fresh fruits contains essentially malic, citric, quinic and tartaric acids (Wu et al., 2022). Moreover, organic

acids have shown an important potential of extending the shelf life of fresh fruits and their processed products, through their ability to stabilize anthocyanins (Ma et al., 2015).

Chen et al. (2007) evaluated the chemical composition of eight pear cultivars from China, and described that malic, citric, quinic and shikimic acids were the main organic acids, with malic acid the predominant one in the studied pears. Those results were consistent with the present study where malic acid were the most abundant organic acid in the pulp and skin of both pear's varieties.

Wu et al. (2022) have quantified the organic acids of 193 pear cultivars using UPLC method and have demonstrated that malic and citric acid contributed to about 70% of the total organic acid content, and that shikimic acid had the lowest concentration. These results were partially in accordance with the present study, as shikimic, oxalic and fumaric acids presented the smallest values, and the differences may be caused by the differences of the species as well as the largest number of pears studied in the previously mentioned study.

A study developed by Kolniak-Ostek (2016a), focused on the assessment of the chemical composition and antioxidant capacity of different anatomical parts of *Pyrus communis* L., the results of the detected malic acid were important in the peels when comparing to the pulp. The oxalic acid presented quite similar values in both pulp and peel. Regarding citric acid, it presented higher value in the pulp. Shikimic acid was mostly detected in the pulp. However, fumaric acid was totally absent in both pulp and peel. Those results were partially consistent with the result in present study, and there were considerable differences among the values. In fact, the values were expressed as g/Kg of dry matter, and dry tissues always present higher values, because of the freeze-drying process, which increases the concentration of the substances in fruit tissues.

Table 5 - Composition of free sugars, fatty acids and organic acids in pulp and peel of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak pears (mean±sd).

	Pulp			**Peel**		
	P. communis	***P. pyrifolia***	***p*-value**	***P. communis***	***P. pyrifolia***	***p*-value**
Free Sugars (g/100 g fw)						
Fructose	35.6 ± 0.3	27 ± 2	0.016	22 ± 1	18.2 ± 0.4	0.02
Glucose	8.11 ± 0.04	12.0 ± 0.7	0.02	7.1 ± 0.4	7.4 ± 0.4	0.6
Sucrose	8.36 ± 0.04	2.5 ± 0.1	<0.001	3.0 ± 0.2	1.7 ± 0.02	0.01
Trehalose	0.23 ± 0.02	0.29 ± 0.04	0.2	0.20 ± 0.03	0.18 ± 0.04	0.6
Total sugars	52.3 ± 0.4	41.8 ± 2.3	0.02	32 ± 1	28 ± 1	0.04
Fatty acids (%)						
C11:0	2.21 ± 0.01	1.501 ± 0.002	<0.001	n.d.	n.d.	n.a.
C12:0	n.d.	n.d.	n.a.	n.d.	0.46 ± 0.01	n.a.
C14:0	2.97 ± 0.04	1.66 ± 0.05	<0.001	1.15 ± 0.02	0.65 ± 0.02	0.002
C15:0	1.87 ± 0.03	1.34 ± 0.03	0.003	0.87 ± 0.02	1.29 ± 0.04	0.004
C16:0	32 ± 2	24 ± 1	0.02	16.1 ± 0.4	16.51 ± 0.02	1
C17:0	6.1 ± 0.3	0.98 ± 0.01	0.002	n.d.	0.347 ± 0.004	n.a.
C18:0	8.0 ± 0.1	5.0 ± 0.2	0.002	5.6 ± 0.2	3.6 ± 0.1	0.006
C18:1n9	15.6 ± 0.2	6.6 ± 0.1	<0.001	51 ± 1	22.9 ± 0.1	<0.001
C18:2n6	25 ± 1	49 ± 1	0.001	9.1 ± 0.1	36.1 ± 0.4	<0.001
C18:3n6	n.d.	n.d.	n.a.	n.d.	3.37 ± 0.04	n.a.
C18:3n3	n.d.	3.16 ± 0.05	n.a.	5.0 ± 0.1	1.76 ± 0.01	<0.001
C20:0	n.d.	1.27 ± 0.04	n.a.	2.0 ± 0.1	1.07 ± 0.04	0.003
C20:1	n.d.	n.d.	n.a.	n.d.	1.21 ± 0.01	n.a.
C20:4n6 + C20:3n3	n.d	n.d.	n.a.	6.5 ± 0.1	5.5 ± 0.1	0.01
C22:0	2.49 ± 0.04	2.00 ± 0.04	0.006	2.85 ± 0.04	1.61 ± 0.03	<0.001
C22:2	n.d.	1.0 ± 0.1	n.a.	n.d	n.d.	n.a.
C23:0	1.25 ± 0.02	n.d.	n.a.	n.d	n.d.	n.a.
C24:0	1.88 ± 0.03	1.64 ± 0.04	0.02	n.d.	2.5 ± 0.1	n.a.
C22:6n3	n.d.	0.42±0.01	n.a.	n.d.	n.d.	n.a.
SFA	59 ± 1	39 ± 1	0.002	29 ± 1	28.0 ± 0.4	0.02
MUFA	15.6 ± 0.2	6.6 ± 0.1	0.0004	51 ± 1	25.3 ± 0.1	0.0002
PUFA	25 ± 1	54 ± 1	0.001	20.7 ± 0.1	46.8 ± 0.3	<0.001
Organic Acids (mg/100 g fw)						
Oxalic acid	2.17 ± 0.03	2.96 ± 0.02	<0.001	1.80 ± 0.01	1.39 ± 0.03	<0.001
Quinic acid	2.1 ± 0.1	11.3 ± 0.1	<0.001	7.9 ± 0.3	2.6 ± 0.2	<0.001
Malic acid	19.3 ± 0.2	15±1	<0.001	15.4 ± 0.2	8.6 ± 0.1	<0.001
Shikimic acid	0.20 ± 0.01	1.64 ± 0.04	<0.001	0.569 ± 0.004	0.129 ± 0.004	<0.001
Citric acid	0.96 ± 0.02	8.7 ± 0.4	<0.001	7.0 ± 0.4	0.66 ± 0.03	<0.001
Fumaric acid	0.042 ± 0.001	0.083 ± 0.001	<0.001	0.061 ± 0.003	0.028 ± 0.001	<0.001
Total	24.7 ± 0.3	40 ± 1	<0.001	33 ± 1	13.5 ± 0.4	<0.001

n.d: not detected; n.a.: not applied. Undecylic acid (C11:0); Lauric acid (C12:0); Myristic acid (C14:0); Pentadecylic acid (C15:0); Palmitic acid (C16:0); Margaric acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6c); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3n3); Arachidic acid (C20:0); eicosenoic acid (C20:1c); Arachidonic acid + Eicosatrienoic acid (C20:4n6 + C20:3n3); Behenic acid (C22:0); cis-13. 16- docosadienoic acid (C22:2); Tricosylic acid (C23:0); Lignoceric acid (C24:0); cis-4. 7. 10. 13. 16. 19- docosahexaenoic acid (C22:6n3). SFA – Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids.

4.3 Total phenols and flavonoids

The concentration of total phenols and total flavonoids detected in all samples (pulp and peels of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak) is presented in **Table 6**.

The *P. pyrifolia* peel stands out as the sample that presented the highest content of total phenols and, consequently, the highest content of total flavonoids, with values of 7.2 ± 0,1 GAE/g of extract and 3.3 ± 0,1 CE/g of extract, respectively. The highest concentration of total phenols in the peels was transversal for both species. Considering the statistical treatment applied, significant differences ($p < 0.05$) were detected between the shells of the different species analyzed (both for phenols and for total flavonoids).

Table 6 – Total phenols and flavonoids of the extract of of *P. communis* and *P. pyrifolia* pulps and peels.

Sample		**Total phenols** (GAE/g extract)	**Total flavonoids** (CE/g of extract)
Pulp	***P. communis***	0.57 ± 0.02	0.42 ± 0.01
	P. pyrifolia	0.57 ± 0.02	0.53 ± 0.05
	***p*-value**	0.978	0.245
Peel	***P. communis***	3.3 ± 0.1	tr
	P. pyrifolia	7.2 ± 0.1	3.3 ± 0.1
	***p*-value**	0.02	n.a

n.a: non applied ; tr: traces; GAE: galic acid; CE: catechin equivalentes.

Öztürk et al. (2015) studied the phenolic compounds and the chemical characteristics of the peel and pulp in some pear cultivars (*Pyrus communis* L.) and have found that peels are richer in phenolic compounds than the flesh, with arbutin and chlorogenic acid as the major phenolics in the peels. Chlorogenic acid is known as a powerful antioxidant preventing of many diseases such as cardiovascular diseases and cancer with the enhancement of the immune system also.

Another study made by Lee et al. (2015), in which bioactive compounds contents an antioxidative activities in the peel and pulp of *Pyrus pyrifolia* Nakai were compared, has showed that total phenolics and flavonoids were more concentrated in the peels than in the flesh. In addition, the peels exhibited higher antioxidative activity which is strongly related to the presence of phenolic compounds.

So, consider the literature data, the results obtained in the present study are in accordance with the other authors.

4.4 Evaluation of bioactive properties of peel and pulp of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak pears

4.4.1 Antioxidant activity

The antioxidant potential of the two pears varieties: *P. communis* and *P. pyrifolia* (pulp and peel) was evaluated using two *in vitro* assays: inhibition of lipid peroxidation – TBARS and Cellular Antioxidant Activity – CAA. The results are shown in **Table 7** (TBARS) and **Table 8** (CAA).

In the TBARS assay, the hydroethanolic extracts of the pulp and peel have shown antioxidant potential, especially in *P. communis* pears' peels. The EC_{50} values were as following: 2 ± 1 mg/mL in *P. communis* pulp, 3.7 ± 0.3 mg/mL in *P. pyrifolia* pulp, 0.16 ± 0.02 mg/mL in *P. communis* peel and 0.30 ± 0.05 mg/mL in *P. pyrifolia* peel. The significant difference in the results between both pulps and peels is confirmed statistically ($p < 0.05$).

However, only the peel's extract of *P. communis* have shown a great antioxidant potential, as the EC_{50} value were lower than the sodium metabisulfite (E223) control, and the calcium ascorbate (E302) control. This could be a good alternative for the use of *P. communis* pear peels as additive in the food sector as antioxidant, increasing the shelf life of foods.

In the CAA assay, only the *P. pyrifolia* pear's peel has shown an antioxidant potential when using a maximum extract concentration of 2000 µg/mL which gives 25 ± 1% of oxidation inhibition. The remaining tested samples required a concentration higher than 2000 µg/mL.

The antioxidants naturally present in fruit and vegetables have benefits in human health, through scavenging free radicals from damaging biological organs, tissues and cells. In addition, the antioxidant activity is strongly correlated to the presence of phenolic compounds (Li et al., 2012).

Patricia et al. (2020) evaluated the antioxidant properties of *Pyrus communis* and *Pyrus pyrifolia* peel extracts, and found that the peels of both varieties have antioxidants potentials, but *P. communis* peel extract showed a higher antioxidant content, which agrees with the present study.

Lee et al. (2015) compared bioactive compound content and antioxidative activities of the peel and flesh *Pyrus pyrifolia* Nakai using the free radical scavenging activity (DPPH), $ABTS^{+}$,

nitric radicals and reducing capacity. They have demonstrated that the peels revealed higher free radical scavenging and reducing activities compared to the pulp. Moreover, the authors have also performed *ex vivo* assays, administrating peel and flesh extracts to rats and the peel extract have shown greater antioxidant activity in their blood plasma.

In another study performed by Kolniak-Ostek (2016a), antioxidant activity of different anatomical parts of pear (*Pyrus communis* L.) were compared. DPPH method and ferric reducing capacity by the FRAP method were used, and the highest DPPH and FRAP values were detected in leaves, followed by seeds and peel, while the pulp had the lowest DPPH and FRAP antioxidant values. The antioxidant activity of the different anatomical parts of the pear fruit was linked to polyphenolic compounds.

Table 7 - Antioxidant activity of the extract of pulp and peel of *P. communis* and *P. pyrifolia* using TBARS assay.

Sample		TBARS (EC_{50} value, mg/mL)
Pulp	***P. communis***	2 ± 1
	P. pyrifolia	3.7 ± 0.3
	***p*-value**	0.05
Peel	***P. communis***	0.16 ± 0.02
	P. pyrifolia	0.30 ± 0.05
	***p*-value**	0.01

EC_{50} values: Extract concentration corresponding to 50% of antioxidant activity.
Positive control: Trolox EC_{50} value: 0.023 ± 0.0001 mg/ml; E223 EC_{50} value: 0.228 ± 0.00009 mg/ml and E302 EC_{50} value: 0.289 ± 0.00001 mg/ml

Table 8 - Antioxidant activity of the pulp and peel of *P. communis* and *P. pyrifolia* using CAA assay.

Sample	[ ] max tested (µg/mL)	% inhibition [ ] max tested
***P. communis* pulp**	2000	>2000
***P. communis* peel**	2000	>2000
***P. pyrifolia* pulp**	2000	>2000
***P. pyrifolia* peel**	2000	25 ± 1

Positive control: Quercetin: 95 ± 5% oxidation inhibition at 0.3 µg/mL.

4.4.2 Antimicrobial activity

The antibacterial and antifungal potential of peel and pulp of both pear varieties are shown in **Table 9** and **Table 10,** respectively. The hydroethanolic extracts were tested against a variety of bacteria and fungi, selected according to their impact in food sector as well as human health.

Concerning the antibacterial activity, the inhibitory and bactericidal capacity were obvious in all the bacterial strains used. The best inhibitory capacity was obtained with *Staphylococcus aureus* and *Bacillus cereus*, since all the minimal inhibitory concentrations (MIC) were lower or the same as the control (E211 and E224). In the case of *Staphylococcus aureus*, the MIC values of the extracts varied between 2 and 4 mg/mL with *P. communis* having a better antimicrobial activity (pulp and peel). The MIC values against *Bacillus cereus*, ranged between 0.5 and 1 mg/mL, and extracts of *P. communis* pear showed again higher antimicrobial potential. For the remaining strains, all the MIC values were 2 mg/mL, with a promising inhibitory activity against *Enterobacter cloacae*, since MIC was reached the same way as E211 control, while higher concentration of the hydroethanolic extracts was required to inhibit the remaining bacteria. The best bactericidal activity was also found to be against *Staphylococcus aureus* and *Bacillus cereus*, as the minimal bactericidal concentration was equal or lower than the E211 or E224 controls. All the remaining MBC values were 4 mg/mL, and the best bactericidal activity was against *Enterobacter cloacae*, because the MBC value was reached the same way as E211 control.

Regarding the antifungal activity, all the obtained results including inhibitory and fungicidal potential were evident against the all the tested fungi. The minimal inhibitory concentration (MIC) values ranged between 0.125 and 1mg/mL, and the minimal fungicidal concentration (MFC) varied between 0.25 and 2mg/L which was the same or even lower than the controls (E211 and E224). The best inhibitory and fungicidal capacities were obtained against *Aspergillus fumigatus*, since the values were lower than both controls (E211 and E224), but this does not negate the good antifungal activity against the other strains.

Table 9- Antibacterial activity (MIC and MBC, mg/mL) of peel and pulp extracts of *P. communis* and *P. pyrifolia*.

Samples			*Staphylococcus aureus*	*Bacillus cereus*	*Listeria monocytogenes*	*Escherichia coli*	*Salmonella typhimurium*	*Enterobacter cloacae*
Pulp	***P. communis***	MIC	2	0.5	2	2	2	2
		MBC	4	1	4	4	4	4
	P. pyrifolia	MIC	4	1	2	2	2	2
		MBC	8	2	4	4	4	4
Peel	***P. communis***	MIC	2	0.5	2	2	2	2
		MBC	4	1	4	4	4	4
	P. pyrifolia	MIC	2	0.5	2	2	2	2
		MBC	4	1	4	4	4	4
Control	**E211**	MIC	4	0.5	1	1	1	2
		MBC	4	0.5	2	2	2	4
	E224	MIC	1	2	0.5	0.5	1	0.5
		MBC	1	4	1	1	1	0.5

MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration
Sodium benzoate (E211) and potassium metabisulphite (E224) food additives were used as positive controls.

Table 10- Antifungal activity (MIC and MFC, mg/mL) of peel and pulp extracts of *P. communis* and *P. pyrifolia.*

Samples			*Aspergillus fumigatus*	*Aspergillus versicolor*	*Penicillium funiculosum*	*Penicillium verrucosum var. cyclopium*	*Trichoderma viride*
Pulp	***P. communis***	MIC	0.25	0.5	0.25	0.5	0.25
		MFC	0.5	1	0.5	1	0.5
	P. pyrifolia	MIC	0.125	0.5	0.125	0.5	0.25
		MFC	0.25	1	0.25	1	0.5
Peel	***P. communis***	MIC	0.125	0.5	0.25	0.5	0.5
		MFC	0.25	1	0.5	1	1
	P. pyrifolia	MIC	0.125	1	0.25	1	1
		MFC	0.25	2	0.5	2	2
Control	**E211**	MIC	1	2	1	2	1
		MFC	2	2	2	4	2
	E224	MIC	1	1	0.5	1	0.5
		MFC	1	1	0.5	1	0.5

MIC: Minimal Inhibitory Concentration; MFC: Minimal Fungicidal Concentration
Sodium benzoate (E211) and potassium metabisulphite (E224) food additives were used as positive controls.

Fattouch et al. (2008), compared antioxidant and antimicrobial activities of Tunisian pome fruits' pulp and peel, including apple, pear and quince. In their antimicrobial results, it was found that the active extracts were effective against *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Particularly, the peel extracts have shown a greater antimicrobial activity than the pulp.

Bacillus species and particularly *Bacillus cereus* are known as food poisoning organisms, causing various nongastrointestinal diseases, and other symptoms linked to food poisoning such as diarrhea or emesis (Rowan et al., 2003). In this way, the promising results found in the present study could be exploited in order to control those food-poisoning strains.

Güven et al. (2006) used the agar-well diffusion assay to evaluate the antimicrobial activities of fruits of *Crataegus* and *Pyrus* species and found that the extracts of *Pyrus serikensis* had an antimicrobial effect against all the bacteria tested and some fungi and yeasts. However, when compared to *Pyrus communis*, it was shown that *Pyrus serikensis* had a higher activity, which could be used in pharmaceutical field.

The results obtained in present study are in agreement with the literature, since pear's extract have shown a promising antimicrobial activity, especially in the peels. The few differences detected may be due to the difference of the studied varieties, or to the methods used to evaluate the antimicrobial potential.

4.4.3 Cytotoxicity and anti-inflammatory activity

The cytotoxicity and anti-inflammatory activity of all samples analyzed are presented in **Table 11.** Taking into consideration the values, the hydroethanolic extracts obtained from pulp and peel of *Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak had not shown anti-proliferative activity in all tumor cell lines. However, both pears' varieties extracts from pulp and peel had no cytotoxicity against VERO non-tumor cell lines.

Regarding the anti-inflammatory activity, none of the extracts had shown anti-inflammatory potential in RAW 264.7 mouse macrophage cell line.

Table 11- Cytotoxic and anti-inflammatory activities of pulp and peel extracts of *P. communis* and *P. pyrifolia*.

Samples		Cytotoxicity					Anti-inflammatory (values IC_{50}, µg/mL)
		Tumor cell lines (values GI_{50}, µg/mL)				Non tumor cell lines (values GI_{50}, µg/mL)	
		AGS	**Caco2**	**MCF-7**	**NCI-H460**	**VERO**	**RAW 264.7**
P. communis	Pulp	>400	>400	>400	>400	>400	>400
	Peel	>400	>400	>400	>400	>400	>400
P. pyrifolia	Pulp	>400	>400	>400	>400	>400	>400
	Peel	>400	>400	>400	>400	>400	>400
Control	Ellipticine (µg/mL)	1.23 ± 0.03	1.21 ± 0.02	1.02 ± 0.02	1.01 ± 0.01	1.41 ± 0.06	
	Dexamethasone (µg/mL)						6.3 ± 0.4

GI_{50} – concentration that inhibited 50% of cell growth. IC_{50}-concentration of extract causing 50% inhibition of NO production- IC_{50}.Ellipticine and Dexamethasone were used as positive control.
GI_{50} and IC_{50} > 400 µg/mL- does not have activity.

Kolniak-Ostek et al. (2020), studied the antiproliferative activity of five pear cultivars (*Pyrus communis* L.) against six tumor cell lines and, they observed an important cytotoxic activity with the cultivar 'Radana' and 'Conference' especially against tumor cells, with the higher activity against HCV-29T (bladder cancer).

El-Hawary et al. (2018), have also confirmed the cytotoxic activity of volatile compounds of pear cultivar 'Le-Conte' as well as apple cultivar 'Anna' with the highest value against A-549 (Human lung carcinoma) tumor cell line, followed by M-NFS-60 (Mouse myelogenous leukemia cell).

In 2014, Li et al. demonstrated that most of the methanolic extracts of 10 pear varieties (*Pyrus spp*.) had an important anti-inflammatory activity. In this sense, they have used Carrageenan-induced mice hind paw edema and xylene-induced mice ear edema models in order to study the anti-inflammatory activities.

Moreover, Li et al. (2012) have shown that the anti-inflammatory potential was strongly correlated to the content of triterpenes, particularly oleanolic and ursolic acids, and arbutin which is a phenolic compound that is present in the pears' varieties studied.

A more recent study conducted by Sun et al. (2021), determined the anti-inflammatory activity of *Pyrus ussuriensis* pear variety using the anti-denaturation assay since protein-

denaturation is a cause of inflammation. These authors have shown that *Pyrus ussuriensis* methanol extracts exhibited an outstanding anti-inflammatory potential inhibiting and preventing protein denaturation comparing to the diclofenac sodium control.

When comparing the previous results with those obtained in the current study, huge differences can be observed, since our extracts have not shown anti-proliferative and anti-inflammatory activities. The difference of the pears varieties, the type of extracts, the methods used to assess the bioactivities, could affect the obtained results.

5. Concluding remarks

The large amount of bio-waste generated by industries, especially the food industry has led the scientific community to take urgent decisions to minimize the negative impact caused by these residues.

In this sense, a complete characterization of two pears varieties (*Pyrus communis* L. and *Pyrus pyrifolia* (Burm.) Nak) bio-wastes was done. Those bio-wastes consists of peels, pulp, and the entire fruit when it doesn't have the necessary marketing standards to proceed to the market such as the shape, colour or size. Therefore, discarding those bio-wastes lead to the loss of its commercial value and contributes to the degradation of the environment through the mismanagement of practices when dealing with wastes.

The characterization of both pears' varieties including a nutritional and chemical characterization as well as an evaluation of the bioactive potential of the pulp and peel intends to promote the valorization of its bio-wastes.

The nutritional composition has shown that carbohydrates were the major macronutrient in both pulp and peels of *P. communis* and *P. pyrifolia*, and thus the two pears' varieties are a good source of energy. Adding to that, the chemical characterization of the pulp and skin of the two pears' varieties revealed that fructose was the main sugar, and malic acid had the highest concentration among the six organic acids found. The fatty acid composition has shown the predominance of palmitic acid, oleic acid and linoleic acid among nineteen fatty acids detected.

Regarding the bioactive potential of pulp and peel extracts, only the peels of the two pears' varieties have shown a great antioxidant activity that may be strongly related to the presence of total phenols and total flavonoids. Otherwise, the hydroethanolic extracts of pulp and peel revealed promising antimicrobial and cytotoxic activities.

Considering these results, it is possible to conclude that the biowastes of both pears varieties could be considered as a promising source of bioactive compounds and functional ingredients to be exploited on an industrial scale. In fact, due to their potent antioxidant and antimicrobial activities, the biowastes of both pear varieties studied are an excellent ingredient to incorporate in food industry in order to preserve food, protecting it from oxidation, and microorganism

proliferation, thus prolonging its shelf life. On another side, pharmaceutical and cosmetic field could also incorporate the extract with powerful bioactivities in their formulations.

This study emphasized the importance of the use of bio-wastes and residues to extract biologically active molecules with several health benefits that can be incorporated in different products, thus resolving a global waste problem that weighs on global economy, environment, and health, since we have chosen two pear varieties that belongs to the European and Asian continent. Furthermore, the recovery of bio-wastes in the industrial field will considerably promote the circular economy and contribute to the zero-waste goal.

Finally, as a perspective of this experimental study, the incorporation of the extracts obtained from pulp and peel of *P. communis* and *P. pyrifolia* as functional ingredients in a food product could be evaluated, since the bioactive evaluation have shown great antioxidant and antimicrobial potentials.

6. References

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