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Review

Non-Extractable Polyphenols from Food By-Products: Current Knowledge on Recovery, Characterisation, and Potential Applications

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Abstract: Non-extractable polyphenols (NEPs), or bound polyphenols, are a significant fraction of polyphenols that are retained in the extraction residues after conventional aqueous organic solvent extraction. They include both high molecular weight polymeric polyphenols and low molecular weight phenolics attached to macromolecules. Current knowledge proved that these bioactive compounds possess high antioxidant, antidiabetic, and other biological activities. Plant-based food by-products, such as peels, pomace, and seeds, possess high amount of NEPs. The recovery of these valuable compounds is considered an effective way to recycle food by-products and mitigate pollution, bad manufacturing practice, and economic loss caused by the residues management. The current challenge to valorise NEPs from plant-based by-products is to increase the extraction efficiency with proper techniques, choose appropriate characterising methods, and explore potential functions to use in some products. Based on this scenario, the present review aims to summarise the extraction procedure and technologies applied to recover NEPs from plant-based by-products. Furthermore, it also describes the main techniques used for the characterisation of NEPs and outlines their potential food, pharmaceutical, nutraceutical, and cosmetic applications.

Keywords: non-extractable polyphenols; food by-products; extraction; characterisation; antioxidants

1. Introduction

Non-extractable polyphenols (NEPs), or bound polyphenols, are usually ignored when studies are dealing with the extraction of polyphenols from plant-based food. They remain insoluble in the solvent used for the extraction because of the capacity of their hydroxyl functionalities to form polymers or to link to macromolecules such as polysaccharides and proteins [1]. Compared to extractable polyphenols (EEPs), the high molecular weight, complex structure, and bounding to macromolecules makes NEPs difficult to cross the plant matrix and further be extracted with conventional aqueous-organic extraction [2].

Despite some recent studies suggesting that NEPs are even more abundant than EEPs in many plant foods [3,4], surprisingly, only a minor part of the existing literature on polyphenols in foods deals with NEPs. For instance, Saura-Calixto noticed that studies on EPPs cover only the tip of the iceberg and that, instead, NEPs should be regarded as the primary fraction of polyphenols in plant-based foods [5]. However, the challenging issue is that NEPs isolation requires several steps, which are often not systematically optimised and greatly depending on the chemical and physical nature of the food plants [6]. The analytical methods used to characterise NEPs are various but not specific. There are no standardised methods to analyse the content of NEPs [7]. Meanwhile, although the

functions and properties of the NEPs are discussed in some of the studies, there are currently few developed products including NEPs in their formulation [8,9].

Extracting bioactive compounds from food by-products is considered as a solution to valorise food wastes and mitigate the social, economic, and environmental impact caused by food wastes. Phenolic compounds, which are among the main bioactive compounds recovered from fruit and vegetable by-products, represent a wide group of secondary plant metabolites, arising from phenylalanine or shikimic acid [10,11]. Due to their capacity to prevent oxidation reactions, phenolic compounds have received attention from the food, cosmetic, and pharmaceutical industries [12,13]. As a major category of polyphenols, several studies have been done to reveal the content of NEPs in food by-products including fruit peels [3,14] or pomace [15,16], wheat [17] or rice [18,19] bran, and vegetable process leftovers [20,21]. With the gradually developed extraction methods and hydrolysis strategies, the isolation of NEPs from food by-products could be an economically feasible and environmentally friendly way to recover them.

The present review aims to provide an overview of NEPs extraction from food by-products: their nature, recovery methods, characterisation strategies, and potential applications. To provide new solutions for food by-product valorisation and the recovery of high-value components, this review compares the nature of the EPPs and NEPs, summarises the NEPs extraction process in detail, lists the characterisation methods and reports NEPs' potential applications in the food, pharmaceutical, nutraceutical, and cosmetic areas. The literature of this review is focused mainly on NEPs' extraction from food by-products over the last 10 years (2010–2019). Some previous studies (2000–2009), as well as NEPs extraction from food, are also reported for comparison. In the last part describing the NEPs' application, papers related to the usage of food residues and some clinical researches are summarised.

2. NEPs Recovery from Food By-Products

2.1. Food By-Products

Reducing, reusing, and recovering food by-products are some of the major challenges for most food processing plants. In the last century, modern societies have designed food processing industries that are able to make foods that are safe, high quality, and rich in nutrients. The challenge for the current century is likely to design manufacturing processes that are able to prevent waste, reuse by-products in other manufacturing chains, and recover or (bio)refine the nutrients still contained in the by-products that otherwise would be simply lost. It is estimated that in Europe, 88 million tons of food are wasted annually along the chain, which equals 143 billion euros [22]. The sector contributing the most to the food waste is processing (approximately 17 million tons) [23]. During the processing of the plant-based foods, 20% to 60% (*w*/*w*) of the materials, including peels, seeds, husks, and pomace, are considered as waste and discarded [24]. To mitigate that, in the last two decades, a growing amount of literature has demonstrated the further usage of plant by-products, especially the potential to extract valuable bioactive compounds [22,25].

The term "bioactive compound" indicates a compound having an effect, causing a reaction, or triggering a response in the living tissue [26]. The daily intake of fruit and vegetable products is essential to health maintenance due to their medicinal properties from bioactive compounds rather than macronutrients [27]. Besides fruit and vegetable products, their by-products also contain a high amount of bioactive compounds. Recovering such compounds via tailored biological or chemical processes is considered a cheap and sustainable process for food industries [28]. The bioactive compounds recovered from fruit and vegetable by-products can be roughly clustered into four categories, which are phenolic compounds, vitamins, lipids, and dietary fibers [29]. Table 1 shows some examples of bioactive compounds recovered from fruits and vegetables by-products.

Table 1. Bioactive compounds present in plant-based by-products.

Categories	Bioactive Compounds	Food By-Products Sources	Concentration Range	Extraction Method	Reference
Phenolic compounds	Polyphenols	Apple pomace	12.38–13.76 mg/g GAE	Aqueous methanol extraction with acid hydrolysis	[15]
	Polyphenols	Cantaloupe melons peel	25.58 mg/g GAE [1]	95% ethanol extraction	[30]
	Hesperidin	Citrus peel	Up to 673 mg/g DW [2]	Aqueous ethanol extraction	[31]
	Phenolic acid	Wild carrot pomace	569.2 ± 55.9 mg/g DW	Aqueous methanol extraction	[32]
	Phenolic acid	Pumpkin oil cake meal	Up to 89.52 mg/kg DW	Aqueous methanol extraction	[33]
Lipids	Carotenoids	Tomato waste	36.5 ± 1.1 mg/kg dry waste	Hexane–ethyl acetate (50:50) extraction	[34]
		Peach pulp residue	256.1 mg/kg dry residues	Absolute ethanol extraction	[35]
	β-sitosterol	Grape seed	Up to 11.2 g/kg dry seeds	Supercritical CO_2 extraction	[36]
	Lipids	Mango kernel	Up to 84.6 mg/g dry kernel	Hexane extraction	[37]
Vitamins	Tocopherols	Tomato pomace	Up to 195.99 mg/100 g dry pomace	Supercritical CO_2 extraction	[38]
Dietary fiber	Dietary fiber	Citrus peel	780 mg/g	Subcritical water extraction	[39]
-	-	Apple peel	170–920 mg/g	Blanching or scalding	[40]

[1] GAE: gallic acid equivalent; [2] DW: dry weight.

2.2. Non-Extractable Polyphenols

Phenolic compounds play pivotal roles in the physiological and morphological properties of plants and contribute to the organoleptic properties of plants and plant-derived food, such as colour, aroma, texture, and flavour [11,41]. Due to the capacity to prevent oxidation reaction, phenolic compounds have received attention from the food, cosmetic, and pharmaceutical industries related to structure analysis and potential applications [12]. The basic structure of phenols consists a hydroxyl group (–OH) directly bonded to an aromatic hydrocarbon group, and the often-referred term "polyphenol" means a high molecular weight (500–4000 Da) polymer with more than 12 phenolic hydroxyl groups and five to seven aromatic rings for each thousand of molecular weight [42]. The application of polyphenols is mainly associated with their antioxidant, anti-allergenic, and anti-inflammatory properties. Several studies have been done to quantify the polyphenol content in food waste, maximise the extraction efficiency, and exploit proper applications [43,44].

However, current research related to the recovery of polyphenols from food by-products has been mainly focused on EPPs, which are those that can be recovered directly with aqueous organic solvents. Such extraction methods ignore a significant fraction of polyphenols retained in the residues after the extraction, the so-called bound polyphenols or non-extractable polyphenols (NEPs) [2,45]. With the gradually developed extraction methods and hydrolysis strategies, the recovery of NEPs from food by-products could be an economically feasible and environmentally friendly way to recover them [2].

The term "bound polyphenols" or "non-extractable polyphenols" indicates polyphenols bound to the residual matrix, even after the conventional aqueous organic extraction [3]. The high molecular weight, complex structure, or their bond to other macromolecules—for example, polysaccharides or

proteins—makes it difficult for them to be extracted conventionally [17]. To determine the content of NEPs in food by-products, chemical or enzymatic treatment is required prior to the extraction to release polyphenols from the matrix [46]. After extraction from the food matrix, analytical analysis can be done to determine their chemical structure and physicochemical properties. Currently, based on the molecular structure, NEPs can be divided into two groups: condensed tannins and hydrolysable tannins. Typically, condensed tannins (Figure 1) can be classified as flavanols, considering the flavan-3-ol moieties (Figure 2) in their molecular structure [2,7,47]. They are mainly contained in tegumental tissues, which include fruit skins, seed coats, and plant stems [47,48]. The hydrolysable tannins are polyphenolic acid units or their derivatives, which are usually gallic or ellagic acid, attached to a polyol central core (usually glucose, can also be fructose, xylose, etc.) via ester bonds [2,49]. The simplest hydrolysable tannin is gallotannins, in which the gallic acid is directly attached to the polyol core (Figure 3).

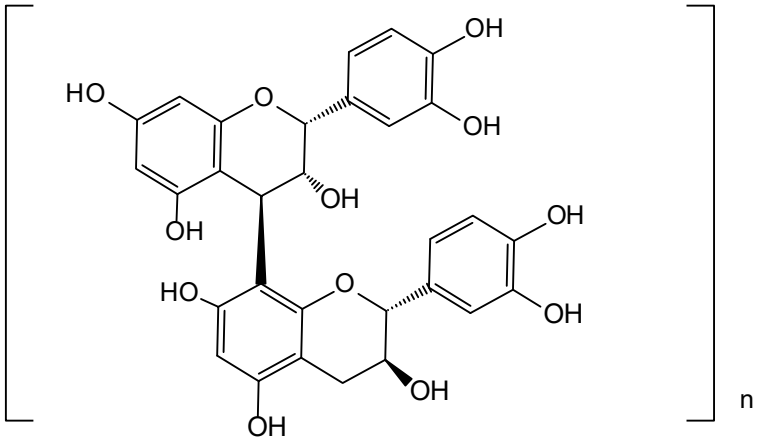

Figure 1. Example structure of proanthocyanidins (Procyanidin B2, (−)-epicatechin-(4β→8)-(−)-epicatechin).

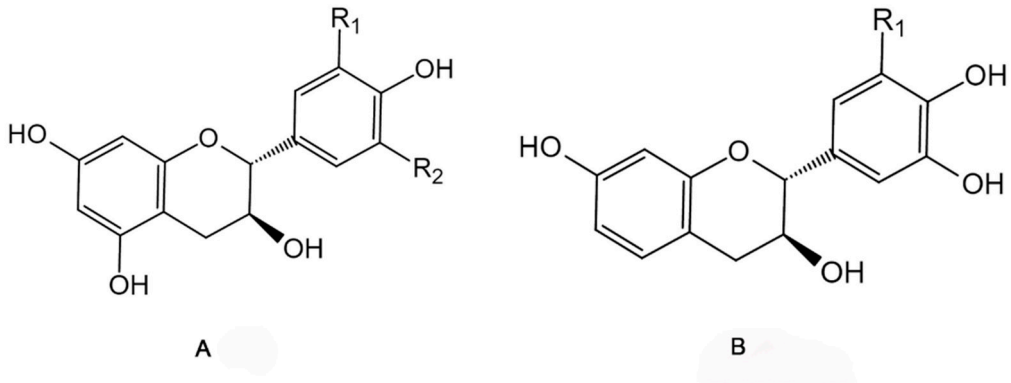

Figure 2. Molecular structure of flavan-3-ol. (**A**): R_1 = OH, R_2 = H, (+)-catechin, R_1 = OH, R_2 = OH, (-)-gallocatechin. (**B**): R_1 = OH, (-)-Robinetinidol, R_1 = H, (−)-Fisetinidol).

2.3. NEPs Recovery Process

NEPs are mainly found in fruit and vegetable residues, such as peels, husks, pomace, and skins. These plant by-products are comparably cheap and contain an abundant quantity of accessible ingredients during food processing. Thus, utilising residues as NEP resources is a value-added and economically feasible way for the recovery of food by-products [2].

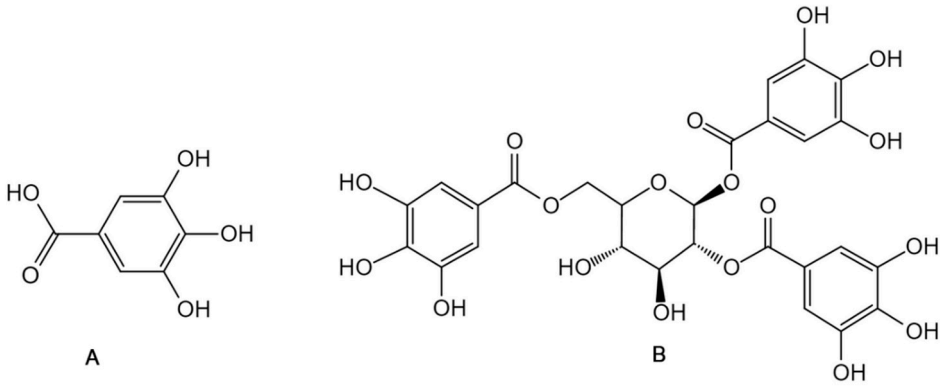

Figure 3. Molecular structure of (**A**): gallic acid and (**B**): gallotannins (1,2,6-Trigalloyl glucose).

However, as mentioned above, NEPs are retained in the plant by-products matrix during aqueous organic extraction. This is mainly due to the molecular size of NEPs and the chemical interactions between NEPs and the matrix compounds [50]. The interaction is further affected by the percentage of galloylation in the phenolic molecules and the aggregation with the polysaccharides [50,51].

To minimise the effect of the interaction and facilitate the NEPs release, different hydrolysis methods can be applied to cleave the bonds [7]. Moreover, the development of environmentally friendly innovative extraction technologies such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE) provide further solutions for NEPs extraction. Figure 4 presents a flowchart usually followed to extract and characterise NEPs from food by-products. The extraction of NEPs from food by-products can be divided into six steps. The first is drying the food by-products, followed by the milling or grinding of the samples into small particles. Then, conventional extraction with aqueous organic solution can be performed to obtain EEPs. After EEPs extraction, the hydrolysis is performed on the residue material to cleave the interaction between NEPs and the plant matrix with a chemical solvent (acid or alkaline) or enzyme. Afterwards, conventional or innovative extraction methods can be applied to isolate NEPs. After the extraction, the characterisation of NEPs can further be performed with different spectroscopic and chromatographic techniques. The characterised NEPs can be further utilised for food, pharmaceutical, nutraceutical, and cosmetic applications.

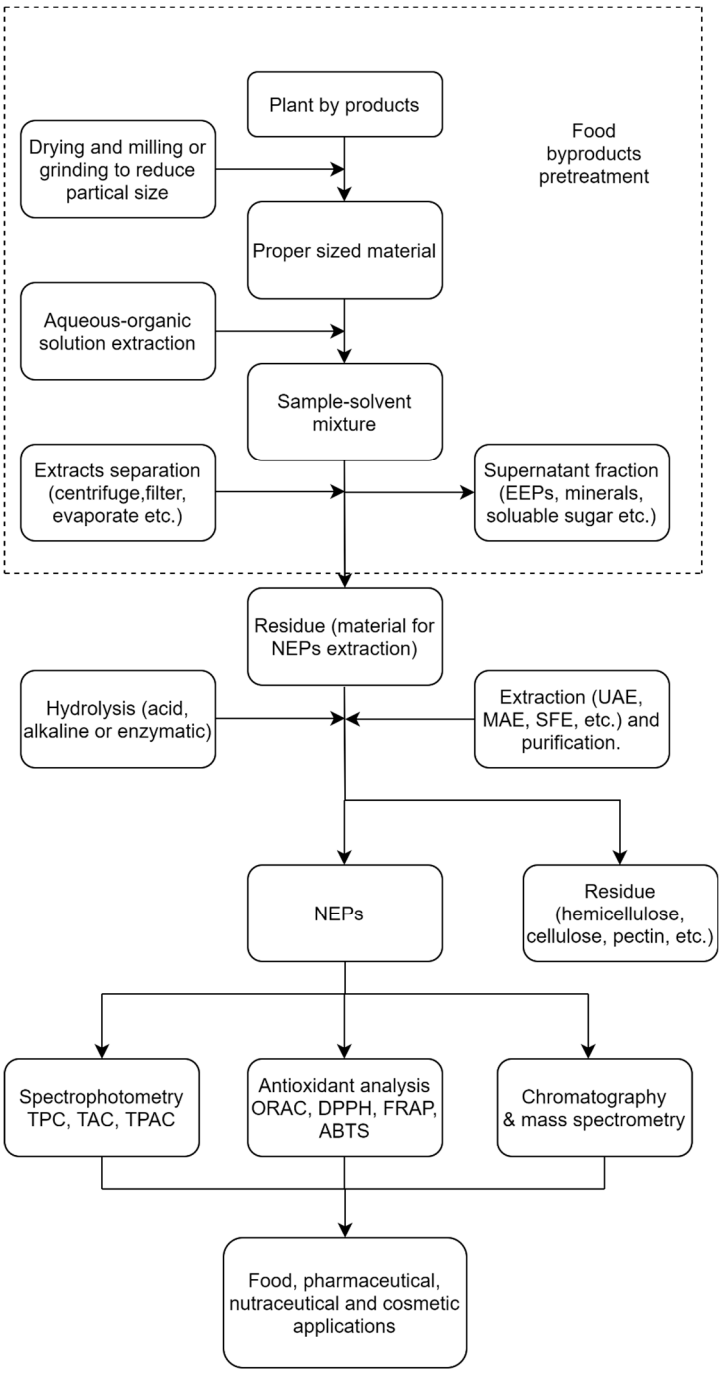

Figure 4. Procedure to extract and characterise non-extractable polyphenols (NEPs) from food by-products (revised from Dzah et al. [2] and Domínguez-Rodríguez et al. [7]).

3. Steps for the Extraction of NEPs from Food By-Products

3.1. By-Products Pre-Treatments

Before the NEPs extraction, the pre-treatment of food by-products is required to increase its efficiency. The choice of the pre-treatment process should be specific for each by-product material and tailored to maximise the efficiency of the follow-up extraction strategies [7]. Despite the many possible pre-treatment procedures available, two major operations seem essential to any biorefinery process: dehydration and size reduction.

Dehydration is a key pre-processing step with the aim of removing moisture. This step is essential because food by-products, especially plant peel or pomace, have relatively high water content and moisture levels, which can promote the occurrence of undesirable chemical or enzymatic

reactions, and further decrease the overall stability of the by-products [52]. However, sample drying can also pose an adverse influence, such as bioactive decomposition and cell contraction, which may decrease the extraction efficiency [2]. Therefore, a proper dehydration method is required.

Concerning the recovery of NEPs, the most applied drying methods are air drying and freeze-drying (see Table 2). Air drying is a comparably cheap and accessible dehydration method. The operation can be run at ambient conditions [53] and high temperatures [16,54,55] by using dried air [56] or even by the natural heating of the sun [14]. Based on research performed by Esparza-Martínez et al., the drying temperature can significantly influence the extraction of non-extractable phenols in mandarin juice waste. Although all samples were dehydrated to 4.5% moisture, higher NEPs yield could be achieved with a higher drying temperature (74.56 mg gallic acid equivalent (GAE)/g dry weight (DW) under 120 °C compared to 47.47 mg GAE/g DW under 60 °C and 49.04 mg GAE/g DW under 90 °C [55].

Since air drying is a relatively long process, this method is mainly used for low water content by-products, such as grape seeds, blueberry leaves, and black tea leftover. Instead, for high water content by-products, freeze-drying seems the preferred drying technology [20,57–59]. Freeze-drying, also called as lyophilisation, is a drying technique with the highest ability to maintain the product quality (structure, colour, nutritional value, etc.) of fruit and vegetable materials [52]. The water inside the sample is removed by sublimation with low processing temperature and pressure. Nevertheless, the freeze-drying suffers from high energy costs and long processing times, which limit it to high-value products [60].

Behind drying, the most common pre-treatment used on food by-products is size reduction. Size reduction is generally obtained by grinding or milling. This operation aims to increase the surface area of the by-products, which is a prerequisite for maximising the subsequent extraction of bioactive compounds [7]. Milling [18,19,61], grinding [16,20,21], and crushing [62] are the main methods to reduce particle size. Landbo and Meyer summarised the influence of sample size on the extraction yields of phenols. The particle size reduction from 500–1000 μm to 250–500 μm significantly increased the yield of phenols up to 3.5 times (from 78 to 256 mg GAE/L) and 3.1 times (from 80 to 247 mg GAE/L) in the methanol and water extraction, respectively [63]. Research carried out by Speroni et al. also outlined that the yield of polyphenols extracted from olive pomace was significantly influenced by the particle size of the samples. The yield of polyphenols ranges from 1.68 g GAE/100 g dry olive pomace (with particle size> 2 mm, micronised at 541.4 rpm for 15 min) to 2.51 g GAE/100 g dry olive pomace (with particle size< 2 mm, micronised at 541.4 rpm for 15 min) [20].

Besides individual drying and milling pre-treatments, the cryogenic grinding (freezer milling) with liquid nitrogen is a very promising technology that can achieve size reduction and dehydration in one step. The liquid nitrogen provides a low-temperature condition for milling and facilitates the sublimation of water [60]. Li et al. pre-treated the citrus peel sample with liquid nitrogen (at −195.6 °C and ambient pressure) and crushed it with a pre-chilled mortar and pestle to achieve a high-quality peel powder [62]. The research reported by Saxena et al. proved that a higher level of total phenolic content (TPC) was measured from cryogenically ground coriander seeds (92.66 mg GAE/g crude seed extract) compared to the ambient condition of grinding (48.78 mg GAE/g crude seed extract) [64].

After the drying and grinding steps, samples are subsequently sieved to reach a specific particle size for research reproducibility and extract efficiency [2]. Samples with high lipid content need to be stored in low water content and low-temperature condition to avoid lipid oxidation and the further generation of free radicals that may degrade phenols [65]. In NEPs extraction experiments, food by-product samples are stored at −20 °C [16,20,66,67], −40 °C [61], or some low lipid content samples at ambient temperature [56,63].

3.2. Isolation of Extractable Polyphenols (EPPs)

In some NEPs extraction protocols [3,18–20], EEPs are preliminarily removed from the by-product. This is achieved by solid–liquid extraction, and it is performed with the purpose to better quantify NEPs. The most used method to extract EEPs is originated from the sample extraction method used for TPC analysis with Folin–Ciocalteu reagent. In principle, the dried sample is added to a methanol/water (50:50 *v*/*v*) solvent that is acidified to pH = 2 with HCl. The mixture is shaken, and afterwards, the solid residual is filtered for a second extraction procedure with acetone/water (70:30 *v*/*v*) [56,68]. This method is widely used to extract EEPs from fruit or fruit peels [3,4], olive pomace [20] and roselle calyx by-products [56]. Other EEPs isolation solvents include chilled 80% (*v*/*v*) acetone [18,69], chilled acidified methanol (95% methanol:1 M HCl 85:15 *v*/*v*) (52,53), or acetic acidified acetone (acetone:0.5% acetic acid 70:30 *v*/*v*) [58].

3.3. Extraction of NEPs

To extract NEPs from food by-products, it is fundamental to decrease the interaction between NEPs and the plant matrix [50]. Currently, this can be achieved by breaking down cellular or molecular structures with acid, alkaline, or enzymatic hydrolysis [70].

3.3.1. Acid Hydrolysis

Currently, publications focused on NEPs extraction from food by-products mainly apply two acid hydrolysis methods to break the glycosidic bond, which are hydrolysis with methanol/H_2SO_4 to recover NEPs-hydrolysable polyphenols (HPP), and hydrolysis with butanol/HCl/$FeCl_3$ to determine the content of non-extractable proanthocyanidins [70]. The first one, which is carried out to determine the hydrolysable tannins, has been described by Hartzfeld et al. in 2002 [71]. Briefly, the residues after EPPs extraction were hydrolysed with methanol/H_2SO_4 (90:10 *v*/*v*) at 85 °C for 20 h. Then, the content of hydrolysable tannins was determined by analysing the supernatants combined with two times residues washing with distilled water. This method has been extensively used in recent years to determine the NEPs content from fruit peels or pulps [3,55,72,73], rice bran [61], palm kernels [74], and olive pomace [59]. To maximise the efficiency of acid hydrolysis, in 2014, Cheng et al. optimised the treatment condition based on a response surface methodology. Several factors, including extraction solvent type (methanol or ethanol), organic solvent/H_2SO_4 ratio, liquid/solid ratio, extraction time, and temperature were considered, and the highest yield was achieved at 23 h of extraction with methanol/H_2SO_4 (90:10 *v*/*v*) solvent under a liquid/solid ratio of 21:1 at 75 °C. The NEPs extracted from pulped blueberry reached the value of 27.25 mg/g with HPLC analysis [46].

The second acid hydrolysis method has been reported for the first time by Porter et al. in 1985 to cleave the interflavonoid bond by acid catalysis [75]. In general, the EEPs were preliminarily removed by classical solid–liquid extraction with water:methanol mixtures. Afterwards, the residue was mixed with *n*-butanol/HCl (95:5 *v*/*v*) and $FeCl_3$ at 100 °C for 60 min. The mixture was centrifuged, and the residues were washed two times with butanol. The analysis of the NEPs content was based on the supernatants collected and the washing butanol [72,75]. This method was also extensively applied to extract NEPs from several by-products, including fruit peel and pomace [3,72], black rice bran [61], fruit kernels and seeds [73,74], and olive pomace [20]. In 2019, Mahmud et al. carried out one-factor-at-a-time study to maximise the extraction yield based on butanol/HCl hydrolysis on date palm kernels. Three factors were considered, namely extraction temperature (70, 85, 100 °C), time (1, 2, 3, 4, and 5 h), and the solid/solvent ratio (5:1, 10:1, 15:1, 20:1, and 25:1). The maximum recovery of total phenol compounds (TPC) (10.83 mg GAE/g) was achieved with a sample/solvent ratio equal to 1:20 under 85 °C for three hours [74].

In some studies, these two acid hydrolysis methods have been applied in series for the extraction of different NEP groups. The first hydrolysis method with H_2SO_4 has been applied to recover the NEPs-hydrolysable polyphenols (HPP), or hydrolysable tannins, while the other one with butanol and HCl has been used to collect the non-extractable proanthocyanidins or condensed tannins. Based on the research carried out by Pérez-Jiménez and Saura–Calixto in 2018, significantly different results

were obtained applying the two hydrolysis methods. The polyphenol content in banana peel was 1961.3 mg/100 g DW based on the first method, while it was 7667.2 mg/100 g DW using the second hydrolysis method [3]. The results published by Speroni et al. in 2019 also showed that the two acid hydrolysis methods can provide significantly different results on NEPs yield from olive pomace (6.4 g and 3.2 g GAE per 100 g DW, respectively) [20]. However, in most of the published studies, experiments were performed applying just one of the two hydrolysis methods to determine the content of the NEPs [46,72,74]. Besides choosing the suitable hydrolysis method, attention also needs to be paid to the analytical techniques used for NEPs quantification. Samples obtained from the acid hydrolysis by H_2SO_4 can be measured with both spectrophotometric and chromatographic methods, while those obtained from the hydrolysis with HCl and butanol can only be determined by spectrophotometry [3].

3.3.2. Alkaline Hydrolysis

Recovery of the NEPs can be effectively obtained also by alkaline hydrolysis. The alkaline pH can induce cleavage of the ester bonds between phenolic acid and the cell wall, which further increases the release of phenolic compounds from polysaccharide structures [70]. The alkaline hydrolysis was applied for the first time by Naczk and Shahidi in 1989. Briefly, the residues after EPPs extraction were mixed with 2 M NaOH at room temperature for 1 h under nitrogen conditions with continuous shaking. Quantitative analysis was performed after neutralisation with concentrated hydrochloric acid [76]. In recent years, several studies tried to maximise the efficiency of the hydrolysis with different food by-products, for example, chestnut peels [57], fruit and vegetable waste [21,77,78], and brown rice bran [18,19]. To increase the extraction efficiency, studies have been carried out to define the optimum hydrolysis condition. In research performed by White et al., three factors were considered for the alkaline hydrolysis of NEPs extraction from cranberry pomace: NaOH concentration (2 M, 4 M, and 6 M), temperature (25, 40, and 60 °C) and time (from 5 min to 24 h). The highest yield was achieved when hydrolysing the sample with 2 M NaOH for 15 min under 60 °C (690 mg/100 g DW) [77]. Gonzales et al. also gave a similar result in 2014 by extracting NEPs from cauliflower waste. The optimal condition for the hydrolysis was 2 M NaOH for 30 min at 60 °C, and the yield was 9.82 mg GAE/g dry residue [78]. Aires et al. compared the alkaline hydrolysis efficiency on chestnut waste using sodium hydroxide and sodium carbonate. The result showed that sodium carbonate could barely pose any effect to the hydrolysis compared to the sodium hydroxide, and the highest yield was achieved when 8% NaOH was used for 2 h [57].

Several studies indicated that the alkaline hydrolysis performs better compared to the acid hydrolysis in extracting NEPs [46,79]. Kim et al. in 2005 used wheat by-products to compare acid (6 M HCl, 1 h at 95 °C) and alkaline (2 M NaOH, 4 h at room temperature) hydrolysis methods. The result by high-performance liquid chromatography (HPLC) showed that the total yield of phenolic acids from the alkaline hydrolysis (2.02 mg/g defatted dry bran) was significantly higher than the one obtained with the acid hydrolysis (0.32 mg/g defatted dry bran) [79]. Cheng et al. also carried out a similar research focusing on the acid (methanol:H_2SO_4 90:10 *v*/*v*, 23 h at 75 °C) and alkaline (3 M, 4.5 h at room temperature) hydrolysis performance on blueberries. The NEPs yield from alkaline hydrolysis (54.33 mg/g) was much higher than the one from the acid hydrolysis (27.25 mg/g) [46].

3.3.3. Enzymatic Hydrolysis

Apart from chemical hydrolysis, carbohydrate-hydrolysing enzymes can effectively release NEPs bound to sugar, fibre, or proteins inside the plant matrix [70]. With the gradually stricter environmental regulations regarding the industrial extraction of bioactive compounds, enzyme hydrolysis is becoming more popular in both small- and large-scale food by-products processing industries [80]. Enzyme hydrolysis is attractive because it is convenient and environment-friendly. In addition, it uses mild extraction conditions of temperature and pH. Recent works focused on the enzymatic hydrolysis of fruit peels and pomace. Mango [81], apple [58], citrus [62] peels, and grape pomace [16,63,82] were hydrolysed by a different variety of enzymes including pectinases, cellulases, hemicellulases, and ligninases [80]. The optimum condition of enzyme hydrolysis was argued by

Meini et al. in 2019 with grape pomace. In their research, the influence of the variety and concentration of enzyme (pectinase, tannase, or cellulase), pH, temperature, and incubation time were analysed, and the response surface methodology was used to obtain the highest efficiency. The results showed that the optimal hydrolysis condition was reached at 45 °C under pH = 2 with 188 enzyme units per gram of grape pomace of cellulase and 198 enzyme units per gram of grape pomace of tannase for 2 h. The TPC increased by 66% compared to the control sample (from 0.49 g to 0.81 g GAE/100 g grape pomace) [16]. Similar results were provided by Fernández et al. in 2015. They reported that grape pomace with enzyme hydrolysis (solid/liquid ratio 1:10, enzyme/substrate percentage 0.01 g/mL) released higher TPC compared to conventional extraction (from 0.28 to 0.84 mg catechin equivalent/mg grape) [82]. However, when referring to enzyme hydrolysis, the high cost of the enzyme and long processing time are the main hurdles for commercialised applications. Recent research on hyphenated extraction [83] to minimise the extraction process period and immobilised enzymes [84,85] to reduce the costs are important for further industrial-scale applications.

3.4. Innovative NEPs Extraction and Purification

The efficiency of the chemical and enzyme hydrolysis of food by-products can be greatly enhanced by several innovative techniques. Examples of novel technologies include ultrasounds, microwaves, solid phase extraction, and supercritical fluids extraction. This part of the review introduces the main pros and cons of these innovative techniques, with special emphasis on their effect on the resulting yield of extraction.

3.4.1. Ultrasound-Assisted Extraction (UAE)

In recent years, ultrasounds have been extensively applied in extraction procedures because the ultrasound waves can facilitate the penetration of the solvent into the sample and cause a cavitation effect by the generation, growth, and implosion of gas bubbles [86]. The collapsing of the bubbles on the surface of samples allows the formation of hot spots of high pressure and temperature and the subsequent formation of micro-fractures. The mechanical effect of the ultrasounds also facilitates the penetration of the extracting solvent into the sample by the disruption of cell walls with the increase of the contact surface [87,88]. Such a mechanical effect can efficiently save extraction time and reduce the amount of solvent used during NEPs extraction [6].

Several studies have been done to analyse the influence that ultrasounds have during the hydrolysis to recover NEPs from food by-products [21,78,89,90]. In 2014 and 2015, Gonzales et al. performed two studies to evaluate the effect of ultrasounds during the alkaline hydrolysis of red cabbage, brussels sprouts [21], and cauliflower waste [78]. The study first compared the conventional NEPs extraction under UAE at the same extraction condition (15 min, 60 °C, and 2 M NaOH) with cauliflower waste. The results showed that the combination of ultrasound and alkaline hydrolysis (7.3 mg GAE/g DW) has a positive influence compared to solely alkaline hydrolysis (6.5 mg GAE/g DW). Subsequently, a 3^3 factorial design based on temperature, NaOH concentration, and sonication time was applied, and the optimum condition corresponding to a sonication of 30 min with 2 M NaOH under 60 °C was discovered [78]. The research group further determined the optimum conditions for the ultrasonic alkaline hydrolysis of Brussels sprouts stalks (60 °C, 4 M NaOH for 30 min, TPC = 5.0 mg GAE/g DW) and red cabbage wastes (80 °C, 4 M NaOH for 45 min, TPC = 17.9 mg GAE/g DW) [21]. A study carried out by Machado in 2017 also analysed the influence of UAE and combined it with pressurised liquid extraction (PLE) to extract polyphenols from blueberry pomace. The results showed that the combination of PLE and UAE contributed to the highest TPC yield (TPC= 8.84 mg GAE/g DW), while the ultrasound solely had a comparably lower yield (TPC = 5.86 mg GAE/g DW) [89].

3.4.2. Microwave-Assisted Extraction (MAE)

Although MAE is widely applied to extract polyphenols from plant-based ingredients [14,91,92], there is currently only one research from Chiremba et al. that analysed the combined effect of MAE

and hydrolysis to extract NEPs from sorghum bran samples [93]. In this research, the bran samples were hydrolysed with 2 M NaOH in a 1400 W domestic microwave for 45 s at 190 °C. The results showed that the samples were completely solubilised in 45 s, which was significantly quicker compared to conventional alkaline hydrolysis (which needs at least 2 h). The bound phenolic acids released by the alkaline MAE reached 1114 μg/g dry bran [93]. Besides this study, a sequential extraction including the facilitation of MAE was performed by Fernandez et al. in 2019 to extract polyphenols from apple pomace. After two times acidic solvent extraction, a 5 min MAE extraction with acetone/8 M Urea/acetic acid (60:39:1 *v*/*v*/*v*) at 140 °C allowed obtaining the maximum amount of phenolic compound equal to 55 mg GAE per gram of dry apple pomace [66]. Besides facilitating the extraction procedure, microwave can also be used to heat the sample as a pre-treatment to extract NEPs from food by-products. The studies of Hayat et al. in 2010a [14] and 2010b [94] showed that the microwave pre-treatment cleaved and liberated the phenolic compounds from the plant matrix, which increased the extraction efficiency of bound polyphenols.

3.4.3. Supercritical Fluid Extraction (SFE)

SFE relies on the use of a solvent at conditions of pressure and temperature above the critical values. In this condition, the supercritical fluid reaches a characteristic liquid-like density and gas-like viscosity, which makes it suitable for various extraction applications [92]. Although recent research mainly focused on the extraction of EPPs by SFE [95], it is possible and feasible to extract NEPs also [6]. Research by Mushtaq et al. applied the SFE system to extract NEPs from enzyme hydrolysed black tea leftover [53]. Supercritical carbon dioxide combined with ethanol was used to extract NEPs with a 0.2 g/min flowrate at 55 °C and 300 bar pressure for 120 min. The comparison between conventional extraction and SFE showed that the SFE extracted a significantly higher amount of TPC (521 mg GAE/g) compared to the conventional extraction (283 mg GAE/g) [53].

3.4.4. Solid Phase Extraction (SPE)

After the NEPs extraction process, some studies proposed the purification of NEPs [7]. SPE is an extractive technique that separates the compound dissolved or suspended in a liquid mixture according to their physical or chemical properties [96]. In NEPs extraction from food by-products, the SPE purification is mainly used before TPC analysis [62] or before HPLC analysis [3,66,77,78]. This purification can effectively remove sugars and organic acids from the extraction solvent to increase the accuracy and avoid contaminations of the final extract. Sep-Pak C_{18} (vinyl polymers) and Sephadex LH-20 (hydroxypropylated dextran gel) cartridges are mostly used for the elution combined with acidified organic solvent (formic acid with methanol or ethanol) [3,66,78]. Jiangsu University designed a purification system to produce NEPs powder starting from neutralising the hydrolysed solvent. Then, NEPs extracts are further purified with a pre-treated microporous resin column eluted with 50% ethanol. The solvent can be further evaporated with the facilitation of vacuum evaporator and freeze drying to achieve a lyophilised NEPs powder for further analysis [2].

Table 2. NEPs extraction with different hydrolysis from food by-products.

Hydrolysis Methods	Food By-Product	Pretreatment	Protocol	Reference
Acid Hydrolysis	Apple, mango, papaya, pineapple, etc., left over	Freeze-dried.	1.5 M HCl in 85% ethanol, extracted under refrigeration for 13 h in the dark.	[12]
	Apple pomace	Liquid nitrogen dried and milled. Defatted.	Sequence extraction with acetone/water/acetic acid and acetone/8 M urea/acetic acid. Facilitated by microwave and purified by SPE [3] (C_{18} Sep-Pak cartridges).	[66]
	Apple waste	Freeze-dried. Extracted with 80% ethanol and 0.18 M HCl.	200 mg sample (DW) extracted with 10 mL butanol/HCl/$FeCl_3$ (97.5/2.5 *v*/*v*, 0.7 g) at 100°C for 60 min.	[72]
	Blueberry leaves	Oven dried. Extracted with different concentration of methanol/water solvent.	200 mg sample (DW) extracted with 10 mL butanol/HCl/$FeCl_3$ (97.5/2.5 *v*/*v*, 0.7 g) at 100°C for 60 min. 100 mg sample (DW) extracted with 10 mL methanol/H_2SO_4 (90:10 °C) at 85°C for 20 h.	[54]
	Blueberry pomace	Freeze-dried. Extracted with acidic ethanol/water (50:50), pH adjusted to 2 by HCl.	Extracted with acidified water at pH = 2.0. Facilitated by PLE [4] (10.0 MPa, 80 °C for 30 min), UAE [5] (37 KHz, 580 W, for 90 min) and two methods combined.	[89]
	Date palm kernels	Extracted for 2.37 h at 43.23 °C in 75.39% methanol/ethanol concentration and 54.57 mL/g of solvent/sample ratio.	Acid hydrolysis with butanol/HCl (97.5:2.5, *v*/*v*) and heated at 100 °C. Continuously stirred for three hours.	[74]
	Fruit peels (apple, kiwi, banana, melon)	Freeze-dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid hydrolysis with methanol and sulphuric acid for 20 h at 85 °C. Then subjected to SPE [5] treatment (Oasis HLB cartridges). Acid hydrolysis with butanol/HCl/$FeCl_3$ at 100 °C for 1 h.	[3]
	Grape peels	Freeze-dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid hydrolysis with HCl/butanol/$FeCl_3$ (5:95 *v*/*v* containing 0.7 g/L $FeCl_3$) solution in a boiling water bath for three hours. Acid hydrolysis with methanol/H_2SO_4 (90:10 *v*/*v*) at 85°C under slight shaking for 20 h. Purified with a SPE-C_{18} cartridge.	[59]
	Grape pomace	Freeze-dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid hydrolysis with methanol/H_2SO_4 (10:1 *v*/*v*) at 85 °C under slight shaking for 20 h. Acid hydrolysis with HCl/butanol (97.5:2.5 *v*/*v* with 0.1% $FeCl_3$) solution at 100 °C for one hour.	[67]
	Grape seed	Air dried. Extracted with 60% methanol.	Acid hydrolysis with HCl/butanol (5:95 *v*/*v* containing 2% ferric reagent). Performed with liquid–liquid extraction.	[73]
	Mandarin waste	Oven dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid hydrolysis with methanol/H_2SO_4 (90:10 *v*/*v*).	[55]

	Olive pomace	Freeze-dried. Defatted. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*). Lipid removed by hexanes.	Acid hydrolysis with methanol/H_2SO_4 for 20 h at 85 °C. Acid hydrolysis with butanol/HCl (95:5 *v*/*v*, containing $FeCl_3$) at 100 °C for 1 h.	[20]
	Roselle by-products	Forced circulation dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid hydrolysis with butanol/HCl (95:5 *v*/*v* containing 2% *w*/*v* $NH_4Fe(SO_4)_2 \cdot 12\ H_2O$ in 2 M HCl) at 100 °C for 1 h.	[56]
Alkaline hydrolysis	Black rice bran	Air dried. Extracted with chilled acidified methanol (95% methanol:1 M HCl 85:15 *v*/*v*). Lipid removed with hexanes.	Alkaline hydrolysis with 2 M NaOH at room temperature for 1 h.	[61]
	Brown rice bran	Extracted with 80% chilled acetone. Lipid removed by hexanes.	Alkaline hydrolysis with 2 M NaOH at room temperature for 1 h with continuous shaking under nitrogen gas.	[18]
	Cauliflower waste	Freeze-dried. Extracted with pure methanol.	Alkaline hydrolysis with 2 M NaOH for 15 min at 60 °C in a screw-capped test tube previously flushed with nitrogen. The extraction was facilitated with ultrasound (37 KHz, 180 W). The NEP went through SPE (C_{18} SPE cartridge) prior to analysis.	[78]
	Citrus peels	Sun dried. Extracted with 80% methanol.	Alkaline hydrolysis (4 M NaOH) at ambient temperature for 1 h. Facilitated with microwave (for heating) and ultrasound.	[14]
	Chestnut peel	Freeze-dried.	Optimise the alkaline hydrolysis condition with different concentrations of Na_2SO_3 and NaOH (from 1% to 8%) for a different period (from 30 to 960 min) at 85 °C.	[57]
	Cranberry pomace	Lipid removed by hexane.	Optimise the alkaline hydrolysis condition with NaOH (2 M, 4 M, and 6 M) with water shaking bath (200 rpm) at different times (from 5 min to 24 h) under 25, 40, or 60°C. NEPs isolated with SPE (Sephadex LH-20).	[77]
	Mango peels	Extracted with 80% chilled acetone. Air dried.	Alkaline hydrolysis with 1 M NaOH (containing 0.5% $NaBH_4$) under nitrogen atmosphere.	[81]
	Red cabbage and Brussels sprouts waste	Freeze-dried. Extracted with methanol.	Optimise the alkaline hydrolysis condition with NaOH with different temperatures and incubation time. Extraction facilitated with ultrasound. Purified with SPE (C_{18}-cartridge).	[21]
	Rice bran	Air dried. Extracted with chilled acidified methanol (95% methanol:1 M HCl 85:15 *v*/*v*). Lipid removed with hexanes.	Alkaline hydrolysis with 2 M NaOH at ambient temperature for 1 h under nitrogen condition.	[19]
	Sorghum bran	Air dried.	Alkaline hydrolysis with 2 M NaOH facilitated by microwave oven (1400 W, 45 s).	[93]
Sequential hydrolysis or acid and alkaline	Apple pomace	Freeze-dried. Extracted with methanol/water (80:20 *v*/*v* with 1% formic acid).	Multistep sequential extraction with acid, alkaline and combined hydrolysis (2 M NaOH and 2 M HCl).	[15]

hydrolysis comparison study	Wheat bran	Freeze-dried. Extracted with acidic methanol/water (50:50), pH adjusted to 2 by HCl. Washed by acetone/water (70:30 *v*/*v*).	Acid (methanol/H_2SO_4 90:10 *v*/*v* at 85 °C for 20 h) and alkaline hydrolysis (2 M NaOH at room temperature for 4 h).	[17]
	Wheat bran	Defatted with hexane. Extracted with 80% methanol.	Acid (6 M HCl at 95 °C for 20 h) and alkaline (2 M NaOH at room temperature for 4 h) hydrolysis.	[79]
Enzyme Hydrolysis	Black tea leftover	Washed thoroughly and dried under ambient condition.	Enzyme hydrolysis (Kemzyme, Alcalase, Pectinex, etc.). Applied ultrasound for degas. Facilitated by SFE [6].	[53]
	Citrus peels	Liquid nitrogen dried and milled.	Enzyme hydrolysis with Cellulase MX, Cellulase CL, and Kleerase AFP. Purified by SPE (C_{18} Sep-Pak cartridge).	[62]
	Grape pomace	Oven-dried.	Enzyme hydrolysis with pectinase, cellulase, tannase, etc.	[16]
	Grape skin and seeds	Freeze-dried.	Enzyme hydrolysis with pectinase, cellulase, and tannase.	[82]
	Juice pomace	Freeze-dried.	Enzyme hydrolysis with Pectinases Macer 8 FJ, 8R, and Novozym 89 acid protease.	[63]
	White grape pomace	Oven-dried.	Enzyme hydrolysis with Pectinex 3X L, Pectinex Ultra SPL, Termamyl, Fungamyl, Pentopan, 500B G, and Celluclast.	[97]

[3] SPE: solid phase extraction; [4] PLE: pressurised liquid extraction ; [5] UAE: ultrasound-assisted extraction; [6] SFE: supercritical fluid extraction.

4. NEPs Characterisation Methods

Based on the potential applications, several analytical techniques are applied to characterise the NEPs extracted from food by-products. Generally, in vitro analysis of NEPs is based on three main approaches. The first is based on the determination of the total amount of NEPs, which is generally achieved by spectrophotometric methods. The second approach aims to determine the profile of individual constituent of NEPs by liquid or gas chromatography. Finally, the third approach for NEP analysis is focused on the determination of their antioxidant activity.

4.1. Spectrophotometry

Table 3 shows the main methods used for the determination of the total amount of NEPs. Spectrophotometric assays, such as the Folin–Ciocalteu, are the most common, which is likely because of their low cost and simple procedure of analysis [6].

The working principle of Folin–Ciocalteu is that phenol compounds produce a blue colour complex by reacting with phosphomolybdate and phosphotungstate reagent under alkaline conditions. Gallic acid standard is used as a comparison; it is detected and quantified at 765 nm by spectrophotometry, and the result is presented as gallic acid equivalent [98]. However, a drawback of the assay is that the reagent can react also with any reducing substances (sugars, proteins, etc.) present in the NEPs extracts, resulting in overestimations. To mitigate that, a pre-treatment of the NEPs, such as SPE [77,78], is often required.

A second widely used method to characterise the flavonoid content of NEPs is the vanillin assay [99]. The assay is used specifically to determine the proanthocyanidin content. The assay is based on the reaction between vanillin and the 6′ or 8′ position of the flavonoid ring under strong acid conditions. The occurrence of the reaction produces a coloured compound, which can be further detected and quantified at 500 nm by spectrophotometry. The epicatechin standard is used for equivalency [54,74]. However, the acid condition and substrate concentration may influence the accuracy of the results [7]. Comparably, dimethylaminocinnamaldehyde (DMAC) assay, which is based on the reaction with phenolic compounds, measured at 640 nm, excludes the interferences with the solvent condition and is more accurate and sensitive [6,100].

The anthocyanidin content is determined by a differential pH assay [54,61]. The assay measures the absorption of the extract at 510 nm and 700 nm at two pHs, respectively, pH = 1 and pH = 4.5. Since anthocyanins present a highly coloured oxonium form at pH = 1 and colourless hemiketal form at pH = 4.5 [101], the resulting anthocyanidin content can be calculated [102]. Meanwhile, some studies also estimated the anthocyanins content by directly measuring the absorbance at 535 nm or 555 nm after NEPs extraction and compared it with the standard calibration curve [12,72].

4.2. Liquid Chromatography and Mass Spectrometry

The polyphenol profile of NEPs is generally determined by HPLC. The efficiency of the HPLC method depends on the stereochemistry, polarity, or molecular weight of the individual compounds [103]. NEPs are strong chromophores, since the molecular structure contains multiple conjugated double bonds. HPLC is currently the most extensively applied instrumental technique to separate and quantify NEPs [6] (see Table 3). Reverse phase HPLC [3,4,72,81] and normal phase HPLC [77] can both be implemented for NEPs identification, while the reverse phase column is dominating in NEPs research. That is because the highly polymerised procyanidins cannot entirely be eluted from a silica-based normal phase column [104]. HPLC systems are often coupled to mass spectrometers (MS) in order to identify the unknown compounds. White et al. analysed the NEPs content of cranberry pomace with a normal phase HPLC coupled to a fluorescence detector eluted by acetone, water, and acetic acid (70:29.5:0.5). The mass spectral data indicated that A-type dimers and oligomers were the dominant procyanidins presented in the cranberry pomace [77,105]. However, the increasing degree of polymerisation may negatively influence the accuracy of the fluorescence detector [7].

Table 3. NEPs characterisation and main results.

Food By-Product	Featured Extraction/Purification Method	Characterisation (Spectrophotometry or Chromatography)	Antioxidant Capacity	Main Compounds	Reference
Apple, mango, papaya, pineapple etc., left over	Acid hydrolysis	TAC [7], TPC [8]	-	Cherry left over has the highest TPC (12,696.03 mg GAE [9]/100 g DW [10]), followed by cashew apple (6588.41 mg GAE/100g DW) and pineapple (2787.09 mg GAE/100g DW).	[12]
Apple pomace	Chemical hydrolysis	TPC, UHPLC [11]-MS [12](Q-Exactive Orbitrap MS, ESI [13], 80–1200m/z), ORAC [14]	Highest antioxidant capacity achieved by alkaline hydrolysis (478.66 μmol TE [15]/g DW).	Sequence extraction with both acid and alkaline hydrolysis achieved the highest TPC (12.38–13.76 mg GAE/g DW). Quercetin-3-O-galactoside is the main compound identified by MS.	[15]
Apple pomace	Sequential extraction facilitated by microwave	UHPLC-DAD [16]-ESI-MS (RP [17], Ultra ion trap MS, ESI, 100–1000 m/z)	-	Flavan-3-ols are the major class of polyphenols (2.88 g/kg of dry apple pomace), with an average DP [18] of 4.7. Main compounds after alkaline hydrolysis of apple procyanidins are 3,4-dihydroxybenzoic acid (0.67 M/kg) and catechol (0.15 M/kg).	[66]
Apple waste	Acid hydrolysis	TAC [19], TPC, DPPH [20], ABTS [21]	NEPs has a significantly higher rate of radical scavenging capacity than EEPs.	The NEPs content ranges from 18.40 to 23.48 mg anthocyanidins equivalent/100g DW, which occupies 64.07% of TPC in apple waste.	[72]
Black rice bran	Alkaline hydrolysis	TPC, TAC, ORAC	The antioxidant ability ranges from 47.91 to 79.48 μM of TE/g DW.	The NEPs content ranges from 221.2 to 382.7 mg GAE/100 g DW.	[61]
Black tea leftover	Enzyme hydrolysis, facilitated with SFE [22]	TPC, UHPLC-DAD- MS (RP, Triple quadrupole MS, ESI, 100–1200 m/z), DPPH, FRAP [23], ABTS	Enzymatically hydrolysed sample has liberated a greater amount of ABTS radical cations comparative to Trolox (1,156.56 ± 46.88 μM TE/g).	The optimised condition achieved 521.44 mg GAE/g of TPC during supercritical fluid based extraction. *p*-coumaric acid (208.33 μg/mL) is the major phenolic acid in the black tea left over.	[53]
Blueberry leaves	Acid hydrolysis	TPC, TAC, TPAC	-	The NEPs occupies 2.81% to 3.73% of TPC. The non-extractable proanthocyanidins content ranges from 10.06 to 11.69 mg/g with different extract conditions.	[54]
Blueberry pomace	Acid hydrolysis, facilitated by ultrasound	TPC, HPLC-MS (Q-TOF-MS, ESI, 100–800 m/z)	-	UAE [24] + PLE [25] is the most efficient method to extract polyphenols (8.54 mg GAE/g DW). Main compound after UAE is cyanidin-3-O-galactoside (0.32 mg/g DW).	[89]

Brown rice bran	Alkaline hydrolysis	TPC, HPLC-PDA (RP), FRAP. ORAC	The antioxidant ability of NEPs ranges from 207 to 267 mg of TE/100 g DW, which is significantly lower than EPPs (range from 452 to 589 mg TE/100 g DW).	The TPC after alkaline hydrolysis reaches 276 mg GAE/100 g DW. Ferulic acid (1,617 μg/g DW) and *p*-coumaric acid (394 μg/g DW) are the main compounds in NEPs after hydrolysis.	[18]
Cauliflower waste	Alkaline hydrolysis, facilitated with ultrasound	TPC, HPLC-ESI-MS (HDMS [26]-TOF [27], ESI, 100-1500 Da) (qualify), HPLC-DAD (RP) (quantify)	-	Alkaline and UAE achieves the highest TPC extraction (7.3 ± 0.17 mg GAE/g DW). Kaempferol-3-O-diglucoside-7-O-glucoside is the most abundant flavonoid present in the NEP fraction (2.4 ± 0.1 mg/g DW).	[78]
Chestnut peel	Alkaline hydrolysis with Na_2SO_3 and NaOH	TPC, HPLC-DAD (RP)	-	The highest TPC achieved by extracting with 1% NaOH for 4 h (4,112.1 μg/g DW). The ellagic acid is the main compound after hydrolysis (3,542.6 μg/g DW).	[57]
Citrus peels	Acid and alkaline hydrolysis in a sequence	HPLC-PDA [28] (RP), DPPH	The antioxidant capacity of the extract increased with microwave power after alkaline hydrolysis (the maximise scavenging activity reaches 26.30%).	Microwave treatment of citrus peels cleaves and liberates phenolic compounds (maximum TPC is 3,583.5 μg/g DW). The ferulic acid is the main compound after hydrolysis (2,162.6 μg/g DW).	[14]
Citrus peels	Enzyme hydrolysis	TPC, FRAP, HPLC-PDA (RP)	Grapefruit has the highest total antioxidant activity (1.719 ± 0.075 mM $FeSO_4$/100 g fresh peel).	The grapefruit peels contain the highest amount of TPC after enzyme hydrolysis, which vary from 90 to 162 mg GAE/100 g fresh peel.	[62]
Cranberry pomace	Alkaline hydrolysis	HPLC-DAD (NP [29]), HPLC-MS (Q-ion trap MS, ESI), MALDI [30]-TOF-MS (TOF-MS, MALDI)	-	Alkaline hydrolysis resulted in a 30% increase in total procyanidins compared to conventional extraction (1,685 mg/100 g DW and 1,292 mg/100 g DW, respectively).	[77]
Date palm kernels	Acid hydrolysis	TPC, TPAC [31], DPPH	The antioxidant capacity (IC_{50}) ranges between 58.12 ± 3.32 and 70.5 ± 9.66 μg/mL.	Maximum extract yield of NEPs (14.2%) achieved at 85 °C extracted for 3 h with 1:20 solid to liquid ratio (g/mL).	[74]
Fruit peels (apple, kiwi, banana, melon)	Acid hydrolysis	TPC, HPLC-DAD (RP)	-	NEPs contribute from 7% (mango) to 82% (banana) of TPC. The highest concentration is 9.62 mg/g (banana) among fruit peels. Hydroxycinnamic acids are detected as main compounds in the hydrolysable part of polyphenols in melon, orange, and pear.	[3]
Grape peels	Acid hydrolysis	HPLC-PDA-MS/MS (RP, Q-TOF analyser, ESI, 100–1000m/z)	-	The major phenolic compounds found in the NEPs are procyanidin, syringic acid, *p*-coumaric acid, and hydroxybenzoic acid derivatives.	[59]

Grape pomace	Enzyme hydrolysis	TPC, HPLC-DAD (RP), ABTS	The highest antioxidant capacity is 5.58 mM TE/100 g DW.	With the optimised condition (188 U/g DW of cellulase and 198 U/g DW of tannase at 45 °C), the TPC content reaches 0.81 g GAE/100 g DW. Gallic acid is the main compound after hydrolysis (0.16 g/100 g DW).	[16]
Grape pomace	Acid hydrolysis	TPC, HPLC-ESI-MS (Q-TOF, ESI, 100-1200 Da &TOF, MALDI)	The antioxidant ability is 440 and 324 μM TE/g DW by FRAP and ABTS, respectively.	The NEPs occupy 14.4% of dry weight in grape skin while the EPPs occupy 3.5%. Dihydroxybenzoic acid is the main compound determined after hydrolysis (74.3 mg/100 g DW).	[67]
Grape seed	Acid hydrolysis, facilitated by SFE	TPC	-	The TPC ranges from 1.23 to 2.37 mg GAE/100 g DW.	[73]
Grape skin and seeds	Enzyme hydrolysis	TPC, HPLC-DAD (RP)	-	The three enzymes used individually are able to increase the total phenol release of grape seed by 1.26, 1.32, and 1.34 times, respectively.	[82]
Juice pomace	Enzyme hydrolysis	TPC	-	The maximum TPC yield achieved when adding 10% Macer8 FJ and Grindamyl pectinase enzyme (383 mg GAE/L DW).	[63]
Mandarin waste	Acid hydrolysis	HPLC-DAD (RF), ABTS, ORAC, DPPH, FRAP	The antioxidant ability ranges from 333.43 to 351.55 (μM TE/g DW).	The highest TPC achieved when the sample was dried at 120 °C (74.56 mg GAE/g DW). Non-extractable hesperidin occupies 35.7% of phenolics in fresh mandarin waste.	[55]
Mango peels	Acid hydrolysis	HPLC-DAD (RP)	-	The bound phenolic content in mango peel dietary fibre ranges from 8.12 to 29.52 mg/g, while the bound flavonoids content ranges from 0.101 to 0.392 mg/g. Gallic acid is the major phenolic acid in both raw and ripe mango peel (6.29 and 16.60 mg/g DW, respectively).	[81]
Olive pomace	Acid hydrolysis	TPC, HPLC-PDA(RP)	-	More NEPs released with granulometric fractionised and micronised samples (maximum 13.2 g GAE/100 g DW).	[20]
Red cabbage and Brussels sprouts waste	Alkaline hydrolysis, facilitated by ultrasound	TPC, HPLC-MS (HDMS-TOF, ESI, 100-1500 Da)	-	The maximised condition to extract NEPs from red cabbage waste achieved when extracting with 4 M NaOH at 80 °C for 40 min (7.8 mg GAE/g DW).	[21]

Rice bran	Alkaline hydrolysis	TPC, HPLC-PDA (RP), FRAP, ABTS, ORAC	The IC_{50} values of the bound fraction ranged from 78.7 to 153.6 mg TE/100 g DW with the percentage contribution to the total antioxidant ability ranging from 10.5% to 21.1% by ORAC assay.	The bound phenolic content ranges from 91.1 to 126.8 mg GAE/100 g with the percentage contribution to the total ranging from 10.8% to 14.5%. Ferulic acid is the main component after hydrolysis (1.24 mg/g DW).	[19]
Roselle by-products	Acid hydrolysis	TPC, UHPLC-MS (Q-TOF, ESI, 50–1800 Da)	-	The hydrolysable polyphenols and proanthocyanidins content of roselle calyx by-products are 6.18 mg GAE/g and 6.67 mg proanthocyanidins equivalent/g. The NEPs content occupied 71.2% of the TPC.	[56]
Sorghum bran	Alkaline hydrolysis	HPLC-PDA (RP), HPLC-MS (Q-TOF-MS,100–1500 m/z)	-	The ferulic acid and *p*-coumaric acid are the main compounds (1,189 and 179 μg/g DW) in maize bran.	[93]
Wheat bran	Acid and alkaline hydrolysis	HPLC-MS -DAD (RP, ESI, 100–1000 m/z)	-	Caffeic, ferulic, and cinnamic acids are the main hydroxycinnamic acids in bran (43% of total hydrolysable polyphenols).	[17]
Wheat bran	Acid and alkaline hydrolysis	TPC, HPLC-DAD (RP)	-	The TPC content of wheat bran ranges from 654 to 2,326 μg GAE/g. Ferulic acid is the predominant phenolic acid after acid hydrolysis.	[79]
White grape pomace	Enzyme hydrolysis	TPC, HPLC-DAD (RP), ABTS	The highest antioxidant activity is detected in the dried powder extraction for 6 h with 2% Celluclast at 37 °C (7.82 g ascorbic acid equivalent/L).	For wet pomace, the optimised TPC extraction condition reaches at 2 h of incubation with enzyme hydrolysis (1,316 mg GAE/L). For dry pomace, the incubation time poses no effect to the TPC content (2,636 mg GAE/L).	[97]

[7] TAC: total anthocyanins content; [8] TPC: total phenolic content; [9] GAE: gallic acid equivalent; [10] DW: dry weight; [11] UHPLC: ultra-high-performance liquid chromatography; [12] MS: mass spectrometry; [13] ESI: electrospray ionisation; [14] ORAC: oxygen radical absorbance capacity; [15] TE: Trolox equivalent; [16] DAD: diode-array detector; [17] RP: reverse phase; [18] DP: procyanidins average degree; [19] TAC: total anthocyanins content; [20] DPPH: antioxidant test with 2,2-diphenyl-1-picrylhydrazyl; [21] ABTS: antioxidant test with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; [22] SFE: supercritical fluid extraction; [23] FRAP: ferric reducing antioxidant power; [24] UAE: ultrasound-assisted extraction; [25] PLE: pressurised liquid extraction; [26] HDMS: high definition mass spectrometry; [27] TOF: time of flight; [28] PDA: photodiode array; [29] NP: normal phase; [30] MALDI: matrix-assisted laser desorption/ionisation; [31] TPAC: total proanthocyanidins content.

Reverse-phase HPLC is the most implemented instrument for detecting NEPs extracted from food by-products. Compared to normal phase HPLC, where the compounds are eluted based on the degree of polymerisation, the reverse phase HPLC separates NEPs according to the overall polarity [106]. However, peak overlapping may occur in complex extractions. Hallström et al. observed the retention times overlapping between procyanidins, which made it challenging to separate large polymers (degree of polymerisation > 4) [58].

To avoid overlapping and increase the accuracy, thiolysis [58] or phloroglucinolysis [82] pre-treatments could be used [7,104]. By cleaving the inter-flavane bounds, such a reaction can facilitate the stoichiometry determination of degree of polymerisation [58,107]. Fernández et al. analysed the NEPs content in enzyme-hydrolysed grape skin with a phloroglucinolysis pre-treatment. In detail, a solution of NEPs in methanol was treated with 0.1 mol/L HCl in methanol and 100 g/L of phloroglucinol for 20 min at 50 °C. The solution, after adding sodium acetate to terminate the reaction, was analysed by HPLC with two RP-18e columns. The extract characterisation by phloroglucinolysis pre-treatment presented values of mean degree of polymerisation, percentage of galloylation, as well as the proportion of components [82].

Ultraviolet diode array detector (UV-DAD) and electrospray ionisation mass spectrometry (ESI-MS) are common detectors coupled to HPLC to analyse NEPs [6]. The molecular structure of NEPs possesses at least one aromatic ring, which exhibits at least one absorption maxima in the UV region. Pérez-Jiménez and Saura-Calixto in 2018 summarised the absorption maxima of flavanols (214 nm), hydroxybenzoic acid and flavanones (280 nm), hydroxycinnamic acids (320 nm), flavonols (365 nm), and anthocyanins (520 nm). The results of each polyphenol class were expressed by proper standard equivalents. The total concentration of the NEPs from fruit peels were calculated by combining the non-extractable proanthocyanidins (detected from spectrophotometry) with the hydrolysable tannins (hydroxybenzoic acids, hydroxycinnamic acids, and flavanols) [3]. This method was also used to determine the content of NEPs in wheat bran [17], grape pomace [16,59], and chestnut waste [57]. Since both the proanthocyanidins and hydrolysable tannins exhibited an absorption peak from 270 to 280 nm, and bound flavonoids at 320 nm [6], several studies also determined the content of NEPs with wavelengths at 280 nm [14,18,19,62] or 320 nm [79,81]. However, the UV/Vis or DAD detector was not able to detect procyanidin monomers, oligomers, and their degradation products separately.

To carry out an individual molecular structure analysis of NEPs, HPLC can be coupled to a mass spectrometry (MS) detector [108]. According to the mass-to-charge ratio and the exact masses provided by the high-resolution mass spectra, the HPLC-MS system can deduce the elemental formulas of the respective compounds from the NEPs extract [6,108]. An MS system involves mainly three parts, which are the ion source, the mass analyser, and the detector [106]. The proper selection of the first two parts is crucial for NEPs characterisation [6]. In studies related to NEPs extracted from food by-products, electrospray ionisation (ESI) is the most used instrument for ionisation (see Table 3). ESI is very efficient for detecting high molecular weight compounds by analysing multicharged ions [108]. Comparably, it allows the detection of thermosensitive compounds, and it can separate between different polyphenol classes. In this case, the phenol compounds are usually detected in negative ionisation mode [109,110]. Li et al. used ESI with Q-Exactive Orbitrap MS to analyse NEPs extracted from apple pomace. With several external standards, the study identified and quantified the dominant individual phenolic compounds in apple pomace as quercetin-3-O-galactoside and quercetin-3-O-glucoside [15]. However, the ESI is not suitable for detecting NEPs with a high degree of polymerisation, since it is difficult to interpret multiply charged ions [6,56,111]. Meanwhile, a lack of external standard and limited mass range may also decrease the accuracy [7]. Therefore, to determine the high degree of polymerisation NEPs, matrix-assisted laser desorption/ionisation (MALDI) can be used for ionisation [111]. It provides higher resolution results than ESI, since it has a higher tolerance for impurities and does not form multiply charged ions [48,111].

Another MS system used for NEPs detection is time of flight (TOF) MS, which is usually coupled with the matrix-assisted laser desorption ionisation (MALDI) system [56,77,105]. White et al. characterised procyanidins with MALDI-TOF-MS and HPLC-ESI-MS from cranberry pomace dietary

supplements [77,105]. The results showed that the ESI-MS was able to identify procyanidins from monomers to hexamer (degree of polymerisation = 6). At the same time, the MALDI-TOF-MS qualified high molecular weight procyanidins until the degree of polymerisation = 13, which significantly increased the accuracy of the identification [77,105]. Moreover, HPLC-quadrupole-time of flight (Q-TOF)-MS coupled with ESI can also be applied to analyse the NEPs, especially hydrolysable tannins [56,59,93]. A study carried out by Maurer et al. reported the identified and quantified NEPs profile after sequential acid and alkaline hydrolysis. Syringic acid and hydroxybenzoic acid (which are derivates of hydrolysable tannins), as well as procyanidin (degree of polymerisation not mentioned), were detected in the grape peel powder [59]. In some research, triple quadrupole (QQQ) MS [53] and ion-trap MS [15,66] were also used to determine the derivates of NEPs after hydrolysis. To obtain more specific structure information, MS analyser can be also combined as tandem MS (MS-MS or MS^n) [56,59,90]. Research by Montero et al. developed and optimised a new chromatography system to analyse the procyanidins from grape seed based on tandem MS. This system combined hydrophilic interaction × reversed phase liquid chromatography (HILIC×RP-LC) with a diode array detector and tandem mass spectrometry detector (DAD-MS/MS). It allowed the direct identification of 43 monomer, oligomer, and polymer (until polymerisation degree 7) flavan-3-ols with different galloylation degrees [90].

4.3. Antioxidant Activity of NEPs

The capacity of delaying or inhibiting the oxidation process under the presence of oxygen or reactive oxygen species is one of the main properties of NEPs. Several methods based on the competitive probe reaction could be applied to test the antioxidant ability of NEPs [72,112]. Currently, mainly, four spectrophotometric tests have been applied for the NEPs quantification of the antioxidant activity, which are DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric-reducing antioxidant power), oxygen radical absorbance capacity (ORAC), and ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (see Table 3).

The measurement of DPPH free radical scavenging activity relies on the hydrogen donor reaction with DPPH, which fades the violet colour measured at 517 nm by spectrophotometry. The result can be appraised as maximal inhibitory concentration (IC_{50}) [20,53] or expressed as the Trolox equivalent [55] or scavenging activity of a specific standard antioxidant compound [14,72,74]. Research by Tow et al. analysed the antioxidant activity of both EEPs and NEPs extracted from apple waste. The result by DPPH demonstrated that the NEPs (80.54%) had a significantly higher rate of radical scavenging capacity compared to EPPs (16.20%) [72]. The research also tested the antioxidant ability by ABTS, which relies on measuring the bluish-green colour decolourisation at 743 nm when the hydrogen donating antioxidant is present [72,112]. Similar results were obtained also using this assay. A study carried out by Esparza-Martínez et al. demonstrated the antioxidant activity by ABTS, expressed as Trolox equivalent, and proved that mandarin waste dried at 120 °C had an antioxidant capacity equal to 574.71 μM Trolox equivalent/g DW [55].

The FRAP assay relies on the reaction between antioxidants and a complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine). The ligand and Fe^{2+} binding create a navy-blue colour measured by spectrophotometry at 700 nm [112]. FRAP was applied to analyse the reducing power of NEPs from black tea leftover [53], mandarin waste [55], and brown rice bran [18,19] based on Trolox equivalent.

The ORAC assay, which measures the reaction between antioxidants and a peroxyl radical induced by AAPH (2,2′-azobis-(2-amidino-propane) dihydrochloride) by a fluorescent probe, is also widely implemented to analyse the antioxidant activity of NEPs extracts [15,18–20,55]. A study carried out by Zhang et al. applied both FRAP and ORAC to analyse the antioxidant capacity of extracts from brown rice and its bran. The results showed that the bran had the highest antioxidant activity by both methods compared to polished rice [18]. Esparza-Martínez et al. applied all four antioxidant tests on mandarin waste dried at different temperatures [55]. By comparing the results, it was concluded that the four methods provided significantly different results. This was associated to the different ways antioxidants can scavenge radicals, which occurs by mainly two mechanisms:

hydrogen atom transfer (HAT) and single electron transfer (SET). The ORAC is based on the HAT to analyse the total antioxidant equivalents while FRAP, ABTS, and DPPH are based on SET from free radical scavenging capacity (ABTS and DPPH), and ferric reducing power (FRAP) perspective [19,53,55]. The selection of the proper assay should be based on the characteristics of NEPs extract such as the colour or content of other bioactive components.

5. Potential Applications

The increasing knowledge and research on NEPs from food by-products leads to further discussion and studies on their potential applications (Table 4). Currently, the existing and potential applications of NEPs from food by-products can be mainly divided into two categories: as food additives or ingredients, and as human health supplements [45].

The studies about solely utilising NEPs from by-products as food ingredients or additives is currently insufficient [6]. There are only two patents licensed to food companies for the extraction of NEPs from food by-products [113,114]. The first patent recovers dried ingredient powder with up to 35% antioxidants from fruit pulps with organic solvent extraction, sugar removal, dehydration, and micronation. The final products, which have a high antioxidant ability and low-calorie content (less than 20 kcal/100 g), are preferred for the production of functional beverages, juices, milkshakes, or other products with an antioxidant claim [113]. The second patent applies enzymatic treatment to produce polymeric proanthocyanidins without organic solvent. Under optimum conditions, this patent achieves a production of polymeric proanthocyanidins with a concentration of 90% and 80% from persimmon and banana, respectively. Such concentrates can be used as functional food or dietetic product ingredients [114]. Meanwhile, research by Zhang et al. showed that NEPs from black rice bran could potentially be extracted as natural colourants to substitute artificial black, purple, or red pigments in the food industry [61].

In most of the current research, NEPs from food by-products are studied in combination with EEPs [8]. To minimise the production cost and increase efficiency, the dried fruit or vegetable pomaces, which have a high content of both EEPs and NEPs, are directly added to food products as additives or ingredients without further extraction [115]. Adding processed pomace as an ingredient is able to increase the shelf life, nutritional content, or fibre content to the final food products. Marchiani et al. analysed the effect of adding dried grape skin flour to yoghurt production in 2015. Adding grape skin pomace during the yogurt fermentation process can significantly increase the TPC without sensory changes of the product [116]. The added pomace posed no side effect to the yoghurt storage. Similar research was carried out by Frumento et al. in 2013, who proved that the addition of dried grape pomace powder accelerated the milk fermentation process and increased the antioxidant activity of the final products [117].

Besides milk-related applications, dried apple, tomato, and grape pomaces can also serve as ingredients in meat and fish products [118]. In 2013, Ribeiro et al. compared the functionality of a conventional stabiliser (inner pea dietary fibre) with the grape dietary fibre added to meagre sausage. The results showed that both sausage samples were stable during the preservation period, while the sample with grape fibre presented a higher nutritional value (balanced amino acid composition and fatty acid profile, high dietary fibre content with low caloric content) [119]. Sánchez-Alonso et al. also performed a research in 2007 to analyse the influence of grape pomace powder in minced fish muscle. Water retention capacity (WRC), storage time, and sensory evaluation were carried out to compare fish muscle mixed with 0%, 2% and 4% of grape pomace. It was concluded that the addition of grape pomace significantly increased the water and oil retention capacity. In addition, 2% grape pomace in fish muscle received the highest acceptance in sensory tests [118]. Yadav et al. compared the properties of chicken sausage with the addition of dietary fibre from corn bran, dried apple, and tomato pomace. The sensory, texture, and microbiological tests showed that sausages with 6% apple or tomato dietary fibre, which meets 1/6 of recommended daily fibre intake, were microbiologically safe along the preservation period (two weeks) and feasible for industrialised production [120].

Adding fruit or vegetable pomace to cereal products can provide natural NEPs and EEPs and improve the taste [115]. Lohani and Muthukumarappan added apple pomace in sorghum and maize

flour blends to increase the content of bioactive compounds. Liquid CO_2 extrusion was applied in this research to retain the polyphenolic content and antioxidant activity while avoiding the Maillard reaction. The cereal and fruit pomace combination had the potential to be used for snack production for consumers suffering from celiac disease [121]. Berry pomace, which is rich in vitamin, polysaccharides, organic acid, and polyphenols, was found suitable as an ingredient in wheat flour dough based on research carried out by Rohm et al. in 2015 and Struck et al. in 2018 [122,123]. The added berry pomace affected the rheological properties of the dough and thus the stiffness of the final products. Optimum dough microstructure and texture was achieved with 10% of berry pomace added to the wheat flour [122]. To increase the nutrition value of cookies, a study has been carried out to analyse the possibility of adding grape or blueberry pomace as an ingredient. The research by Acun and Güi in 2013 discovered that 10% of grape pomace in cookies could significantly increase the total polyphenol content, while 5% pomace in cookies received the highest acceptance in sensory evaluation. However, with the increasing amount of grape pomace in the cookies (from 5% to 15%), the colour and the acceptability of the product significantly decreased [124]. Similar studies were done by Mildner-Szkudlarz et al. in 2012 and Aksoylu et al. in 2015. They showed that biscuits with blueberry or grape pomace reached a high nutrition value (especially mineral content), dietary fibre, and antioxidant activity compared to biscuits without fruit pomace [125,126].

Table 4. Food related applications of NEPs.

Food By-Products	Final Product	Purpose	Reference
Fruit pulps residual	Functional beverage, juice, milkshake, etc.	As antioxidant ingredients with low calorie content (<20 kcal/100 g)	[113]
Fruit peels	Polymer proanthocyanidins in different food varieties	Food supplements, functional ingredient, pharmaceutical or cosmetic products	[114]
Black rice bran	Different food varieties	As food colourants	[61]
Grape skin	Yogurt products	Increase the TPC without sensory and storage changes	[8]
Dried grape pomace	Yogurt products	Accelerate the milk fermentation, increase antioxidant ability	[117]
Grape dietary fiber	Meagre sausage	Increase nutritional value	[119]
Grape pomace powder	Minced fish muscle	Increase water and oil retention capacity	[118]
Corn bran, dried apple, and tomato pomace	Chicken sausage	As microbiological preservative	[120]
Apple pomace	Sorghum and maize flour blends	Increase the content of bioactive compounds	[121]
Berry pomace	Wheat flour dough	Improve microstructure and texture	[122,123]
Grape or blueberry pomace	Cookies	Increase TPC	[124]
White grape pomace	Wheat biscuits	Increase texture and total dietary fibre	[125]
Blueberry and grape seed powder	Biscuits	Increase antioxidant ability, increase TPC	[126]

Besides serving as food ingredients or additives, NEPs from food by-products also have a potential in the pharmaceutical area. The consuming of NEPs mainly influences gastrointestinal health due to their metabolic property. They are extensively transformed in the colon, and the several absorbed metabolites have been reported to have different health effects [9]. Specifically, the NEPs will pass through the gut and small intestine without digestion or absorption. When the NEPs reach the colon, they are absorbed, and new small molecular compounds will be formed with the action of

local microbiota. Some of these metabolites will be absorbed by the portal vein and subsequently reach the target issue with the bloodstream [45].

Several studies have been done both in vivo and in vitro to analyse the health effect possessed by NEPs from food by-products. In 2019, Amaya-Cruz et al. analysed the anti-obesity effect of roselle calyx pomace with high caloric diet rats. After 18 weeks of experiments, the rats fed with 4% of pomace from roselle calyx generated a reduction of 10% body weight and 17% of adipocytes hypertrophy compared to the solely high caloric diet group. The insulin resistance and hepatic steatosis index also significantly decreased by 48% and 15%, respectively [56]. Rodríguez-González carried out a similar research in 2018 to analyse the effect of 8% peach juice pomace on the anti-obesity effect on a high fat and fructose diet. The results showed that the supplementation of peach juice by-products improved the hepatic steatosis. The effects of the pomace were related to the reduced absorption of fat and glucose and a decrease of hepatic gluconeogenesis and lipogenesis [127]. Ferri et al. extracted NEPs with enzymatic hydrolysis and analysed the anti-tyrosinase and anti-inflammatory effect with an optimised tyrosinase enzyme inhibition assay and embryonic kidney cell, respectively. The tyrosinase was able to catalyse the mammalian melanogenesis and further induce skin pigments as a multifunctional oxidase. The grape peel possessed an inhibition of both significant tyrosinase and inflammatory effect, which could be furthermore applied in supplements or skincare products [97].

Maurer et al. carried out research to analyse the beneficial effect of grape peel powder on intestinal barrier homeostasis during acute 2,4,6 trinitrobenzene sulfonic acid (TNBS) colitis. NEPs-rich grape peel powder supplement was applied in rat diet. Rats received 8% of the supplement for the first 15 days and colitis induction on the 16th day. Then, the rats were raised for seven days, and colonic tissue was removed for further analysis. The results showed that rats with NEPs-rich grape peel powder supplement had significant better barrier functions. The polyphenols in the supplements effectively reduced the colonic lesion and activation of unfolded protein response [59]. Bowser et al. compared the influence on the insulin-related activities by high and low molecular weight procyanidins in human primary skeletal muscle cells. By analysing the glycogen synthesis and glucose uptake, it was observed that the procyanidins with high molecular weight possessed an enhanced ability to improve glucose utilisation in skeletal muscle [128]. Sánchez-Tena analysed the relationship between grape dietary fibre with intestinal polyposis in mice. The mice were treated with a standard diet or 1% (*w*/*w*) grape fibre supplement diet, which mimic the recommended human daily fibre intake. The intestinal polyps were measured, and RNA samples were isolated for Affymetrix microarrays. The results showed a significant reduction of intestinal tumorigenesis in mice both in the number (−76%) and the size (−65% to −87%) [129]. Moreover, some studies also indicated that the consumption of NEPs by humans prevented cardiovascular diseases [45]. Specifically, the research proved that consuming NEPs-rich matrix product might mitigate hyperlipidaemia or hypertension [130], reduce lipid biosynthesis or oxidation, and increase the excretion of cholesterol [131].

As can be concluded, NEPs and their small molecular weight bioactive metabolites possess significant benefits associated with gastrointestinal health, or even systematic effect. Further potential applications of NEPs from food by-products can focus on the pharmaceutical aspect to transfer such bioactive effects to diet supplements or medicines.

6. Conclusions

The growing demand for the recovery of bioactive compounds from food by-products encourages continuing the studies on the improvement of the entire process from the extraction to the final applications. NEPs, as an essential phenolic fraction found mainly in the plant-based products, are not taken into enough consideration for the food by-product recovery aspect. For this reason, it is vital to understand all the aspects along the food by-products recovery process related to NEPs.

To develop research for the extraction of NEPs from a particular food by-product, several methodological issues need to be considered. Sample pre-treatment measurement, hydrolysis, and

the application of innovative extraction technologies are the main issues that need to be assembled properly based on the sample biological properties and potential final NEPs usage. Prior to application, extracted NEPs should be analysed with different analytical techniques to characterise the chemical composition along with their antioxidant and functional properties. Based on the results, NEPs can be then furthermore applied in both food and pharmaceutical products.

However, the current methodological knowledge is not enough for the proper extraction and utilisation of NEPs from food by-products. More specific extraction and application need to be developed on an industrial basis. To achieve that, the research ought to focus not only on the highest recovery rate but also on the cost-effective balance. Some techniques, such as freeze-drying, need to be reconsidered for industrial extraction. More innovative extraction methods, for example supercritical fluid extraction or high hydrostatic pressure, can be applied combined with hydrolysis to increase the NEPs yield. In the meanwhile, there is no standard protocol to analyse NEPs content in food by-products. Current measurements are either taken from EPPs evaluation (TPC) or allow evaluating only part of the NEPs properties (total anthocyanins content (TAC), total proanthocyanidins content (TPAC)). Improvements on characterisation techniques can furthermore facilitate the related research and help determine the proper food by-product ingredients for NEPs extraction.

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