

Bio-Residues Analysis of Fruit Crops

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Contributor: Lillian Barros

Food processing generates a large amount of bio-residues, which have become the focus of different studies aimed at valorizing this low-cost source of bioactive compounds. High fruit consumption is associated with beneficial health effects and, therefore, bio-waste and its constituents arouse therapeutic interest.

bio-residues

Portuguese fruit crops

bioactive compounds

apple

orange

pear

1. Introduction

Recent statistics showed that European food processing units might generate approximately 100 Mt of waste and by-products each year, mostly comprising the production of drinks (26%), dairy and ice cream (21.3%), and fruit- and vegetable-derived products (14.8%) ^[1]. According to the Food and Agriculture Organization of the United Nations (FAO), one-third of the world's food production (1.3 billion tons) is lost or wasted ^[2].

The term food loss is associated with food spoilage before reaching its final destination; in turn, food waste consists of food that is not consumed and is discarded or left to spoil by retailers or consumers. Despite being different concepts, food loss and food waste both cause a decrease in the availability of food for human consumption along the whole supply chain. However, food waste can still be suitable for human consumption. In this sense, the terms bio-waste and bio-products also arise. In the present review, the term bio-waste or bio-residue will be adopted, under different circumstances, to refer to waste, i.e., any product/compound without economic value generated from any process (e.g., apple pomace). On the other hand, the term by-product will be generally used to refer to products that are only discarded because they do not meet a specific production/consumption requirement. However, they maintain their physical-chemical or quality properties (e.g., a fruit that does not have the appropriate size). The FAO report showed that more than 40% of food losses in developed countries occur in retail and consumer markets ^{[2][3]}.

Regarding fruits, there is a considerable percentage that reaches the consumer not as the whole fruit itself, but in processed formulations, such as juices or pulps. For example, tomatoes are frequently sold in the form of tomato paste, juice, or sauce. In some of these formulations, seeds, skin, and pomace must be separated, resulting in fruit bio-residues, commonly used in low-value applications such as feed or fodder ^{[4][5]}. As fruit bio-residues are considered food waste, their economic value is low. In this way, they may represent a financial problem for companies, especially those producing them in large quantities. In most cases, given the lack of applicability of these bio-residues, they do not present any economic advantage for the manufacturing units that need to dispose of them ecologically and responsibly. To reduce the potential environmental impact of these residues while

providing additional economic benefits, the scientific community has been focused on this subject in the last years, seeking different valorization alternatives [3][4][6][7]. The food industry's bio-residues have been identified as an excellent source of bioactive and functional compounds, with possible applications in nutraceutical and pharmaceutical formulations [6]. It is important to highlight that these residues can also be used to generate energy, either as heat, steam, or electricity, helping to reduce the energy invoice [7]. Biorefineries have stood out in this context, mainly concerning biomass conversion processes for the production of fuels, energy, heat, and value-added chemicals from biomass residues [8]. Furthermore, biorefineries have many environmental and economic advantages compared to traditional technology [9][10][11]. These aspects demonstrate the great importance and need for innovative research to discover suitable and under-valued agro-industrial bio-residues and by-products, as well as developing the most sustainable and efficient extraction methodologies to obtain bioactive compounds of interest [8].

This paradigm is also associated with currently relevant concepts, such as bioeconomy and circular economy [12], since the drastic increase in energy consumption and the deterioration of the environment forced us to retreat and move from a linear economy (dependent on fossil fuels) to a sustainable circular bioeconomy (based on green resources, energy, and methodologies, with zero-waste generation) [13][14]. Fundamentally, the intention is to replace the orthodox idea of the end of life with the concept of regeneration, increase the use of renewable energy sources, minimize the use of toxic chemicals and, in general, eliminate waste [8]. Given the world's challenges related to climate change, resource depletion and energy, and food security, the circular economy is expected to develop sustainably [15].

It is a fact that the modern world has a severe problem with wasting food and by-products. Therefore, it is necessary to find sustainable solutions for these residues to be used at their full potential. Following this line of thought, this review's main objective is to summarize the bioactive compounds present in the main Portuguese fruit crops and their biological activities, further evaluating their potential applications.

The Current Status of Fruit Production in Portugal

In Portugal, the estimated average production of apples is 265,000 t/year, being the main permanent crop in mainland Portugal, followed by orange and pear [16]. Currently, 14,580 ha are used for apple production in the country. Data indicate that in 2018 apple production was around 264,000 tonnes (Figure 1) [17]. Due to favorable weather conditions, in 2019 there was an increase in production, more significantly in the region of Trás-os-Montes (+65%) [18], reaching a national production of 355,700 tonnes.

Apple (*Malus* spp.) is one of the most popular fruits in the world. More than 95 countries have apple crops, mostly meant to meet the domestic needs of the population [19]. This high production may also be due to the fact that the beneficial effects of this fruit have been validated in the prevention of chronic heart and vascular diseases, respiratory and pulmonary disorders, diabetes, obesity, or cancer, among many others [20]. According to FAO statistics, apple is cultivated worldwide, and its global production exceeded 86 million tons in 2018, in an estimated area of 5 million hectares.

A significant part of apple production is processed and converted into juice or cider. The extraction of apple juice generates a solid residue, *apple pomace*, which is the main bio-residue obtained by crushing and pressing apples during the juice-making process and represents around 30% of the original fruits. This apple industry bio-residue consists basically of 94.1% of peels and 4.1% of seeds (data on a wet weight basis) [19][21][22]. Therefore, this industrial activity generates large quantities of underused bio-residues, which can be expensive and complex to remove. Thus, adding value to these materials might produce economic benefits, while reducing the huge volume of bio-residues demanding suitable disposal strategies [12].

Orange (*Citrus sinensis* (L.) Osbeck) production in Portugal reached 340,000 t/year in 2018 and 2019, which corresponds to almost 18,000 ha in production area (Figure 2) [17]. In Portugal, citrus is mainly grown in the country's southern region, namely in Algarve, where oranges are classified as a Protected Geographical Indication (PGI) fruit, granting a significant economic impact. As non-climacteric fruit, "Algarve Citrus" ("*Citrinos do Algarve*") are harvested at their optimal ripe stage, when fruit internal quality attributes (IQA) comply with the requirements of the respective PGI normative reference [23][24]. During orange juice production, only about half the weight of fresh orange is transformed into juice. Meanwhile, the other half of the fruit's weight generates large amounts of waste, including the peel, pulp, seeds, orange leaves, and whole orange fruits that do not meet the quality requirements [7][25]. It is important to highlight that these fruits are also known for their antioxidant properties that have beneficial effects on human health [26].

The production of pear (*Pyrus communis* L.) in Portugal in recent years has reached around 157,000 t/year, which corresponds to almost 12,500 ha in production area (Figure 3) [17]. It is the fifth most widely produced fruit in the world, being harvested mainly in China, Europe, and the United States. Pears are typically eaten fresh and also used to produce juice, puree, and jam [27][28][29]. The pear's popularity among consumers is due to its desirable flavor, high digestibility, attractive color, soft pulp, sweet taste, and subtle floral aroma. Regarding their chemical composition and related bioactive properties, pears' phenolic composition and antioxidant activity vary widely between different cultivars, which is also due to the stage of ripeness and storage conditions [30]. Studies have shown that a more varied and much higher phenolic content was found in the pear's skin than in the fruit's pulp. Additionally, a higher content of total soluble phenolics, together with high antioxidant activity, was observed in bark extracts from different pear cultivars [31]. Phenolic compounds are also associated with the nutritional quality of fruits, as they contribute (directly or indirectly) to modulate taste and aroma and to protect fruits from oxidative deterioration, besides interacting with proteins, carbohydrates, and minerals [30].

2. Bio-Residues from Main Portuguese Fruit Crops Processing as Sources of Bioactive/Functional Compounds

Fruits' residues, such as peel, pulp, seeds, pomace, oil cake, etc., are readily available and make up about 30–50% of the total weight. The usefulness of food waste can be assessed by its composition and the cost of extracting valuable compounds. Residual products can consist of bioactive compounds such as polyphenols or glucans [32][33].

Apple pomace has been suggested as a source of bioactive molecules such as dietary fiber, protein, biopolymers, and natural antioxidants [19][22][34]. Solid waste is a rich and heterogeneous mixture composed mainly of apple peel, seeds, and pulp [21][34][35]. Most apple compounds remain in the pomace, including insoluble carbohydrates (cellulose, hemicellulose, pectin and lignin), simple sugars (glucose, fructose, and sucrose), as well as small amounts of acids, minerals, proteins, and vitamins, among others [19]. Moreover, apple pomace has also been reported as a good source of a range of phenolic constituents with antioxidant potential and human health beneficial effects [21][36]. Other constituents less studied in apples, especially in the cuticle wax layer, such as terpenoids, have been associated with antioxidant, antibacterial, and antitumor properties [19][34][35].

As is common knowledge, the color of the apples can vary between green, yellow, and red (Figure 4). These colors are related to the type of compounds present, namely chlorophylls, carotenoids, and anthocyanins. These type of compound compounds may therefore vary according to the cultivar [37].

Generally, apple pomace represents a low-cost source of phytochemicals and bioactive compounds, such as polyphenols, dietary fiber, pectin, triterpenoids, and volatiles [38].

Orange is a citrus fruit consumed in high quantities all over the world because it contains many nutrients including vitamin C, A, and B, minerals (calcium, phosphorus, potassium), dietary fiber, amino acids, and many phytochemicals, including flavonoids, triterpenes, phenolic acids, and carotenoids [7][39][40]. The valorization of residues requires knowledge of their chemical composition. Figure 5 shows the different types of products obtained from orange residues. Orange waste contains soluble sugars, cellulose, hemicelluloses, and pectin, its most important component [7]. The soluble sugars present in the orange peel are glucose, fructose, and sucrose. The insoluble polysaccharides of the orange peel's cellular wall are composed of pectin, cellulose, and hemicelluloses. Pectin and hemicelluloses are rich in galacturonic acid, arabinose, and galactose, and also contain small amounts of xylose, rhamnose, and glucose [7][41].

Pear has high nutritional value with reasonable amounts of sugars, amino acids and minerals like sodium, potassium, calcium, magnesium and iron [42]. It also has a higher dietary fiber level than most common fruits and vegetables, giving excellent results in constipation and intestine inflammation [28]. Pears contain other nutritional and bioactive components as polyphenols [29]. There is little data available in the literature on pear pomace. Thus, it is assumed that the same bioactive compounds present in the fruit are present in its bio-residues. Figure 6 represents the main components of the three fruits covered.

The foremost biomolecules that may be found and contribute to bio-residues' valorization are shown in Table 1.

As shown in Table 1, some biomolecules may be obtained from the residues of the industrial processing of the three fruits included in this review, sometimes being exclusive to just one of the three species. Below, the main bioactive compounds obtained from these residues are described.

2.1. Polyphenols–Biomolecules Common to the Three Fruit Crops

Polyphenols are an important group of secondary metabolites widely distributed in the plant kingdom [29]. Plants produce phenolic structures, mostly flavonoids and tannins, phenolic acids, lignans, and stilbenes as a defense mechanism against UV-light, parasites, other pathogens, and herbivores. Some of these molecules are pigments, attracting pollinators and thus contributing to plant seeds' dispersion [3][13]. When consumed as part of the diet, these compounds are important to protect enzymes; provide vitamins, taste, and color; and avoid lipid peroxidation [43], having great utility as functional ingredients.

Flavonoids are among the most consumed polyphenols by humans. Given their wide presence in various fruits, vegetables, legumes, grains, and nuts, these compounds represent approximately two thirds of the phenols consumed, which is why the class is predominantly described. Flavonoids are polyphenols (phenolic compounds with more than one hydroxyl group linked with an aromatic ring) present in most plants. Currently, there are more than 8000 flavonoid structures identified. This subclass of phenolic compounds includes flavonols (Figure 7A), flavanols (Figure 7B), flavones (Figure 7C), flavanones (Figure 7D), isoflavones (Figure 7E), and anthocyanins (Figure 7F) [3][44].

Flavonoids are associated with many health benefits. They are potent natural antioxidants that can improve human health by preventing oxidative stress, (neuro)degenerative diseases, and cardiovascular diseases [45][46]. Phenolic compounds contribute also to the sensory quality of fruit (color, astringency, bitterness, and flavor) [29][47]. Generally, phenolic compounds are more concentrated in fruit skin than fruit pulp [48][49].

Apple pomace, which includes soft tissues, core, stems, seeds, and peels, is a good source of polyphenols such as chlorogenic acid (Figure 8A), hydroxycinnamates (Figure 8B), quercetin (Figure 8C), and catechin (Figure 8D) [13][50]. Studies show a wide variation in the polyphenolic content of different apple cultivars. The main compounds present in the polyphenolic fraction of apples were catechins and proanthocyanidins followed by hydroxycinnamates, flavonols, dihydrochalcones, and anthocyanins [51]. However, the number of individual compounds varies up to 30% from one year to another in the same cultivar [52].

The profile of total flavanone of sweet and bitter oranges investigated by Farag et al. contained hesperidin (Figure 9A) and narirutin (Figure 9B) for sweet oranges and naringin (Figure 9C), neoeriocitrin (Figure 9D), and neohesperidine (Figure 9E) (hesperetin-7-neohesperidoside) for bitter oranges. A second class of citrus flavonoids, polymethoxy flavones (PMF), which are considered to be more biologically active than flavanones, although less abundant, have also been identified [53]. Fruit peels are richer in PMF than juice [54]. Likewise, it has been reported that citrus peel contains more polymethoxylated flavonoids than other parts of edible fruits [55]. Another study reported flavonoids as the most discriminating phytochemicals among citrus fruits, including orange, analyzed using hierarchical cluster analysis [56].

Pear polyphenols have diverse structures and belong to different classes, namely flavonoids (flavan-3-ols (Figure 10A) and flavonols (Figure 10B)), phenolic acids (hydroxycinnamic acids derived from caffeic acid and *p*-coumaric acid), and simple phenolics (glycosylated hydroquinone: arbutin (Figure 10C)) [49][57][58]. Many studies on

polyphenol composition conclude that hydroxycinnamic acids and arbutin are the main phenolic compounds in pear [29][42][59][60][61].

2.2. Pectins–Biomolecules Common for Apple and Orange Crops

Pectin (Figure 11) is a structural heteropolysaccharide of D-galacturonic acid that can be organized into three different structures, namely homogalacturonane, rhamnogalacturonane I, and rhamnogalacturonane II [1][12]. Despite integrating soluble dietary fiber in several fruits and vegetables [62], the main sources of pectin at the industrial level are represented by apple pomace [63], citrus peel [7][64], and sugar beet pulp [65]. Dried apple pomace, citrus peel, and sugar beet treated with hot diluted mineral acid resulted in the production of 10–30% of pectins and flavanones [66]. The content of pectin (powder) in apple pomace, spent guava extract, and lemon peel in optimized conditions was found between 0.05 and 0.06% [67].

Apples contain approximately 2.21 g/100 g of total fiber. Of that, 70% is insoluble fiber, including cellulose and hemicellulose, and 30% is soluble fiber, mainly pectin [68]. Pectins are complex polysaccharides present in the cell wall of higher plants, which are not metabolized in the upper digestive tract in humans [69]. Beneficial health effects of pectin are attributed to its ability to lower cholesterol [70], slow down glucose absorption, and increase colonic short-chain fatty acid (SCFAs) production [68][71].

Pectin can be obtained from pomace generated as a residue from the processing of citrus fruits, grapes, and apples with a wide range of uses in the pharmaceutical, cosmetic, and food industries as thickening and gelling agents to improve food texture [7][66][72]. For pectin extraction, two general processes are used: (1) those that separate pectins from most of the other materials by precipitation with alcohol and (2) those that precipitate pectins as an insoluble salt with suitable multivalent metal ions (Figure 12) [73].

Among the health-promoting properties, the immunomodulatory activity of polysaccharides has been specified [74]. More and more polysaccharides isolated from medicinal plants are reported for their potent immunomodulating activities [74][75]. Acidic heteropolysaccharides could mediate the activity of certain immunological factors. Important factors from innate immunity are the complement system, NK-cells, phagocytes (macrophages, neutrophils, and dendritic cells), and their metabolites (monokines) and mediator cells (mast cells, basophils, eosinophils, and thrombocytes), producing pro-inflammatory molecules [74]. Immunostimulating polysaccharides modulate the intestinal immune system, which has been known to direct not only defensive but also regulatory functions of both mucosal and systemic immune systems through lymphocyte migration depending on homing receptor pattern [76]. Furthermore, the proposed therapeutic perspective of herbal polysaccharides, activating the complement system and immune cells, is an expression of tumor-preventing, antitumor, and antimetastasis activities [77].

2.3. Triterpenoids–Biomolecules Common for Apple and Orange Crops

Terpenoids are the most significant and widespread class of plant secondary metabolites synthesized from isopentenyl pyrophosphate oligomers [78], being composed of different isoprene units (C₅H₈) that may undergo chemical rearrangement [12][19][79][80]. The production of isoprene seems to be related to the mechanism that plants

use to combat thermal and oxidative stress. Compounds derived from isoprene often undergo additional chemical modifications, such as oxidation or rearrangement of carbon structures. Triterpenoids are a group of compounds with 6 rearranged isoprene units (C₃₀) with several beneficial biological activities and have, therefore, attracted research attention [19][79][81][82]. Regarding their application in food products, pentacyclic triterpenoids, particularly those with lupane, ursane, or oleanane structures, display several pharmacological activities, not to mention the fact of being devoid of prominent toxicity [19]. Among the ursan-based triterpenoids, ursolic acid (Figure 13a) is the main compound present in apple, followed by its oleanolic isomers (Figure 13b) and betulinic acids (Figure 13c) [12][79][82].

2.4. Carotenoids–Biomolecules Common for Apple and Orange Crops

Carotenoids are widespread secondary metabolites, biosynthesized by plants, algae, and some fungi and bacteria [83]. In addition to their relevance in terms of sensory quality, carotenoids are important from a nutritional point of view, being among the main precursors of vitamin A [84]. Moreover, there is a great deal of evidence associating high levels of carotenoid intake and a lower risk of developing chronic diseases such as heart disease [85], cancer, and macular degeneration [86], among other health benefits [87][88].

The color of orange varieties, one of the main attributes that affect customer acceptance, is mainly due to carotenoid pigments, of terpenoid origin (except for the shade of blood orange, which originates from anthocyanin pigments) [40][53]. The carotenoid profile of most sweet orange varieties is dominated by 5,6-epoxycarotenoid (Figure 14a) and xanthophyll (Figure 14b) [53][88].

Madrera et al. (2015) studied the characterization of aroma compounds from apple pomace and several uncommon terpenes were detected, all considered products of oxidative degradation of structural carotenoids (e.g., β -ionone, pseudoionone, β -damascenone, 6-methylhept-5-en-2-one, 6-methylhept-5-en-2-ol, 6-methylhepta-3,5-dien-2-one, 2,6-dimethylhept-5-enal, 2,6-dimethylhept-5-en-1-ol, nerylacetone, and 6,10-dimethylundeca-5,9-dien-1-ol). Cyclic carotenes from apple, such as β -carotene, produce the cyclic terpenoids ionone, pseudoionone or damascenone, while acyclic carotenes, such as lycopene, produce linear derivatives. As in the autoxidation of unsaturated fatty acids, oxidative activity occurs due to fruit damage during processing. Hence, these components are found in apple pomace both before and after fermentation [89].

2.5. Limonoids–Biomolecules Common for Orange Crops

Limonoids are a prominent group of oxygenated triterpenoids, present in the pulp, seed, and peel tissues of oranges [90]. Both lactones and glucosides are soluble in water but do not contribute much to citrus fruit taste. In contrast, limonoid aglycones, such as limonin (Figure 15a) and nomilin (Figure 15b), impart the sour taste of bitter orange [53].

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