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INFLUÊNCIA DA HIDRÓLISE ÁCIDA NA OBTENÇÃO DE COMPOSTOS FENÓLICOS NÃO EXTRAÍVEIS DA CASCA E SEMENTE DE UVA

Porto Alegre

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos como um dos requisitos para obtenção do grau de Mestre em Ciência e Tecnologia de Alimentos.

Orientadores: Prof. Dr. Eliseu Rodrigues e Profª. Drª. Giovana Domeneghini Mercali
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DISSERTAÇÃO

INFLUÊNCIA DA HIDRÓLISE ÁCIDA NA OBTENÇÃO DE COMPOSTOS FENÓLICOS NÃO EXTRAÍVEIS DA CASCA E SEMENTE DE UVA

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RESUMO

Diversos estudos têm demonstrado que os compostos fenólicos exercem benefícios à saúde humana. Grande parte destes estudos foram conduzidos avaliando os compostos fenólicos extraíveis (CFE), sendo ignorados os compostos fenólicos não extraíveis (CFNE). Os CFNE são uma fração de compostos fenólicos que não são extraídos da maneira convencional, uma vez que interagem de diferentes formas com a matriz do alimento, podendo estar ligados a macromoléculas ou a parede celular de produtos de origem vegetal. Atualmente, a sua extração é conduzida aplicando hidrólise ácida ou básica, utilizando concentrações elevadas de ácido ou base, respectivamente. Uma grande limitação associada à aplicação do processo de hidrólise é a degradação dos compostos fenólicos, o que prejudica a interpretação da sua ação biológica em estudos *in vivo* e *in vitro*. Nesse trabalho, o efeito da hidrólise ácida sobre os CFNE de casca e semente de uva em pó foi avaliado, e a aplicação de condições ácidas menos agressivas para a sua obtenção foi sugerida. Diferentes concentrações de ácido clorídrico (0,10 até 15,0 %), temperaturas (50 a 90 °C) e tempos (5 a 20 min) foram avaliados para a obtenção dos CFNE. Os CFNE foram analisados por cromatografia líquida de alta eficiência acoplada à espectrometria de massas de alta resolução (HPLC-DAD-ESI-MS/MS). Os resultados foram analisados considerando como resposta a concentração de CFNE total e a concentração obtida para as classes de CFNE separadamente. Os resultados mostraram que as concentrações de ácido e temperaturas intermediárias estão associadas a uma maior concentração de CFNE para a casca (8,0 % e 65 °C) e a semente (1,0 % e 80 °C). Nessas condições, o teor dos CFNE representou 39 e 22 % (*m/m*) dos CF totais para casca e semente de uva, respectivamente. Avaliando as classes dos compostos fenólicos separadamente para ambas as matrizes, observou-se que estas combinações foram capazes de extrair grandes concentrações de CFNE de taninos condensados, sendo esta a classe majoritária desses compostos nessas matrizes. Os resultados demonstram que as condições de hidrólise podem ser moduladas para a obtenção de extratos ricos em uma determinada classe. Os taninos hidrolisáveis e ácidos hidroxibenzóicos, por exemplo, foram melhor extraídos usando concentrações de ácido e temperaturas mais elevadas. Os taninos condensados, por outro lado, apresentaram maiores concentrações quando valores intermediários de temperatura e concentração de ácido foram utilizados. O maior tempo de hidrólise (20 min) aumentou 1,2 vezes a concentração dos CFNE totais para a casca da uva em pó, mas isso não aconteceu para a semente em pó, sendo 5 min o suficiente para liberá-los da matriz alimentar. Os resultados desse trabalho demostram que a fração dos CFNE é capaz de ser obtida das matrizes estudadas com a aplicação de condições mais brandas de hidrólise, com menor degradação. De uma forma geral, os resultados indicam que a concentração e composição dos CFNE obtidos estão diretamente relacionados com as condições de hidrólise e a matriz estudada.

Palavras-chave: compostos fenólicos ligados, concentração de ácido, hidrólise ácida, temperatura

ABSTRACT

Several studies have shown that phenolic compounds have beneficial effects on human health. Most of these studies were conducted evaluating extractable phenolic compounds (EPC), while non-extractable phenolic compounds (NEPC) were ignored. NEPC are a fraction of phenolic compounds that are not released in the conventional extraction, as they interact in different ways with the food matrix and may be linked to macromolecules or components of the plant cell wall. Currently, their extraction is carried out by applying acidic or basic hydrolysis, using strong acid or base concentrations, respectively. A major limitation of this process is the degradation of phenolic compounds, which impairs the interpretation of their biological action in in vivo and in vitro studies. In this work, the effect of acid hydrolysis on NEPC from grape peel and seed powder was evaluated, suggesting application of less aggressive conditions to obtain them. Different concentrations of hydrochloric acid (0.1 to 15.0 %), temperatures (50 to 90 °C) and times (5 to 20 min) were evaluated to obtain NEPC. NEPC were analyzed by high performance liquid chromatography coupled with high resolution mass spectrometry (HPLC-DAD-ESI-MS/MS). The results were analyzed considering the NEPC total concentration and the concentration obtained for each CFNE class individually. Overall, the results showed that intermediate of acid and temperatures are associated with a higher concentration and higher number of NEPC for grape peel (8.0 % and 65 °C) and seed (1.0% and 80 °C) powders. Under these conditions, the NEPC content represented 39 and 22 % (*m/m*) of the total PC for grape peel and seed, respectively. Evaluating each class of NEPC separately for both matrices, it was observed that these combinations were able to extract large concentrations of condensed tannins, which is the major class of these compounds in both matrices. The results indicate that hydrolysis conditions can be modulated to obtain extracts rich in a specific class. Hydrolysable tannins and hydroxybenzoic acids, for example, were better extracted using higher acid concentrations and higher temperatures. Condensed tannins, on the other hand, showed higher concentrations when intermediate values of temperature and acid concentration were applied. The longer hydrolysis time (20 min) increased 1.2 times total NEPC concentration for the grape peel powder, while for the grape seed powder the shortest time (5 min) was enough to release it from the food matrix. The results of this work demonstrate that the NEPC fraction can be obtained from the studied matrices with the application of milder hydrolysis conditions, with less NEPC degradation. Overall, the results highlight that NEPC concentration and composition are directly related to the hydrolysis conditions and the matrix characteristics.

Keywords: acid hydrolysis, acid concentration, bound phenolic compounds, temperature

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1 INTRODUÇÃO

Os compostos fenólicos (CF) são encontrados em alimentos de origem vegetal, tais como frutas, legumes e cereais. Diversos estudos demonstram que uma dieta rica nesses alimentos possui um efeito positivo à saúde humana, com destaque para a diminuição do risco de desenvolvimento de doenças crônicas não transmissíveis (FRAGA et al., 2019; HERNANDES et al., 2014; LAGE et al., 2021; LUDWIG et al., 2018). Esse efeito positivo está relacionado aos diferentes mecanismos de ação dos CF, incluindo a sua capacidade antioxidante, anti-inflamatória (CHENG et al., 2016), sua interação com a microbiota intestinal (TOMÁS-BARBERÁN; SELMA; ESPÍN, 2016) e seus efeitos dentro das vias de sinalização intra e intercelulares (FRAGA et al., 2019).

A compreensão do papel e dos mecanismos envolvidos na ação dos CF na saúde humana passam, invariavelmente, pelo conhecimento, amplo e acurado, dos CF presentes nos alimentos. Nos últimos 20 anos, foram publicados mais 100 mil trabalhos buscando compreender a relação entre os CF e a saúde humana. Em muitos desses trabalhos, foram aplicadas técnicas avançadas de extração e de análise de CF. Os resultados encontrados permitem explicar as mais diferentes propriedades biológicas encontradas, tanto *in vitro* quanto *in vivo*. Contudo, em grande parte desses trabalhos, um grupo importante dos CF foi ignorado. Essa fração é conhecida como "compostos fenólicos não extraíveis" ou "ligados" à matriz do alimento.

Os CF podem ser divididos em dois grandes grupos: compostos fenólicos extraíveis (CFE) e compostos fenólicos não extraíveis (CFNE). Como indicado pela nomenclatura atribuída, os CFE são todos os CF que são extraídos diretamente da matriz pela aplicação de solventes orgânicos ou misturas de solventes orgânicos e água. Os CFNE, por outro lado, permanecem no resíduo após a extração com solventes orgânicos aquosos (GONZALES et al., 2015). Esses compostos fenólicos ficam retidos devido à forte interação com a matriz ou devido a insolubilidade no solvente de extração (ARRANZ; SAURA CALIXTO, 2010; WANG et al., 2020).

Nos últimos 10 anos, os números de trabalhos com os CFNE têm aumentado, permitindo um maior conhecimento sobre sua representatividade frente aos compostos fenólicos totais. Por exemplo, os CFNE representam aproximadamente 15 % dos CF totais em cascas de jabuticaba (QUATRIN et al., 2019), cerca de 35 % dos CF presentes em araçá amarelo e vermelho (MALLMANN et al., 2020) e 80 a 84 % dos CF encontrados em frações de farelo de trigo enriquecidas em aleurona (MARTÍN-GARCÍA et al., 2021).

Vale ressaltar que esses trabalhos realizaram a extração exaustiva dos CFE, evitando, dessa forma, a superestimação da fração não extraível.

A literatura recente também demonstra que os CF presentes na fração CFNE apresentam um perfil qualitativo distinto da fração extraível. MALLMANN et al. (2020) encontram somente ácido gálico e ácido elágico em ambas as frações em araçá vermelho e amarelo. De forma semelhante, apenas os ácidos cafeico, vanílico e sinápico foram encontrados nas frações CFE e CFNE obtidas a partir de trigo (MARTÍN-GARCÍA et al., 2021). Outro ponto de destaque na literatura é que esses compostos chegam ao cólon praticamente intactos, onde passam por uma extensa transformação pela microflora. No cólon, os CFNE são liberados de sua matriz em metabólitos com potencial de absorção por meio da fermentação colônica (PÉREZ-JIMÉNEZ; DÍAZ-RUBIO; SAURA-CALIXTO, 2013).

O estudo da fração CFNE enfrenta uma grande barreira que impede um conhecimento mais aprofundado da natureza desses compostos nos alimentos vegetais. A grande maioria dos estudos realizados utilizou os métodos de hidrólise ácida ou alcalina para extração dos CF ligados à matriz. No método da hidrólise ácida, os CFNE são comumente liberados pela aplicação de ácido clorídrico (HCl) ou ácido sulfúrico (H_2SO_4), em altas temperaturas e longos tempos de hidrólise. Pode-se observar uma grande variação dos parâmetros utilizados na hidrólise ácida, sendo comumente encontrado a utilização de solventes metanólicos com H_2SO_4 10 % (BALLI et al., 2020; DOMÍNGUEZ-RODRÍGUEZ; PLAZA; MARINA, 2021; SU et al., 2021) e HCl 2 M (DZAH et al., 2021; NARVEKAR; THARAYIL, 2021; YOU et al., 2021). Nesses estudos, o tempo variou de 30 min até 24 horas e a temperatura de 62 °C à 85 °C. Essas condições são capazes de promover a liberação dos CFNE, porém, promovem também a sua posterior sua degradação (MOUSSA-AYOUB et al., 2011; PÉREZ-JIMÉNEZ; SAURA-CALIXTO, 2015).

A hidrólise química gera também uma grande quantidade de resíduos, que precisa ser descartada de forma adequada. Dessa forma, as condições da hidrólise, como concentração de ácido ou base, temperatura, tempo, razão amostra/solvente, entre outras, precisam ser otimizadas para reduzir as perdas por degradação e os resíduos gerados. Além disso, os métodos necessitam ser padronizados para permitir a comparação dos CFNE em diferentes matrizes. Neste contexto, o objetivo desse trabalho é avaliar o efeito de diferentes condições de hidrólise ácida da fração CFNE em casca e semente de uva, propondo modificações no método que permitam a identificação e quantificação dos

compostos com o menor grau de degradação possível. As amostras de casca e semente de uva foram escolhidas como objeto de estudo devido ao conhecimento prévio de que elas são ricas em diferentes classes de compostos fenólicos, o que irá facilitar, posteriormente, a identificação dos compostos e a discussão dos resultados obtidos.

O presente texto está estruturado da forma descrita a seguir. No Capítulo 2 são apresentados os objetivos gerais e específicos do trabalho. Posteriormente, o Capítulo 3 apresenta os fundamentos teóricos sobre os compostos fenólicos, com ênfase nos CFNE, na forma de um artigo de revisão. No Capítulo 4, os materiais e métodos utilizados no desenvolvimento deste trabalho são apresentados. Os Capítulos 5 e 6 apresentam os resultados e discussão, contemplados em formato de artigo, e as conclusões gerais do trabalho, respectivamente.

2 OBJETIVOS

2.1 Objetivo Geral

O objetivo geral desse trabalho é avaliar o efeito das condições de hidrólise na composição dos CFNE da casca e semente de uva e desenvolver um método de extração menos agressivo que evite a degradação desses compostos, possibilitando sua correta caracterização.

2.2 Objetivos Específicos

Os objetivos específicos desse trabalho são:

- extrair a fração CFE dos compostos fenólicos presentes na semente e na casca de uva, utilizando um método de extração exaustiva com solventes orgânicos;
- identificar e quantificar os CFE por HPLC-DAD-ESI-MS/MS;
- obter exaustivamente os CFNE presentes na semente e na casca de uva pela aplicação da técnica de hidrólise ácida, método tradicionalmente utilizado;
- identificar, quantificar e caracterizar os CFNE por HPLC-DAD-ESI-MS/MS.
- avaliar o efeito de diferentes concentrações de ácido, temperatura e tempo da hidrólise ácida na composição do CFNE;
- propor um método alternativo de hidrólise ácida menos agressivo que evite a degradação desses compostos, possibilitando sua correta caracterização.

3 REVISÃO BIBLIOGRÁFICA

Nesse capítulo é apresentada uma revisão sobre os CF, com ênfase nos CFNE, foco do presente trabalho. O conteúdo será apresentado na forma de artigo de revisão. O artigo será posteriormente submetido a um periódico internacional indexado.

3.1 New insights into non-extractable phenolic compounds: how to overcome the analytical challenges

Abstract

Although phenolic compounds (PC) have been studied for many years, some unanswered questions concerning non-extractable phenolic compounds (NEPC) need to be elucidated. These compounds remain in food-matrix after the conventional extraction, and their importance has been shown in recent studies with fruit and vegetables. In this review, 182 studies published in the last five years about NEPC in foods were evaluated, focusing on critical points of the applied extraction methods. Analytical pitfalls were pointed out, and strategies to overcome them were proposed. First, free PC exhaustive extraction should be performed before hydrolysis processes to avoid overestimation of the NEPC fraction. NEPC extraction by aggressive methods modifies their original structure and makes their complete elucidation difficult. These methods must be optimized considering the research objective, as different conditions may result in different amounts and profile of compounds. Concerning quantification and identification, the widely used spectrophotometric method is not able to identify individual compounds and leads to overestimation. Liquid chromatography can be improved, especially regarding standards availability. Identification through mass spectrometry combined with bioinformatics may be used to explore data generated.

Keywords: bioactive compounds, bound phenolic, macromolecular antioxidants, fruits.

3.1.1 Introduction

Phenolic compounds (PC) are secondary metabolites of plants, covering many structures, some of which are ubiquitous in plants. In contrast, others are restricted to specific families or species (Tarascou et al., 2010). They can be synthesized via the phenylpropanoid pathway and have at least one aromatic ring attached to one or more

hydroxyl groups (Wang et al., 2020). Over 10,000 PC have been reported in plants, consisting of approximately 6500 flavonoids and 1000 hydrolysable tannins (Tarascou et al., 2010). More than 500 compounds were found in food and beverages (Neveu et al., 2010). These molecules range from simple, low molecular weight, single-aromatic-ring compounds to large and complex tannins and derived polyphenols (Crozier et al., 2009).

Regarding PC extraction, two fractions can be distinguished: extractable (EPC) and non-extractable phenolic compounds (NEPC - also called bound phenolic compounds). EPC are found mainly in plant vacuoles (Li et al., 2012), considered free in the food matrix; they are known as "extractable" because they are more easily removed from food through extractions with organic solvents and/or water and agitation. The NEPC, on the other hand, are known as "non-extractable" because their extraction cannot be performed only using organic solvents or mixtures of solvents and water, requiring a hydrolysis step before extraction to release them from the food matrix. This hydrolysis is necessary due to the interaction of NEPC with structural components of the cell wall (such as cellulose, hemicellulose, pectin, and lignin) through covalent bonds and with components physically incorporated as plant matrices (such as polysaccharides, proteins, and dietary fiber). Moreover, they may interact with insoluble complexes due to food processing (Hamauzu, 2018; Saura-Calixto, 2018). NEPC stand out due to their health-related potential, with diverse properties, especially their high antioxidant capacity when interacting with the gastrointestinal tract and gut microbiota (González-Sarrías et al., 2017; Lucas-González et al., 2021; Pérez-Jiménez et al., 2013).

There are thousands of works about PC in foods in the literature, which can be seen by a simple search carried out in the ScienceDirect database, with the keywords "phenolic/phenols/polyphenol compounds" and "food". The search resulted in approximately 22,000 research articles and short communications from the last five years. Among them, the vast majority evaluated only the EPC fraction, leaving behind an important portion of bounded PC. A similar search was performed in the ScienceDirect database with the keywords "phenolic/phenols/polyphenol compounds" and "bound/non-extractable", and only 578 research articles were found. These studies represent around 3 % of those performed with PC in foods, considering research articles and short communications from the last five years.

In this framework, it is possible to state that some limitations promote a lack of background knowledge about NEPC, namely: (1st) the absence of exhaustive extraction of free PC, overestimating the NEPC fraction; (2nd) lack of NEPC extraction methodology

standardization; (3nd) the use of aggressive solvents for NEPC extraction, causing degradation; (4th) inadequate quantification methods; and (5th) compound identification little explored. In this context, this review focus on NEPC extraction methods, aiming to detect critical analytical pitfalls that prevent the correct characterization of NEPC compounds in foods. For this, 182 studies about NEPC published in the last five years were revised, and quality information was gathered as starting point to propose strategies to overcome these drawbacks. **Table 1** summarizes the approaches discussed, with advantages, disadvantages, and strategies of each topic.

Table 1. Summary of the different approaches discussed in this review for phenolic compounds characterization in food.

NEPC release methods	Advantages	Disadvantages	Improvements
Acid hydrolysis	Can more effectively release NEPC. Cleavage of glycosidic bonds in the cell wall.	Use of high temperatures and acid concentrations, waste generation, degradation of some compounds.	Use of milder conditions, optimization of extraction method.
Alkaline hydrolysis	Can break ester bonds in cell wall, releasing phenolic polysaccharide compounds.	Waste generation. It requires a complex pre-treatment process. It must be carried out in the dark, under an inert gas atmosphere to prevent NEPC oxidation. Need to adjust the pH after the process.	Use of milder conditions, optimization of extraction method.
Enzymatic hydrolysis	Specificity, breaking specific links, reduction of process time and temperature. Green extraction alternative.	High cost. Specificity can be a challenge because enzyme preparations with different enzymes are necessary.	Further studies involving the methodology, recovery, and recycling of enzymes.
Emerging technologies	Can break bonds with mechanical actions, increasing the solubility of the mixture, increasing the efficiency of the extraction process, reducing time and costs in the long run. Green extraction alternative.	Thermal degradation, current high cost of methodologies, lack of low-access equipment and consumables.	Larger studies involving methodologies; it is a new field to be explored.
Quantification methods	**Advantages**	**Disadvantages**	**Improvements**
Spectrophotometric methods	Low cost, simple technique, widely applied for analytical quantification of total tannins and phenolics.	Overestimation of compounds due to interaction with other compounds present in extracts, lack of selectivity.	Use of the technique as an indication of the reduction capacity, possibility of discounting interfering compounds.
HPLC-DAD / HPLC-MS	Specificity, individually PC quantification and identification.	High cost, difficult access to oligomeric and polymeric analytical standards, errors involved in quantification using monomeric standards. Matrix effect.	Purification of compounds to use them as a standard, expansion of compound libraries, systematization of the generated data analysis to facilitate identification.

3.1.2 Literature search strategy

A comprehensive literature search of current articles (from 2018 to 2022), published in journals of relevant impact, related to the topic “NEPC” was performed. Several databases were used, such as Scopus, ScienceDirect, PubMed, and Google Scholar. The main keywords entered the database search boxes, combined in several

ways, were: “phenolic/phenol/polyphenol”, “non-extractable”, “bound” or “insoluble” compounds. The articles were selected following a procedure in three stages. First, the title and abstract were analyzed. Then, the full text was used to evaluate the extraction approach and the methodologies carried out, selecting articles with information in the scope of this review. Finally, the reference list of the selected articles was revised to retrieve additional studies. Some studies published before 2018 were included in the discussion due to their relevance to the research field. A total of 182 articles were included and analyzed in the current literature review article.

3.1.3 Phenolic compounds

3.1.3.1 Chemical nature

PC can be classified according to their chemical structures into different groups, such as phenolic acids, flavonoids, tannins, and lignins (Gan et al., 2018). Phenolic acids comprise hydroxybenzoic acids (C6–C1) and hydroxycinnamic acids (C6–C3). The flavonoids (C6–C3–C6) comprise a three-ring structure, including different classes of compounds, such as flavonols, flavones, flavanones, flavanonols, isoflavones, flavanols, and anthocyanidins. Tannins can be of two types: hydrolysable tannins formed by gallic acid or ellagic acid and condensed tannins (also known as proanthocyanidins), which are polymers of catechin and epicatechin. Lignins are the polymer of *p*-coumaric acid and sinapic acid.

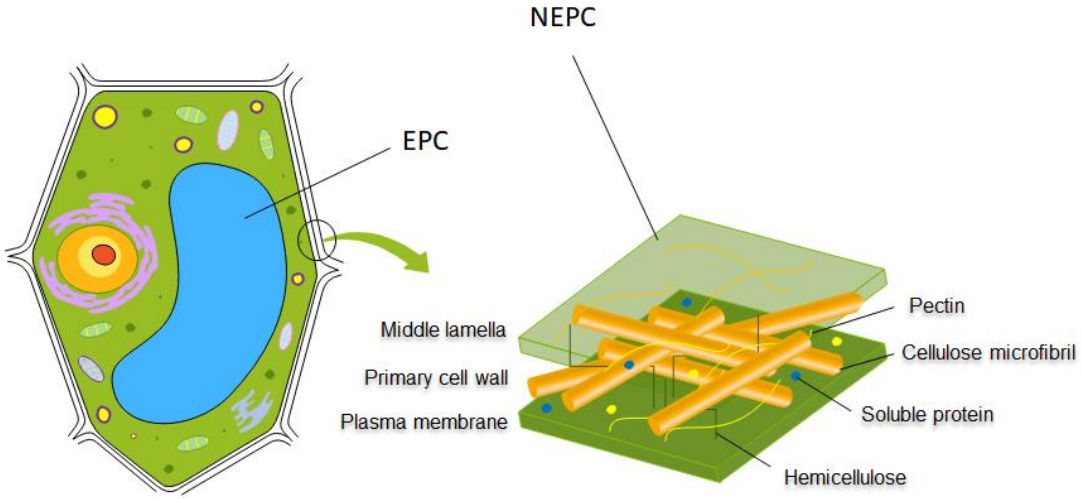
PC can be divided based on their solubility in organic solvents into two groups: extractable phenolic compounds (EPC) and non-extractable phenolic compounds (NEPC). EPC are known as “extractable” because they are more easily removed from food through extractions with organic solvents and/or water and agitation. The NEPC, on the other hand, are known as “non-extractable” because their extraction cannot be performed only using organic solvents or mixtures of solvents and water. EPC are synthesized mainly in the endoplasmic reticulum of plants and stored in vacuoles. NEPC, are formed and transported into the cell wall, being then conjugated with cell wall macromolecules, such as cellulose and protein, through ester and glycosidic bonds, thus contributing to the formation of the cell wall structure (Agati et al., 2012; Gan et al., 2018; Giada, 2013).

The plant cell wall is built as a series of layers, as depicted in **Figure 1**. The first layer, identified by electron microscopy, is the middle lamella. The next main layer is the

primary cell wall. In fruits and vegetables, it consists mainly of cellulose, hemicellulose, pectic polysaccharides (which may be covalently linked to phenols, cellulose, and proteins), and other cell wall components found at a similar or lower concentrations. Cellulose is the cell wall component in the microfibrillar phase, and pectin, hemicellulose, proteins, and phenolics (lignin, ferulic acid, among others) are found in the matrix (Brett & Waldron, 1990).

NEPC are bound to the food matrix through weak or covalent bonds (Pérez-Jiménez et al., 2013) (**see section 3.1.4.2**). This fraction is composed of molecules with diverse molecular weights, such as phenolic acids (low), hydrolysable tannins (intermediate), and proanthocyanidins (**see section 3.1.4.1**). These compounds were called macromolecular antioxidants (MACAN), a term used by a research group due to the importance of NEPC as antioxidants. However, this term encompasses a broader series of non-phenolic compounds, such as, for example, carotenoids (Saura-Calixto, 2018).

Figure 1. Plant cell anatomy and cell wall structure.



Source: Prepared by the authors in the EdrawMax program.

NEPC interact with the food matrix, especially polysaccharides and proteins, through different mechanisms: (I) hydrogen bonding (between the hydroxyl groups of phenols and the oxygen atoms of the ether crosslinks); (II) hydrophobic interactions; and (III) covalent bonds (to form esters and ether bonds) (Le Bourvellec et al., 2005). Hydrogen bonds are found between the food matrix and non-extractable proanthocyanidins and hydrolysable tannins (Brook & Munday, 1970; Le Bourvellec et

al., 2005; Ligny, 1979). Covalent bonds are present bonding phenolic acids (Bunzel, 2010) and possibly non-extractable proanthocyanidins and hydrolysable tannins.

Research with NEPC arose from the analysis of dietary fiber (DF) in some plant materials because they are associated with the cell wall. In fruits, most of the PC attached to DF are tannins (Saura-Calixto, 2011). Currently, DFs are considered edible parts of vegetables, resistant to digestion and absorption in the human small intestine, which undergo complete or partial fermentation in the large intestine. DF contains polysaccharides as main constituents. The free PC and the soluble fraction of the DF can be solubilized during digestion, while the insoluble fraction of the DF and the NEPC remain insoluble. In the colon, the soluble fraction of the fibers and phenolics that are not absorbed in the small intestine are fermented by the action of the microbiome, while the insoluble fraction is only partially fermented (Saura-Calixto, 2018). NEPC are not significantly released from the food matrix by chewing, acidic pH in the stomach, or action of digestive enzymes. These compounds reach the colon almost intact, where they undergo an extensive transformation by the colon microflora (Pérez-Jiménez et al., 2013). As a result, dietary phenolics (or fiber-bound phenolics) have been suggested to be responsible for health effects previously related to fibers (Maurer, Cazarin, Quatrin, Nichelle, et al., 2020; Saura-Calixto, 2018). Recent work has shown that the soluble and fiber-bound polyphenols of grape are more effective than the fiber fraction to reduce matrix metalloproteinase 9 expression (Maurer et al., 2019), usually associated with tumor invasion and metastasis (Paez-Pereda et al., 2005). However, fiber-bound polyphenols chemical characterization, bioavailability, and health effects are still poorly understood because this residue is still little explored. Thus, these topics will be covered in the following sections as well as methods of extraction and analysis.

3.1.3.2 Sources

PC are ubiquitous, found in plant tissues, including edible parts, such as fruits, flowers, seeds, leaves, stems, roots, among others (Crozier et al., 2009; Harborne, 1983). **Table 2** describes different sources of NEPC in studies carried out in recent years. It is possible to observe that NEPC were studied in all types of plant foods (fruits, vegetables, cereals, legumes, and nuts), concluding that this fraction of PC is a natural constituent of plant foods.

Processed foods and beverages, such as black tea, wine, coffee, and cocoa, may contain phenolic transformation products best described as “derived polyphenols”.

During food processing, flavanols monomers (flavan-3-ol) are largely converted into other products, as, for example, the production of theaflavins, theacitrins, and thearubigins in black tea (Crozier et al., 2009).

Regarding gallic acid, the main source of this compound in the diet is the esters of gallons, without sugars, in grapes, wine, mangoes, green tea, and black tea. Ellagic acid and related ellagitannins are found in raspberries, strawberries, pomegranates, blackberries, persimmons, walnuts, hazelnuts, and oak-aged wines. Derivatives of phenylvaleric acid, phenylacetic acid, phenyl propionic acid, phenylmandelic acid, and phenylhydracrylic acid hardly occur preformed in food but are metabolites of the microflora of many dietary PC that are easily absorbed, being partially responsible for specific biological effects related to PC rich diets (Crozier et al., 2009).

Flavonols are widely found throughout the plant kingdom, except algae. They are also not found in fungi. Flavones have been reported in celery, parsley, and certain herbs. Polymethoxylated flavones, such as tangeretin and nobiletin, have been found in citrus. Anthocyanidins are widely dispersed in plants, especially in the tissue of fruits and flowers, where they are responsible for the colors red, blue, and purple (Crozier et al., 2009). Isoflavones are found almost exclusively in legumes, with the highest concentrations in soy.

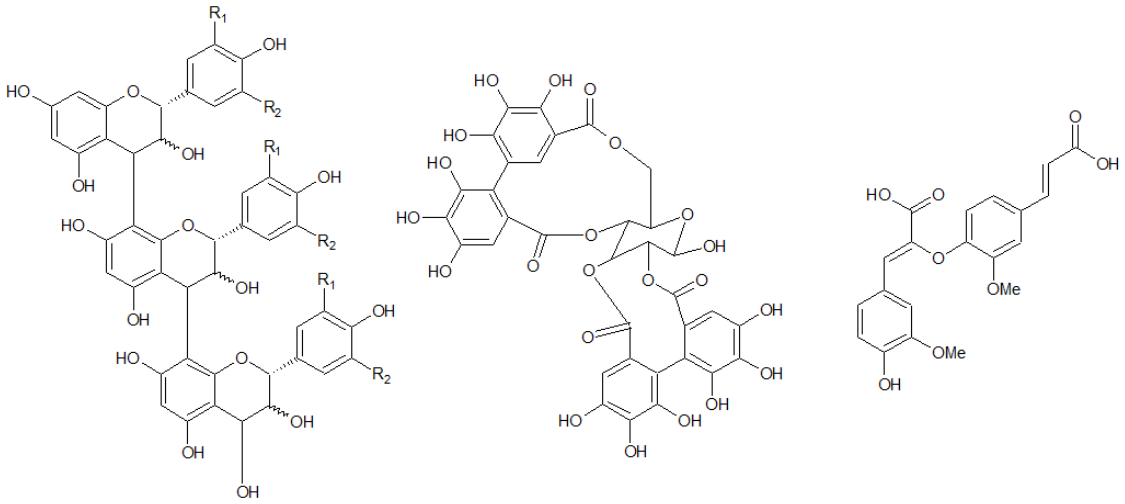
3.1.4 Non-extractable phenolic compounds

3.1.4.1 Classes

NEPC fraction includes macromolecules, such as high molecular weight proanthocyanidins, and simple PC, such as phenolic acids, associated with macromolecules, especially constituents of fiber polysaccharides (Pérez-Jiménez et al., 2013). These compounds can also be divided into two classes: non-extractable proanthocyanidins (NEPA) and hydrolysable polyphenols (HPP) (Saura-Calixto, 2018). Pérez-Jiménez et al. (2013), on the other hand, adopt a distinct classification: class I, molecules of high molecular weight or weakly linked to the plant matrix; and class II, compounds strongly linked to different constituents of plant tissue, such as pectin and structural proteins. **Figure 2** shows the basic structures of NEPC, according to the classification proposed by Pérez-Jiménez & Torres (2011): proanthocyanidins with the radicals R1 and R2, which can be H and/or OH, hydrolysable tannins and phenolic acid oligomers.

Proanthocyanidins comprise a group of PC made up of subunits of flavanols with a wide structural range and a wide degree of polymerization. The most abundant proanthocyanidins are procyanidins, consisting of (epi)catechin units. Propelargonidins and prodelfinidines are less abundant and contain (epi)afzelechin and (epi)gallocatechin units, respectively (Ludwig et al., 2018). Hydrolysable tannins, which include gallotannins and ellagitannins, are possibly the most complex group of tannins with molar mass ranging from 300 to 3000 Da (Mena et al., 2015). Hydrolysable phenolics, on the other hand, comprise low molecular weight PC (mainly hydroxycinnamic acids, such as ferulic acid, caffeic acid, and sinapic acid) and hydroxybenzoic acids, esterified with cell wall polysaccharides (Ludwig et al., 2018).

Figure 2. Basic chemical structures of NEPC.



3.1.4.2 NEPC extraction methods

Methods used for NEPC extraction have been widely discussed in recent reviews (Acosta-Estrada et al., 2014; Domínguez-Rodríguez et al., 2017; Pérez-Jiménez & Torres, 2011; Z. Wang et al., 2020). Overall, these studies state that only an extraction with an organic solvent, such as methanol, is not able to release these compounds because they can be in esterified or conjugated forms. Thus, some steps should be followed to release and extract these compounds.

Figure 3 shows the main steps that should be followed to conduct PC extraction. Before NEPC release and extraction, it is important to perform the exhaustive extraction of free PC (EPC fraction). Several procedures have been reported for solid-liquid EPC

extraction from vegetable foods, with different combinations of organic solvents (methanol, ethanol, or acetone) and/or water (Pérez-Jiménez et al., 2013). After this extraction (**Figure 3a**), the supernatant is analyzed for free phenolic identification and quantification. As previously stated, this fraction must be extracted exhaustively so, there is no interpolation between the EPC and NEPC fractions, thus avoiding the overestimation of NEPC content. A recent review of the literature on this topic is shown in **Table 2**. As can be seen, only 8 % of the studies described an exhaustive EPC extraction before conducting NEPC extraction. The spectrophotometric method widely used to determine the total phenolic content (TPC) is the Folin-Ciocalteu. This can best be used as an indication of the reducing capacity of the sample, as well as an indication of the number of extractions necessary for the complete EPC removal. Alternatively, high-perfomance liquid chromatography (HPLC) can be used for this purpose. Tang et al. (2016) confirmed by HPLC that the residue of quinoa used in the NEPC hydrolysis was free of soluble phenolics. Exhaustive EPC extraction is of paramount importance to avoid overestimation of the NEPC which generates erroneous results, hindering a complete quantitative and qualitative understanding of the NEPC fraction.

Several factors affect the efficiency of the extraction, including the number of extractions. EPC extraction was performed only once in 34 % of the studies listed in **Table 2**. Złotek et al. (2016) evaluated the effect of different solvents and the number of extraction steps on polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. The researchers performed extraction procedures with acetone:H_2O:CH_3COOH (three concentrations), MeOH:H_2O, MeOH:H_2O:CH_3COOH, and MeOH:CH_3COOH: (1) once shaking for 60 min and (2) three times shaking for 20 min. The PC contents of the extracts obtained by the different procedures were not significantly ($p \geq 0.05$) different; however, the extraction was more effective with the solvents containing acetone with the highest addition of acetic acid in fresh and freeze-dried material. Regarding the second parameter evaluated, the procedure with the highest number of extraction repetitions were able to extract the highest amount of PC in fresh basil leaves, contrary to extracts derived from frozen material. Therefore, even if a single extraction is able to extract all EPC, descriptions and methods that prove this are essential. In this context, standardization of extraction methods is extremely important to discuss and understand the results of different studies with greater clarity.

NEPC extraction must follow two stages due to the nature complexity of these compounds: hydrolysis (**Figure 3b**); and extraction (**Figure 3c**) itself. After the EPC

exhaustive extraction, processes capable of releasing NEPC from sample residue, such as chemical (acidic or basic) or enzymatic hydrolysis, should be conducted. Hydrolysis must take into account the type of binding between the compound and the matrix (Pérez-Jiménez et al., 2013). According to Durazzo (2018), the residue from EPC extraction must be subjected to two different acid treatments to isolate the fractions of hydrolysable PC and non-extractable proanthocyanidins. For non-extractable proanthocyanidins, the process is based on depolymerization by butanolysis, which leads to monomers of anthocyanidins. In this process, the samples are treated with butanol-HCl containing $FeCl_3$ (100 °C, 60 minutes), and absorbances at 555 nm (anthocyanidins) and 450 nm (xanthyl compounds) are measured in the hydrolysates (Porter et al., 1985). The NEPC values may vary slightly, depending on the type of solvent used and the extraction conditions (temperature, particle size, agitation, among others).

Considering the physiological approach, NEPC are insoluble PC in intestinal fluids after the action of digestive enzymes (instead of aqueous organic solvents, as in chemistry). Thus, these structures are simple PC covalently bound to each other or to polysaccharides and proteins, along with small amounts associated with the DF matrix by weak links (H-bonds and hydrophobic and Van der Waals bonds). Taking this into account, a specific methodology for NEPC determination in foods was developed from the residue obtained in the dietary fiber analysis. This method uses enzymes, such as pepsin, pancreatin, and alpha-amylase, generating a residue composed of insoluble DF and NEPC associated compounds (Saura-Calixto, 2018).

Thus, the chemical approach for a complete analysis of PC should include EPC and NEPC extractions, being the total content of PC in foods the sum of these two fractions. Overall, PC extraction from plant foods is influenced by the chemical nature of the food and the compounds, by the method of extraction employed, by the size of the sample particles, by the time and storage and processing conditions, and by the presence of interfering substances.

As shown in **Table 2**, different extraction methods were applied to remove this bound fraction of phenolics. However, chemical hydrolysis was the most used procedure in these studies, mainly acid hydrolysis, a technique that will be covered in the next section. In general, for EPC and NEPC extractions, the aqueous-organic solvents used are gauged for acidic pH since PC are generally more stable at low pH. As stated in **Table 1**, acid hydrolysis usually requires the use of high temperatures, which can lead to PC degradation. For example, Matsumura et al. (2016) extracted NEPC from dried

persimmon with a solution of HCl 1.2 N, at 90 ° C, for 3 h. On the other hand, alkaline hydrolysis requires a longer time for the hydrolysis process. Bueno-Herrera & Pérez-Magariño (2020) carried out NEPC extraction from a residue of purple wheat (*Triticum aestivum* L.) with 2 M NaOH at room temperature for 16 h. Each approach has its advantages and disadvantages (described in Table 1). Thus, the method for NEPC release and extraction must be selected based on the matrix and objectives.

Figure 3. Mains steps for EPC extraction, chemical hydrolysis, and NEPC extraction.

Table 2. Recent literature (from 2018 to 2022) regarding extractable (EPC) and non-extractable phenolic compounds (NEPC) in food matrices.

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Wheat flours (*Triticum aestivum*) genotypes	Two times	-	70 mg, EtOH (80 %)	Alkaline	Ultrasonic bath (42 kHz), 40 °C, 90 min	Residue, NaOH (4 M)	Three times with ethyl acetate	Folin–Ciocalteu UPLC-ESI-QTOF-MS	(Barros Santos et al., 2022)
Tropical fruit peels	Two times	Shaking, room temperature, 1 h	15 g, 20 mL MeOH:H_2O (50:50, v/v) with HCl (2 M) / 20 mL, acetone:H_2O (70:30, v/v)	Alkaline and enzymatic - assisted extractions	Shaking, room temperature, 4 h	Residue, 25 mL NaOH (2 M) / Enzyme preparations (details in the original article)	-	DART-HRMS	(Domínguez-Rodríguez et al., 2022)
Sourdough-wheat bread	Three times	Ultrasonic bath (60 Hz) / Shaking, room temperature, 5 min / 1 h	50 mg, 1.5 mL aqueous MeOH (80 %, v/v)	Alkaline	Sonicated in water bath / Shaking, room temperature 30 min / 150 min	Residue, 0.9 mL NaOH (2 M)	Ethyl acetate	Folin-Ciocalteu AC: DPPH, FRAP	(Graça et al., 2022)
Wheat bran	Three times	-	1 g, 50 mL MeOH (80 %)	Alkaline	3 h, with N_2	Residue, 40 mL NaOH (2 M)	Three times with ethyl acetate	Folin-Ciocalteu HPLC-ESI-MS AC: DPPH, ABTS	(Li et al., 2022)
Red rice bran	-	Shaker, room temperature, 1 h	0.1 g, 8 mL MeOH	Acid	-	Residue, 8 mL HCl:MeOH (1 %)	-	Folin-Ciocalteu (TPC) Absorbance (TFC) HPLC-DAD AC: DPPH, FRP, superoxide anion radical, hydroxyl radical, nitric oxide	(Sapna & Jayadeep, 2022)
Probiotic goat milk yogurt (Isabel grape)	Two times	Ultrasonic bath (40 kHz), 25 °C, 30 min	1 g, 5 mL MeOH:H_2O (85:15, v/v)	Alkaline	Orbital shaker (250 rpm), 4 h	Residue, 20 mL NaOH (4 M)	Five times with ethyl acetate	HPLC-DAD AC: ABTS, DPPH, ORAC	(Silva et al., 2022)
Mango leaves	Three times	Ultrasound bath, room temperature, 30 min	50 g, 1000 mL MeOH (70 %)	Alkaline	-	Esterified: Residue, NaOH (4 M) / Insoluble: Fitrate residue, NaOH (4 M)	Five times with ethyl acetate / According to EPC.	Folin-Ciocalteu (TPC) Absorbance (TFC) UHPLC-Q-Orbitrap-MS/MS	(Zhang et al., 2022)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: ABTS, DPPH, FRAP	
Foxtail millet bran	Two times	-	1 g, 20 mL MeOH solution (80 %)	Alkaline and ultrasonic-assisted deep eutectic solvent extraction	-	Residue, NaOH (2 mol L^{-1}) / Ultrasonic-assisted deep eutectic solvent extraction (details in the original article)	Ethyl acetate	Folin-Ciocalteu (TPC) Absorbance (TFC) UPLC-QqQ-MS /MS AC: ABTS, DPPH, FRAP	(Zheng et al., 2022)
Date palm fruit (Phoenix dactylifera L.)	Three times	Ultra-turrax, 5 min	2 g, 50 mL MeOH (80 %)	Alkaline	Water bath, 20 °C, 4 h	Residue, 10 mL NaOH (3 M)	Three times with ethyl acetate	Folin–Ciocalteu (TPC) AC: ABTS, DPPH, FRAP	(Alam et al., 2021)
Barley	Three times	Mixed, 10 min / Anthocyanins: 15 min	50 mg, 1 mL MeOH:H_2O: CH_2O_2 (79.5:19:5:1) / 100 mg, 1.6 mL EtOH:HCl 1 M (85:15)	Alkaline	Room temperature, 12 h	Residue, NaOH (2 M)	Two times. MeOH: CH_2O_2 (99.9:0.1)	Folin-Ciocalteu (FPC and BPC) Spectrophotometric (Anthocyanins) Near-infrared spectroscopy (NIRS)	(Albanell et al., 2021)
Mopan persimmon fruit	Three times	Magnetic stirring, room temperature, 20 min	20 g, 200 mL MeOH:H_2O (8:2) with NaF 40 mM	Acid	Various times at the required temperature	Residue, 50 mL HCl (different pH)	-	Folin–Ciocalteu HPLC	(An et al., 2021)
Cooked Rice Dyed with Sorghum-Leaf Bio-Colorants	-	Sonication, 1 h	0.5 g/2.5 mg, 6 mL MeOH 2 % CH_2O_2 / acetone:H_2O: CH_2O_2 (70:29:1)	Alkaline	4 h	Residue, NaOH (4 M)	Three times with ethyl acetate	Folin-Ciocalteu (TPC) HPLC-DAD-Q-TOFMS AC: DPPH, ORAC, TEAC	(Apea-Bah et al., 2021)
Avocado peels	20 mL of 80 % EtOH (remove residual PC)	Microwave-assisted extraction (2.45 GHz, 600W), 3 stages of time	1 g, 20 mL acetone:H_2O (70:30 v/v) or EtOH	Alkaline	Room temperature, 4 h	Residue, 20 mL NaOH (4 M)	Five times with ethyl acetate	Folin-Ciocalteu (TPC) RP-HPLC-ESI-MS AC: ABTS, DPPH, ORAC	(Araujo et al., 2021)
Whole grain highland barley	Two times	5 min	2 g, 20 mL chilled acetone (80 %)	Alkaline	Shaking under N_2, room temperature, 1 h	Residue, 20 mL NaOH (2 M)	Five times with ethyl acetate	Folin-Ciocalteu (FPC, BPC) Spectrophotometric (TPC, TFC) AC: DPPH	(Bai et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Grains of carioca beans	Two times. Three times with ethyl acetate	Sonicated, room temperature (25 °C), 10 min	1 g, 15 mL EtOH (40 %)	Alkaline	Room temperature, 4 h	Water phase, 20 mL NaOH (4 M) / Residue, 20 mL NaOH (4 M)	Three times with ethyl acetate	Folin–Ciocalteu (TPC) Spectrophotometric (TAC) UPLC-QTOF-MS	(Bento et al., 2021)
Terminalia ferdinandiana Exell fruit	Separation by classes (extracts)	Agitated and sonicated	Different solvent mixtures for each fraction	Alkaline	Reciprocating shaker (200 rpm), 1 h	Residue, NaOH (3 M)	Ethyl acetate	Folin–Ciocalteu (TPC) UHPLC–Q/Orbitrap/MS/MS UPLC-PDA analysis AC: DPPH	(Bobasa et al., 2021)
Pequi (*Caryocar brasilense* Camb.) peel flours	Two times	25 °C, 60 min	1 g, 40 mL MeOH (50 %) and acetone (70 %)	Acid	Dubnoff bath (85 °C), 0.5-12 h	Residue, 10 mL MeOH with HCl (pH 2)	-	$AlCl_3$ (TFC) HPLC-UV	(Brito Cangussu et al., 2021)
Snack (Pigmented whole maize grains)	-	Shaking water bath, room temperature, 1 h	0.5 g, 5 mL acetone:H_2O (1:1)	Alkaline	Shaking water bath, room temperature, 4 h	Residue, 40 mL NaOH (2 M)	Five times with diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) Spectrophotometric (TAC) AC: DPPH, FRAP, TEAC	(Çetin-Babaoğlu et al., 2021)
Varieties of fresh waxy corn (whole grain)	Two times	Water bath, room temperature, 2 h	5 g, 100 mL chilled acidified MeOH 95 % and HCl 1 M (85:15)	Alkaline	Shaking with N_2, room temperature, 1 h	Residue, 200 mL NaOH (2 M)	Five times with ethyl acetate	Folin-Ciocalteu (Phenolic acid) Spectrophotometric (TFC, TAC) AC: DPPH, FRAP	(Chen et al., 2021)
Pine (*Pinus yunnanensis*) pollen	Three times	Ultrasound-assisted, room temperature, 30 min	2 g. 30 mL MeOH:acetone: H_2O (7:7:6)	Alkaline	Magnetic stirrer (150 rpm), room temperature, 4 h	Esterified and glycosylated (details in the original article). Insoluble PC: Residue, NaOH 4 M with 10 mM EDTA and 1 % ascorbic acid	Five times	Folin–Ciocalteu (TPC) Colorimetric (TFC) HPLC-PDA UHPLC–ESI-QTOF–MS AC: ABTS, DPPH, FRAP	(Cheng et al., 2021)
Sorghum [*Sorghum bicolor* (L.) Moench] genotypes (Tannin and tannin-free)	Two times	Stirred, 25 °C, 10 min	EPC: 70 mg, 1 mL EtOH. Flavonoids (details in the original article).	Alkaline and acid	(Barros Santos et al., 2019)			Folin-Ciocalteu (TPC) Vanillin (TPAC) Spectrophotometric (TFC) UPLC-ESI-QTOF-MS	(D'Almeida et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: DPPH, FRAP	
Atemoya fruits and by-products	Three times	Ultrasonicated (50/60 Hz), 15 min	1 g, 20 mL different solvents	Acid	Refluxed system of heating bath wit ball condensers, 80 °C, 90 min	Freeze-dried, 5 mL EtOH:H_2O (70:30) or EtOH:MeOH with HCl (1.2 mol L^{-1})	-	Folin-Ciocalteu (TPC) UPLC-ESI-QqQ-MS/MS AC: ABTS, ORAC	(Moraes et al., 2021)
Zooxanthellate jellyfish (*Cassiopea Andromeda*)	Two times	Rotary tube mixer (25 rpm), 4 °C, 2 h / 4 °C, 16 h	16 volumes (w/v) PBS / EtOH (80 %)	Enzymatic	Stirred, 4 °C, 48 h / Stirred, 37 °C, 5 h	Residue, pepsin (1 mg mL^{-1}) in acetic acid (0.5 M) / bacterial collagenase (1 mg mL^{-1}) in TES buffer (50 mM)	Washed two times with H_2O	Folin-Ciocalteu (TPC) AC: TEAC	(Rinaldis et al., 2021)
Irradiated pineapple (*Ananas comosus* L.) snack-bars	Two times	Shaken (250 rpm), 25 °C	1 g, 10 mL EtOH:H_2O (80:20)	Alkaline	Shaken (250 rpm), 2 h	Residue, NaOH (2 M)	Two times, washed ethyl acetate	HPLC-DAD-ESI-MS	(Del Juncal-Guzmán et al., 2021)
Grains of hulless barley varieties	Three times / Three times with ethyl acetate	Ultrasound, 35 °C, 40 min	1 g, 8 mL MeOH (80 %)	Alkaline	Shaking water bath (150 rpm), room temperature, 2 h	Esterified (details in the original article). Insoluble PC: Residue, 20 mL NaOH (2 mol L^{-1})	Three times with ethyl acetate	Folin-Ciocalteu (TPC) HPLC-DAD UPLC-DAD-ESI-QTOF-MS/MS AC: ABTS, DPPH	(Deng et al., 2021)
Artichokes (*Cynara Scolymus* cv. Blanca de Tudela)	Two times	Sonicated, 90 min	25 mg, 0.5 mL MeOH:H_2O (80:20) with formic acid (0.1 %)	Alkaline	Room temperature, 1 h	Residue, 0.75 mL NaOH (2 M)	0.5 mL MeOH:H_2O (80:20) with formic acid (0.1 %)	Folin-Ciocalteu (TPC) HPLC-QqQ(LIT)-MS/MS AC: ABTS, DPPH	(Domínguez-Fernández et al., 2021)
Passiflora species, mangosteen, and cherimoya peels	Two times	Shaking, room temperature, 1 h	15 g, 20 mL MeOH:H_2O (50:50) with HCl (2 N) / 20 mL acetone:H_2O (70:30)	Alkaline, acid and enzymatic	Shaking, room temperature, 4 h / 85 °C, 20 h / 70 °C, pH 10, 18.4 or 40 min	Residue, 25 mL NaOH (2 M) / Residue, 10 mL MeOH:H_2SO_4 (90:10) / Residue, sodium phosphate buffer (100 mM) with different enzyme preparations	-	HPTLC DART-HRMS Folin-Ciocalteu (TPC, total PA) AC: TEAC	(Domínguez-Rodríguez et al., 2021)
Tartary buckwheat (*Fagopyrum tataricum* L. Gaerth) hulls	-	Hot water, ultrasound, subcritical water,	100 g, 50 mL	Alkaline and acid	Stirrer ater bath, 25 °C / Magnetic	100 mL NaOH (2 M) / 100 mL HCl (2 M)	-	Folin-Ciocalteu (TPC) HPLC-ESI-MS	(Dzah et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
		ultrasound-assisted subcritical water			stirrer water bath (130 rpm), 1 h, 80 °C			AC: FRAP, TEAC	
Snack produced from maize/common bean mixture	Three times	Stirred (300 rpm), 10 min	0.5 g, 10 mL chilled EtOH (80 %)	Alkaline	95 °C, 30 min	Residue, 10 mL NaOH (2 M)	Five times with ethyl acetate	UPLC-DAD-ESI-MS AC: ABTS, DPPH	(Félix-Medina et al., 2021)
Whole grain brown rice	Three times	48 h	95 % EtOH	Alkaline	Room temperature, 4 h, with N_2	Residue, NaOH (2 M)	Five times with ethyl acetate	LTQ- Orbitrap-MS HPLC-DAD AC: ORAC, peroxyl radical scavenging capacity (PSC), cellular antioxidant activity (CAA)	(Feng et al., 2021)
Brown, black, and red rice	(Alves et al., 2016)							LC-ESI-QTOF-MS	(Ferreira et al., 2021)
White, gray, red, and black quinoas (*Chenopodium quinoa* Willd.)	-	Ultrasonic bath, 30 min	0.5 g, 50 mL MeOH with HCl (1 M) (85:15)	Alkaline	1 h, with N_2	Residue, 40 mL NaOH (2 M)	Three times with ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric (TFC) HPLC AC: ABTS, DPPH	(Gu et al., 2021)
Whole corn slurry	Three times	-	EtOH (80 %)	Alkaline	Room temperature, 1 h	Residue, NaOH (2 mol L^{-1})	Four times with ethyl acetate	Folin-Ciocalteu (TPC) Spectrophotometric (TFC) AC: ABTS, DPPH	(Guo et al., 2021)
Mango (*Mangifera indica* L.) bagasse	-	Stirred in orbital shaker (250 rpm), 10 min	1 g, EtOH:H_2O (80:20)	Alkaline	Stirring (250 rpm), 25 °C, 60 min, with N_2	Residue, 10 mL NaOH (2 M)	Ethyl acetate	LC/MS- TOF-ESI AC: ABTS, DPPH	(Herrera-Cazares et al., 2021)
Navel orange at different growth stages	Three times and fractionation	Ultrasound, 30 °C, 30 min	1 g, 15 mL MeOH (80 %)	Alkaline	25 °C, 6 h, with N_2	Residue, NaOH (4 M)	Ethyl acetate and ether	Folin-Ciocalteu (TPC, TFC) HPLC–QQQ–MS AC: DPPH, FRAP	(Hou et al., 2021)
Grape pomace	Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF): enzymatic methods			Alkaline	90 min	NaOH (4 mol L^{-1})	Two times with H_2O	Folin-Ciocalteau (TPC) HPLC FT-IR AC: ABTS, FRAP	(Jiang et al., 2021)
Wheat bran	Two times	Ultrasonicated, 1 h	0.1 g, 1.5 mL MeOH (80 %)	Alkaline	2 h	Residue, 6 mL NaOH (0.4 M)	-	Folin-Ciocalteu (TPC)	(Kaur et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: DPPH	
Jaboticaba (*Myrciaria cauliflora*) Peel	-	-	0.5 g, sequential solvent extractions: 3 mL MeOH acidified and 3 mL acetone:H_2O	Alkaline		(Luo et al., 2016)		Folin-Ciocalteu (TPC) pH-differential (Total extractable monomeric anthocyanin) Spectrophotometric (NEPA) HPLC-MS/MS AC: DPPH	(Lage et al., 2021)
Hempseed (*Cannabis sativa* L.) hull	Three times	Shaken, room temperature, 2 h, with N_2	4 g, 35 mL MeOH (80 %)	Acid	Boiled, 100 °C, 1 h, with N_2	Residue, 30 mL HCl (2 M)	Partitioned / Four time with ethyl acetate	Folin-Ciocalteu (TPC) Spectrophotometric (TFC) HPLC-ESI-QTOF-MS/MS HPLC-DAD AC: ABTS, DPPH	(Leonard et al., 2021)
Bamboo (*Phyllostachys edulis*) shoot tips	Four times / Fractionated	Different drying process methods underwent an ultrasonic bath (600 W), 30 min	20 g, 300 mL MeOH (80 %)	Acid or alkaline	85 °C, 30 min	Residue, 150 mL HCl (6 M) / Residue, 150 mL HCl (4 M)	Partitioned	Folin-Ciocalteu (TPC) $Al(NO_3)_3$ chromogenic agent (TFC) HPLC−ESI-QqQ-MS/MS AC: ABTS, DPPH, FRAP	(J. Li et al., 2021)
Red sorghums		Room temperature, overnight	600 mg, 18 mL acetone:H_2O (60:40)	Alkaline	Stirred, 2 h	Residue, NaOH (2 M)	Ethyl ether/ethyl acetate	Folin-Ciocalteu (TPC) Spectrophotometric (TFC) UPLCQ-TOF-MS AC: ABTS, DPPH	(M. Li et al., 2021)
Pepper (*Capsicum annum* L.) Miguel	-	-	1 g, 10 mL chilled EtOH	Alkaline	Ultrasonic bath, room temperature, 1 h	2 mL KOH (6 M)	Ethyl acetate	HPLC-DAD	(Lobato Ureche et al., 2021)
Wine lees	10 mL of wine lees were mixed with the commercial enzyme solution Flavourzyme® (enzyme/substrate ratio, 80 LAPU/g protein). Hydrolysis was carried out at 25 °C for 2 h at pH 4.0 and 250 rpm in a shaker.							Folin–Ciocalteu (TPC) Q-TOF/MS	(López-Fernández-

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: DPPH	sobrino et al., 2021)
Spaghetti with persimmon (*Dyospyros kaki*) flours	-	Sonication, room temperature, 2 h	2 g, 10 mL MeOH acidified HCl (1 %)	Alkaline	4 h, with N_2	Residue, 60 mL NaOH (4 M)	Three times with ethyl acetate	HPLC AC: ABTS, FRAP	(Lucas-González et al., 2021)
Grape pomace	-	Shaken, room temperature, 1 h / Boiling water, 1 h	0.5 g, 20 mL MeOH:H_2O acidified HCl (50:50) and 20 mL of acetone:H_2O (70:30) / 2.5 mL butanol:HCl (97.5:2.5, v/v) with 0.1% $FeCl_3$	Butanolysis and acid	Boiling water, 1 h / 85 °C, 20 h	Residue, 10 mL butanol:HCl (97.5:2.5, v/v) with 0.1% $FeCl_3$ / Residue, 2 mL MeOH and 200 µL H_2SO_4	Two times	Folin-Ciocalteu pH-differential (Total monomeric anthocyanins) Spectrophotometric (TPA) UHPLC-QqQ-ESI-MS AC: ABTS, DPPH, nitric oxide	(Martínez-Meza et al., 2021)
Aleurone fractions from wheat bran (*T. aestivum* L.)	Exhaustive extraction, three times	Ultrasonic bath, 10 min	1 g, 10 mL EtOH:H_2O (4:1)	Alkaline	Shaking, 18 h, with N_2	Residue, 25 mL NaOH (1 M)	Three times with diethyl ether:ethyl acetate	HPLC-DAD-ESI-TOF-MS	(Martín-García, Gómez-Caravaca, et al., 2021)
Buckwheat flour fractions	Two times	Ultrasonic bath, 40 °C, 10 min	1 g, 10 mL EtOH:H_2O (4:1)	Alkaline	Shaking, room temperature, 18 h, with N_2	Residue, 25 mL NaOH (1 M)	Five times with diethyl ether:ethyl acetate	RP-HPLC-ESI-TOF-MS AC: DPPH, FRAP	(Martín-García, Verardo, et al., 2021)
Black, green, and pink pepper	Two times	Sonicated, 90 min and 30 min	50 mg, 1 mL MeOH:H_2O:CH_2O_2 (80:19.9:0.1)	Alkaline	40 °C, 1 h	Residue, 1.5 mL NaOH (2 mol L^{-1})	-	Folin-Ciocalteau LC-ESI-IT-MS AC: ABTS, FRAP	(Martini et al., 2021)
Raw cocoa nibs and husk	Three times	Shaking water bath, 30 °C, 20 min	Acetone (70 %)	Alkaline	40 °C, 4 h	Residue, NaOH (4 M)	Five times with diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) UHPLC-DAD AC: ABTS, DPPH, FRAP, ORAC	(Mudenuti et al., 2021)
Cupcake with red rice grains	-	-	MeOH (80 %)	Alkaline	-	Residue, NaOH (2 M)	Ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric reaction (Flavonoids) UFLC AC: ABTS, DPPH	(Müller et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Alcoholic fermentation of citrus flavedo and albedo with pure and mixed yeast strains		Fermentation juice		Alkaline	Stirred, room temperature, overnight	Residue, 3 mL NaOH (1 mol L^{-1}) with EDTA (10 mmol L^{-1})	Two times with ethyl acetate	UPLC-MS/MS	(Multari et al., 2021)
Mexican red wines and their by-products	One time	sonicated for 30 min extracte	10 g, MeOH (80 %) acidified HCl (1 %) / extracted using acetone 70% in a 1:25 (solid: solvent) ratio	Alkaline	Refluxed, 80 °C (H_2O bath) with agitation, 4 h	Residue, NaOH (3 M)	-	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) DMAC (TPAC) pH shift (Anthocyanin content) HPLC- ESI-MS/MS	(Muñoz-Bernal et al., 2021)
Strawberries	Three times, completely extract the vacuolar metabolites.	4 °C	600 mg, 4 mL of MeOH / acetone (80 %)	Acid	85 °C, 4 h	Residue, HCl (2M) with 0.04% ascorbic acid	-	Butanol-HCl (PA) HPLC-MS AC: DPPH	(Narvekar & Tharayil, 2021)
Dehulled, whole, and hulls of green and black lentils	Two times	Sonicated (180 W, 50hz), 40 °C, 20 min	1 g, 10 mL of MeOH:acetone: H_2O (1:1:1, v/v/v)	Alkaline	Stirring, room temperature, 4 h	15 mL, NaOH (2 M)	Four times with diethyl ether:ethyl acetate	Folin-Ciocalteau (TPC) Spectrophotometric (TFC) (ESI)-MS-MS AC: DPPH, TEAD, hydroxyl radical scavenging capacity	(Paranavitana et al., 2021)
Brewers' spent grain	Three times	Shaking, room temperature, 2 h/30 min	MeOH (80 %)	Alkaline	60 °C, 30 min	Residue, 30 mL, NaOH (2 M)	Two times with ethyl acetate	Folin-Ciocalteu HPLC-DAD AC: ABTS, DPPH, FRAP	(Patrignani et al., 2021)
Grapefruit peel	-	Thermostatic H2O bath, 60 °C	Enzymatic extraction	Microwave, enzymatic and combined microwave and enzymatic treatments				Folin–Ciocalteau (TPC) UPLC-ESI-QTOF-MS/MS LC-ESI-QqQ-MS/MS AC: ABTS, DPPH, ORAC	(Peng et al., 2021)
Sourdough fermentation of whole and sprouted lentil flours	Two times	Ultrasonic bath, 10 min	4 g, EtOH:H_2O (4:1)	Alkaline	Room temperature, overnight with N_2	Residue, 300 mL, NaOH (2 M)	-	LC-ESI-QTOF-MS AC: DPPH	(Perri et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Mangosteen peel	Two times	Agitated, room temperature, 1 h	15 g, 20 mL MeOH:H_2O (50:50) acidified with HCl (2 N) / 20 mL of acetone:H_2O (70:30)	Emerging methods	Ultrasound with 30 % amplitude, 1 min	Residue, different deep natural eutectic solvents. 1 mL of NaDES:H_2O (70:30; v/v)	-	DMAC (TPAC) Butanol-HCl HPLC-SEC HPLC-DAD AC: ABTS, FRAP, hydroxyl radical	(Plaza et al., 2021)
Coffee husk	Two times	Ultrasonic bath, 30 min / Stirring, 40 °C, 16 h	1 g, 50 mL MeOH:HCl (0.1%):H_2O (80:20)	Alkaline	Shaking, 25 °C, 1 h, with N_2	Residue, NaOH (4 mol L^{-1})	Three times with diethyl ether:ethyl acetate	Folin–Ciocalteu (TPC) $AlCl_3$ (TFC) Vanillin adapted (Flavanols) Bate-Smith (TPAC) Spectrophotometric (Phenolic acids) UPLC-ESI-MS/MS	(Rebollo-Hernanz, Cañas, Taladrid, Benítez, et al., 2021)
Cocoa shell	Two times	Ultrasonic bath, 30 min / Orbital shaker, 40 °C, 16 h	1 g, 50 mL MeOH:HCl (1%):H2O (80:20)	Alkaline	Shaking, 25 °C, 1 h, with N_2	Residue, 20 mL NaOH (4 mol L^{-1})	Three times with diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) Vanillin adapted (Flavanols) Bate-Smith (TPAC) Spectrophotometric (Phenolic acids) UPLC-ESI-MS/MS	(Rebollo-Hernanz, Cañas, Taladrid, Segovia, et al., 2021)
Strawberry, raspberry, blueberry, and blackberry decoctions	4 mL of acetone:H_2O (70:30)	Stirring, room temperature, 1 h	200 mg, 4 mL MeOH:H_2O (50:50)	Alkaline and acid	Stirring, 16 h / 85 °C, 30 min	Residue, 12 mL H_2O and 5 mL NaOH (10 M) / Residue (alkaline), 2.5 mL of HCl	Distilled H_2O	Folin-Ciocalteu (TPC) Spectrophotometric (TFC, TAC) Butanol-HCl (TPAC) Phloroglucinol reaction UPLC-ESI-Q-ToF MS MALDI-ToF MS	(Reynoso-Camacho et al., 2021)
Winemaking by-products: skins, stems, and lees	0.5 g, 20 mL MeOH:H_2O solution (50:50) acidified HCl. The mixture was incubated with stirring for 1 h. This process was repeated with an acetone:H_2O solution (70: 30)							Folin-Ciocalteu AC: ABTS, DPPH	(Rivas, Casquete, Córdoba, Ruíz-

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
									Moyano, et al., 2021)
Pomegranate (*Punica granatum* L.) peel	Two times	Magnetic mixer, room temperature (25 °C), 1 h	10 g, 60 mL EtOH:H_2O (80:20)	Acid	Stirring, 1 h	0.5 g, 20 mL MeOH:H2O solution (50:50) acidified with HCl / acetone:H_2O solution (70:30)	-	Folin-Ciocalteu (TPC) AC: DPPH	(Rivas, Casquete, Córdoba, Benito, et al., 2021)
Bread dough (with yerba mate leaves)	Two times	10 min	1 g, 20 mL chilled EtOH (80 %)	Alkaline and acid	Agitation, room temperature, 22 h / H_2O bath, 85 ± 2 °C, 30 min	Residue, 34 mL NaOH (4 mol L^{-1}) / Residue, 2.5 mL concentrated HCl	Two times / Ethyl acetate	Folin-Ciocalteu AC: ABTS, FRAP LC-MS/MS	(Santetti et al., 2021)
Red rice bran	-	Pre-treatment: Enzymatic hydrolysis. Mixing, 1 h	0.1 g, 8 mL MeOH	Acid	-	Residue, 8 mL of MeOH acidified HCl (1 %)	-	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) HPLC AC: DPPH, FRAP, superoxide anion, hydroxyl radical, nitric oxide	(Sapna & Jayadeep, 2021a)
Red rice bran	-	Pre-treatment: Enzymatic treatment. Room temperature (27 ± 2 °C), 1 h	0.1 g, 8 mL MeOH	Acid	Room temperature (27 ± 2 °C), 1 h	Residue, 8 mL of MeOH acidified HCl (1 g 100 mL^{-1})	-	Folin-Ciocalteu (TPC) HPLC AC: DPPH, FRAP, superoxide anion, hydroxyl radical, nitric oxide	(Sapna & Jayadeep, 2021b)
Purple sweet potato	Exhaustive extraction	H_2O bath, 50 °C, 2 h	10 g, 250 mL of EtOH (60 %)	Acid, alkaline, and enzymatic	62 °C, 22 h / Stirred, 25°C, 5 h / 53 °C, 18 h	Residue, 35 mL of H_2SO_4 (10 %) / 30 mL NaOH (2 M) / 30 mL phosphate buffer (0.02 M, pH 6) with enzyme preparation	Partitioned with ethyl acetate	LC-MS/MS	(Su et al., 2021)
Pitahaya fruit peel from two red-skinned species (*Hylocereus polyrhizus* and *Hylocereus undatus*)	Two times	Ultrasonic (400 W), 25 °C, 30 min	0.5 g, 15 mL MeOH (80 %) with CH2O2 (1 %)	Acid, alkaline, and enzymatic	H_2O bath, 85 °C, 60 min, with N_2 / Shaking H_2O bath, 30 °C, 4	Residue, 15 mL of HCl (3 M) / 15 mL of NaOH (3 M) with EDTA (10 mM) and ascorbic acid (1 %) /	Four times ethyl acetate	Folin–Ciocalteu (TPC) $AlCl_3$ (TFC) UPLC-TOF-MS	(Tang et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
					h, with N_2 / Shaking H_2O bath, 50 °C, 2 h	Residue, 0.03 g of composite enzymes 10 mL of H_2O		AC: ABTS, DPPH, FRAP	
Infant cereal formulation	Two times	Ultrasonic bath, 20 min	20 mL acetone:H_2O (4:1)	Alkaline	Shaking, room temperature, 20 h	Residue, 100 mL NaOH (2 M)	Three times with diethyl ether:ethyl acetate	HPLC-TOF-MS	(Verardo et al., 2021)
Tea seed oil	Three times	Stirred, 3 min	6 mL MeOH:H_2O (60:40) / Deep eutectic solvents (DES), mixture choline chloride (ChCl) and glycerol (1:2)	Alkaline and emerging method	Room temperature, 12 h / Vortexed, 3 min / H_2O bath, 50 °C, 1 h	Residue, NaOH (0.2 mol L^{-1}) / The oil phase, 6 mL MeOH H_2O (60:40) / 6 g DES	Two times	UHPLC-QqQ-MS/MS AC: ABTS, DPPH, FRAP, ORAC	(Wang et al., 2021)
Walnut kernel	Three times / Ethyl acetate (several times)	-	1 g, 50 mL MeOH:H_2O (7:3)	Alkaline	1 h, inert atmosphere	NaOH (2 M) / EtOH:H_2O (7:3) and acetone:H_2O (7:3)	Ethyl acetate	Folin-Ciocalteu (TPC) $Al(NO_3)_3$ (TFC) UPLC-MS/MS AC: ABTS	(Wu et al., 2021)
Soybean sprouts	Three times	Shaken (200 rpm), 25 °C, 1 h, with N_2	MeOH (80 %)	Alkaline	Shaker (200 rpm), 25 °C, 4 h	NaOH (2 N)	Three times with ethyl acetate	Folin-Ciocalteu (TPC) HPLC	(Xie et al., 2021)
Lychee juice by-products	Two times	-	MeOH:H_2O (8:2)	Alkaline	Magnetic stirrer, room temperature, 18 h	NaOH (2 mol L^{-1})	-	Folin–Ciocalteu (TPC) LC-ESI-MS/MS HPLC-DAD	(Xiong et al., 2021)
Co-extruded wheat/okra composite blends	Three times	-	0.25 g, MeOH (80 %) with CH_2O_2 (2 %)	Alkaline	37 °C, 2 h	Residue, 10 mL NaOH (2M)	Three times with ethyl acetate	Folin-Ciocalteu UPLC-MS/MS AC: DPPH	(K. Xu et al., 2021)
Defatted adlay (*Coix lachryma-jobi* L.) bran	Three times	25 °C, 2 h	1 g, 20 mL MeOH (80 %) with HCl (0.15 M)	Alkaline	Shaking, 25 °C, 2 h, with N_2	Residue, 40 mL NaOH (2M)	Five times with ethyl acetate	Folin–Ciocalteu HPLC-DAD AC: ABTS, FRAP	(L. Xu et al., 2021)
Raspberry pomace	Three times	-	Ethyl acetate	Alkaline	Bath shaker (150 rpm), room temperature, 4 h, with N_2	Esterified and glycosylated (details in the original article) / Residue, NaOH (4 M) with	Ethyl acetate	Folin-Ciocalteu HPLC-MS AC: DPPH	(Yao et al., 2021)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
						EDTA (10 mmol L^{-1}) and vitamin C (1 %)			
Brown rice	Remove free phenol	12 h	1 g, EtOH (95%)	Alkaline	Shaking, room temperature, 1 h, with N_2	Residue, 20 mL NaOH (2 M)	Five times, ethyl acetate	HPLC	(Ye et al., 2021)
Prunus mume fruits	-	Ultrasonic sonication (24 kHz) H_2O bath, 30 min	0.5 g, 15 mL MeOH (80 %) with CH_2O_2 (1 %)	Alkaline and acid	Shaking, 30 °C, 4 h, with N_2 / 85 °C, 1 h	Residue, sequential base hydrolysis (2 M NaOH) and then acid hydrolysis (2 M HCl)	Three times ethyl acetate	Folin-Ciocalteu UPLC–Q–Exactive Orbitrap/MS AC: ABTS, DPPH	(You et al., 2021)
Brown rice	-	Shaken (250 rpm), room temperature, 2 h	1 g, 20 mL EtOH (80 %)	Alkaline	Shaken (250 rpm), room temperature, 4 h	Residue, 20 mL NaOH (4 M)	Three times ethyl acetate	Folin-Ciocalteu UPLC-PDA-MS/MS AC: DPPH, FRAP	(Yu et al., 2021)
Milling fractions of foxtail millet	-	-	1 g, 20 mL MeOH (80 %)	Alkaline	-	Residue, 40 mL NaOH (2 M)	Ethyl acetate	Folin-Ciocalteu $AlCl_3$ (TFC) HPLC- DAD-Q-TOF-MS^2 UPLC AC: ABTS, DPPH, FRAP	(Zhang et al., 2021)
Millet	-	Sonicated (30 min), and stirred (12 h) / Sonicated (30 min), and stirred (2 h)	2 g, 20 mL/25 mL MeOH with HCl (1 %) or EtOH:H_2O (7:3) with CH_2O_2 (1 %)	Alkaline and acid	Sonicated, 60 °C, 1 h / Stirring, room temperature for 4 h	1 g, 25 mL of MeOH:H_2O (7:3) with NaOH (0.1 M) / Residue, 40 mL NaOH (2-4 M) / 1 g, 25 mL of MeOH:H_2SO_4 (9:1)	-	HPLC-DAD HPLC-DAD-MS	(Balli et al., 2020)
Sprouts and wheatgrass of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and emmer ([(*Triticum turgidum* L. spp. *dicoccum*, (Schrank ex Schübler) Thell.)])	Two times	Ultra-Turrax / Sonicated, room temperature, 40 min	1 g, 20 mL MeOH:acetone: H_2O (7:7:6) / 5 mL MeOH:H_2O:acetic acid (70:29.5:0.5)	Alkaline and acid	1 h / Sonication, room temperature, 40 min / Room temperature, overnight	Residue, NaOH (5 M) and HCl (5 M)	Ethyl acetate	Folin-Ciocalteu (Polyphenols, tannins - subtraction of the phenolic content- and flavonoids - subtracting the non-flavonoid content from polyphenols-) HPLC (Phenolic acids)	(Benincasa et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: DPPH, FRAP	
Purple wheat (*Triticum aestivum* L.)	Three times	Shaking (150 rpm), room temperature, 30 min	1 g, 8 mL MeOH:HCl 1 M (85:15) / 8 mL aqueous acetone (70 %).	Alkaline	Shaking (150 rpm), room temperature, 16 h	Residue, 10 mL NaOH (2 M)	Three times diethyl ether:ethyl acetate	HPLC-DAD LC-MS	(Bueno-Herrera & Pérez-Magariño, 2020)
Common wheat (*Triticum aestivum* L.) and millet (*Panicum Miliaceum*, L.)	Two times	Ultra-Turrax, 1 min on ice, ultrasonicated for 40 min at room temperature	1 g, MeOH:H_2O:CH_3COOH (70:29.5:0.5)	Alkaline	Sonicating, room temperature, 40 min and left overnight	Residue, NaOH (4 M)	Three times ethyl acetate	Folin-Ciocalteu (TPC) HPLC (Phenolic acids)	(Ceccaroni et al., 2020)
Ripe carob (*Ceratonia siliqua* L.) pods	Three times	Magnetic stirring, room temperature, 1 h	1 g, 20 mL or 800 mL L^{-1} acetone / Diethyl ether:ethyl acetate	Alkaline	Stirring, room temperature, 4 h	Residue, 40 mL NaOH (2 mol L^{-1})	Diethyl ether:ethyl acetate	Adapted Folin-Ciocalteu (TPC) Spectrophotometric (TFC) HPLC AC: DPPH, ORAC, ABTS	(Chait et al., 2020)
Green tea leaves (*Camellia sinensis* O. Kuntze)	Ultrasound, agitation, and conventional extraction techniques. Three individual replications. Extraction with ethyl acetate (3 times)			Alkaline	Shaking (100 rpm) H_2O bath, room temperature, 4 h	Soluble esterified and glycosylate (details in the original article). Residue, NaOH (4 M) with EDTA (10 mM) and ascorbic acid (1 %)	Three times ethyl acetate	Folin-Ciocalteu (Total polyphenols) $AlCl_3$ (TFC) UPLC-DAD-QToF	(Das et al., 2020)
Fruit peelings: Mango (*Mangifera indica*), apple (*Malus sylvestris*), banana (*Musa acuminate*), and orange (*Citrus sinensis*)	-	1 h	2 g, 20 mL EtOH (80 %)	Alkaline	Shaking, room temperature, 1 h	Residue, 20 mL NaOH (4 M)	Six times ethyl acetate	Folin-Ciocalteu (TPC) HPLC-DAD AC: DPPH, Oyaizu (reducing power)	(Feumba Dibanda et al., 2020)
Einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*) bulgur	Two times	Sonication, 10 min	0.5 g, 5 mL EtOH (80 %)	Alkaline	Shaking, 60 °C, 90 min	Residue, NaOH (2 N)	Five times ethyl acetate	HPLC LC-MS	(Giambanelli et al., 2020)
Rice (*Oryza sativa* L.), i.e., Heizhenmi (black rice), Liushuihong (red rice), and Wuyouhuazhan (brown rice, polished rice)	Two times	10 min	2 g, 45 mL acetone (80 %)	Alkaline	Shaking, 1 h, with N_2	Residue, 20 mL NaOH (2 M)	Five times ethyl acetate	Folin-Ciocalteu (TPC) Sodium borohydride/chloranil-based (SBC) (TFC)	(Gong et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								pH-differential (TAC) DMAC (TPAC) UPLC-ESI-Q-TOF-MS HPLC AC: Peroxyl radical scavenging capacity (PSC) and cellular antioxidant activity (CAA)	
Loquat fruits (cvs. 'Karantoki' and 'Morphitiki')	Two times	-	5 g, 15 mL of 950 mL L^{-1} cold EtOH and 10 mL of 800 mL L^{-1} cold EtOH.	Alkaline	Sonicated, 40 °C, 90 min	Residue, 10 mL NaOH (4 M)	Three times ethyl acetate	Folin-Ciocalteu AC: DPPH, FRAP	(Hadjipieri et al., 2020)
Phoenix dactylifera L. (date) seeds	-	Sonicated, 5 min	150 mg/50 mg, 10 mL MeOH:H_2O (70:30) with HCl (0.1 %)	Depolymerisation of date seed polyphenols by phloroglucinolysis				HPLC-ESI-UV/MS/MS (IT) UPLC-QTOF (MS/MS)	(Hilary et al., 2020)
Apple pomace	Two times	Ultra-turraxed (16,000 rpm), 30 min	0.5 g, 15 mL chilled MeOH:H_2O (80:20) with CH_2O_2 (1 %) / 40 mL MeOH:H_2O	Alkaline and acid	Method 1: Residue was subjected to sequential base (NaOH 2 M) and acid hydrolysis (HCl 2 M). Method 2: Method 1, except that the acid hydrolysis was carried out before base hydrolysis. Method 3: 0.5 g was mixed with 15 mL of NaOH 2 M, with N_2. Method 4: 0.5 g was hydrolyzed by adding 15 mL HCl 2 M. The mixture was incubated in a H_2O bath at 85 °C for 1 h.		Three times diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) UPLC-Q-Exactive Orbitrap/MS AC: ORAC	(Li et al., 2020)
Berry fruit (*Crataegus pinnatifida*)	Two times	Ultrasonic processing. 25 °C, 30 min	1 g, 30 mL EtOH (50 %)	Alkaline	H_2O bath shaker (150 rpm), room temperature, 4 h	Soluble esterified and glycosylate (details in the original article) / Residue, 12 mL NaOH (4 mol L^{-1}) with EDTA (10 mol	5 times by diethyl ether-ethyl acetate	HPLC-ESI-MS/MS AC: ABTS, DPPH, FRAP	(Lou et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
						L^{-1}) and ascorbic acid (1 %)			
Red and yellow araçá (*Psidium cattleianum* Sabine)	Exhaustively extracted	Vortexing, 3 min	0.5 g, 5 mL MeOH:H_2O (8:2) acidified CH_2O_2 (0.35 %)	Acid	H_2O bath, 90 °C, 15 min	Residue, 20 mL MeOH acidified with HCl (15 %)	Two hydrolysis / Eight times MeOH exhaustive extraction	Folin-Ciocalteu LC-DAD-ESI-MS/MS AC: Peroxyl radical scavenging	(Mallmann et al., 2020)
Grape peel powder	-	-	MeOH:H_2O (50:50) / acetone:H_2O (70:30)	Acid	100 °C, 3 h / 85 °C, 20 h	Butanol:HCl (95:5) / MeOH:H_2SO_4 (90:10)	-	HPLC-PDA-MS/MS Spectrophotometric	(Maurer, Cazarin, Quatrin, Minuzzi, et al., 2020)
Grape peel powder	-	-	MeOH:H_2O (50:50) / acetone:H_2O (70:30)	Acid	100 °C, 3 h / 85 °C, 20 h	Butanol:HCl (95:5, v/v) with 0.7 g/L of $FeCl_3$ / MeOH:H_2SO_4 (90:10)	-	HPLC-PDA-MS/MS Spectrophotometric	(Maurer, Cazarin, Quatrin, Nichelle, et al., 2020)
Grape pomace and sorghum bran	5 g, 12.5 mL aqueous EtOH (50 %) with NaOH (3 %) and with or without 0.25 g (dry weight basis, 5 % w/w starch) freeze-dried extract were mixed at 50 °C and 150 rpm in a shaking H_2O bath for 6 or 12 h. The slurry was either washed with aqueous EtOH 6 times or left unwashed before oven drying (12 h, 50 °C).							Folin-Ciocalteu modified (TPC) Vanillin-HCl (TPAC) (FTIR)spectroscopy UFLC AC: ABTS	(Oladele et al., 2020)
Baru (*Dipteryx alata* Vog.) nuts	-	Ultrasound, room temperature	10 g, 100 mL MeOH:H_2O (80:20)	Acid	Shaking, room temperature / Ultrasonic H_2O bath (40 kHz. 50 W), 25 ± 3 °C, 120 min	10 g, 100 mL MeOH:H_2O:HCl (80:16:4)	-	Folin-Ciocalteu (TPC) HPLC-DAD-ESI-MS/MS HPLC-DAD-ED HPLC-DAD AC: ORAC and HOSC	(Oliveira-Alves et al., 2020)
Couscous	Two times	Ultrasonic bath, 15 min	3 g, 30 mL EtOH/H_2O (4:1)	Alkaline	Shaking, room temperature overnight, with N_2	Residue, 100 mL NaOH (1 mol L^{-1})	Two times ethyl acetate	HPLC-ESI–TOF-MS	(Oriente et al., 2020)
Mutamba (*Guazuma ulmifolia* Lam.)	Two times	Ultrasonicated, room temperature, 30 min	1 g, 15 mL MeOH:acetone: H_2O (7:7:6)	Alkaline	H_2O bath shaker (150 rpm), room	Residue, NaOH (4 M) with EDTA (10	Diethyl ether-ethyl acetate	HPLC-ESI-MS/MS	(Pereira et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
					temperature, 4 h	mM) and ascorbic acid (1 %)			
Guarana Powder	Four times	Homogenization (14.000 rpm), 3 min	0.8 g, 20 mL of H_2O, MeOH, and acetone (20:56:24)	Alkaline	Ultrasound-assisted (37 kHz and 100 W), 60 ºC, 15 min, with N_2	Residue, 2 mL NaOH (2 M)	Three extraction cycles. acetone:H_2O:CH_3COOH (70:29.5:0)	Folin-Ciocalteu (TPC) MALDi-TOF-MS	(Pinaffi et al., 2020)
Mustard grains namely black (*Brassica nigra*) and white (*Sinapsis alba*)	Three times	Stirring (150 rpm), 25 °C, 20 min	1 g, 30 mL H_2O:acetone (1:1)	Alkaline	Stirring, 4 h	Residue, 15 mL NaOH (mol L^{-1})	Five times ethyl acetate	Folin-Ciocalteu modified (TPC) Colorimetric (TFC, TPAC) AC: ABTS, DPPH, ORAC, FRAP UPLC-MS/MS	(Rasera et al., 2020)
Pigmented Thai rice, Mali Dang (red), Hom Nil (purple), and Riceberry (purple) varieties	Three times	Shaken (180 rpm), room temperature, 2 h.	1 g, 10 mL MeOH (80 %) acidified with HCl (1.0 N) (85:15)	-	-	1 g, 10 mL MeOH (80 %)	Five times with ethyl acetate	Folin–Ciocalteu (TPC) Colorimetric (TFC) AC: DPPH, FRAP HPLC	(Ratseewo et al., 2020)
Yellow Guava (*Psidium cattleianum* Sabine)	1.0 g of the sample was mixed with ascorbic acid and stirred for 1 min. An aliquot of HCl (5 mL, 6 mol L^{-1}) was added to the mixture and incubated at 85 ± 2 °C in an oven for 30 min. After cooling, the extract was sequentially extracted three times with 5 mL of ethyl acetate for 1 min.							LC-MS/MS AC: DPPH, FRAP	(Schulz et al., 2020)
Coffee pulp (*Coffea arabica* L.)	Three times	Agitation (100 rpm), room temperature, 15 min	80 mL H_2O:MeOH (20:80) acidified with CH_2O_2 (2 %)	Alkaline	Agitation (150 rpm), room temperature, 30-180 min	Residue, 10 mL NaOH (1-2 M) with EDTA (10 mM) and ascorbic acid (1 %) / PA (details in the original article) (Phloroglucinolysis)	-	HPLC-DAD HPLC-MS/MS	(Silveira et al., 2020)
116 commonly consumed food in five regions of China	Two times	Vortex oscillation for 10 s, ultrasonicated, room temperature, 30 min	2 g, 20 mL MeOH (80 %) with vitamin C (0.2 %)	Alkaline	Gas bath with shaking, 40 °C, 2 h, with N_2	Residue, 20 mL NaOH (4 M)	Ethyl acetate	HPLC-MS	(Song et al., 2020)
Triticum spp. L. whole grains and milling by-products	-	Shaker (200 S/min), room temperature, 10 min	1g, 4 mL MeOH:H_2O (7:3)	Alkaline	Room temperature, 1 h	Residue, 20 mL NaOH (2 N)	Ethyl acetate	UHPLC-MS/MS	(Spaggiari, Calani, et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Solid-state fermentation using a *Lactobacillus rhamnosus* strain was applied to wheat bran	Two times	10 min	1 g, cold EtOH (80 %)	Alkaline and acid	Shaken (2000 rpm), 4 h	Residue, 75 mL HCl (6 M) / NaOH (2 M)	Diethyl ether	Folin-Ciocalteu (TPC) UHPLC-ESI-MS/MS AC: DPPH, FRAP, ABTS	(Spaggiari, Ricci, et al., 2020)
Blackberry fruits	Two times	Ultrasonic bath, 15 min / Ultra-turrax (5000 g), 3 min	2 g, 5 mL MeOH (75 %) with CH_2O_2 (0.1 %) / 1 g, 10 mL MeOH (80 %) acidified with CH_2O_2 (0.1 %)	Alkaline	Room temperature, 1 h	Residue, 3 mL NaOH (2 M)	Ethyl acetate	Folin-Ciocalteu (TPC) pH-differential (monomeric anthocyanin) HPLC-PDA UHPLC-QTOF-MS AC: CUPRAC, DPPH	(Tomas et al., 2020)
Carrots	-	Vortex (0.14×g), 3 min, and shaker (0.07×g), 10 min	1 g, 10 mL EtOH (80 %)	Alkaline	H_2O bath, 95 °C, 30 min, with N_2	10 mL NaOH (2 M)	Ethyl acetate	HPLC-DAD HPLC-ESI-MS	(Viacava et al., 2020)
Sorghum grain genotypes	Three times	Shaken (150 rpm), 25 °C, 2 h, with N_2	4 g, 30 mL MeOH: H_2O (80:20) / 35 mL MeOH	Acid	100 °C, 1 h, with N_2	Residue, 30 mL HCl (2 M)	Six times ethyl acetate	HPLC-DAD HPLC-DAD-ESI-QTOF-MS/MS	(Xiong et al., 2020)
Lychee (cv. *Jizui*, *Lizhiwang*, *Guiwei*, *Yuhe*, *Nuomici*, and *Guihong*)	Methodology for soluble (SDF) and insoluble dietary fiber (IDF) content.			Alkaline	90 min	The dried IDF (or SDF), NaOH (4 mol L^{-1})	H_2O	Folin–Ciocalteu (TPC) Colorimetric (TFC) AC: FRAP, ABTS HPLC-DAD	(Z. Xu et al., 2020)
Coarse flours from okra seeds and seedless pods	The total content of EPP, HPP, and NEPA of flours and breads as well as their antioxidant activities (scavenging activity of DPPH) were measured according described by Pico et al. (2019).							The total content of EPP, HPP, and NEPA: antioxidant activities (scavenging activity of DPPH) were measured according described by Pico et al. (2019).	(K. Xu et al., 2020)
Blank tea seed oil	Two times	Vortexed and then immersed in a H_2O bath at 50 °C for 1 h with agitation	5 g, 5 g of deep eutectic solvent (DES)	Alkaline	Room temperature, 12 h	Residue, 12 mL NaOH (0.2 M)	Two times, 6 g of DES	UHPLC-QqQ-MS AC: DPPH	(Wang et al., 2020)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Lentils: CDC green land (green), CDC invincible (green), 3493–6 (green), and maxim (red)	Three times	20 min	1 g, 10 mL MeOH:H_2O:acetone (1:1:1)	Alkaline	Stirring, 4 h	Residue, 15 mL NaOH (2 M)	Five times ethyl acetate	Folin-Ciocalteu (TPC) HPLC-DAD-ESI-MS^n AC: DPPH using EPR, reducing power and hydroxyl radical	(Yeo & Shahidi, 2020)
Green and red pickled olives (*Olea europaea* L.; cv. Improved Nabali), fresh ripe tomatoes (cv. Sunrise), and fresh peppers (green hot (cv. HY), yellow sweet (cv. YouKy), red hot (cv 4501), green sweet (cv. Jor2), and red sweet (cv. J21))	-	Shaking H_2O bath, 30 °C, 1 h / 60 °C, 1 h	1 g, 25 mL MeOH	Alkaline and acid	Shaking H_2O bath, 30 °C, 24 h	Residue, 25 mL NaOH (0.1 M) / Residue, 25 mL HCl (0.1 M)	MeOH for 1 h at 30 °C	Folin-Ciocalteu (TPC) LC-MS/MS AC: β-carotene bleaching	(Alu'datt et al., 2019)
Roselle (*Hibiscus sabdariffa* L.) calyx and its by-product	Two times	Shook, 1 h	0.25 g, 10 mL MeOH/H_2O (50:50) acidified with HCl / 10 mL of acetone/H_2O (70:30, v/v)	Acid	100 °C, 60 min / 85 °C, 20 h.	n-butanol:HCl (95:5) and iron reagent (2 % ferric ammonium sulfate in 2 mol L^{-1} HCl) / MeOH/H_2SO_4 (90:10)	-	Folin-Ciocalteu (TPC) UPLC-QTOF MS^E	(Amaya-Cruz et al., 2019)
Bread wheat doughs fermented with different lactic acid bacteria strains	-	Mixing 30 s, 10 min	2 g, 20 mL MeOH:acetone:H_2O (7:7:6)	Alkaline	Three fractions (free: F, soluble-conjugated: SC, insoluble-bound: IB) were extracted from the sample supernatant (F, SC), and the pellet (IB). The F fraction was obtained by analyzing the supernatant as such, while the SC fraction was obtained by the difference after alkaline hydrolysis.			Folin-Ciocalteu (TPC) HPLC AC: FRAP	(Antognoni et al., 2019)
White, red, or black quinoa flours	-	Shaking, room temperature, 60 min	MeOH:H_2O (50:50) / acetone:H_2O (70:30)	Acid	85 °C for 20 h	Residue, MeOH:H_2SO_4 (90:10)	-	Folin-Ciocalteu (TPC) AC: DPPH, FRAP and total antioxidant activity	(Ballester-Sánchez et al., 2019)
Fermented whole wheat	-	Magnetic stirring in an ultrasonic bath, 15 min	250 mg, EtOH:H_2O 80:20 / H_2O acidified with HCOOH 1 %	Alkaline and acid	Sonicated (1 or 2 h, 60 °C)	1 g, 25 mL of NaOH 0.1 M in MeOH:H_2O (7:3) / 100 mL NaOH 0.1 M in MeOH:H_2O (7:3) / 25 mL of	-	HPLC-DAD	(Balli et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
						MeOH:H_2O (7:3) with NaOH 4 M / MeOH:H_2SO_4 9:1			
Coffee parchment	Three times	5 min and vortex for 10 min.	2 g, 30 30 mL of 80% chilled acetone (1:2, w/v)	Alkaline	Shaking, room temperature for 1 h	20 mL of NaOH 4 M	Three times with ethyl acetate	Folin-Ciocalteu (TPC) AC: ABTS	(Benitez et al., 2019)
Tartary buckwheat (*Fagopyrum tataricum*) varieties	Two times	Ultrasonicated, 1 h	.5 g in 80% MeOH (8 mL)	Alkaline	-	Residue, NaOH 0.4 M (25 mL) for 2 h.	-	Folin-Ciocalteu (TPC) Absorbance (TFC) HPLC-DAD AC: DPPH	(Bhinder et al., 2019)
Wheat *(Triticum aestivum* L.) seedlings	-	25 °C, 1 h	2 g, 80% MeOH (20 mL)	Alkaline	25 °C for 4 h, with N_2	40 mL of NaOH 2 M	Three times ethyl acetate	Folin-Ciocalteu (TPC) AC: DPPH, ABTS	(Chen et al., 2019)
Wheat seeds (*Triticum aestivum* L. 'Ceyhan-99′)	-	Boiled	2 g, aqueous MeOH (80%, v/v)	(Gruz et al., 2008) for the extraction, further fractioning, and determination of free, esterified, glycoside, and ester-bound forms.				Folin-Ciocalteu (TPC) UPLC-MS/MS AC: DPPH	(Colak et al., 2019)
Black lentils, pinto beans, ruviotto beans, black beans, black chickpeas, and desi chickpeas	Two times	Ultrasonic bath for 1 h at 40 °C	1.5 g, 7.5 mL of extraction solvent (EtOH and H_2O in the ratio of 70:30	Alkaline	1 h at room temperature	Residue, 7.5 mL of NaOH 2 N added with 0.01% EDTA 10mM and 0.1% ascorbic acid	Two times diethyl ether-ethyl acetate	HPLC-DAD	(Giusti et al., 2019)
Apple peel, apple pomace, pomegranate peel, pomegranate seed, black carrot pomace	-	Ultraturrax for 45 s at 10000 rpm	2 g, 15 mL/10 mL of 80% MeOH in H_2O	Alkaline	Sonicated H2O bath at 100 % amplitude at 60°C for 30 min	Residue, 2 mL of NaOH 2 N	Three times MeOH 100% containing HCOOH 0.1 %	Folin-Ciocalteu (TPC) Colorimetric (TFC) HPLC AC: DPPH and CUPRAC	(Gulsunoglu et al., 2019)
Dried persimmon (*Diospyros kaki*) fruit	Two times	-	5 g, 70% (v/v) acetone (20 mL)	Acid hydrolysis method, non-destructive method, thiolytic degradation of NEPAs, alkaline hydrolysis for the analysis of hydrolyzable bound phenolic.				Folin-Ciocalteu (TPC) Butanol-HCl (TPAC) LC-MS	(Hamauzu & Suwannachot, 2019)
Cranberries	Two times	Sonicated for 30 min at room temperature	100 g, 70 % (v/v) acetone aqueous (1% CH_3COOH)	Alkaline	37 °C for 2 h with N_2	Residue, NaOH 2 M	Three times diethyl ether-ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) Vanillin–H_2SO_4 (Tannin content) LC-MS	(Han et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: ORAC	
Sunflower and rapeseed meals	-	75 °C for 20 min	Meal-to-MeOH ratio of 1:100	Alkaline and enzymatic	30 °C for different times (15, 30, 60, and 120 min)	50 mL of the meal were mixed with 1.5 mL of MeOH / 6 mL of NaOH 2 M / Enzymatic hydrolysis (details in the original article)	Three times with ethyl acetate	UFLC-DAD	(Laguna et al., 2019)
Black rice (*Oryza sativa* L.)	Three times	Mechanical shaker (150 rpm, 1 h)	2 g, 15 mL of acetone:H_2O solution (70:30)	Alkaline and enzymatic	Shaker for 4 h / 15 min at 37 °C	Residue, enzymatic hydrolysis (α-amylase) followed by alkaline hydrolysis (NaOH 4 M).	Three times with ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) Spectrophotometric (TAC) LC-ESI-qToF-MS	(Lang et al., 2019)
Red sweet peppers	Two times	Shaker (200 rpm, 10 min). Ultrasonication was applied to the samples at room temperature for 30 min	500 mg 5 mL 80% MeOH containing HCl 0.01 N	Alkaline	Shaken in a reciprocating shaker (200 rpm, 1 h).	Residue, NaOH 2 M	Three times ethyl acetate	Folin-Ciocalteu (TPC) UPLC-PDA UHPLC-ESI-MS/MS AC: ORAC	(Lekala et al., 2019)
Hulless barley	Two times	Shaker (100 rpm/min)	IDF and SDF (2 g) were mixed with 40 mL of 70% (v/v) acetone / diethyl ether-ethyl acetate	Alkaline	Ambient temperature for 4 h	Esterified phenolic (details in the original article). Residue, 40 mL of NaOH 4 mol L^{-1}	Five times diethyl ether-ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric (TFC) HPLC-DAD AC: DPPH	(Q. Li et al., 2019)
Loquat Fruit	Ten times	Ultrasound-assisted, 40 °C for 30 min	80% EtOH (1:25, w/v)	Alkaline and acid	85 °C for 1 h / 40 °C for 4 h	2 mol L^{-1} HCl / 2 mol L^{-1} NaOH	Two times ethyl acetate:H_2O	Folin-Ciocalteu UHPLC-QqQ-MS/MS	(Wenfeng Li et al., 2019)
Fresh lotus seeds	-	-	Papain and α-amylase, followed by 50% EtOH (v/v)	Alkaline	Room temperature, 3.7 h	NaOH 2.8 M (solid-liquid ratio of 1–25)	-	HPLC	(Lin et al., 2019)
Fruit On-tree Ripening in Five Peach Cultivars	-	Mechanical shaking for 1 h, room temperature	MeOH (50:50) / 20 mL of acetone:H_2O (70:30	Acid	85 °C for 20 h	Residue, MeOH:H_2SO_4 (90:10, v/v)	-	Folin-Ciocalteu (TPC) AC: DPPH, FRAP	(H. Liu et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Carrot	-	The carrot was dried, ground, and sieved.	-	Alkaline	Shake for 4 h, with N_2 / 30 min	200 mg, NaOH (2 M) / 60 % EtOH	H_2O, EtOH (95%), and acetone (95%)	Folin-Ciocalteu (TPC) AC: DPPH, hydroxyl radical scavenging, and superoxide anion radical scavenging	(S. Liu et al., 2019)
Grape peel powder	Exhaustive extraction	60 min room temperature under stirring	1 g, 40 mL of MeOH:H_2O solution (50:50) / 40 mL of acetone:H_2O solution (70:30)	Acid	100 °C for 3 h / 85 °C for 20 h	Butanol:HCl (95:5, v/v) containing 0.7 g/L of $FeCl_3$ / MeOH:H_2SO_4 (90:10, v/v)	-	Spectrophotometric HPLC-PDA-MS/MS	(Maurer et al., 2019)
Pigmented rice (*Oryza sativa* L.) varieties	-	Magnetic stirring	MeOH (50:50 v/v) and acetone:H_2O (70:30 v/v)	Extracted by ethyl acetate from the pellet and that had undergone saponification with NaOH 2 M under ultrasonic irradiation at 40 °C for 90 min.				Folin-Ciocalteu (TPC) HPLC	(Melini et al., 2019)
Raw and roasted beans of two *Theobroma cacao* L. varieties	-	Orbital shaker at room temperature for 30 min	0.1 g, 150 g with a solution of NaCl 1 M	Alkaline and acid	Heating/Stirring Module 75 °C for 150 min with stirring at 150 g / 90 min under agitation at 30 °C.	20 mg, 5 mL of MeOH (50% v/v), 1 mL of HCl (10.2 M), and 150 µL of 1% (w/w) ascorbic acid / 20 mg, 2 mL of NaOH 2 M solution containing 2% (w/w) ascorbic acid and EDTA 20mM	-	UHPLC-DAD UHPLC-DAD-ESI-HR-MS^n	(Oracz et al., 2019)
Banana (*Musa Cavendish*) flour	Two times	15 min using the wrist shaker	2 g, 8 mL of MeOH:H_2O (50:50) / 8 mL of acetone:H_2O (70:30)	Acid	22 h at 85 °C, with magnetic stirring / 1 h at 100 °C with magnetic stirring	Residue, MeOH:H_2SO_4 (90:10) / Butanol:HCl (97.5:2.5) and 0.7 g of $FeCl_3$	Two times 1-butanol	Folin-Ciocalteu (TPC) Colorimetric determination (Anthocyanins) UPLC/MS AC: DPPH, ABTS	(Pico et al., 2019)
Kainth (*Pyrus pashia* Buch.-Ham. Ex D.Don) fruit pulp	Three times with separated with ethyl acetate (6 times)		100 g, 150 mL of 70% EtOH	Alkaline	2 h, with N2	Esterified phenolics (details in the original article) / Residue, NaOH 2 M	Six times ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) UPLC-ESI-HRMS/MS	(Prakash et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
								AC: DPPH, ABTS, total AC, FRAP	
Jaboticaba fruit peel	Exhaustive extraction	Stirred (2000 rpm, 30 s), and sonicated for 5 min	0.5 g, 7.5 mL of MeOH:H_2O:HCOOH solution (85:15:0.5)	Alkaline and acid	Room temperature (25 ± 2 °C) for 16 h under agitation / stirring 30 s, 2000 rpm, 30 min, 85 ± 3 °C	12 mL of H_2O and 5 mL of NaOH 10 M / 2.5 mL of concentrated HCl	Two times H_2O	HPLC-DAD HPLC-ESI-MS/MS	(Quatrin et al., 2019)
Peanuts (*Arachis hypogaea* L.), peeled hazelnuts (*Corylus avellana* L.), pistachios (*Pistacia vera* L.), almonds (*Prunus dulcis* Mill.), and natural walnuts (*Juglans regia* L.)	-	Ultra-turrax	1 g, 10 mL of 1% HCOOH in 70% MeOH	Alkaline	Room temperature for 1 hour	Residue, 3 mL of NaOH 2 M	Ethyl acetate.	UHPLC-ESI-QTOF	(Rocchetti et al., 2019)
Prosopis alba flours	Six times	Ultrasonic bath for 90 min at 25 °C	10 g, MeOH	Alkaline	Shaking at 30 °C for 30 min	20 mL of NaOH 2 M, ascorbic acid 1%, and EDTA 10mM	Six times ethyl acetate	Folin-Ciocalteu (TPC) HPLC-DAD HPLC-ESI-QTOF-MS/MS AC: ABTS, β-Carotene-linoleic acid, hydroxyl radical, hydrogen peroxide	(Rodriguez et al., 2019)
Common wheat (*Triticum aestivum*), genotypes (Campeiro, ORS25, ORS1401, ORS1402, Marfim, Jadeite and Ametista)	Two times	Homogenized (200 rpm, 10 min, 25 °C) in a shaker	70 mg, 80% EtOH	Alkaline and acid	Ultrasonic bath (42 kHz) for 90 min at 40 °C	3.5 mL of NaOH 4 M /	Three times ethyl acetate	Folin-Ciocalteu (TPC) UPLC-QTOF-MS^E	(Barros Santos et al., 2019)
Cagaitas (*Eugenia dysenterica*)	-	Room temperature, 1 h	0.5 g, 1 mL of MeOH:H_2O (50:50) / acetone:H_2O (70:30)	Acid	85 °C for 20 h / 100 °C for 60 min	200 mg, MeOH:H_2SO_4 90:10 (v/v) / 10 mL of butanol:HCl (97.5:2.5 v/v) and 0.7 g of $FeCl_3$	Butanol	Folin-Ciocalteu (TPC) MS LCQ Fleet AC: ABTS, DPPH, FRAP	(Silva et al., 2019)
Olive pomace	-	Shaken for 1 h	MeOH:H_2O solution (50:50)	Acid	85 °C, 20 h / 100 °C for 1 h	MeOH and H_2SO_4 / n-butanol:HCl (95:5,	Two times H_2O	Folin-Ciocalteu (TPC)	(Speroni et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
			/ acetone:H_2O solution (70:30)			v/v) containing $FeCl_3$		HPLC AC: ABTS, DPPH, ORAC	
Rubus idaeus L. leaves and seeds	Two times	Ultrasonic (320 W), 2 h at room temperature	2 g, 20 mL 70% MeOH containing 0.1 % HCl (v/v)	Alkaline, acid, enzymatic	Ultrasonic extraction	H_2O or MeOH	-	Folin-Ciocalteu HPLC-ESI-TOF/MS AC: ABTS, DPPH, FRAP	(Wang et al., 2019)
Finger millet varieties	-	-	80% MeOH	Alkaline	Room temperature	Residue, NaOH	Ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) Vanillin (TPAC) HPLC-DAD HPLC-DAD-Q-TOF-MS^2 AC: DPPH, ABTS, ORAC	(Xiang et al., 2019)
Flowers, leaves, and stems of *Lonicera japonica* and L. *macranthoides*	Two and three times	Ultrasonicated, 30 min at room temperature	1.0000 g, 15 mL of aqueous MeOH (70 %) / Ethyl acetate	Alkaline	4 h at room temperature	Esterified phenolic (details in the original article). Residue, 20 mL of NaOH 4 M	Three times ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) UPLC-DAD–QTOF-MS/MS AC: DPPH, ABTS, ORAC	(Yu et al., 2019)
Vegetables (44) commonly consumed in China	Two times	Ultrasonicated for 30 min at room temperature	2 g, 20 mL of 80% MeOH containing 0.2% vitamin C	Alkaline	40 °C, 2 h in a gas bath with shaking	Residue, 20 mL of 4 M aqueous NaOH	Two times ethyl acetate	HPLC-MS	(Zhang et al., 2019)
Australian sweet lupin (*Lupinus angustifolius*)	Two times	Stirred (10 s vortex and 2 h shaker)	80 % MeOH (v/v)	Alkaline	Stirred, 2 h, with N_2	Residue, NaOH (2 mol L^{-1})	Five times ethyl acetate	HPLC-DAD-ESI-MS/MS	(Zhong et al., 2019)
Oil palm (*Elaeis guineensis* Jacq.) fruits	Two times and five times diethyl ether-ethyl acetate	Ultra-high pressure processing (500 MPa, 10 min, temperature of H_2O 25 °C) / 30 min at room temperature	50.0 g ultrasonically extracted with 250 mL mixture solution of 70% aqueous acetone and 70% aqueous MeOH (1:1, v/v)	Alkaline	Room temperature for 4 h	Esterified phenolic (details in the original article). Residue, 4 mol/L NaOH (1:10, v/v)	Five times diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) Absorbance $Al(NO_3)_3$ (TFC) UHPLC-ESI-HRMS/MS AC: DPPH, ABTS, FRAP	(Zhou et al., 2019)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Three durum wheat cultivars named Duilio, Sant'Agata and Simeto: Grains, flour, semolina and bread	-	Magnetic stirring at room temperature for 1 h.	0.8 g, 20 mL of acidified aqueous MeOH (MeOH:H_2O), acetone:H_2O (70:30 v/v)	Alkaline	Magnetic stirring for 4 h	EtOH 95 % and NaOH 2 M	Ethyl acetate	Folin-Ciocalteu TEER measurements (Millicell ERS apparatus)	(Acquistucci et al., 2018)
Terminalia sericea Burch. ex DC.	-	Orbital shaker for 1 h	50 % EtOH for 24 h / Acidified with HCl (6 M; pH 2) and was partitioned with diethyl ether-ethyl acetate	Alkaline	1 h	Esterified and glycosided phenolic (details in the original article) / NaOH (4 M; 40 mL)	Diethyl ether.	Folin-Ciocalteu (TPC) Nuclear Magnetic Resonance Spectroscopy (NMR) AC: DPPH	(Anokwuru et al., 2018)
Araticum fruit (*Annona crassiflora* Mart.)	Three times	Ultrasonicated (30 min at room temperature)	1 g, 15 mL of a mixture of MeOH:acetone: H_2O (7:7:6)	Alkaline	4 h at room temperature using a H2O bath shaker (150 rpm).	Esterified and glycosided phenolic (details in the original article) / Residue, NaOH 4M containing 10mM EDTA and 1% ascorbic acid	Three times	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) Vanillin-HCl (TPAC) HPLC-ESI-MS/MS AC: DPPH, Trolox equivalent AC (TEAC), $ORAC_{FL}$	(Arruda et al., 2018)
Thirty flowers	Two and three times	Ultrasound for 10 min (23–25 °C)	1 g, 20 mL of MeOH:acetone: H_2O (7:7:6) / Ethyl acetate	Alkaline	4 h at room temperature under a stream of N_2	Esterified phenolic (details in the original article) / Residue, 20 mL of NaOH 4 M	Three times ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric (Flavonoid-aluminum complex) (TFC) ACQUITY UPLC H-Class AC: FRAP, DPPH, TEAC	(Chen et al., 2018)
Maize, sorghum (red and white), pearl millet, and finger millet (red and white)	-	Ultraturrax (10,000 rpm) for 45 s	2 g, 15 mL of MeOH / 80% MeOH	Alkaline	15 min at 60 °C, N_2	0.1 g of residue was hydrolyzed using 2 mL of NaOH 2 M	Two times 4 mL of MeOH (containing 0.1% HCOOH)	Folin-Ciocalteu (TPC) Vanillin (PA)	(Gabaza et al., 2018)
Ligusticum chuanxiong	-	10 min using high-speed homogenizer	2 mL, 80% cold acetone (1:5, v/v)	Alkaline	1 h at room temperature	Solution, NaOH 2 M	-	Folin-Ciocalteu (TPC) AC: DPPH	(Ge et al., 2018)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
					with shaking and N_2				
Yellow corn (*Zea mays* L.)	Two times	Room temperature for 2 h with continuous shaking in a H_2O bath	5 g 100 mL of chilled acidified MeOH (95% MeOH and 1 M HCl (85:15)	Alkaline	Room temperature for 1 h, with N_2	Residue, 200 mL of NaOH 2 M	Five times ethyl acetate	Folin-Ciocalteu (TPC) $AlCl_3$ (TFC) HPLC	(Gong et al., 2018)
Pseudocereals-enriched water biscuits	0.5 g of samples underwent the first phase of solvent extraction with MeOH:acetone:H_2O (7:7:6); supernatant and sediment were hydrolyzed with NaOH 4 M. Total polyphenols: 0.5 g of ground H_2O biscuits, gastric and intestinal sediments were weighted and extracted twice with 5 mL of MeOH acidified with an aqueous solution of HCl 1% (80:20, MeOH:HCl) under dark and refrigeration.							Folin-Ciocalteu (Total polyphenols) HPLC (Phenolic acids) AC: FRAP	(Hidalgo et al., 2018)
Wheat bran and white flour	Two times / three times	1 h at room temperature	80% MeOH / 4 mL of acidified H_2O and partitioned with ethyl ether	Alkaline and acid	Method 1 started with alkaline hydrolysis (1 g residue in 40 mL NaOH 2 M, 4 h, at room temperature) and was subsequently followed by acid hydrolysis of sample residue (HCl 6 M, 1 h at 95 °C), resulting in two fractions. In method 2, hydrolysis by acid was done first and subsequently followed by alkaline hydrolysis, resulting in two fractions.		Three times ethyl ester	Folin-Ciocalteu (TPC) AC: DPPH, ABTS	(Hu et al., 2018)
Rice (*Oryza sativa* L.)	-	Sonicated, different extraction times and temperatures	200 mg, 5 mL of different EtOH:H_2O mixtures	Alkaline	Sonication at different times and temperatures	Residue, 10 mL NaOH different concentrations	Three times ethyl acetate	Folin-Ciocalteu (TPC) HPLC AC: ABTS, FRAP	(Irakli et al., 2018)
Pistachio green hull (*Ohadi* variety)	Two times	30 min on a magnetic stirrer (300 rpm) at 25 °C	1 g, 20 mL of 80% (v/v) aqueous MeOH acidified	Crude pistachio green hull extract powder was dissolved in 80% EtOH and fractionated through Sephadex LH-20 (50 cm× 3 cm). 50% acetone was then used to elute the bound material (tannin fraction).				Folin-Ciocalteu (TPC) HPLC-DAD AC: DPPH, ABTS	(Lalegani et al., 2018)
Fermented quinoa (*Chenopodium quinoa* Willd.) seeds	-	-	25 mL 80% MeOH	Acid	Boiling in H_2O under N_2 for 1 h	Residue, 15 mL of HCl 2 M	Ethyl acetate	Folin-Ciocalteu (TPC) AC: DPPH	(Li et al., 2018)
Peach (fruit and peel)	-	1 h with stirring	MeOH:H_2O solvent (50:50, pH 2)	Acid	85 °C for 20 h	Residue, MeOH:H_2SO_4 (90:10, v/v)	-	Folin-Ciocalteu (TPC) AC: DPPH, FRAP, TEAC	(Liu et al., 2018)
27 barley genotypes (*Hordeum vulgare* L.)	Three times	Sonicated for 30 s	150 mg, 79.5% MeOH, 19.5% H_2O, and 1% HCOOH. 1 mL	Alkaline	12 h at room temperature	6 mL of NaOH 2 mol L^{-1}	-	UPLC-MS/MS	(Martínez et al., 2018)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Raw and traditional chocolate	Three times	H_2O bath shaker at 30 °C for 20 min.	70% acetone (solid:solvent, 1:40, w/v)	Alkaline	40 °C for 4 h	Residue, NaOH 4 M (1:1, v/v)	Five times diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) UPLC-DAD AC: ABTS, DPPH, ORAC, FRAP	(Mudenuti et al., 2018)
Black tea (*Camellia sinensis*)	Washed with H_2O	Boiled in H_2O (1 g/100 mL) for 5 min	-	Extraction with non-ionic compounds by maceration. Neutralized with 20 % Na_2CO_3 (w/v) and incubated at 41 ° C for 30 min.				Folin-Ciocalteu HPLC DAD AC: TEAC, radical scavenging capacity, and linoleic acid peroxidation inhibition capacity	(Mukhtar et al., 2018)
Quinoa seeds (*Chenopodium quinoa*)	Three times	Stirred for 10 min and sonicated in an ultrasonic bath for 5 min	0.1 g HCl 0.2 M (3 mL), extracted into 6 mL of ethyl acetate	Alkaline and acid	Stirred at room temperature for 4 h with N_2 / 95 °C for 30 min with intermittent mixing	Residue, NaOH 1 M (3 mL) / HCl 2 M (3 mL)	Two times ethyl acetate	UPLC-PDA-ESI-MS	(Multari et al., 2018)
Hungarian Sour Cherry	Then two different solvents were used to extract antioxidant compounds			Acid Hydrolysis (HT and condensed tannins). Alkaline hydrolysis. Enzymatic hydrolysis (Protease; Pectinase; α-amilase).				Folin-Ciocalteu (TPC) Spectrophotometric (Total procyanidin content) UHPLC UHPLC-MS AC: FRAP, DPPH, TEAC, photochemiluminescence	(Nemes et al., 2018)
Whole grain and bran of white, red, and black rice	Two times / Three times	Mechanical shaker for 30 min at room temperature	1 g, 80% MeOH (40 mL) / Ethyl acetate / 15 mL MeOH:HCl 1 M (85:15, v/v)	Alkaline	Room temperature on a shaker for 2 h	Residue, NaOH 4 M (20 mL)	Three times ethyl acetate	Folin-Ciocalteu (TPC) pH-differential (TAC) HPLC-UV AC: DPPH, ABTS	(Pang et al., 2018)
Calabura (*Muntingia calabura* L.) fruit	Extraction and purification of anthocyanins: MeOH 0.20% HCl (1,15, w/v) and left in an ultrasonic bath for 10 min at 20 °C. This procedure was repeated three times and the supernatants were combined. Aqueous extract was washed five times with diethyl ether:ethyl acetate.							Folin-Ciocalteu (TPC) Spectrophotometric (TAC)	(Pereira et al., 2018)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
	Extraction of non-colored soluble and insoluble-bound PC: Soluble and insoluble-bound PC from the calabura fruit were extracted and fractionated according to the method described by B. Wang et al. (2016), with some modifications (Arruda et al., 2018).							Vanillin (TPAC) UHPLC-MS/MS AC: DPPH, TEAC, ORAC	
Peels: Apple, banana, kiwi, mandarin, mango, melon, nectarine, orange, pear, watermelon	Successive extraction	Stirred for 1 h	0,5 g, MeOH:H_2O:HCl (50:50. v/v; pH 2) / acetone:H_2O (70:30, v/v)	Acid	85 °C for 20 h / 100 ° C for 1 h	Residue, MeOH:H_2SO_4 (pH 5.5) / butanol:HCl:$FeCl_3$	-	Folin-Ciocalteu (TPC) Spectrophotometric HPLC-DAD	(Pérez-Jiménez & Saura-Calixto, 2018)
Pomegranate juice pomace and grape wine pomace	Successive extraction	-	0,5 g, MeOH:H_2O (50:50) and then with acetone:H_2O (70:30, v/v)	Acid	85 °C for 20 h / 100 ° C for 1 h / 90 °C for 24 h	Residue, MeOH:H_2SO_4 (10:1, pH 5.5) / butanol:HCl (97.5: 2.5, v/v) with 0.1 % $FeCl_3$ / H_2O and HCl	-	Folin-Ciocalteu (TPC) Spectrophotometric HPLC−ESI−QTOF MALDI-TOF-MS AC: ABTS, FRAP	(Pérez-Ramírez et al., 2018)
Defatted camelina (*Camelina sativa*) and sophia (*Descurainia sophia*) seeds	Three times and five times	Ultrasonic bath and sonicated (high speed, 20 min, 30 °C).	5 g, 200 mL 70% (v/v) acetone / diethyl ether:ethyl acetate	Alkaline and acid	30 min at 60 °C / Microwave (600 W), 100 °C, 10 min	Residue, 30 mL of 2 M NaOH / 1 % HCl in MeOH	Two times ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric ($AlCl_3$) (TFC) Vanillin-HCl (PA content) HPLC-DAD-MS/MS AC: TEAC, reducing power, FIC	(Rahman et al., 2018)
Teff (*Eragrostis tef*)	Three times	2 h with shaking at 200 cycles/min at room temperature / 30 min	80 % MeOH (1:5 w/v)	Alkaline	Room temperature for 1 h	Residue, 3 mL of NaOH 2 M	Ethyl acetate	Waters-ACQUITY-UPLC-TQD-MS/MS	(Ravisankar et al., 2018)
Pigmented maize	-	Ultra-turrax (20,000 rpm, 5 min)	1 g using a hydro-alcoholic solution consisting of 80% MeOH (v/v) acidified with 1% HCOOH	Alkaline and acid	Shaken in a H_2O bath at 85 °C for 10 h / Ultrasonic H_2O bath (60 °C, for 45 min).	Residue 20 mL MeOH and 2 mL H_2SO_4 (18 M) / 2 mL NaOH (4 M)	-	UHPLC-ESI/QTOF-MS AC: FRAP, ORAC	(Rocchetti et al., 2018)

Sources	EPC extraction			NEPC extraction				Analytical methods	References
	Type of extraction	T (°C) and time	Mass, solvent type and concentration	Type of hydrolysis	T (°C) and time	Solvent type and concentration	Type of extraction		
Fruits of a Chinese Wild *Passiflora foetida*	Two times	1 h in an ultrasonic H_2O bath at room temperature	4 g, 20 mL MeOH:H_2O (50:50 v/v)	Alkaline	4 h at room temperature, with N_2	Esterified and conjugated (details in the original article) / Residue, 15 mL NaOH 4 M	Two times diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) Colorimetric modified (TFC) UPLC-Q-TOF-MS AC: ABTS, DPPH	(Song et al., 2018)
Colored barley (*Hordeum vulgare* L.)	-	Ultrasonic bath for 30 min, with N_2	0.5 g, 8 mL of MeOH acidified with HCl 1 N (85:15; v/v)	Alkaline	Overnight (18 h) at room temperature	Residue, 10 mL and 5 mL of NaOH 3 M	Three times diethyl ether:ethyl acetate	Folin-Ciocalteu (TPC) Vanillin (TPAC) UHPLC AC: DPPH, ABTS	(Suriano et al., 2018)
Colored oats (*Avena sativa*)	-	10 min by sonication at room temperature	300 mg, 3 mL of MeOH:H_2O (80:20, v/v)	Alkaline	Room temperature for 4 h by shaking with N_2.	200 mL of NaOH 2 mol L^{-1}	Five times diethyl ether:ethyl acetate	HPLC-DAD HPLC-ESI-IT-MS AC: DPPH	(Varga et al., 2018)
Buckwheat flour in white wheat bread	Two times	Ultrasonic bath	2 g, EtOH:H_2O (4:1)	Acid	20 h at 85 ° C	H_2SO_4 and H_2O (1:10)	H_2O	HPLC-ESI-MS AC: DPPH, ABTS	(Verardo et al., 2018)

AC = Antioxidant Capacity; EPC = Extractable phenolic compounds; HPP = Hydrolyzable polyphenols; HT = Hydrolyzable Tannins; NEPA = Non-extractable proanthocyanidins; NEPC = Non-extractable phenolic compounds; NEPP = Non-extractable polyphenols; TAC = Total anthocyanin content; TFC = Total flavonoid content; TPAC = Total proanthocyanidin content; TPC = Total phenolic content; PC = Phenolics compounds; PA = Proanthocyanidins.

3.1.4.2.1 Acid hydrolysis

The acid hydrolysis is performed using inorganic substances, such as hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) (Arranz & Saura Calixto, 2010). During this process, hydrolysis of the glycosidic bonds of cellulose and hemicellulose, in addition to the solubilization of sugars, tend to occur. Usually, after acid hydrolysis, the pH is adjusted with the addition of NaOH (**Table 2**). Acid hydrolysis may be occasionally more effective in releasing NEPC from matrix than other techniques. According to Arranz & Saura Calixto (2010), cereal products showed higher values of the non-extractable fraction in acid hydrolysates than alkaline hydrolysates; the extracts were composed of several PC as hydroxybenzoic, caffeic, cinnamic, and protocatechuic acids. Balli et al. (2020) compared acid and basic hydrolysis and found that acid hydrolysis was able to extract the largest amount of TPC from millet (up to 178 mg 100 g^{-1}), while basic hydrolysis underestimated the phenolic concentration. Likewise, Oliveira-Alves et al. (2020) reported that the acidic hydrolysis promoted significantly higher extraction of phenolic acids from ground Baru nuts; the hydrolyzed methanolic extract had higher total phenolic content and antioxidant activity than the crude methanolic extract. However, the authors observed a higher number of PC in the chromatographic profile in the crude methanolic extract.

Table 2 shows that only 30 % of the studies used acid hydrolysis to release NEPC. Among these studies, the technique was applied alone, in parallel, or sequentially with other techniques, such as basic hydrolysis, enzymatic hydrolysis, and butanolysis process. It is also possible to observe different methods for each fraction of these compounds, with the use of acid. Pérez-Ramírez et al. (2018) used methanol and concentrated sulfuric acid at 85 °C for 20 h for hydrolysable PC, butanol:HCl with 0.1% $FeCl_3$ at 100 °C for 1 h for nonextractable proanthocyanidins, and H_2O:HCl at 90 °C for 24 h for nonextractable ellagitannins. The process parameters influence the qualitative and quantitative yield of the extraction. Factors, such as the type of food matrix and the structure of phenolics, must be considered based on the bonds that are broken during this technique. High acid concentrations, high temperatures, and long treatment times can cause degradation and must be highly controlled for an efficient treatment.

3.1.4.2.2 Alkaline hydrolysis

In alkaline hydrolysis, the ester bound between the phenolic compound and the plant tissue breaks, causing the release of the polysaccharide chain compound. The most used

bases for alkaline treatment are sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), and calcium hydroxide ($Ca(OH)_2$) (Wu et al., 2018). Similar to acid hydrolysis, the use of bases can promote PC degradation. Tang et al. (2016) evaluated NEPC release from quinoa and observed that ferulic acid release occurred more readily through alkaline hydrolysis when compared to the use of the enzyme pectinase; however, loss of ferulic acid and other PC tend to occur when alkaline hydrolysis is performed.

Most of the studies (about 76 %) listed in **Table 2** used alkaline hydrolysis. Like acid hydrolysis, it was performed alone or in conjunction with other techniques. The reaction is commonly carried out in the dark under an atmosphere of inert gas (common N_2), and ascorbic or ethylenedinitrilotetraacetic acid (EDTA) is generally used to prevent the degradation of compounds, as performed by Tang et al. (2021). In addition, after hydrolysis, acids are added to decrease the pH because it is known that low pH promotes PC stabilization.

Free and bound phenolics were extracted from solids of the tortilla industry wastewater, known as nejayote (Acosta-Estrada et al., 2015). Alkaline hydrolysis (NaOH, 4 M) was performed to release the NEPC. The results showed a greater concentration of coumaric acid (7 times higher) and ferulic acid (24 times higher) in the NEPC extract when compared to the concentration found in EPC extract. Zhang et al. (2021) optimized the extraction of NEPC from jackfruit pulp by alkaline, acid, and enzymatic hydrolysis. Alkaline hydrolysis showed a greater diversity of extracted compounds and greater antioxidant activity (ABTS and ORAC).

Irakli et al. (2018) evaluated EPC and NEPC extractions from rice by-products. For EPC, the effects of ethanol concentration, extraction time, and temperature (ultrasonic bath) were evaluated; for NEPC, the influence of NaOH concentration, hydrolysis time, and temperature (under sonication) was investigated. The optimal conditions for the EPC extraction were 41–56 % of ethanol concentration, 40 °C, and 10 min of ultrasound application; for NEPC extraction, the best conditions were NaOH 2.5–3.6 M, 80 °C, 120 min of sonication. The NaOH concentration (0.1 and 4 M) was also evaluated by Balli et al. (2020) during NEPC extraction from millet; results showed that the use of the highest NaOH concentration induced a partial PC degradation, with complete disappearance of methyl feril that was entirely converted to ferulic acid.

3.1.4.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis is another specific and effective way to release NEPC. In this process, enzymes, such as cellulases, hemicellulases, pectinases, amylases, and glucanases, are used, isolated or mixed (Wu et al., 2018). Cellulases, for example, are enzymes responsible for the hydrolysis of cellulose, the main compound present in plant cells. Pectinases are enzymes that break down pectin, a polysaccharide found in the cell wall of plants. Thus, each enzyme acts as a catalyst in the hydrolysis of specific bonds.

About 5 % of the studies compiled in **Table 2** evaluated enzymatic hydrolysis for NEPC release, so this technique can be considered an alternative, while chemical hydrolysis is the classic procedure. For different quinoa cultivars, ferulic acid was the most easily released compound by alkaline hydrolysis and pectinase, obtaining yields comparable to acid hydrolysis. The researchers concluded that enzymatic (pectinase) and alkaline hydrolysis acted similarly when breaking glycosidic bonds that link phenolic acids to galacturonic acid residues or cell wall polysaccharides; however, unlike the enzymatic hydrolysis, the alkaline condition can cause loss of ferulic acid and other PC (Tang et al., 2016).

Domínguez-Rodríguez et al. (2022) evaluated the enzymatic hydrolysis with different enzymatic preparations, in comparison and together with the alkaline hydrolysis, in the peels of four different passion fruit. Despite the results being dependent on the type of passion fruit and the enzyme preparation evaluated, it was possible to observe the release of a greater number of NEPC with the application of enzymatic hydrolysis.

Thus, the effectiveness of enzymatic hydrolysis depends on factors such as the type of NEPC, type of enzyme, or mixture of enzymes, treatment time, sample particle size, and type of extraction solvent later used (Meyer et al., 1998).

3.1.4.2.4 Use of emerging technologies

Some physical methods have been studied to increase extraction process efficiency, to reduce time or temperature, and to decrease costs in the long run, as alternative NEPC extraction techniques. Among these techniques, ultrasound (Barros Santos et al., 2019; Irakli, Chatzopoulou, et al., 2018; Oliveira-Alves et al., 2020; Pinaffi et al., 2020), supercritical fluids (Mushtaq et al., 2017; Rivas, Casquete, Córdoba, Benito, et al., 2021), pulsed electric field (Lohani & Muthukumarappan, 2016), and microwave (Araujo et al., 2021; F. Chen et al., 2016; Patrignani et al., 2021; Ravisankar et al., 2018) have been already evaluated.

High-intensity ultrasound is widely used as a simple, efficient, and reproducible alternative method to improve the extraction of compounds of interest from the food matrix. The effects of ultrasound in liquid systems are related especially to the phenomenon of cavitation (Soria & Villamiel, 2010), defined as the process of production, growth, and collapse of bubbles. Several studies used ultrasound to extract NEPC (**Table 2**). Barros Santos et al. (2019) evaluated the phenolic profile of seven different wheat genotypes using alkaline hydrolysis assisted by ultrasound. Oliveira-Alves et al. (2020) investigated ultrasound-assisted acid hydrolysis to extract EPC and NEPC in-ground Baru nuts; gallic acid, gallic acid ester derivatives, and gallotannins were the predominant PC found in the extracts.

The mild intensity pulsed electric field was studied to release bound phenolics from sorghum flour and apple pomace. At optimized conditions, the TPC and antioxidant capacity of apple pomace were 37.4 % and 86 % higher than the control, respectively. The study suggested that this treatment could be useful for preparing processed foods with increased levels of phenolic antioxidants (Lohani & Muthukumarappan, 2016). The microwave-assisted method for distillation and double extraction was applied to obtain essential oil, oligomeric proanthocyanidins, polymeric proanthocyanidins, and polysaccharides from Cinnamomi Cortex; the developed technique produced higher yields of the four constituents in a shorter time (F. Chen et al., 2016).

Studies involving microwave-assisted extraction of bioactive compounds are increasing, especially in recent years. Araujo et al. (2021) evaluated the technology applied on avocado peels, testing different extraction solvents. They observed that microwave-assisted extraction was able to release bound PC from the residues. Furthermore, HPLC-MS analysis showed the presence of several phenolic acids, numerous A and B dimer procyanidins in different forms of isomers, catechin, epicatechin and perseitol. Combination of technologies has been evaluated as an alternative to increase yield and quality of the extracted compounds (Peng et al., 2021; Zhu et al., 2016). Peng et al. (2021) studied microwave extraction, enzymatic hydrolysis, and combination of both methods to extract NEPC from grapefruit peel. The combined use of the techniques allowed a greater extraction yield (148.87 GAE mg/100 g). Moreover, the extract showed higher antioxidant capacity, directly associated with the NEPC content.

3.1.5 Methods for quantification and identification

Spectrophotometry and chromatography procedures with different detection methods can identify and quantify PC linked to the matrix. Spectrophotometric methods contemplate the main techniques for NEPC analysis due to their simplicity and low cost. However, these methods lack selectivity, as the reagent reacts with all reducing substances, leading to an overestimation of the PC content (Wu et al., 2018). Moreover, spectrophotometric methods are simpler and cannot quantify and classify these compounds properly. Therefore, they are more suitable for indicating the reducing capacity than measuring the PC content.

The spectrophotometric method more commonly used for TPC quantification is the Folin-Ciocalteu method. This method does not allow the quantification of individual PC. As shown in **Table 2**, almost 10 % of the studies performed used only the Folin-Ciocalteu method for TPC quantification. This method has a major limitation since the reagent Folin-Ciocalteu can also react with other oxidizable compounds present in the matrix. Compounds, such as ascorbic acid, bisulfite, and ferrous ions, can interfere and generate overestimated results (Singleton & Rossi, 1965). Pérez-Jiménez & Saura-Calixto (2015) evaluated NEPC content and profile for the 24 most consumed fruits and vegetables in four European countries (France, Germany, The Netherlands, and Spain). Among the experiments carried out, they quantified hydrolysable polyphenols (HPP) in bananas, using the Folin-Ciocalteu method (1,415.1 ± 206.5) and HPLC (98.34 ± 15.56) (mg/100 g dw). As expected, the Folin-Ciocalteu method detected higher contents than the HPLC methodology. A significant correlation was observed of HPP content between both methods ($p < 0.01$) despite the differences. These results are in agreement with findings reported by other studies. Escarpa & González (2001) demonstrated that PC concentrations were up to five times higher than those found by HPLC in fruit and vegetable samples.

The Folin-Ciocalteu method is frequently used as a standard procedure for comparing distinct methodologies. Almost 60 % of works listed in **Table 2** used this methodology for this purpose. In addition, standards for all compounds present in a sample are hardly available for quantification by HPLC. Thus, it is common to use techniques in parallel: Folin-Ciocalteu to determine TPC and HPLC to quantify PC individually. Yao et al. (2021) quantified free, soluble-bound, and insoluble-bound phenolics and evaluated their bioactivity in raspberry pomace using Folin-Ciocalteu (TPC content) and liquid chromatography coupled with high-resolution mass spectrometry (HPLC-MS, quantitation

of 18 phenolics). Regarding the insoluble-bound PC fraction, 6.39 mg GAE/g (dw) and 1323.96 μg/g (dw) were found for Folin-Ciocalteu and HPLC-MS, respectively.

HPLC is the predominant method used for free or bound PC separation and quantification. Around 66 % of the studies listed in **Table 2** used HPLC, alone or coupled to MS. Reverse phase chromatographic columns are widely used, although limited to high molecular weight PC. Therefore, NEPC hydrolysis is essential before injection into the liquid chromatography. The diode array detector (DAD) is highly employed, as PC have a characteristic spectrum. The different subclasses can be identified through the wavelengths and retention times. A disadvantage of this technique is the impossibility of distinguishing monomers, oligomers, polymers, or derivatives of the same class, as they all exhibit the same absorption bands (Wu et al., 2018). Thus, more robust techniques are necessary for NEPC analysis, such as HPLC-MS.

MS is an analytical technique that generates multiple ions from the sample under investigation, separating them according to their specific mass-to-charge ratio (m/z), providing structural information. For this, compounds are ionized through an ionization source. The generated ions pass through a mass analyzer where they are separated according to their mass/charge ratio (m/z) and go to a detector (El-Aneed et al., 2009; Hoffmann, 2005). There are different ionization techniques, and each one has its advantages and ideal applications. Among the ionization methods, electrospray ionization (ESI) sources are extremely versatile and, therefore, are suitable for a wide range of compounds with high to moderate polarity, and variable molecular weights (Awad et al., 2015). Thus, they are usually applied for EPC and NEPC analysis. Among the studies evaluated, almost 62 % used MS to tentatively identify NEPC.

3.1.6 Strategies to overcome analytical drawbacks

Table 2 shows the main drawbacks of the methodologies used to extract, identify, and quantify PC in plant materials. These pitfalls were pointed out and discussed through the article, and strategies to overcome them need to be established. The first point to be highlighted is the necessity of conducting exhaustive EPC extraction to avoid the superestimation of the NEPC fraction. In this step, spectrophotometric assays, such as the Folin-Ciocalteu, are interesting to guarantee their complete extraction.

Regarding NEPC extraction, hydrolysis (acidic, basic, or enzymatic) and/or emerging technologies can be applied. The methodology choice should take into account the

structure of the matrix and the interactions between NEPC and the food matrix. For each method, it is possible to observe advantages and disadvantages. Acid and basic hydrolysis must be optimized, exploring experimental parameters, to reduce degradation, to diminish concentrations of acid or base, and to reduce time and/or temperature of hydrolysis. This optimization needs to be conducted ensuring the efficient NEPC release from the matrix.

The use of enzymes and emerging technologies, alternative extraction methodologies, have interesting advantages to be explored, such as less waste generation and the possibility of using milder temperatures and lower times. These alternatives need to be better investigated to understand their effects on PC structure. Moreover, research is required to reduce the development costs, making them more affordable. The possibility of reusing enzymes and the development of more accessible emerging technologies, such as ultrasound, are interesting approaches.

The techniques used to quantify CFNE should be reassessed. There is a huge difference between the real PC concentration and the values assayed through spectrophotometric methods. This technology has been used as a standard, but this practice should be re-evaluated. Liquid chromatography, widely applied and indicated for quantification, still presents possibilities for improvement, especially regarding the availability and use of standards. Purified compounds, obtained through widely characterized matrices, may be alternatives to analytical standards, facilitating the identification and quantification process.

Identification through mass spectrometry is another point that can show great improvements in the long term. Thousands of data generated are hardly explored as they could be. Thus, bioinformatics can be an ally for CFNE analysis and characterization. Manual screening of the generated data combined with bioinformatics can be explored and used in the food area.

3.1.7. Concluding remarks

NEPC are an important fraction of PC that are frequently ignored in studies conducted to evaluate this class of compounds in the food matrices For their correct identification and quantification, it is necessary to perform the exhaustive extraction of free PC with organic solvents. Without this initial step, NEPC fraction may be overestimated, which causes erroneous results and hinders a complete quantitative and qualitative understanding of this class of compounds.

Another point that needs to be highlighted is that an acceptable method for NEPC extraction is unavailable. Currently, the most applied method for NEPC extraction is chemical hydrolysis, which can be acidic or basic. This technique can generate large amounts of waste and degradation, altering their original structure. These structural changes during extraction hampers their correct characterization since the PC obtained will be, in majority, derived from phenolic acids and other degradation products. Furthermore, lack of method standardization can lead to different results. Methods must be standardized according to the objective of the study and the composition of the food matrix. Operational conditions, such as acid or base concentration, temperature and time, need to be optimized to reduce losses during hydrolysis and extraction.

The use of the Folin-Ciocalteu assay as a methodology for TPC determination generates overestimations due to the reagent ability to act with other reducing compounds present in the food matrix. Thus, quantification methods must be able to consider the classes of compounds analyzed and also individual compounds. HPLC-MS is a good alternative to overcome these drawbacks because it allows identification and quantification of individual compounds with reduced errors. With this technique, thousands of data are generated, opening doors to another great opportunity: systematization of data analysis to better understand the nature of these compounds in food plant materials.

Conflicts of interest

The authors declare that there is no conflict of interest.

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4 MATERIAIS E MÉTODOS

Neste capítulo serão apresentadas as condições experimentais utilizadas no desenvolvimento do trabalho. A parte experimental foi desenvolvida no Laboratório de Antioxidantes Naturais e no Laboratório de Processamento e Química de Alimentos, ambos situados no Instituto de Ciência e Tecnologia de Alimentos (ICTA), na Universidade Federal do Rio Grande do Sul (UFRGS).

4.1 Amostras

As amostras de casca e semente de uva orgânica em pó foram doadas pela empresa Econatura Produtos Ecológicos e Naturais Ltda - Uva'Só Produtos Orgânicos. A Figura 1 apresentam casca e semente de uva em pó, respectivamente. As cascas e sementes são de uvas orgânicas da variedade “bordô”, proveniente da produção de suco de uva da empresa. As uvas foram produzidas em sete municípios da Serra Gaúcha, Rio Grande do Sul. Após a prensagem das uvas para a extração do suco, o bagaço resultante é peneirado para separação das cascas e sementes. As cascas e sementes tiveram a sua umidade reduzida utilizando um secador com circulação de ar quente. Posteriormente, as cascas foram moídas e embaladas. Para as sementes, o óleo foi extraído a frio antes da moagem e embalagem.

Figura 1. Casca e semente de uva em pó.

Fonte: autora.

As amostras foram embaladas na empresa e transportadas até o Instituto de Ciência e Tecnologia de Alimentos (ICTA). No laboratório, as amostras foram fracionadas (porções de 20 g), embaladas à vácuo em embalagens plásticas e armazenadas na geladeira (5 ± 1 °C) até o momento das análises.

4.2 Reagentes e Padrões Analíticos

Os seguintes reagentes e solventes foram utilizados: água ultrapura, acetonitrila grau HPLC (J.T. Baker), metanol grau HPLC (J.T. Baker), metanol P.A. (Dinâmica), ácido clorídrico P.A. (Química Moderna), ácido fórmico P.A. (Neon), carbonato de sódio (Neon) e reagente *Folin-Ciocalteu*. A água ultrapura (Milli-Q) foi purificada por um sistema Millipore. As fases móveis foram filtradas em sistema de filtração com filtros de acetato de celulose e PTFE (Politetrafluoretileno) para fase aquosa e orgânica, respectivamente. Os extratos produzidos foram filtrados em filtros PTFE (hidrofílico) de 0,22 µm.

Para a quantificação, foram obtidas curvas analíticas de 13 padrões de compostos fenólicos (pureza ≥90%, Sigma-Aldrich), nomeadamente: ácido cafeico, ácido 5-cafeioilquínico, ácido elágico, ácido ferúlico, ácido gálico, ácido *p*-cumárico, ácido vanílico, apigenina, catequina, cianidina 3-O-glicosídeo, epicatequina, naringenina e quercetina.

4.3 Métodos de Extração

4.3.1 Extração Exaustiva dos CFE

Os CFE foram extraídos a partir 0,5 g de amostra (casca de uva ou semente de uva em pó) e 10 mL de uma mistura metanol (MeOH) e água (H_2O) (MeOH:H_2O 8:2, v/v) acidificada com 0,35 % de ácido fórmico. Essa mistura foi agitada em vortex (Phoenix Luferco, 3800 rpm) por 3 minutos. O extrato obtido foi centrifugado (Centrífuga HITACHI) a 25.000 *g* por 5 minutos à 4 °C, e o sobrenadante foi retirado. Esse procedimento foi repetido até a extração exaustiva dos compostos fenólicos, e o resíduo sólido (*pellet*) foi armazenado para a extração dos CFNE.

A extração exaustiva foi avaliada pela reação dos sobrenadantes obtidos com o reagente *Folin-Ciolcateu* (SINGLETON e ROSSI, 1965), sendo a não geração de produtos de coloração azul o indicativo de extração exaustiva dos compostos fenólicos da amostra. Um total de nove repetições foram necessárias para retirar a fração extraível da semente de uva em pó e oito repetições para casca de uva em pó. Os extratos obtidos, para cada matriz,

foram combinados, filtrados (PTFE hidrofílico, 0,22 µm) e analisados por HPLC-DAD-ESI-MS/MS. Os extratos foram obtidos, no mínimo, em triplicata.

4.3.2 Obtenção dos CFNE

Os CFNE foram obtidos a partir do resíduo sólido (*pellet*) gerado na extração dos CFE. O método de hidrólise ácida descrito por MALLMANN et al. (2020) foi utilizado como referência para a obtenção do CNFE. Esse método é uma adaptação da proposta por PÉREZ-RAMÍREZ et al. (2018) e foi utilizado como referência para a definição das condições de concentração de HCl, temperatura e tempo de extração a serem estudadas. Destaca-se que, para garantir a temperatura constante durante todo o processo, neste trabalho foram utilizados banhos termostáticos conectados a um sistema *by-pass*, descrito na seção 4.3.2.1.

Resumidamente, as condições para a obtenção dos CFNE foram avaliadas em três etapas: (1°) efeito da concentração de HCl, (2°) efeito da temperatura e (3°) efeito do tempo de extração. Na Figura 2, está representado o diagrama destes experimentos, que serão detalhados nas próximas seções.

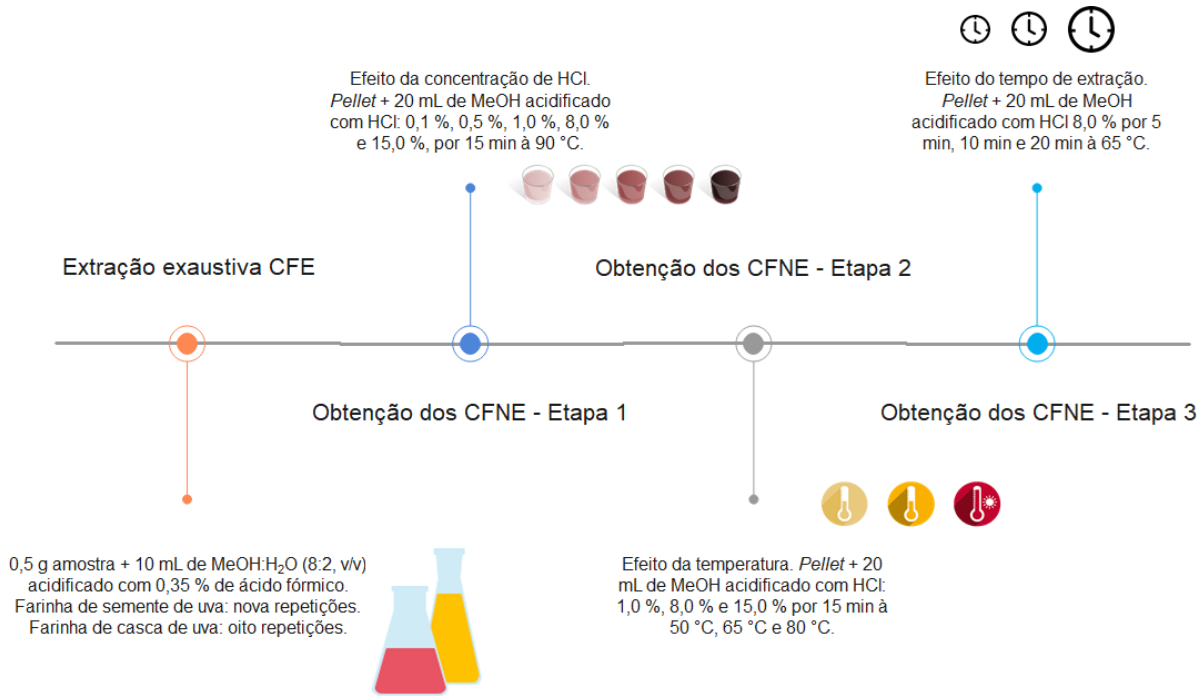

Figura 2. Diagrama das etapas experimentais realizadas no presente trabalho. Fonte: autora.

4.3.2.1 Etapa 1 – Efeito da Concentração de Ácido

Na primeira etapa, o *pellet* resultante da extração do CFE foi submetido à hidrólise utilizando 20 mL de metanol acidificado com quatro diferentes concentrações de HCl (0,1, 0,5, 8,0 e 15,0 %, v/v) por 15 min a 90 °C. Para a realização do processo, um sistema *by-pass* foi utilizado. Esse sistema pode ser visualizado na Figura 3. A amostra com o solvente foi submetida à aquecimento sob agitação até temperatura de 90°C utilizando o banho B, sendo mantida nessa temperatura por 15 min. Após esse período, o banho A foi utilizado para rapidamente diminuir a temperatura da amostra para valores próximos a temperatura ambiente. O banho C foi utilizado conectado ao condensador para permitir a recuperação do solvente utilizado.

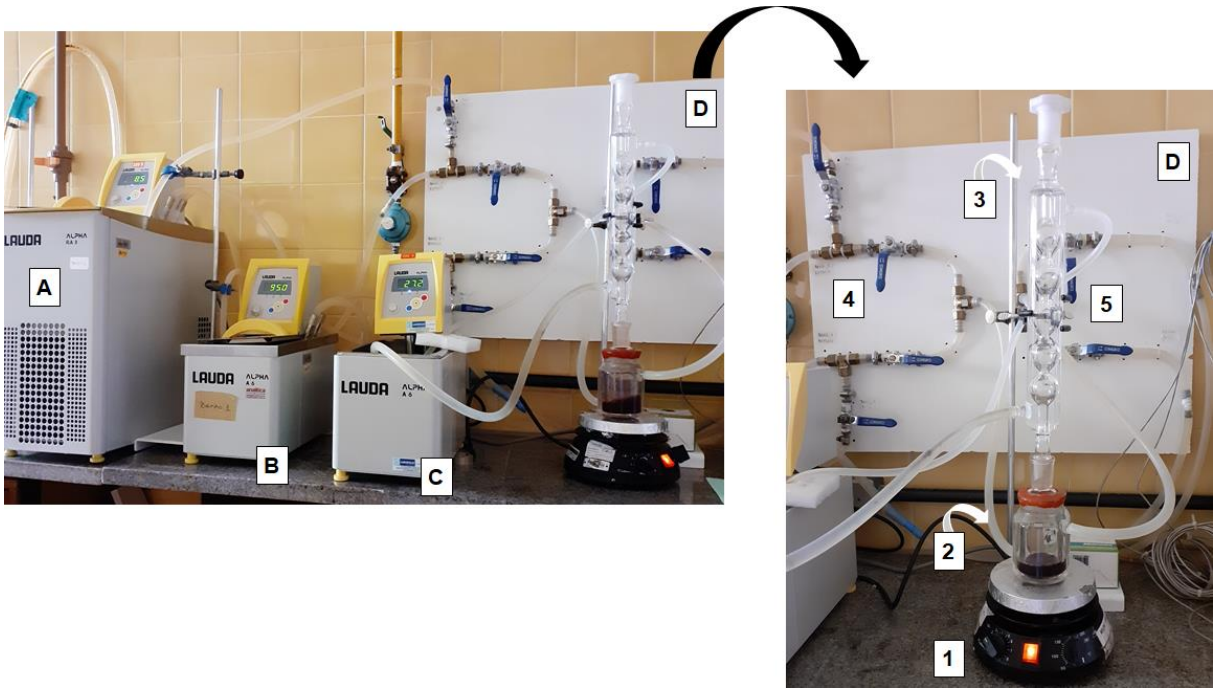

Figura 3. Sistema *by-pass* utilizado na hidrólise ácida: banho de resfriamento (Figura A), banho de aquecimento (B), banho ligado ao condensador (C), painel com o sistema by-pass (D), agitador magnético (1), célula de vidro encamisada (2), condensador (3), mangueiras e válvulas de entrada (4) e saída (5) de água. Fonte: autora.

O extrato obtido foi filtrado em papel filtro qualitativo, o resíduo foi recolhido e, então, o procedimento repetido. Após a segunda hidrólise, o *pellet* foi extraído com metanol até garantir a extração exaustiva dos CFNE, avaliada através do método de *Folin-Ciolcateu*. Os extratos obtidos foram combinados, filtrados em filtro PTFE (hidrofílico, 0,22 µm) e

utilizados para a análise por HPLC-DAD-ESI-MS/MS. O procedimento de hidrólise foi realizado, no mínimo, em triplicata.

Sensores de temperatura do tipo Pt-100 com haste de aço inoxidável foram utilizados para medir a temperatura durante o processo de hidrólise. O perfil de temperaturas obtido é representado na Figura 4. Como pode ser observado, foi necessário um tempo de 07 minutos para o extrato atingir a temperatura desejada de 90 °C, 15 minutos de hidrólise, e por fim, 2 minutos para o resfriamento (20 °C), totalizando 24 minutos de processo.

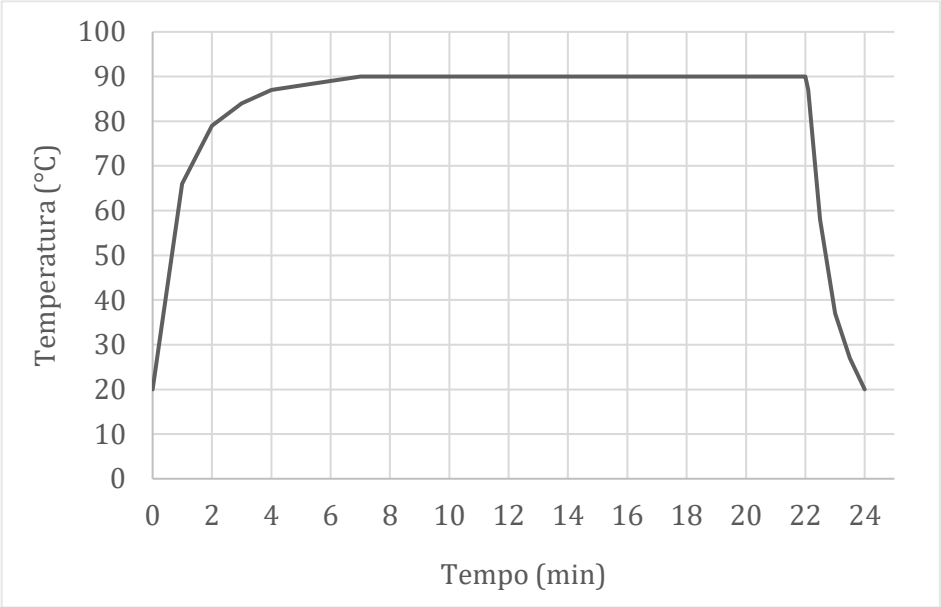

Figura 4. Perfil de temperatura obtido durante a hidrólise ácida dos compostos fenólicos não extraíveis. Fonte: autora.

Com o objetivo de verificar a degradação dos compostos fenólicos no processo de hidrólise, o mesmo procedimento descrito (com exceção da repetição da hidrólise) foi realizado utilizando um sistema modelo com uma mistura de padrões. Essa mistura de padrões foi composta de: ácido ferúlico, ácido gálico, ácido p-cumárico, apigenina, cloreto de cianidina 3-O-glicosídeo, epicatequina, naringenina e quercetina. Essa solução foi produzida na concentração de 100 mg L^{-1}, com exceção da cianidina 3-O-glicosídeo (32 mg L^{-1}). As concentrações iniciais e finais dos padrões (após o procedimento de hidrólise) foram avaliadas utilizando curvas analíticas construídas.

4.3.2.2 Etapa 2 – Efeito da Temperatura

Com base nos resultados da etapa 1, foram definidas três concentrações de ácido (1,0, 8,0 e 15,0 %) para realizar os experimentos da etapa 2. Nesta etapa foram avaliadas três temperaturas inferiores aos 90 °C: 50, 65 e 80 °C.

4.3.2.3 Etapa 3 – Efeito do Tempo

Com base nos resultados da etapa 2, foram definidas as condições de concentração de HCl (8,0 %) e temperatura (65 °C), para avaliar o efeito do tempo de hidrólise: 5, 10 e 20 min.

4.4 Análise dos Compostos Fenólicos por HPLC-DAD-ESI-MS/MS

A determinação dos compostos fenólicos foi realizada utilizando um cromatógrafo a líquido de alta eficiência (HPLC) da Shimadzu (Quioto, Japão), contendo os seguintes módulos: duas bombas LC-20AD, desgaseificador DGU20A, injetor automático SIL20AHT, detector de arranjo de diodos SPD-M20A (DAD) e forno de coluna CTO-20A. Para a identificação, o HPLC foi conectado em série a um espectrômetro de massas (MS) da Bruker Daltonics (micrOTOF-Q III, Bremen, Alemanha) com fonte de ionização por electrospray (ESI) e analisador de massas quadrupolo-tempo de voo (Q-TOF).

As condições de separação cromatográfica dos compostos fenólicos foram descritas previamente por RODRIGUES; MARIUTTI e MERCADANTE (2013). Os compostos fenólicos foram separados em uma coluna Phenomenex C18 de 4 µm (250 x 4,6 mm), fluxo de 0,7 mL min^{-1} a 35 °C, com fase móvel consistindo em água Milli-Q (acidificada com 0,1% de ácido fórmico) (fase móvel A) e acetonitrila (acidificada com 0,1% de ácido fórmico) (fase móvel B) em gradiente linear. Destaca-se que foram realizadas pequenas modificações em relação ao método referenciado, no fluxo da fase móvel (0,9 para 0,7 mL min^{-1}), temperatura (29 para 35 °C) e concentração de ácido fórmico (0,5 para 0,1%).

4.4.1 Identificação

Para identificação, após a separação dos compostos fenólicos no LC, o eluato da coluna foi dividido utilizando uma conexão em forma de “T” de forma que apenas 0,35 mL min^{-1} do fluxo seguiu para o MS. A fonte ESI foi operada nas seguintes condições: modos

negativo e positivo de ionização, voltagem do capilar de 4000 Volts, faixa de varredura (*scan range*) de *m/z* 50 a 1000, temperatura e fluxo do gás (N_2) secante de 310 °C e 8 L min^{-1}, pressão do gás nebulizador de 4 bar. A fragmentação dos compostos fenólicos foi realizada no modo automático com *threshold* de 1500 *counts* e a definição de três íons precursores. A energia de fragmentação para os diferentes compostos fenólicos foi definida previamente em testes com padrões de compostos fenólicos. A calibração do equipamento foi realizada semanalmente com formiato de sódio como calibrante. Além disso, para cada corrida uma calibração interna foi realizada com esse mesmo calibrante.

A identificação foi realizada utilizando duas abordagens. A primeira foi uma análise manual dos resultados, onde a identificação foi realizada considerando os seguintes parâmetros: massa acurada, padrão de fragmentação, ordem de eluição em coluna C18 e características de absorção no UV-vis. Essas informações foram comparadas com resultados de padrões, com a literatura e também com a biblioteca MassBank of North America (MoNA). Na segunda abordagem, utilizou-se o software Sirius 4 para auxiliar na identificação dos compostos fenólicos. Esse software identifica moléculas com base no padrão isotópico e no padrão de fragmentação. As duas abordagens permitiram identificar os compostos com diferentes níveis de confiança (Schymanski et al, 2014), nomeadamente L1 a L5.

4.4.2 Quantificação

A quantificação dos compostos fenólicos foi realizada por padronização externa através da construção de curvas de treze padrões analíticos, nomeadamente: ácido cafeico, ácido clorogênico, ácido elágico, ácido ferúlico, ácido gálico, ácido *p*-cumárico, ácido vanílico, apigenina, catequina, cloreto de cianidina 3-O-glicosídeo, epicatequina, naringenina e quercetina.

Os compostos fenólicos não extraíveis foram separados em cinco grandes grupos: taninos hidrolisáveis, ácidos hidroxibenzóicos, taninos condensáveis (flavan-3-ois, derivados e proantocianidinas), ácidos hidroxicinâmicos e antocianinas. Para cada grupo, os compostos foram quantificados com seu padrão correspondente ou equivalente.

4.4.2.1 Preparo das Soluções-Estoque

As soluções-estoque de compostos fenólicos (1000 mg L^{-1}) foram preparadas pela pesagem de 10 mg de padrão diretamente em balão volumétrico de 10 mL e dissolução com

metanol. O volume de metanol foi adicionado em duas etapas. Após a adição de cerca de 5 mL de metanol, a suspensão obtida foi submetida a 2 min de agitação em ultrassom para auxiliar a dissolução do padrão. Após a completa dissolução, o volume restante de metanol foi adicionado. Devido a sua baixa solubilidade, a solução-estoque do ácido elágico foi preparada em uma concentração dez vezes menor, 100 mg L^{-1} em metanol.

O padrão de cianidina 3-glicosídeo foi preparado de maneira diferente. Primeiramente, todo o conteúdo do padrão foi dissolvido em 10 mL de uma solução metanólica acidificada com HCl (0,10 %). Uma alíquota dessa solução concentrada foi transferida para um balão de 10 mL e diluída com a solução de metanol acidificado. A absorbância dessa solução foi lida a 529 nm e a concentração foi calculada utilizando a Equação 1:

$$Cianidina\ (mg/L) = \frac{A\ x\ MM\ x\ FD\ x\ 1000}{\varepsilon} \qquad (1)$$

onde *A* é a absorbância a 529 nm (deve estar entre 0,2 e 0,9), *MM* a massa molar da cianidina (287 g mol^{-1}), *FD* o fator de diluição e ε a absortividade molar da cianidina (34300 L mol^{-1} cm^{-1}).

A Tabela 1 mostra as concentrações das soluções-estoque e as faixas de concentração das curvas analíticas dos padrões de compostos fenólicos.

4.4.2.2 Preparo das Curvas Analíticas

Para onzes dos compostos, as curvas analíticas foram construídas a partir das injeções de doze (pontos), concentrações na faixa de 0,047 a 96 mg L^{-1}. Para o ácido elágico, a curva foi construída em dez concentrações, na faixa de 0,047 a 24 mg L^{-1}. Para a curva do cloreto de cianidina 3-O-glicosídeo, foram utilizadas nove concentrações entre 0,156 a 25 mg L^{-1}. Estas faixas podem ser observadas na Tabela 1.

Os pontos das curvas analíticas foram preparados a partir da diluição das soluções dos padrões na fase móvel A (água acidificada com 0,1 % de ácido fórmico) para onze dos padrões, mistura de metanol (MeOH) e água (H_2O) (MeOH:H_2O 90:10, v/v) para o padrão ácido elágico, e por fim, solução de metanol acidificado (0,1 % de ácido clorídrico) para o padrão cloreto de cianidina 3-O-glicosídeo.

Tabela 1. Faixas de concentração das curvas analíticas e concentração das soluções-estoque dos padrões de compostos fenólicos.

Padrão	Faixa de concentração (mg L^{-1})	Concentração da solução-estoque (mg L^{-1})
Ácido Gálico	0,047 – 96	1000
Ácido Clorogênico	0,047 – 96	1000
Catequina	0,047 – 96	1000
Epicatequina	0,047 – 96	1000
Ácido Vanílico	0,047 – 96	1000
Ácido Cafeico	0,047 – 96	1000
Ácido p-cumárico	0,047 – 96	1000
Ácido Elágico	0,047 – 24	100
Ácido Ferúlico	0,047 – 96	1000
Quercetina	0,047 – 96	1000
Naringenina	0,047 – 96	1000
Apigenina	0,047 – 96	1000
Cianidina 3-O-glicosídeo	0,156 – 25	320

Os limites de detecção (LD) e quantificação (LQ) foram determinados de acordo com a metodologia do ICH (2005). Este método calcula os limites com os resultados obtidos das curvas analíticas através da relação entre o erro padrão e a inclinação da curva, de acordo com as Equações 2 e 3:

$$LD = 3{,}3 \times \frac{s}{b} \qquad (2)$$

$$LQ = 10 \times \frac{s}{b} \qquad (3)$$

onde *s* é o erro padrão da curva analítica e *b* a inclinação da curva analítica.

Os dados obtidos com a construção das curvas analíticas e parâmetros da espectrometria de massas avaliados estão apresentados como anexo (Apêndice A).

4.5 Análise Estatística

Os experimentos foram realizados, no mínimo, em triplicata para cada parâmetro avaliado no processo de hidrólise ácida. A comparação dos valores médios entre os tratamentos foi realizada por análise de variância (ANOVA) com teste de Tukey ($p < 0{,}05$).

As análises estatísticas foram realizadas utilizando o software Statistica® (versão 14.0.0.15, TIBCO Software Inc, CA).

5 RESULTADOS E DISCUSSÃO

Nesta seção, estão apresentados os resultados obtidos no estudo da hidrólise ácida. O conteúdo será apresentado na forma de artigo. O artigo será posteriormente submetido a um periódico internacional indexado.

5.1 *Acid hydrolysis conditions affect the non-extractable phenolic compounds composition from grape peel and seed*

Abstract

Phenolic compounds are present as extractable or non-extractable phenolic compounds. The non-extractable fraction is usually obtained through the application of chemical, acidic or basic hydrolysis. The major limitation of the hydrolysis process is the degradation of the compounds, impairing their complete elucidation. In this work, were evaluated the effect of acid hydrolysis on non-extractable phenolic compounds and evaluated the application of less aggressive conditions to obtain it. Different concentrations of hydrochloric acid (0.1 to 15.0 %), temperatures (50 to 90 ºC) and times (5 to 20 min) were evaluated by HPLC-DAD-ESI-QTOF. The use of concentrations of 1.0 and 8.0% of HCl (v/v) and temperatures (65 and 80 °C) were able to obtain higher concentrations and a larger set of compounds. Under these conditions of concentration and temperature, NEPC represented 39 and 22 % (*m/m*) of the total PC for the grape peel and seed powder, respectively. NEPC had a 1.2 times increase in time from 5 to 20 min of hydrolysis, however, degradations were observed in the grape peel and seed powder. The results allowed us to conclude that the acid hydrolysis method should be optimized according to the objective of the study, such as the NEPC class of interest, and the composition of the food matrix.

Keywords: bioactive compounds, bound phenolic compounds, mass spectrometry, bioinformatics

5.1.1. Introduction

Phenolic compounds (PC) found in fruit and vegetables are associated with a reduced risk of developing certain non-communicable diseases, such as cardiovascular diseases and some types of cancer (Crozier et al., 2009). This effect is hypothesized due

to their ability to attenuate oxidative and/or nitrosative reactions induced by reactive oxygen species (ROS) and nitrogen species (RNS), especially when the endogenous antioxidant defense system cannot remove them in adequate amounts (Halliwell & Gutteridge, 2015; Pérez-Jiménez & Saura-Calixto, 2018). In recent years, other mechanisms have already been studied to elucidate the beneficial health effects of PC, with emphasis on their interactions with the gut microbiota (Tomás-Barberán et al., 2016). Some PC reach the colon almost unchanged (Hilary et al., 2020), being released from their matrix and fermented by the action of the microbiome into metabolites that are potentially absorbed through colonic fermentation (Pérez-Jiménez et al., 2013).

Although PC have been studied for many years, most studies focused on the PC that are readly solubilized in water and organic solvents. Data on the content and composition of the non-extractable PC (NEPC) remain scarce even if NEPC are gaining greater biological relevance due to their interactions with gut microbiota. NEPC remains in the solid residue after the extraction of PC with organic solvents or aqueous-organic solvent mixtures. Generally, this solid residue has been ignored as a source of PC. However, NEPC can represent between 15 % and 90 % of PC content of foods (Mallmann et al., 2020; Martín-García et al., 2021; Quatrin et al., 2019). For example, our research group showed that araçá (*Psidium cattleianum* Sabine) fruit present an NEPC fraction that represents 35 % (*m/m*) of its total phenolic content (Mallmann et al., 2020).

NEPC comprise different types of PC, including (1) hydrolysable tannins (HT), (2) condensed tannins (CT) or proanthocyanidins (PA), and (3) low-molecular-weight PC, such as phenolic acids and some flavonoids linked to cell wall constituents. NEPC fraction has been analyzed by different methods (Acosta-Estrada et al., 2014; Pérez-Jiménez et al., 2013); most of them follow a procedure in three stages due to their complexity: hydrolysis, extraction, and identification and quantification. NEPC release from food matrix is usually performed by enzymatic, alkaline, or acid hydrolyses. Acid hydrolysis, used in several studies, combine high temperatures (62 °C to 90 °C), high acid (H_2SO_4 or HCl) concentrations (usually 10 % and 2-6 M) variable processing times (15 min to 24 h) to release and extract these compounds. However, due to the drastic conditions applied, some PC can be lost during the hydrolysis, such as *trans*-sinapic acid (92%) and caffeic acid (87%) (Wu et al., 2018).

The most suitable acid hydrolysis method to obtain the NEPC should release them (HT, PA, and other NEPC) in high concentrations and with low degradation levels. Operational conditions, such as acid concentration, temperature, and time, need to be optimized to reduce losses during hydrolysis and extraction. Moreover, methods must be standardized according to the objective of the study and the composition of the food matrix. In this context, this study aims to evaluate the effect of hydrolysis conditions (acid concentration, temperature and time) on the NEPC composition of grape peel and seed powder.

5.1.2. Material and methods

5.1.2.1. Chemicals

Standards of apigenin, caffeic acid, catechin, 5-caffeoylquinic acid, cyanidin 3-O-glucoside chloride, ellagic acid, (-)epicatechin, ferulic acid, gallic acid, naringenin, *p*-coumaric acid, quercetin, and vanillic acid were purchased from Sigma-Aldrich (St. Louis, MO). HPLC-grade solvents acetonitrile and methanol were acquired from J.T. Baker (Phillipsburg, NJ). Hydrochloric acid was purchased from Química Moderna (São Paulo, Brazil). Methanol was purchased from Dinâmica (São Paulo, Brazil). Formic acid and sodium carbonate were purchased from Neon (São Paulo, Brazil). Folin-Ciocalteu reagent were purchased from Synth (São Paulo, Brazil). Ultrapure water (MilliQ) was generated by the Millipore System (Molsheim, FR). The mobile phases were filtered through cellulose acetate and hydrophilic PTFE (Polytetrafluoroethylene) membranes for aqueous and organic phases, respectively.

5.1.2.2. Samples

The peel and seed grape powders (PGP and SGP, respectively), shown in **Figure 1**, were donated by the Econatura company (https://www.econatura.com.br/). They are co-products of the juice production from organic grapes (“bordô” variety). The grape juice was produced with the use of pectolytic enzyme preparation, and the resulting bagasse was sieved to separate the peels and seeds. The moisture content of the peels and seeds was reduced by hot air circulation drying, resulting in moisture about 4 and 6 g/100g, for PGP and SGP, respectively. Finally, the dry peels were ground and packed, and the seeds were cold defatted, ground and packed.

Figure 1. Peel and seed grape powders.

5.1.2.3. Extraction of phenolic compounds

5.1.2.3.1. Extractable phenolic compounds

Extractable phenolic compounds (EPC) were exhaustively extracted from the sample (PGP or SGP) (0.5 g) using 10 mL of a mixture of methanol (MeOH) and water (H_2O) (MeOH:H_2O 8:2, v/v) acidified with 0.35% formic acid. This mixture was vortexed for 3 minutes. The obtained extract was centrifuged at 25,000 x g for 5 minutes (4 °C), and the supernatant was removed (Rodrigues et al., 2013). This procedure was repeated until EPC exhaustive extraction, and the solid residue (pellet) was stored for NEPC extraction. The extracts obtained were combined, filtered (hydrophilic PTFE, 0.22 µm), and analyzed by HPLC-DAD-ESI-QTOF. The extraction procedure was carried out in triplicate.

5.1.2.3.2. Extraction of non-extractable phenolic compounds

The non-extractable phenolic compounds (NEPC) were obtained from the solid residue (pellet) by acid hydrolysis and extraction. Experimental conditions were carried out based upon the method previously described by Mallmann et al. (2020). NEPC were extracted using methanol acidified with HCl (15.0 %, *v/v*) for 15 minutes in a water bath at 90 °C. In the present study, five HCl concentrations (0.1 to 15.0 %), four temperatures (50 to 90 ºC), and three hydrolysis times (5 to 20 min) were tested in 3 steps. Figure 2 shows the experiments carried out in each step. The apparatus used in the experimental procedure is shown in Figure 3. The equipment consists of three thermostatic baths, a

bypass system, a jacketed glass vessel of 250 mL, thermocouples, a magnetic stirrer (C-MAG HS 10, IKA), a condenser, a data acquisition system, and a computer for the data acquisition.

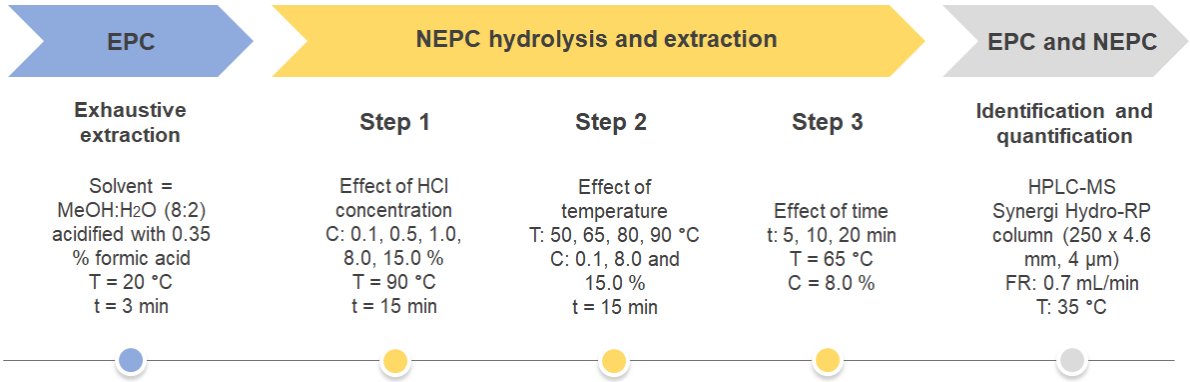

Figure 2. Experiments carried out to extract the EPC and NEPC fractions.

Samples were heated using the hot thermostatic bath, were kept at the target temperature during hydrolysis and extraction during a pre-determined time, and were cooled to room temperature using the cold bath. After this procedure, the extract was filtered (qualitative filter paper), and the solid residue was collected. The supernatant was transferred to a flat bottom flask, and the procedure was repeated (step 1 and 2). After the second hydrolysis, the solid residue was extracted with methanol until NEPC exhaustive extraction. The extracts obtained were combined, filtered (hydrophilic PTFE, 0.22 µm), and analyzed by HPLC-DAD-ESI-QTOF. For step 3, hydrolysis was conducted only once, and methanol extraction at the end of the process was not performed. Each experiment was carried out in triplicate.

5.1.2.4. A model system with analytical standards

A mixture of seven PC standards (cyanidin 3-O-glucoside, epicatechin, ferulic acid, gallic acid, naringenin, *p*-coumaric acid, and quercetin) was submitted to the same hydrolysis conditions (procedure described in section 2.3.2) to evaluate their level of degradation.

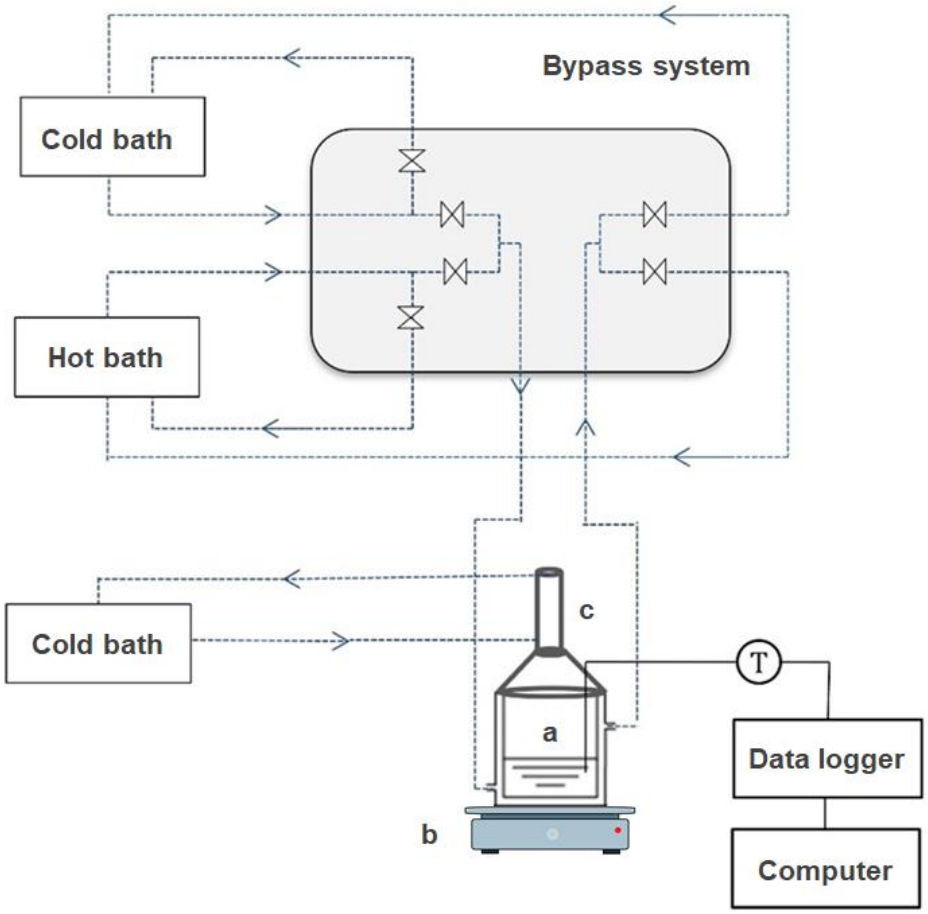

Figure 3. Schematic diagram of the equipment used to conduct the NEPC hydrolysis and extraction: a) jacketed glass cell; b) magnetic stirrer; and c) condenser. Adapted from (Brochier et al., 2018).

5.1.2.5. HPLC-DAD-ESI-QTOF analysis

PC determination was performed using a Shimadzu high-performance liquid chromatography (HPLC) (Kyoto, Japan), connected in series to a DAD detector (SPD-M20A) and a mass spectrometer (MS) with Quadrupole-Time-of Flight (QTOF) analyzer and an electrospray ionization source (ESI) (Bruker Daltonics, micrOTOF-Q III model, Bremen, Germany).

The PC chromatographic separation conditions were previously described by Rodrigues et al. (2013). PC were separated in a Synergi Hydro-RP column (250 x 4.6 mm, 4 µm, Phenomenex, Torrance, CA) at 0.7 mL min^{-1} at 35 °C, using as mobile phase a linear gradient of water:formic acid (99.9:0.1, v/v, solvent A) and acetonitrile:formic acid (99.9:0.1, v/v, solvent B). The column eluate was split using a "T" connection to allow 0.35 mL min^{-1} entering the MS interface. The UV-vis spectra were obtained

between 200 and 900 nm, and the chromatograms were processed at 280, 320, 360 and 520 nm. Instrument control and data processing were carried out using the software Esquire Control and Data Analysis 4.3, respectively (Bruker Daltonik). The MS parameters were set as follows: scan range from *m/z* 50 to 1000, ESI source in negative and positive modes, capillary voltage of 4000 V, drying gas temperature of 310 °C, flow rate of 8 L min^{-1}, and nebulizer gas pressure of 4 bar. PC fragmentation was performed in automatic mode with a threshold of 1500 counts and the definition of three precursor ions. The fragmentation energy was set based upon tests with PC standards using the software Esquire Control optimization tool. External MS calibration with 10 mM sodium formate solution was performed using a syringe pump directly connected to the interface, equipped with a syringe (500 μL). Moreover, the same calibration solution was injected at the beginning of the run, and all the spectra were recalibrated prior to compound identification.

PC identification was performed applying two approaches: (1) traditional and (2) using a bioinformatics tool. In the traditional one, the MS and MS^2 spectra were manually analyzed, and the identification was performed considering the following parameters: accurate mass, fragmentation pattern, elution order in C18 column, and UV-vis absorption characteristics. This information was compared with the PC standard results and literature data. Moreover, the MassBank of North America (MoNA) library was used. In the second approach, the SIRIUS software (https://bio.informatik.uni-jena.de/software/sirius/) was used to assist PC identification (Dührkop et al., 2019). The two approaches allowed to identify compounds with different levels of confidence (Schymanski et al., 2014), namely L1 to L5.

PC were quantified by external standardization using curves of thirteen analytical standards. The method was validated considering the following performance parameters: linearity (R^2), limit of detection (LD), and limit of quantification (LQ) (**Supplementary Table S1)**.

5.1.2.6. Statistical analysis

All experiments were performed in triplicate. Thus, data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA), performed using the Statistica® (version 14.0.0.15, TIBCO Software Inc, CA), was applied to determine

differences between treatments. Differences between means were evaluated using the Tukey test with 95 % of confidence level ($\alpha = 0.05$).

5.1.3. Results and discussion

5.1.3.1. Effect of hydrolysis conditions on NEPC total content

The solid residue (*pellet*) from the EPC exhaustive extraction was treated with varying concentrations of HCl (0.1 – 15.0%) at different temperatures (50 – 90 °C) for different amounts of time (5 – 20 min). EPC and NEPC concentrations of peel and seed grape powders are presented in **Table 1**. NEPC total content was affected by HCl concentration and temperature. The best hydrolysis condition was different for PGP and SGP, namely: HCl concentration of 8.0 % at 65 °C and concentration of 1.0 % at 80 °C, respectively. In these conditions, it was extracted the highest NEPC content, which represents 39 and 22 % (*m/m*) of total PC for PGP and SGP, respectively. It has been shown in the literature that NEPC fraction contributes between 35 and 90 % of total PC content in several types of foods representing a relevant portion of these compounds. For instance, considering the NEPC fraction, the total PC contents in PGP and SGP are 1.3 and 1.6 times higher than the EPC fraction alone, respectively. For PGP, the NEPC percentage was higher (1.6 times) than the value reported in the literature (Maurer et al., 2020), which can be partially attributed to the hydrolysis conditions utilized in this work.

The results suggest that HCl concentration is an important factor for NEPC release from the PGP and SGP, and its effect is matrix-dependent. An increase of 10 times at HCl concentration (0.1 to 1.0%, 90 °C) increased twice NEPC content for PGP; for SGP, on the other hand, this effect was not observed. Moreover, high HCl concentrations at 90°C promoted a decrease of approximately 2 times in the NEPC content for SGP, and no effect was observed for PGP. These findings suggest that these variations are attributed to differences in matrix composition. NEPC are associated with macromolecules, mainly polysaccharide constituents of dietary fiber (DF) and proteins. Both matrices (PGP and SGP) have similar protein (9-11 %) and dietary fiber (46-50 %) contents. However, the proportion of soluble (SDF) and insoluble (IDF) fibers is different, and this may be the reason for distinct behaviors during NEPC release. PGP has SDF content 7 times higher than SPG (Costa et al., 2019; Maurer et al., 2020). Studies have shown that the content and composition of PC bounded in the DF fractions (SDF and IDF) can be different (Guo & Beta, 2013).

Temperature also played a key role in NEPC release and extraction. Higher temperatures usually increase the rate of the hydrolysis reaction and improve and speed up the extraction process. Nevertheless, it may also promote PC degradation. The effect of temperature was evaluated in step 2 in three HCl acid concentration: 1.0, 8.0 and 15.0 %. Results from step 2 show that NEPC concentration in the extracts increased up to a certain temperature was reached. However, an increase of temperature beyond this temperature (65 ºC for PGP and 80 ºC for SGP) dropped significatively NEPC content by 1.4 and 1.2 times for PGP and SGP, respectively. It is important to highlight that for both matrices the temperature was lower than those used by most of the research conducted so far for NEPC extraction (Hu et al., 2018; Wu Li et al., 2020; Mallmann et al., 2020; Matsumura et al., 2016; Peng et al., 2017; Pérez-Ramírez et al., 2018).

Table 1. EPC and NEPC concentrations (μg g^{-1}) in peel and seed grape powders submitted to different hydrolysis conditions (steps 1 and 2).

Source	EPC concentration (μg g^{-1})	NEPC concentration (μg g^{-1})				
		HCl (%)	Temperature			
			50 °C	65 °C	80 °C	90 °C
PGP	11871 ± 216	0.1	n.t.	n.t.	n.t.	2593 ± 270 ^{e}
		0.5	n.t.	n.t.	n.t.	4944 ± 163 $^{b, c, d}$
		1.0	2469 ± 64 ^{e}	6023 ± 639 ^{b}	1718 ± 543 ^{e}	5327 ± 190 $^{b, c}$
		8.0	3829 ± 681 ^{d}	**7596 ± 566** ^{a}	5591 ± 335 $^{b, c}$	5127 ± 382 $^{b, c}$
		15.0	2334 ± 72 ^{e}	5400 ± 317 $^{b, c}$	4748 ± 182 $^{c, d}$	5310 ± 164 $^{b, c}$
SGP	13299 ± 255	0.1	n.t.	n.t.	n.t.	3199 ± 191 $^{a, b}$
		0.5	n.t.	n.t.	n.t.	2999 ± 71 $^{a, b}$
		1.0	1227 ± 58 $^{d, e}$	1925 ± 77 $^{c, d}$	**3732 ± 699** ^{a}	3231 ± 413 $^{a, b}$
		8.0	1720 ± 348 ^{d}	2701 ± 335 $^{b, c}$	2726 ± 196 $^{b, c}$	1495 ± 42 $^{d, e}$
		15.0	859 ± 83 ^{e}	1917 ± 119 $^{c, d}$	3434 ± 252 $^{a, b}$	1740 ± 100 ^{d}

Results are the average values of three independent experiments (values are shown as mean ± SD). Means with the same lowercase letters are not significantly different ($p > 0.05$). n.t.: not tested.

Overall, the results show that the acid hydrolysis method must be optimized according to the objective of the study and the composition of the food matrix. Lack of optimization and standardization can lead to different results. The best condition will be achieved when an equilibrium between release/extraction and degradation occur, which is dependent on the hydrolysis conditions.

5.1.3.2. Effect of hydrolysis conditions on NEPC composition

Phenolic compounds found in PGP and SGP by HPLC-DAD-ESI-QToF are presented in **Tables 2-5** and **Figure 10**. These compounds were manually or software-assisted identified (**see section 5.1.2.5**), and the confidence level of the identification was attributed considering the five levels described by Schymanski et al. (2014). A comprehensive discussion about the PC identification was not addressed in the present work because it has been already described in detail by our research group or other studies (Dal Magro et al., 2016; Escobar-Avello et al., 2019; Goufo et al., 2020; Mallmann et al., 2020; Pérez-Ramírez et al., 2018; Rodrigues et al., 2013; Vargas et al., 2018). Nevertheless, in **Tables 2-3** it is possible to check all parameters utilized for PC identification.

The HPLC-DAD chromatogram, processed at 280 nm, shows the separation of PC candidates. Thereafter, the analysis showed that although some peaks have UV-spectrum related to PC, they did not have mass spectra compatible with PC. An example is an intense peak found in NEPC 15.0 % for SGP observed in **Figure 10**. This compound has a mass to charge ratio (deprotonated molecule) at *m/z* 410.1285, which indicates that this molecule has one or more nitrogen atoms and, thus, probably it is not a PC. Moreover, this molecule did not fragment under different tested fragmentation energies, making its identification a task impossible.

A total of 41 and 25 PC were identified in the NEPC fraction of the PGP and SGP, respectively. Few PC (<10 %) were found in both EPC and NEPC fractions. Catechin and epicatechin were found in both fractions and matrices. Other PC, found in both fractions, differ according to the matrix: gallic acid and malvidin hexoside for PGP, and proanthocyanidin dimer IV for SPG. This finding agrees with literature data for different foods, in which the majority of PC are either present in EPC or NEPC (Mallmann et al., 2020; Martín-García et al., 2021)**.**

NEPC from PGP and SGP belong to five PC classes, namely: hydrolysable tannins (HT), condensed tannins (CT), hydroxybenzoic acids (HA), and hydroxycinnamic acids (HCA). These classes responded differently to hydrolysis and extraction because they are bonded in a distinct way to the matrix and also have different stabilities. **Figures 4-7** show the results separated for each NEPC class. For HT, higher contents were observed at higher acid concentrations and higher temperatures. Combinations of 8.0 % HCl (80 °C) and 15.0 % (90 °C) for PGP, and 15.0 % HCl (80

and 90 °C) for SGP, were statistically similar, showing the highest HT content (**Figure 4**). This class was represented by gallic acid and methyl gallate, products from the depolymerization of HT under HCl methanolysis conditions. Thus, NEPC from PGP and SGP are mainly gallotannins, which need more drastic conditions to break down their hydrogen and covalent bonds (Brook & Munday, 1970; Bunzel, 2010; Le Bourvellec et al., 2005; Ligny, 1979) with the matrices and glycoside and ester bonds with glucose. In the experiment conducted with gallic acid standard submitted to the same hydrolysis conditions, 35 and 72 % of gallic acid was degraded. This indicates that drastic conditions are releasing these compounds from the food matrix and depolymerizing them but are also partially degrading them after hydrolysis.

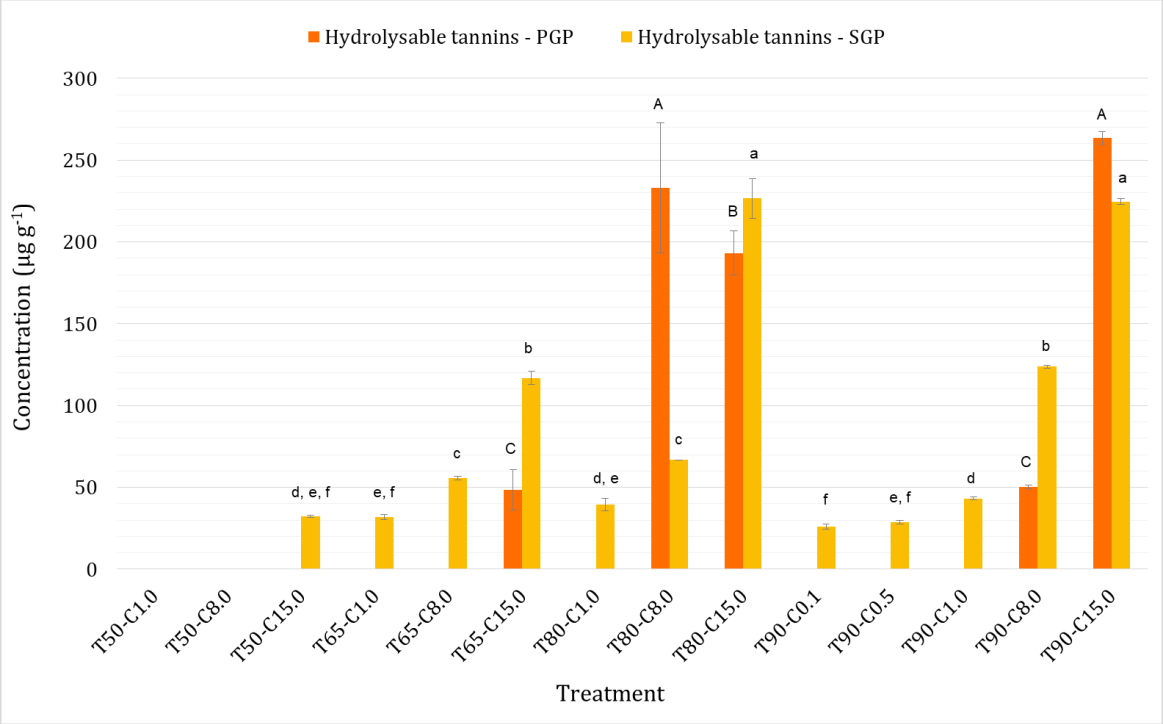

Figure 4. Hydrolysable tannins of PGP and SGP extracted under different condition of temperature and HCl concentration.

Results are the average values of three independent experiments (values are shown as mean ± SD) (**Supplementary Table S4**). Means with the same letters are not significantly different ($p > 0.05$). <LQ: Below limit of quantification. The limits of quantification were between 0.08-1.95 (**Supplementary Table S1**). n.d.: not detected.

Of course, the increased yield of HT compensates for the losses that occurred due to degradation. Finally, it is necessary to point out that the hydrolysis conditions utilized

may underestimate the content of ellagitannins since cross-linked and condensed gallic acid structures can be missed (Pérez-Jiménez & Torres, 2011).

Regarding HA (**Figure 5)**, it was observed that the combination of 8.0 % of HCl concentration with 80 and 90 °C of temperature for PGP were statistically similar, showing the highest HA content. For SGP, the best condition was 15.0 % with 80 °C. This class is represented by hydroxybenzoic, vanillic, syringic, and protocatechuic acids. For PGP, even low acid concentrations (0.5 and 1.0 %) were able to extract high concentrations of these compounds at 90 °C. For SGP, this behavior was observed only in the combination of high acid concentrations and temperature. Even at mild conditions it was possible to extract these compounds, indicating that these molecules are interacting through different types of bonds, hydrogen and covalent, and also with different molecules.

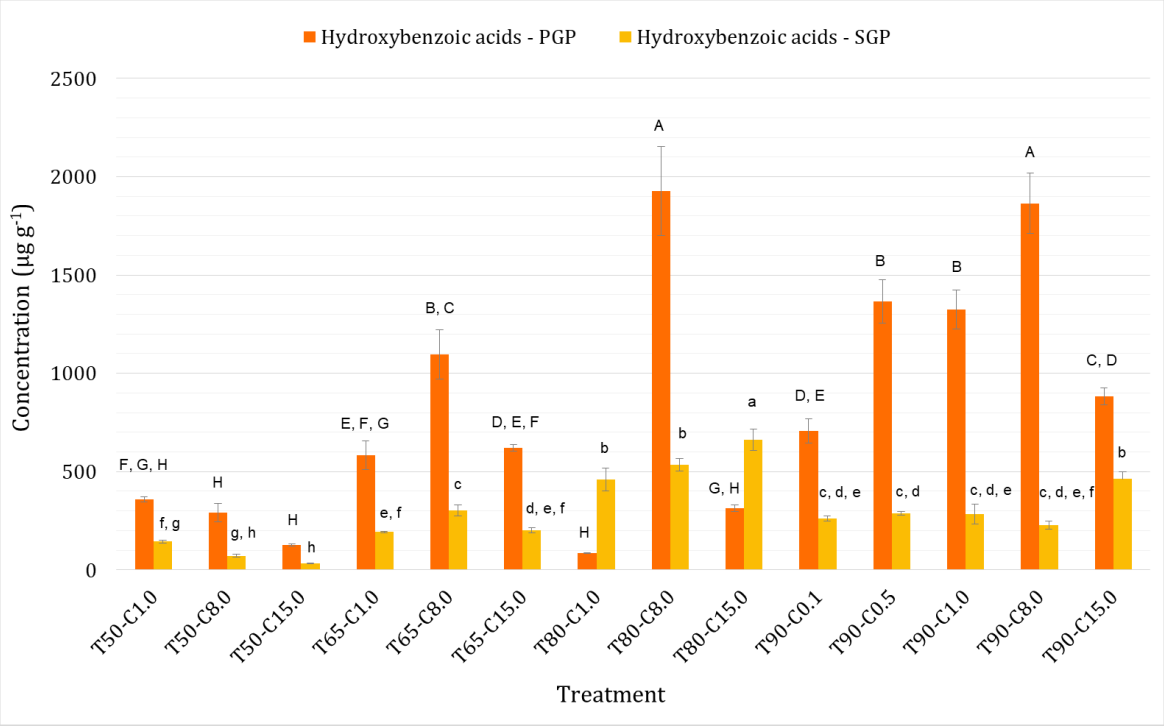

Figure 5. Hydroxybenzoic acids of PGP and SGP extracted under different condition of temperature and HCl concentration.

Results are the average values of three independent experiments (values are shown as mean ± SD) (**Supplementary Table S4**). Means with the same letters are not significantly different ($p > 0.05$). <LQ: Below limit of quantification. The limits of quantification were between 0.08-1.95 (**Supplementary Table S1**). n.d.: not detected.

Concerning CT (**Figure 6**), which were evaluated as flavan-3-ols monomers and anthocyanidins, the best conditions for their release and extraction was 8.0 % HCl and

65 °C for PGP. In this condition of acidity, increasing temperature to 80 or 90 °C decreased 2.1 and 2.2 times the content of CT, respectively. Moreover, lower acid concentrations may not be adequate for their complete release from the food matrix. For SGP, a distinct behavior was observed: low temperatures combined with low acid concentration and high temperatures combined with high acid concentrations showed the lowest values of CT. The highest result was found combining 8.0 % HCl, and 80 °C. Low temperature and acid concentration may not well extract these compounds from the matrix, and drastic conditions may promote their degradation. This class represented the largest fraction of NEPC in SGP compared to the other classes (**Figure 6**) since grape seed is an abundant source in nature of proanthocyanidins.

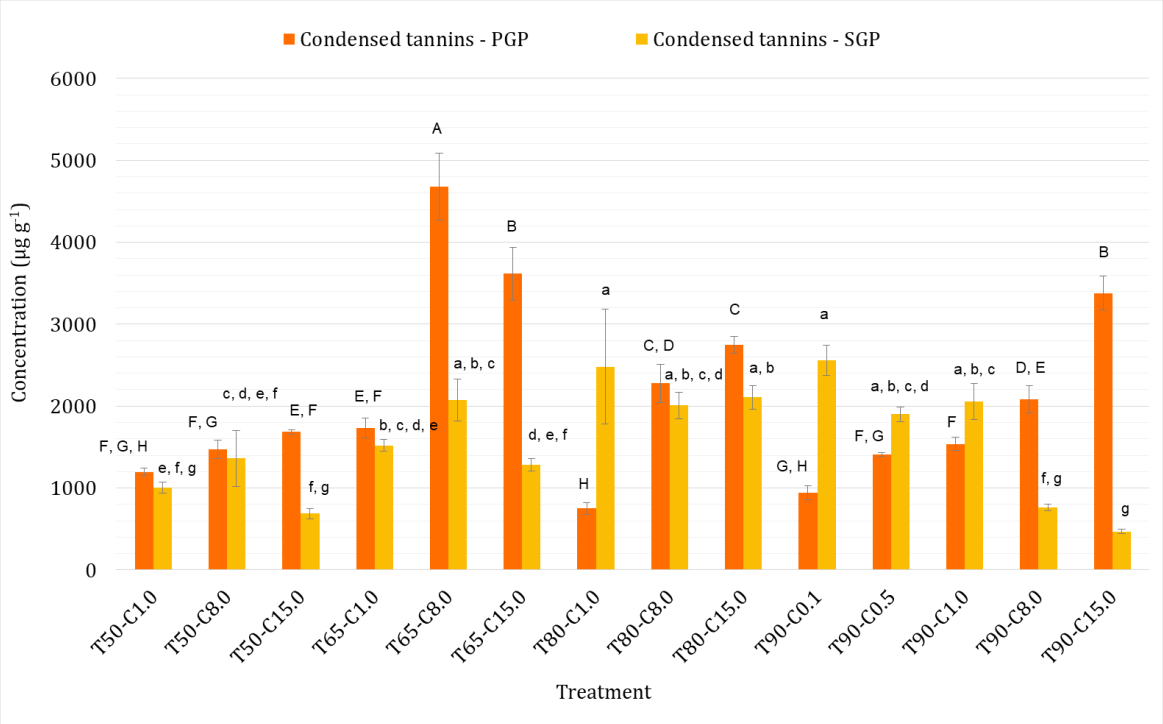

Figure 6. Condensed tannins of PGP and SGP extracted under different condition of temperature and HCl concentration.

Results are the average values of three independent experiments (values are shown as mean ± SD) (**Supplementary Table S4**). Means with the same letters are not significantly different ($p > 0.05$). <LQ: Below limit of quantification. The limits of quantification were between 0.08-1.95 (**Supplementary Table S1**). n.d.: not detected

HCA (**Figure 7**) were found in PGP in highest concentrations when HCl concentration of 15.0 % and a temperature of 80 and 90 °C were used. For SGP, on the other hand, this class of compounds was not found probably because the conditions used were not effective to release them form the food matrix. According to Wang et al.

(2020), bonds between ferulic acid and the food matrix, as well as *o*-coumaric acid and the matrix, were broken only after alkaline hydrolysis when comparing it to acidic hydrolysis.

Regarding anthocyanins, in PGP their extraction was possible above the limit of quantification in certain conditions (**Figure 7**). Temperature of 80 °C with 8.0 and 15.0 % of HCl concentrations were best combinations for their release from the matrix. In SGP, this class of compounds were not observed in extracts because this matrix is not a source of free or matrix-bound anthocyanins. Anthocyanins were not observed under less drastic conditions in PGP, indicating that they might be strongly bound to the matrix, being necessary a combination of higher temperatures and concentrations

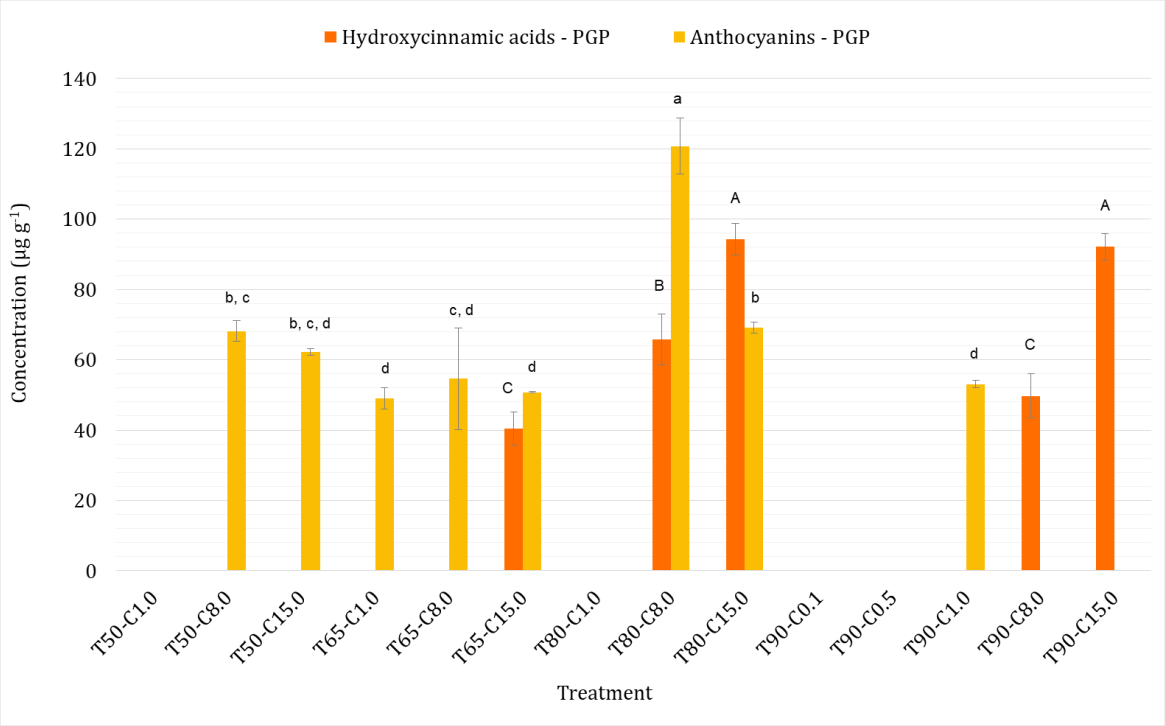

Figure 7. Hydroxycinnamic acids and anthocyanins of PGP in different treatments.

Results are the average values of three independent experiments (values are shown as mean ± SD) (**Supplementary Table S4**). Means with the same letters are not significantly different ($p > 0.05$). <LQ: Below limit of quantification. The limits of quantification were between 0.08-1.95 (**Supplementary Table S1**). n.d.: not detected.

release them. Cyanidin had its highest content values with increasing temperature and concentration. Malvidin was quantified in 8.0 % (90 °C) and 15.0 % (80-90 °C). Delphinidin, cyanidin, petunidin, peonidin, and malvidin anthocyanidins, common in grapes and derivatives, twere observed in the non-extractable fraction of PGP. Acid

treatment can extract proanthocyanidins but can also cause their degradation through their conversion into anthocyanidins, as aforementioned. Furthermore, the drastic conditions applied can also cause anthocyanin partial degradation after their releases. In this case, information about the original polymer can be lost.

5.1.3.3. Effect of hydrolysis times on NEPC

In step 3, different hydrolysis times (5, 10 and 20 minutes) were evaluated under the condition of 8.0 % HCl and a temperature of 65 °C. These parameters were determined considering results found in steps 1 and 2. **Figures 8 and 9** show the results for PGP and SGP, respectively.

In this step, it was also observed different behaviors for PGP and SGP. While NEPC content changed over time for PGP, this did not happen for SGP, being 5 min enough to release them from this food matrix. Total NEPC increased 1.2 times from 5 to 20 min of hydrolysis, for PGP. Regarding hydroxybenzoic acids and condensed tannins, the same behavior was observed: the best extraction time was 20 min for PGP, and there were no statistical differences between times for SGP. Acid hydrolysis times vary and depend on conditions, such as acid concentration, but can reach times between 20 h (Domínguez-Rodríguez et al., 2021) and 24 h (Alu'datt et al., 2019). For alkaline hydrolysis, 120 min at 80 C were considered as the best treatment to obtain NEPC from rice by-products (Irakli et al., 2018).

Hydroxycinnamic acids and hydrolysable tannins were not found in PGP extracts. Their concentrations were lower than the limit of quantification. Anthocyanins were found only in PGP and the best extraction time was 20 min. In SPG, hydroxycinnamic acids and anthocyanins were not found. Hydrolysable tannins were found in higher concentrations in 5 and 10 min, decreasing their concentration after this time probably due to degradation.

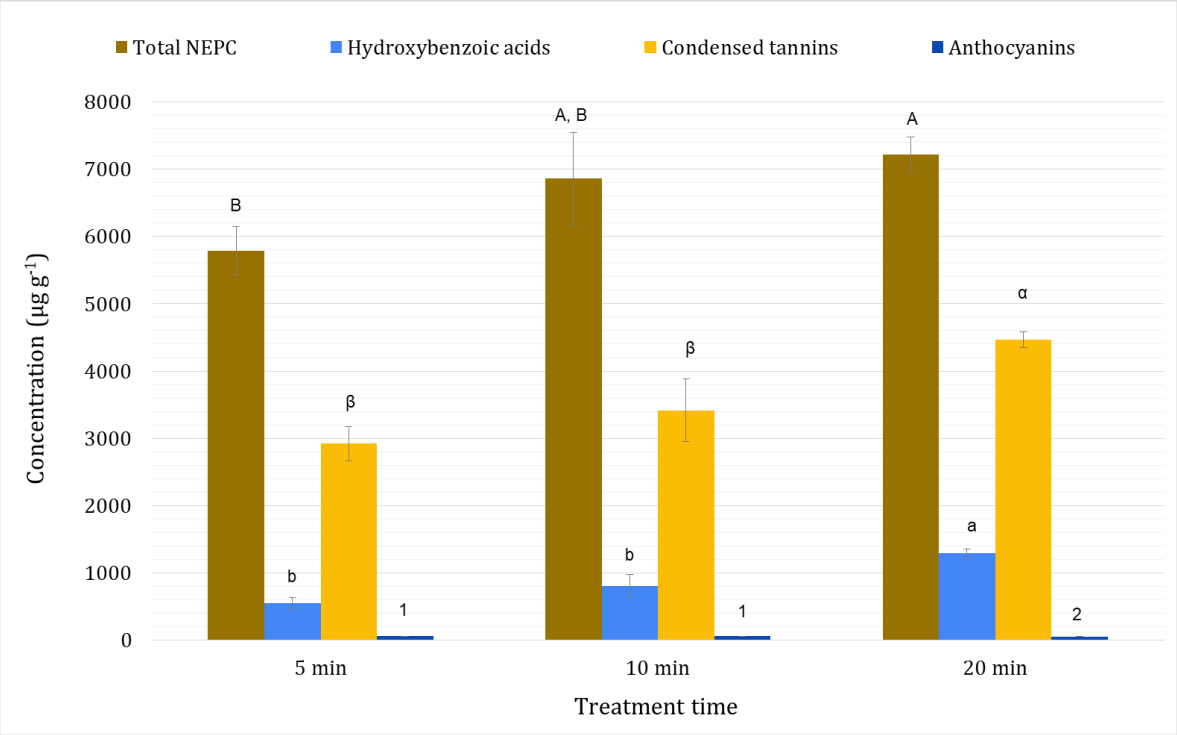

Figure 8. Total NEPC and NEPC classes extracted in different treatment times for PGP.

Results are the average values of three independent experiments (values are shown as mean ± SD). Means with the same letters are not significantly different ($p > 0.05$).

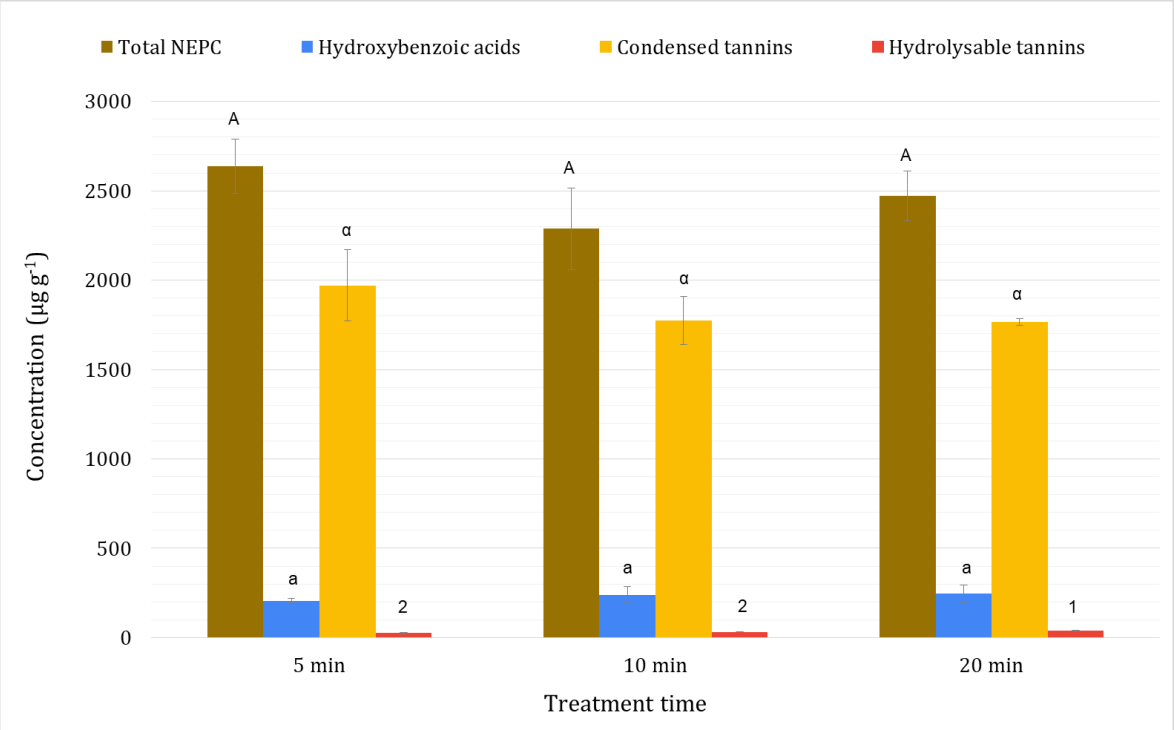

Figure 9. Total NEPC and NEPC classes extracted in different treatment times for SGP.

Results are the average values of three independent experiments (values are shown as mean ± SD). Means with the same letters are not significantly different ($p > 0.05$).

Overall, considering all classes and total NEPC content, the best hydrolysis time was 20 min for PGP. For SGP, 5 min was enough to extract most of the NEPC found in this matrix (except for hydrolysable tannins). Again, a different pattern was observed comparing both matrices, confirming that NEPC extraction is matrix dependent. The type of NEPC and their interaction with other compounds of the matrix will determine the extraction conditions. It is important to optimize these conditions to guarantee maximum extraction with minimum degradation.

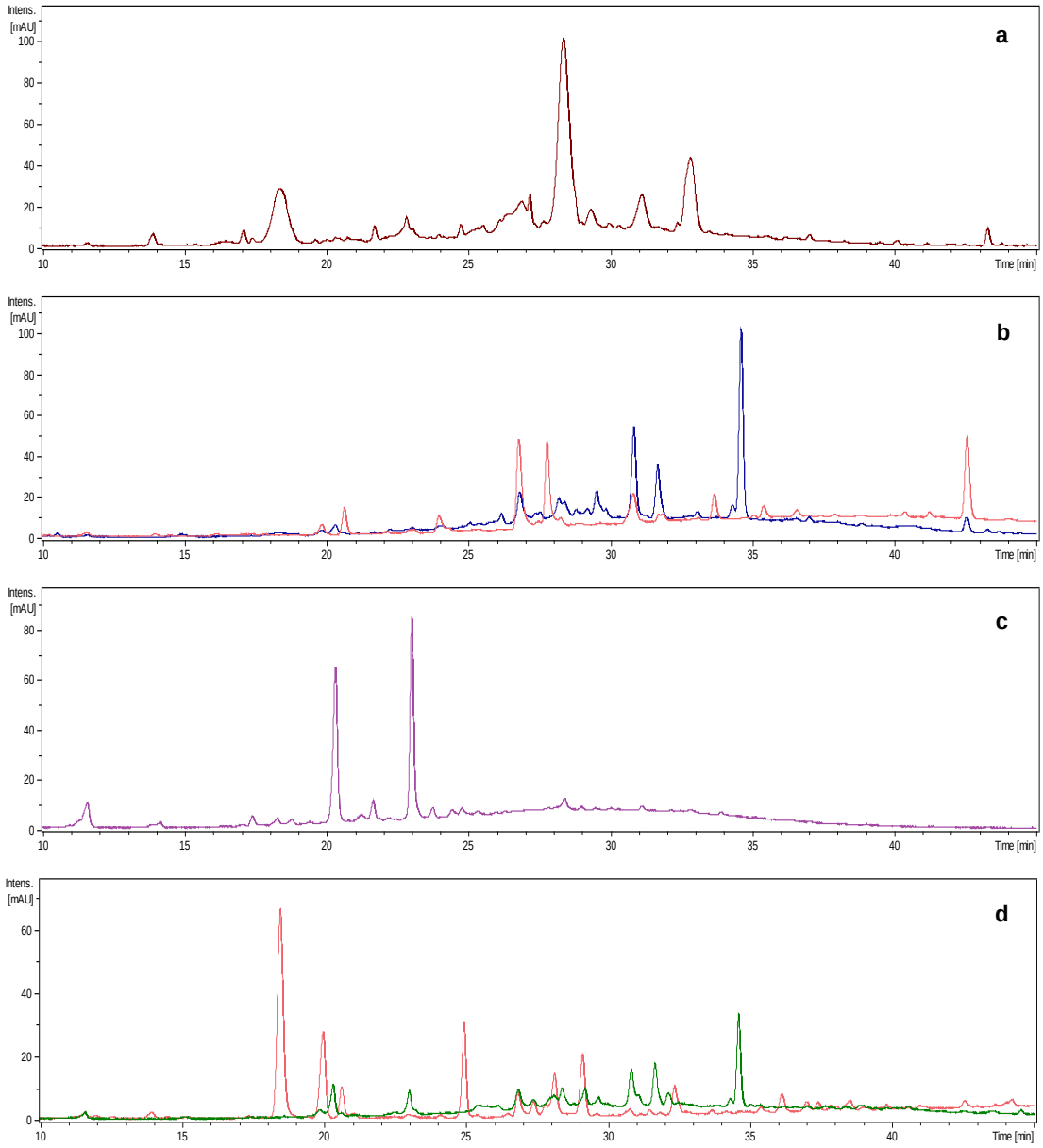

Figure 10. Chromatograms at 280 nm: a) EPC from PGP; b) NEPC from PGP (blue color line: 1.0, and red line: 15.0 % of HCl concentration); c) EPC from SGP; d) NEPC from SGP (green line:1.0, and red line: 15.0 % of HCl concentration).

Table 2. Non-extractable phenolic compounds (NEPC) identified or tentatively in PGP extract by liquid chromatography coupled with electrospray ionization hybrid quadrupole-time of flight mass spectrometry (HPLC-DAD-ESI-QTOF).

Peak[a]	Compound	t_R (min)[b]	λ_{max} (nm)[c]	$[M-H]^-$ or $[M]^+$ (*m/z*)[d]	Molecular formula	Error (ppm)	mSigma	MS/MS (-) (*m/z*)[e]	ID level[f]	Reference
1	gallic acid	11.6	271	169.0150	$C_7H_6O_5$	-4.3	30.3	134.8715 (100), 124.0180 (36)	1	Standard
2	(epi)gallocatechin-(epi)catechin	14.0	279	593.1209	$C_{30}H_{26}O_{13}$	-	-	441.0458 (10), 289.0446 (25), 245.0654 (11), 153.0197 (100)	2	(Goufo et al., 2020)
3	protocatechuic acid	14.7	279	153.0205	$C_7H_6O_4$	-7.5	26.9	n.d.	4	(Escobar-Avello et al., 2019)
4	proanthocyanidin dimer I	14.9	278	575.1289	$C_{30}H_{24}O_{12}$	-	-	575.1289 (24), 413.0809 (26), 287.0483 (85), 125.0183 (100)	2	(Yuzuak et al., 2018)
5	hydroxybenzoic acid I	16.2	279, 310	137.0214	$C_7H_6O_3$	22.0	7.6	n.d.	4	(Escobar-Avello et al., 2019)
6	proanthocyanidin dimer II	18.4	279	577.1313	$C_{30}H_{26}O_{12}$	6.7	11.6	407.0791 (65), 289.0663 (100), 245.0795 (21), 125.0279 (38)	2	(Goufo et al., 2020)
7	(epi)catechin-(epi)gallocatechin gallate	19.0	279	745.1350	$C_{37}H_{29}O_{17}$	-	-	441.0806 (90), 423.0666 (100), 315.0495 (88), 289.0358 (41), 169.0168 (32), 153.0136 (32), 125.0247 (29)	2	(Escobar-Avello et al., 2019)
8	hydroxybenzoic acid II	19.9	278, 310	137.0237	$C_7H_6O_3$	5.0	14.0	136.0173 (100), 108.0224 (83)	2	(Escobar-Avello et al., 2019)
9	catechin	20.3	278	289.0706	$C_{15}H_{14}O_6$	4.2	23.6	123.0417 (100), 109.0190 (58)	1	Standard
9	(epi)gallocatechin	20.4	278	305.0610	$C_{15}H_{14}O_7$	-	-	161.0253 (44), 125.0198 (100)	2	(Escobar-Avello et al., 2019)
10	methyl gallate	20.8	271	183.0294	$C_8H_8O_5$	2.8	1.4	140.0107 (100), 124.0174 (86)	2	(Martini et al., 2021)
11	(epi)gallocatechin gallate I	21.2	279	457.0740	$C_{27}H_{17}O_{11}$	-	-	303.0497 (30), 169.0082 (23), 153.0192 (79), 151.0330 (100), 125.0316 (36)	2	(Escobar-Avello et al., 2019)
12	malvidin hexoside	22.4	276, 530	493.1390	$C_{23}H_{25}O_{12}$	-6.1	41.2	493.1390 (23), 331.0824 (100)	2	(Goufo et al., 2020)
13	peonidin hexoside	22.6	273, 523	463.1270	$C_{22}H_{23}O_{11}$	-7.6	n.a.	463.1270 (24), 301.0697 (100)	2	(Goufo et al., 2020)
14	epicatechin	23.1	278	289.0658	$C_{15}H_{14}O_6$	0.2	81.5	151.0505 (86), 123.0406 (99), 76.0579 (100)	1	Standard

15	(epi)catechin gallate-(epi)catechin	23.4	279	729.1171	$C_{37}H_{29}O_{16}$	-	-	577.1457 (30), 407.0758 (66), 289.0687 (100), 169.0136 (31), 125.0244 (55)	2	(Escobar-Avello et al., 2019)
16	delphinidin	23.9	273, 521	303.0492	$C_{15}H_{11}O_7$	2.5	7.4	303.0492 (100)	2	(Kelebek et al., 2007)
17	proanthocyanidin dimer III	24.8	279	577.1222	$C_{30}H_{26}O_{12}$	-	-	407.0816 (42), 339.0821 (17), 289.0670 (100), 245.0757 (21), 125.0235 (65)	2	(Goufo et al., 2020)
18	cyanidin	26.7	275, 524	287.0536	$C_{15}H_{11}O_6$	5.1	0.7	287.0536 (100)	1	Standard
19	syringic acid	27.4	279	197.0776	$C_9H_{10}O_5$	-	-	150.0255 (100)	4	(Goufo et al., 2020)
20	petunidin	27.4	273, 532	317.0649	$C_{16}H_{13}O_7$	2.2	5.8	317.0649 (100)	2	(Kelebek et al., 2007)
21	peonidin	28.0	276, 526	301.0692	$C_{16}H_{13}O_6$	4.9	1.2	301.0692 (100)	2	(Kelebek et al., 2007)
22	vanillic acid	28.1	n.d.	167.0335	$C_8H_8O_4$	8.9	6.8	124.0149 (100), 108.0218 (76)	1	Standard
23	(epi)catechin gallate II	28.3	279	441.0682	$C_{22}H_{18}O_{10}$	-	-	289.0672 (37), 245.0749 (20), 169.0161 (100), 125.0252 (32)	2	(Escobar-Avello et al., 2019)
24	*p*-coumaric acid	28.4	n.d.	163.0392	$C_9H_8O_3$	5.0	6.2	119.0479 (100)	1	Standard
25	(epi)catechin gallate III	29.2	n.d.	441.0795	$C_{22}H_{18}O_{10}$	-5.9	25.0	289.0661 (38), 169.0125 (100), 125.0273 (38)	2	(Escobar-Avello et al., 2019)
26	not identified VII	29.7	n.d.	741.1303	-	-	-	589.1071 (18), 437.0711 (40), 301.0672 (100), 169.0159 (95), 125.0205 (90)	-	-
27	*p*-coumaric acid derivative I	30.4	n.d.	347.0756	$C_{17}H_{15}O_8$	4.7	47.5	315.0499 (40), 163.0008 (100), 151.0387 (31), 125.0211 (83)	-	-
28	malvidin	30.6	273, 530	331.0801	$C_{17}H_{15}O_7$	3.3	10.1	331.0801 (100)	2	(Kelebek et al., 2007)
29	gallic acid derivative I	31.0	n.d.	333.0935	$C_{16}H_{14}O_8$	-	-	257.0388 (38), 165.0151 (100), 137.0239 (78)	2	-
30	petunidin-(6-coumaroyl)-3-hexoside	31.2	278, 520	625.1525	$C_{31}H_{29}O_{14}$	4.3	n.a.	625.1525 (24), 317.0658 (100), 136.0826 (17)	2	(Goufo et al., 2020)
31	hydroxybenzoic acid II	31.4	n.d.	137.0219	$C_7H_6O_3$	-	-	n.d.	4	(Escobar-Avello et al., 2019)
32	not identified VIII	31.5	279	605.1218	$C_{28}H_{22}O_8$	3.9	15.9	479.1009 (68), 285.0309 (100), 163.0031 (36)	-	-

33	gallic acid derivative II	31.6	286	333.0980	$C_{16}H_{14}O_8$	-	-	257.0481 (62), 165.0234 (100), 137.0185 (70)	-	-
34	not identified IX	32.4	278	491.0850	$C_{22}H_{20}O_{13}$	-3.9	44.7	491.0850 (6), 300.0270 (100)	-	-
35	taxifolin	32.9	279, 310	303.0494	$C_{15}H_{12}O_7$	5.2	16.0	151.0335 (100), 123.0460 (98)	4	(Goufo et al., 2020)
36	not identified X	33.1	276	317.0649	$C_{16}H_{14}O_7$	5.7	8.4	317.0728 (40), 164.0119 (100), 137.0270 (23)	-	-
37	not identified XIV	34.9	280	621.1316	-	-	-	469.1199 (12), 299.0526 (22), 273.0394 (23), 161.0253 (32), 137.0186 (35), 125.0233 (100)	-	-
38	ferulic acid	35.5	n.d.	193.0497	$C_{10}H_{10}O_4$	4.9	5.0	133.0293 (100)	1	Standard
39	not identified XIX	42.0	n.d.	615.1393	-	-	-	301.0668 (100), 164.0070 (5)	-	-
40	not identified XX	42.5	309	177.0551	$C_{10}H_9O_3$	3.3	14.3	117.0337 (100)	-	-

[a] Numbering according to the ion chromatogram extracted from **Figure 10b**; [b]elution time on a C18 column and solvent: gradient of water and acetonitrile with 0.1% formic acid; [c] maximum value of absorption; [d]mass charge ratio. For anthocyanin and anthocyanidins, the molecules are ionized [M]+; [e]relative intensity (%); [f]according Schymanski et al. (2014).

Table 3. Non-extractable phenolic compounds (NEPC) identified or tentatively in SGP extract by liquid chromatography coupled with electrospray ionization hybrid quadrupole-time of flight mass spectrometry (HPLC-DAD-ESI-QTOF).

Peak[a]	Compound	t_R (min)[b]	λ_{max} (nm)[c]	$[M-H]^-$ or $[M]^+$ (*m/z*)[d]	Molecular formula	Error (ppm)	mSigma	MS/MS (-) (*m/z*)[e]	ID level[f]	Reference
1	gallic acid	11.7	271	169.0136	$C_7H_6O_5$	11.9	69.7	132.8680 (100)	1	Standard
3	protocatechuic acid	14.9	278	153.0177	$C_7H_6O_4$	19.0	n.a.	n.d.	4	(Escobar-Avello et al., 2019)
4	hydroxybenzoic acid I	19.9	280	137.0247	$C_7H_6O_3$	-2.3	16.9	108.0253 (100)	2	(Escobar-Avello et al., 2019)
5	catechin	20.5	278	289.0706	$C_{15}H_{14}O_6$	4.0	10.1	221.0777 (30), 123.0436 (100)	1	Standard
6	methylgallate	20.6	273	183.0291	$C_8H_8O_5$	4.3	18.1	140.0053 (100)	2	(Martini et al., 2021)
7	proanthocyanidin dimer IV	21.3	279	577.1228	$C_{30}H_{26}O_{12}$	-	-	407.0739 (28), 289.0713 (100), 245.0742 (16), 161.0140 (6), 125.0216 (90)	2	(Goufo et al., 2020)
8	proanthocyanidin dimer gallate	22.9	278	729.1387	$C_{44}H_{26}O_{11}$	2.2	93.1	559.1135 (29), 407.0724 (91), 289.0649 (100), 169.0151 (46)	2	(Goufo et al., 2020)
9	epicatechin	23.0	278	289.0688	$C_{15}H_{14}O_6$	10.3	19.8	123.0400 (100)	1	Standard
10	*p*-coumaric acid derivative II	25.0	277, 305	195.0627	-	-	-	163.0375 (100), 108.0187 (81)	-	-
11	proanthocyanidin dimer V	25.6	280	575.1151	$C_{37}H_{20}O_7$	-2.6	55.4	449.0859 (78), 289.0698 (100), 125.0268 (83)	2	(Goufo et al., 2020)
12	cyanidin	26.9	273, 524	287.0547	$C_{15}H_{11}O_6$	1.0	5.9	287.0547 (100)	1	(Kelebek et al., 2007)
13	not identified XXII	27.2	278	411.0711	-	-	-	285.0421 (100), 125.0241 (66)	-	-
14	not identified XXIII	27.6	n.d.	639.2123	-	-	-	221.0564 (100), 167.0416 (14)	-	-
15	vanillic acid	28.0	n.d.	167.0345	$C_8H_8O_4$	2.9	21.1	108.0233 (100)	1	Standard
16	peonidin	28.2	279, 310	301.0707	$C_{16}H_{13}O_6$	-0.1	16.5	301.0707 (100)	2	(Kelebek et al., 2007)
17	(epi)catechin gallate I	28.5	278	441.0767	$C_{22}H_{18}O_{10}$	-	-	289.0619 (40), 245.0739 (18), 169.0153 (100)	2	(Escobar-Avello et al., 2019)

18	gallic acid derivative II	29.6	278	333.0898	$C_{16}H_{14}O_8$	-	-	165.0184 (100)	-	-
19	gallic acid derivative III	30.6	278	333.0890	$C_{16}H_{14}O_8$	-	-	161.0115 (100)	-	-
20	proanthocyanidin dimer VI	31.0	278	575.1146	$C_{37}H_{20}O_7$	-1.7	64.0	449.0922 (60), 289.0735 (57), 285.0373 (100), 125.0230 (64)	2	(Goufo et al., 2020)
21	gallic acid derivative IV	31.3	280	333.0915	$C_{24}H_{14}O_2$	1.8	51.7	180.0379 (27), 165.0185 (79), 137.0221 (100)	-	-
22	not identified XXIV	31.5	286	301.0686	$C_{23}H_{10}O$	-9.0	29.6	164.0108 (100)	-	-
23	not identified XXV	31.7	284	603.1413	-	-	-	301.0702 (100), 179.0372 (10)	-	-
24	not identified XXVIII	33.2	278	317.0642	$C_{16}H_{14}O_7$	7.8	21.9	317.0575 (28), 164.0111 (100)	-	-
25	ferulic acid	35.5	279	193.0466	$C_{10}H_{10}O_4$	-	-	133.0335 (100)	1	Standard

[a] Numbering according to the ion chromatogram extracted from **Figure 10d**; [b]elution time on a C18 column and solvent: gradient of water and acetonitrile with 0.1% formic acid; [c] maximum value of absorption; [d]mass charge ratio. For anthocyanin and anthocyanidins, the molecules are ionized [M]+; [e]relative intensity (%); [f]according Schymanski et al. (2014).

Table 4. Concentration (μg g^{-1}) of each compound from the NEPC of PGP in the different treatments performed in step 1 and 2.

Compound	T90-C0.1	T90-C0.5	T50-C1.0	T65-C1.0	T80-C1.0	T90-C1.0	T50-C8.0	T65-C8.0	T80-C8.0	T90-C8.0	T50-C15.0	T65-C15.0	T80-C15.0	T90-C15.0
gallic acid	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	61 ± 3	51 ± 2
protocatechuic acid	237 ± 16	95 ± 9	94 ± 15	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified I	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	50 ± 11	42 ± 9
proanthocyanidin dimer II	218 ± 4	311 ± 10	256 ± 7	227 ± 37	<LQ	197 ± 35	450 ± 11	587 ± 14	<LQ	<LQ	780 ± 122	591 ± 63	<LQ	<LQ
hydroxybenzoic acid II	<LQ	<LQ	<LQ	<LQ	52 ± 8	<LQ	94 ± 32	91 ± 13	166 ± 34	89 ± 3	<LQ	111 ± 11	212 ± 8	140 ± 25
catechin	391 ± 49	459 ± 23	363 ± 44	576 ± 21	<LQ	483 ± 46	<LQ	188 ± 11	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
methyl gallate	<LQ	<LQ	<LQ.	<LQ	<LQ	<LQ	<LQ	<LQ	233 ± 40	50 ± 1	<LQ	48 ± 12	132 ± 10	212 ± 6
not identified II	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	41 ± 3	92 ± 7	<LQ	<LQ	47 ± 4	118 ± 4	<LQ	<LQ
malvidin hexoside	<LQ.	<LQ	<LQ	49 ± 3	<LQ.	53 ± 1	68 ± 3	55 ± 14	53 ± 7	<LQ	62 ± 1	51 ± 0	<LQ	<LQ
peonidin hexoside	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	68 ± 3	<LQ	<LQ	<LQ	69 ± 2	<LQ
epicatechin	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	262 ± 91
delphinidin	<LQ.	63 ± 4	<LQ	75 ± 9	73 ± 13	99 ± 14	178 ± 16	619 ± 71	319 ± 29	259 ± 26	138 ± 22	447 ± 94	336 ± 8	432 ± 23
not identified III	<LQ	59 ± 22	<LQ	109 ± 25	37 ± 17	37 ± 6	81 ± 33	94 ± 42	<LQ	<LQ	55 ± 16	111 ± 21	<LQ	<LQ
cyanidin	<LQ	309 ± 28	61 ± 4	398 ± 58	372 ± 83	548 ± 92	746 ± 58	**2926 ± 310**	1770 ± 170	1661 ± 116	766 ± 113	2466 ± 276	1735 ± 44	1574 ± 94
syringic acid	119 ± 19	118 ± 22	108 ± 3	133 ± 14	33 ± 6	55 ± 2	<LQ	<LQ	179 ± 8	35 ± 1	<LQ	<LQ	<LQ	<LQ
petunidin	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	61 ± 12	<LQ	<LQ	<LQ	<LQ	<LQ	97 ± 3
vanillic acid	106 ± 6	116 ± 1	157 ± 1	118 ± 9	<LQ	119 ± 4	126 ± 1	142 ± 9	127 ± 23	140 ± 9	126 ± 5	126 ± 5	102 ± 10	86 ± 2
(epi)catechin gallate II	181 ± 19	163 ± 5	471 ± 0	165 ± 17	198 ± 26	155 ± 10	<LQ	72 ± 3	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified VI	<LQ	<LQ	119 ± 7	72 ± 2	<LQ	<LQ	31 ± 5	101 ± 13	<LQ	<LQ	<LQ	99 ± 26	<LQ	<LQ
epicatechin gallate III	153 ± 20	106 ± 6	45 ± 7	293 ± 87	108 ± 10	54 ± 3	98 ± 51	227 ± 35	190 ± 50	<LQ	<LQ	<LQ	<LQ	<LQ
not identified VII	82 ± 16	189 ± 11	<LQ	67 ± 21	<LQ	221 ± 4	<LQ	<LQ	<LQ	191 ± 19	<LQ	50 ± 3	36 ± 8	<LQ
p-coumaric acid derivative I	<LQ	<LQ	230 ± 17	958 ± 55	<LQ	<LQ	352 ± 127	43 ± 8	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

Compound	T90-C0.1	T90-C0.5	T50-C1.0	T65-C1.0	T80-C1.0	T90-C1.0	T50-C8.0	T65-C8.0	T80-C8.0	T90-C8.0	T50-C15.0	T65-C15.0	T80-C15.0	T90-C15.0
gallic acid derivative I	205 ± 29	694 ± 114	<LQ	<LQ	<LQ	663 ± 67	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
malvidin	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	161 ± 33	<LQ	112 ± 19	675 ± 72	1015 ± 13
hydroxybenzoic acid II	<LQ	<LQ	<LQ	333 ± 91	<LQ	<LQ	72 ± 15	864 ± 117	1456 ± 249	<LQ	<LQ	<LQ	<LQ	<LQ
not identified VIII	<LQ	<LQ	25 ± 4	<LQ	242 ± 83	<LQ	240 ± 20	437 ± 55	<LQ	<LQ	234 ± 37	331 ± 80	554 ± 59	<LQ
gallic acid derivative II	39 ± 10	343 ± 22	<LQ	<LQ	<LQ	488 ± 58	<LQ	<LQ	<LQ	1601 ± 161	<LQ	384 ± 18	<LQ	656 ± 69
not identified IX	<LQ	<LQ	59 ± 2	<LQ	<LQ	<LQ	51 ± 2	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
taxifolin	<LQ	<LQ	<LQ	87 ± 7	49 ± 28	67 ± 3	<LQ	135 ± 10	189 ± 30	<LQ	<LQ	<LQ	51 ± 18	<LQ
not identified X	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	130 ± 55	85 ± 29	175 ± 5	92 ± 9	<LQ	<LQ	<LQ	<LQ
not identified XI	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	66 ± 48	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XII	<LQ	67 ± 9	480 ± 64	2263 ± 339	147 ± 72	<LQ	845 ± 355	251 ± 132	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XIII	861 ± 114	1813 ± 88	<LQ	<LQ	325 ± 271	1992 ± 41	<LQ	<LQ	<LQ	339 ± 33	<LQ	<LQ	<LQ	<LQ
not identified XIV	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	44 ± 16	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
ferulic acid	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	66 ± 7	50 ± 6	<LQ	40 ± 5	94 ± 4	92 ± 4
not identified XV	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	63 ± 15	67 ± 10	38 ± 18	<LQ	<LQ	<LQ	45 ± 10	46 ± 9
not identified XVI	<LQ	<LQ	<LQ	54 ± 3	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XVII	<LQ	40 ± 4	<LQ	<LQ	<LQ	32 ± 4	<LQ	37 ± 5	31 ± 2	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XVIII	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	42 ± 9	55 ± 5
not identified XIX	<LQ	<LQ	<LQ	45 ± 7	82 ± 16	<LQ	95 ± 4	378 ± 28	531 ± 46	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XX	<LQ	<LQ	<LQ	<LQ	<LQ	63 ± 6	<LQ	<LQ	<LQ	457 ± 42	124 ± 10	312 ± 19	552 ± 26	550 ± 13

<LQ: Below limit of quantification. The limits of detection were between 0.03-0.64 and the limits of quantification between 0.08-1.95 (**Supplementary Table S1**).

Table 5. Concentration (μg g^{-1}) of each compound from the NEPC of SGP in the different treatments performed in step 1 and 2.

Compound	T90-C0.1	T90-C0.5	T50-C1.0	T65-C1.0	T80-C1.0	T90-C1.0	T50-C8.0	T65-C8.0	T80-C8.0	T90-C8.0	T50-C15.0	T65-C15.0	T80-C15.0	T90-C15.0
gallic acid	26 ± 2	29 ± 1	<LQ	32 ± 2	40 ± 4	43 ± 1	<LQ	56 ± 1	67 ± 0	51 ± 2	32 ± 1	54 ± 2	56 ± 2	49 ± 2
not identified XXI	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	37 ± 7	59 ± 5
protocatechuic acid	86 ± 6	<LQ	39 ± 5	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
hydroxybenzoic acid I	<LQ	<LQ	<LQ	28.03 ± 2.27	<LQ	<LQ	<LQ	45 ± 5	140 ± 23	133 ± 7	32 ± 2	202 ± 12	633 ± 54	<LQ
catechin	1163 ± 89	913 ± 41	305 ± 30	296 ± 20	944 ± 323	801 ± 105	293 ± 191	350 ± 99	365 ± 29	<LQ	<LQ	<LQ	<LQ	<LQ
methylgallate	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	73 ± 2	<LQ	63 ± 2	170 ± 14	176 ± 3
proanthocyanidin dimer IV	<LQ	<LQ	124 ± 17	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
epicatechin	1157 ± 82	577 ± 46	344 ± 22	295 ± 18	785 ± 282	706 ± 90	181 ± 120	196 ± 90	160 ± 12	<LQ	<LQ	<LQ	<LQ	<LQ
p-coumaric acid derivative II	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	59 ± 13	99 ± 13
proanthocyanidin dimer V	<LQ	<LQ	56 ± 17	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
cyanidin	<LQ	153 ± 3	<LQ	156 ± 10	292 ± 17	226 ± 22	308 ± 62	**1055 ± 94**	875 ± 129	605 ± 44	390 ± 29	721 ± 76	667 ± 27	469 ± 26
not identified XXII	<LQ	31 ± 1	20 ± 5	35 ± 2	22 ± 10	29 ± 0	<LQ	41 ± 8	59 ± 29	33 ± 2	<LQ	<LQ	31 ± 4	60 ± 6
not identified XXIII	<LQ	<LQ	<LQ	<LQ	32 ± 13	<LQ	62 ± 7	59 ± 8	57 ± 7	<LQ	<LQ	158 ± 39	82 ± 3	63 ± 21
peonidin	<LQ	<LQ	<LQ	320 ± 22	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
vanillic acid	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	113 ± 2
(epi)catechin gallate I	237 ± 13	256 ± 4	174 ± 5	367 ± 16	461 ± 111	323 ± 20	578 ± 51	474 ± 5	607 ± 54	154 ± 8	253 ± 37	564 ± 18	**1439 ± 143**	<LQ
gallic acid derivative II	108 ± 5	108 ± 4	105 ± 7	121 ± 0	62 ± 10	91 ± 12	<LQ	69 ± 6	37 ± 4	95 ± 13	<LQ	<LQ	<LQ	349 ± 37

Compound	T90-C0.1	T90-C0.5	T50-C1.0	T65-C1.0	T80-C1.0	T90-C1.0	T50-C8.0	T65-C8.0	T80-C8.0	T90-C8.0	T50-C15.0	T65-C15.0	T80-C15.0	T90-C15.0
gallic acid derivative III	67 ± 5	179 ± 6	<LQ	43 ± 3	189 ± 40	193 ± 38	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
proanthocyanidin dimer VI	<LQ	<LQ	<LQ	87 ± 2	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	44 ± 4	<LQ	<LQ	<LQ
gallic acid derivative IV	<LQ	<LQ	<LQ	<LQ	210 ± 33	<LQ	712 ± 9	189 ± 24	358 ± 28	<LQ	<LQ	<LQ	29 ± 1	<LQ
not identified XXIV	<LQ	<LQ	<LQ	85 ± 12	<LQ	<LQ	<LQ	100 ± 42	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XXV	<LQ	101 ± 9	<LQ	<LQ	56 ± 14	184 ± 39	24 ± 3	<LQ	<LQ	300 ± 8	108 ± 18	112 ± 10	<LQ	<LQ
not identified XXVI	53 ± 1	56 ± 1	60 ± 1	59 ± 1	<LQ	55 ± 4	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	104 ± 16	<LQ
not identified XXVII	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	58 ± 1	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	149 ± 20
not identified XXVIII	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	144 ± 2	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XXIX	302 ± 26	595 ± 69	<LQ	<LQ	616 ± 18	579 ± 145	<LQ	68 ± 32	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
not identified XXX	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	67 ± 8	<LQ
not identified XXXI	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	100 ± 17
not identified XXXII	<LQ	<LQ	<LQ	<LQ	23 ± 1	<LQ	<LQ	<LQ	<LQ	49 ± 3	<LQ	43 ± 4	58 ± 2	54 ± 3

<LQ: Below limit of quantification. The limits of detection were between 0.03-0.64 and the limits of quantification between 0.08-1.95 (**Supplementary Table S1**).

5.1.4. Conclusion

This study evaluated the effect of hydrolysis conditions (acid concentration, temperature, and time) on the NEPC composition of grape peel and seed powder. Most of these compounds are different from those found in the EPC fraction. Thus, probably part of the health benefits reported for grape peel and seed can be related to these ignored phenolic compounds. Results showed that intermediate values of acid concentrations and temperatures are associated with a higher concentration of NEPC from grape peel and seed powders. These conditions promote a balance between release/extraction and degradation, maximizing NEPC content in the extracts. Evaluating each class of NEPC separately for both matrices, it was observed that these mild conditions were able to extract large concentrations of condensed tannins, which is the major class of these compounds in both matrices. Moreover, results indicate that hydrolysis conditions can be modulated to obtain extracts rich in a specific class. Hydrolysable tannins and hydroxybenzoic acids, for example, were better extracted using higher acid concentrations and higher temperatures. Condensed tannins, on the other hand, showed higher concentrations when intermediate values of temperature and acid concentration were applied. The longer hydrolysis time (20 min) increased 1.2 times total NEPC concentration for the grape peel powder, while for the grape seed powder the shortest time (5 min) was enough to release them from the food matrix. For grape peel powder, 8.0 % of HCl concentration, 65 °C and 20 min were the best hydrolysis conditions. For grape seed powder, the best conditions were 1.0 % of HCl concentration at 80 °C for 5 min. The results of this work demonstrate that the NEPC fraction can be obtained from the studied matrices with the application of milder hydrolysis conditions, with less NEPC degradation. Overall, the results highlight that NEPC concentration and composition are directly related to the hydrolysis conditions and the matrix characteristics.

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Supplementary Material

Preparation of Analytical Curves

PC were quantified by external standardization using curves of thirteen analytical standards. For eleven of them, twelve concentrations points, ranging from 0.047 to 96 mg L^{-1}, were used to obtain the analytical curves. For ellagic acid, the curve was constructed using ten concentrations, from 0.047 to 24 mg L^{-1}. For cyanidin 3-O-glucoside chloride, nine concentrations between 0.156 and 25 mg L^{-1} were used.

Supplementary Table S1. Parameters of the analytical curves of phenolic standards obtained by HPLC-DAD-ESI-QTOF.

Standard	Inclination	Intercept	LD[a] (mg L^{-1})	LQ[b] (mg L^{-1})	R^{2c}
Gallic acid	97266	3887.9	0.05	0.15	0.99
Chlorogenic acid	89881	32854	0.13	0.40	0.99
Catechin	21559	1931.3	0.07	0.20	0.99
Epicatechin	22728	2363.1	0.16	0.49	0.99
Vanillic acid	64384	11603	0.03	0.08	0.99
Caffeic acid	167223	9699.33	0.05	0.14	0.99
p-coumaric acid	201000	22899	0.05	0.15	0.99
Ellagic acid	143636	30872	0.30	0.90	0.99
Ferulic acid	168216	3071.3	0.06	0.19	0.99
Quercetin	113536,24	157893.10	0.64	1.95	0.99
Naringenin	100180,50	6235.49	0.05	0.15	0.99
Apigenin	143975	63815	0.60	1.82	0.99
Cyanidin 3-O-glucoside	117148	21745	0.09	0.28	0.99

[a]LD: limit of detection; [b]LQ: limit of quantification; [c]R^2: determination coefficient.

Table S2. Extractable phenolic compounds (EPC) identified or tentatively identified in PGP extract by liquid chromatography coupled with electrospray ionization hybrid quadrupole-time of flight mass spectrometry (HPLC-ESI-q-TOF-MS).

Peak[a]	Compound	t_R (min)[b]	λ_{max} (nm)[c]	$[M-H]^-$ or $[M]^+$ (*m/z*)[d]	Molecular formula	Error (ppm)	mSigma	MS/MS (-) (*m/z*)[e]	ID level[f]	Reference
1	galloyl hexoside I	10.2	n.d.	331.0687	$C_{13}H_{16}O_{10}$	-5.0	n.a.	n.d.	2	(Martini et al., 2021)
2	galloyl hexoside II	11.1	n.d.	331.0673	$C_{13}H_{16}O_{10}$	-0.8	n.a.	n.d.	2	(Martini et al., 2021)
3	gallic acid	11.7	271	169.0135	$C_7H_6O_5$	4.7	n.a.	125.0282 (100)	1	Standard
4	galloyl hexoside III	12.0	n.d.	331.0653	$C_{13}H_{16}O_{10}$	5.2	n.a.	n.d.	2	(Martini et al., 2021)
5	galloyl hexoside V	14.3	n.d.	331.0659	$C_{13}H_{16}O_{10}$	3.5	18.1	169.0128 (100), 125.0258 (77)	2	(Martini et al., 2021)
1	petunidin dihexoside	16.3	270, 520	641.1654	$C_{28}H_{33}O_{17}$	6.0	157.3	641.1654 (65), 479.1174 (100), 317.0616 (75)	2	(Cheng et al., 2018)
6	caftaric acid	17.1	297, 327	311.0405	$C_{13}H_{12}O_9$	1.0	19.7	179.0342 (23), 149.0121 (17), 135.0450 (100)	2	(Goufo et al., 2020)
2	malvidin dihexoside	17.9	270, 520	655.1923	$C_{29}H_{35}O_{17}$	-	-	655.1923 (98), 493.1339 (100), 331.0744 (55)	2	(Cheng et al., 2018)
3	peonidin dihexoside	18.0	276, 520	625.1799	$C_{28}H_{33}O_{16}$	-	-	625.1799 (86), 463.1197 (100), 301.0733 (58)	2	(Cheng et al., 2018)
4	delphinidin hexoside	18.7	276, 520	465.0973	$C_{21}H_{21}O_{12}$	1.9	26.6	465.1181 (11), 303.0441 (100)	2	(Cheng et al., 2018)
7	caffeic acid-O-hexoside	19.7	288, 323	341.0886	$C_{15}H_{18}O_9$	-2.5	19.0	221.0404 (88), 179.0435 (64), 161.0163 (93), 135.0381 (100)	2	(Goufo et al., 2020)
8	catechin	20.3	278	289.0667	$C_{15}H_{14}O_6$	-3.0	22.3	123.0496 (91), 109.0349 (100)	1	Standard
5	cyanidin hexoside	20.5	280, 310, 520	449.1009	$C_{21}H_{21}O_{11}$	-	-	449.1009 (15), 287.0540 (100)	1	(Pérez-Ramírez et al., 2018)

6	petunidin hexoside	20.5	280, 310, 520	479.1205	$C_{22}H_{23}O_{12}$	-4.3	n.a.	479.1205 (17), 396.2243 (6), 317.0670 (100)	2	(Cheng et al., 2018)
9	proanthocyanidin dimer IV	21.7	294, 310	577.1292	$C_{30}H_{26}O_{12}$	-	-	407.0778 (54), 289.0755 (100), 245.0786 (24), 161.0243 (21), 125.0213 (38)	2	(Goufo et al., 2020)
10	*p*-coumaric acid hexoside	21.8	290, 310	325.0911	$C_{15}H_{18}O_8$	5.5	17.1	205.0466 (11), 163.0297 (25), 145.0248 (100), 119.0478 (27)	2	(Goufo et al., 2020)
7	malvidin hexoside	22.2	278, 520	493.1417	$C_{23}H_{25}O_{12}$	-	-	493.1417 (13), 331.0778 (100)	2	(Cheng et al., 2018)
8	malvidin-3-O-(6-O-acetyl)-dihexoside	22.2	279, 520	697.1935	$C_{31}H_{37}O_{18}$	2.0	17.6	697.1935 (76), 535.1450 (100), 331.0746 (50)	2	(Cheng et al., 2018)
9	peonidin hexoside	22.4	278, 520	463.1234	$C_{22}H_{23}O_{11}$	0.2	27.3	463.1254 (26), 301.0707 (100)	2	(Cheng et al., 2018)
11	epicatechin	23.1	278	289.0724	$C_{15}H_{14}O_6$	-2.0	20.3	151.0377 (49), 123.0495 (89), 109.0304 (100)	1	Standard
12	caffeic acid	23.3	279	179.0367	$C_9H_8O_4$	-9.4	20.9	135.0381 (100)	1	Standard
10	delphinidin-3-(6-O-*p*-coumaroyl),5-O-dihexoside	25.3	279, 310, 530	773.2039	-	-	-	773.2039 (43), 611.1242 (79), 465.0978 (48), 303.0425 (100)	2	(Cheng et al., 2018)
11	petunidin-3-(6-O-*p*-coumaroyl),5-O-dihexoside	26.6	280, 310, 526	787.1949	-	-	-	787.1949 (100), 625.1654 (64), 479.1062 (26), 317.0612 (64)	2	(Cheng et al., 2018)
12	cyanidin-3-(6-O-*p*-coumaroyl),5-O-dihexoside	26.9	280, 310, 530	757.1942	-	-	-	757.1942 (98), 595.1459 (30), 449.1068 (34), 287.0561 (100)	2	(Cheng et al., 2018)
13	malvidin-3-(6-O-*p*-coumaryl),5-O-dihexoside	27.4	282, 310, 530	801.2181	-	-	-	801.2181 (74), 639.1799 (44), 493.1357 (100), 331.0880 (64)	2	(Cheng et al., 2018)
14	peonidin-3-O-(6-O-*p*-coumaryl)-5-O-dihexoside	28.5	282, 310, 530	771.2056	-	-	-	771.2056 (82), 609.1684 (100), 463.1096 (92), 301.0626 (88)	2	(Dal Magro et al., 2016)
15	delphinidin-3-O-(6-O-*p*-coumaroyl)-hexoside	29.2	280, 310, 530	611.1347	$C_{30}H_{27}O_{14}$	-	-	611.1347 (36), 303.0487 (100)	2	(Pérez-Ramírez et al., 2018)
16	petunidin-3-O-(6-O-*p*-coumaroyl)-hexoside	30.1	280, 310, 530	625.1405	$C_{31}H_{29}O_{14}$	-	-	625.1405 (20), 317.0695 (100)	2	(Pérez-Ramírez et al., 2018)
18	cyanidin-3-O-(6-O-*p*-coumaroyl)-hexoside	30.8	280, 310, 530	595.1523	$C_{30}H_{27}O_{13}$	-	-	595.1523 (29), 287.0518 (100)	2	(Dal Magro et al., 2016)
19	malvidin-3-O-(6-O-*p*-coumaroyl)-hexoside	32.0	280, 310, 530	639.1646	$C_{23}H_{13}O_{14}$	-	-	639.1646 (52), 331.0776 (100)	2	(Cheng et al., 2018)

20	peonidin-3-O-(6-O-*p*-coumaroyl)-hexoside	32.7	283, 310, 531	609.1528	$C_{31}H_{29}O_{13}$	2.7	n.a.	609.1528 (43), 301.0717 (100)	2	(Cheng et al., 2018)
21	quercetin	40.2	280	301.0357	$C_{15}H_{10}O_7$	-0.9	28.3	151.0051 (100), 121.0278 (40)	1	Standard

[a] Numbering according to the ion chromatogram extracted from **Figure 10a**; [b]elution time on a C18 column and solvent: gradient of water and acetonitrile with 0.1% formic acid; [c] maximum value of absorption; [d]mass charge ratio; for anthocyanin and anthocyanidins, the molecules are ionized [M]+; [e]relative intensity (%); [f]according to Schymanski et al. (2014).

Table S3. Extractable phenolic compounds (EPC) identified or tentatively identified in SGP extract by liquid chromatography coupled with electrospray ionization hybrid quadrupole-time of flight mass spectrometry (HPLC-ESI-q-TOF-MS).

Peak[a]	Compound	t_R (min)[b]	λ_{max} (nm)[c]	[M-H]$^-$ (*m/z*)[d]	Molecular formula	Error (ppm)	mSigma	MS/MS (-) (*m/z*)[e]	ID level[f]	Reference
1	galloyl hexoside II	11.0	n.d.	331.0674	$C_{13}H_{16}O_{10}$	-1.1	n.a.	n.d.	2	(Martini et al., 2021)
2	gallic acid	11.8	271	169.0138	$C_7H_6O_5$	2.8	4.1	125.0270 (100)	1	Standard
3	galloyl hexoside IV	13.8	279	331.0676	$C_{13}H_{16}O_{10}$	-1.7	24.0	n.d.	2	(Martini et al., 2021)
4	galloyl hexoside V	14.2	n.d.	331.0666	$C_{13}H_{16}O_{10}$	1.3	8.3	169.0125 (91), 125.0237 (100)	2	(Martini et al., 2021)
5	proanthocyanidin dimer II	18.5	279	577.1337	$C_{30}H_{26}O_{12}$	2.6	n.a.	407.0698 (60), 339.0882 (11), 289.0708 (100), 245.0795 (20), 125.0236 (45)	2	(Goufo et al., 2020)
6	catechin	20.2	279	289.0718	$C_{15}H_{14}O_6$	-0.2	n.a.	203.0715 (40), 151.0470 (33), 137.0225 (80), 123.0442 (100), 109.0315 (88)	1	Standard
7	(epi)gallocatechin	20.7	279	305.0659	$C_{15}H_{14}O_7$	-	-	137.0141 (100), 125.0272 (87)	2	(Goufo et al., 2020)
8	proanthocyanidin dimer IV	21.2	279	577.1188	$C_{30}H_{26}O_{12}$	-	-	407.0865 (67), 339.0768 (13), 289.0740 (100), 245.0668 (17), 161.0274 (25), 125.0220 (54)	2	(Goufo et al., 2020)
9	epicatechin	23.0	279	289.0715	$C_{15}H_{14}O_6$	1.0	-	203.0762 (23), 151.0468 (42), 137.0224 (39), 123.0461 (87), 109.0304 (100)	1	Standard
10	proanthocyanidin gallate	23.4	279	729.1462	-	-	-	577.1216 (34), 433.0972 (41), 407.0820 (100), 289.0704 (95), 169.0092 (42), 125.0184 (43)	2	(Goufo et al., 2020)
11	procyanidin trimer	23.9	279	865.1872	$C_{45}H_{38}O_{18}$	-	-	695.1245 (15), 577.1392 (29), 407.0791 (69), 289.0737 (96), 261.0411 (29), 161.0226 (30), 125.0230 (100)	2	(Escobar-Avello et al., 2019)
12	proanthocyanidin dimer III	24.5	279	577.1334	$C_{30}H_{26}O_{12}$	-	-	289.0816 (100), 287.0395 (31), 151.0364 (23), 125.0223 (58)	2	(Goufo et al., 2020)
13	(epi)catechin gallate I	28.4	279	441.0818	$C_{22}H_{18}O_{10}$	-	-	289.0596 (29), 169.0109 (100), 125.0269 (58)	2	(Escobar-Avello et al., 2019)

19	proanthocyanidin dimer VII	29.7	279	577.1153	$C_{30}H_{26}O_{12}$	-	-	289.0630 (100), 245.0741 (22), 125.0216 (32)	2	(Goufo et al., 2020)

[a] Numbering according to the ion chromatogram extracted from **Figure 10c**; [b]elution time on a C18 column and solvent: gradient of water and acetonitrile with 0.1% formic acid; [c] maximum value of absorption; [d]mass charge ratio; [e]relative intensity (%); [f] according to Schymanski et al. (2014).

Table S4. NEPC concentration by classes of PGP and SGP extracts (μg g^{-1}) in the different treatments performed in step 1 and 2.

Source	Class of compounds	T50-C1.0	T50-C8.0	T50-C15.0	T65-C1.0	T65-C8.0	T65-C15.0	T80-C1.0	T80-C8.0	T80-C15.0	T90-C0.1	T90-C0.5	T90-C1.0	T90-C8.0	T90-C15.0
PGP	Hydrolysable tannins	<LQ	<LQ	<LQ	<LQ	<LQ	49 ± 12 ^{C}	<LQ	233 ± 40 ^{A}	193 ± 13 ^{B}	<LQ	<LQ	<LQ	50 ± 1 ^{C}	264 ± 4 ^{A}
	Hydroxybenzoic acids	359 ± 12 $^{F, G, H}$	292 ± 46 ^{H}	126 ± 5 ^{H}	583 ± 72 $^{E, F, G}$	1098 ± 125 $^{B, C}$	621 ± 18 $^{D, E, F}$	85 ± 2 ^{H}	1928 ± 227 ^{A}	314 ± 18 $^{G, H}$	707 ± 62 $^{D, E}$	1364 ± 111 ^{B}	1325 ± 99 ^{B}	1865 ± 152 ^{A}	882 ± 43 $^{C, D}$
	Condensed tannins	1196 ± 44 $^{F, G, H}$	1472 ± 114 $^{F, G}$	1685 ± 24 $^{E, F}$	1734 ± 121 $^{E, F}$	4680 ± 409 ^{A}	3617 ± 324 ^{B}	750 ± 72 ^{H}	2279 ± 233 $^{C, D}$	2747 ± 100 ^{C}	943 ± 88 $^{G, H}$	1411 ± 23 $^{F, G}$	1536 ± 82 ^{F}	2082 ± 169 $^{D, E}$	3380 ± 206 ^{B}
	Hydroxycinnamic acids	<LQ	<LQ	<LQ	<LQ	<LQ	40 ± 5 ^{C}	<LQ	66 ± 7 ^{B}	94 ± 4 ^{A}	<LQ	<LQ	<LQ	50 ± 6 ^{C}	92 ± 4 ^{A}
	Anthocyanins	<LQ	68 ± 3 $^{b, c}$	62 ± 1 $^{b, c, d}$	49 ± 3 ^{d}	55 ± 14 $^{c, d}$	51 ± 0 ^{d}	<LQ	**121 ± 8** ^{a}	69 ± 2 ^{b}	<LQ	<LQ	53 ± 1 ^{d}	<LQ	<LQ
SGP	Hydrolysable tannins	<LQ	<LQ	32 ± 1 $^{d, e, f}$	32 ± 2 $^{e, f}$	56 ± 1 ^{c}	117 ± 4 ^{b}	40 ± 4 $^{d, e}$	67 ± 0 ^{c}	227 ± 12 ^{a}	26 ± 2 ^{f}	29 ± 1 $^{e, f}$	43 ± 1 ^{d}	124 ± 1 ^{b}	225 ± 2 ^{a}
	Hydroxybenzoic acids	144 ± 7 $^{f, g}$	72 ± 9 $^{g, h}$	32 ± 2 ^{h}	193 ± 4 $^{e, f}$	303 ± 27 ^{c}	203 ± 12 $^{d, e, f}$	461 ± 58 ^{b}	535 ± 32 ^{b}	662 ± 55 ^{a}	261 ± 13 $^{c, d, e}$	288 ± 10 $^{c, d}$	284 ± 49 $^{c, d}$	228 ± 19 $^{c, d, e, f}$	462 ± 39 ^{b}
	Condensed tannins	1003 ± 68 $^{e, f, g}$	1361 ± 339 $^{c, d, e, f}$	687 ± 65 $^{f, g}$	1521 ± 69 $^{b, c, d, e}$	2075 ± 258 $^{a, b, c}$	1285 ± 76 $^{d, e, f}$	2482 ± 702 ^{a}	2008 ± 162 $^{a, b, c, d}$	2106 ± 141 $^{a, b}$	2557 ± 183 ^{a}	1899 ± 90 $^{a, b, c, d}$	2056 ± 223 $^{a, b, c}$	760 ± 39 $^{f, g}$	469 ± 26 ^{g}
	Hydroxycinnamic acids	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	Anthocyanins	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Results are the average values of three independent experiments (values are shown as mean ± SD). Means with the same letters in the same line are not significantly different ($p > 0.05$). <LQ: Below limit of quantification. The limits of quantification were between 0.08-1.95 (**Supplementary Table S1**). n.d.: not detected.

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6 CONCLUSÕES

O presente trabalho teve como objetivo estudar métodos de extração de compostos fenólicos não extraíveis (CFNE) e avaliar o efeito de diferentes condições de hidrólise ácida da fração CFNE em casca e semente de uva em pó, propondo modificações no método que permitam a identificação e quantificação dos compostos com o menor grau de degradação possível.

A revisão de literatura indicou que CFNE são uma fração importante de PC na matriz alimentar. Para sua extração, é fundamental garantir a remoção completa dos fenólicos livres, que podem ser extraídos por meio de solventes, como o metanol. Executar a quantificação e identificação sem esta etapa inicial superestima o conteúdo de NEPC. Essa superestimação gera resultados errôneos e dificulta um entendimento quantitativo e qualitativo completo da fração de NEPC.

Outro ponto a ser destacado é que não existe um método adequado para extração de CFNE. Atualmente, o método mais aplicado para extração de CFNE é a hidrólise química, que pode ser ácida ou básica. Essa técnica pode gerar grandes quantidades de resíduos e degradação dos compostos. Essas mudanças estruturais durante a extração dificultam a correta obtenção desses compostos, uma vez que os compostos fenólicos obtidos serão, em sua maioria, derivados de ácidos fenólicos e outros produtos de degradação. Além disso, a falta de padronização do método pode levar a resultados diferentes. Os métodos devem ser padronizados de acordo com o objetivo do estudo e a composição da matriz alimentar. As condições operacionais, como concentração de ácido ou base, temperatura, tempo, entre outras, precisam ser otimizadas para reduzir as perdas durante a hidrólise e extração.

Os métodos de quantificação de compostos fenólicos devem ser capazes de considerar as classes de compostos analisados, bem como os compostos individuais. HPLC-MS é uma boa alternativa para quantificação desses compostos, pois permite a identificação e quantificação de compostos individuais com erros reduzidos. Metodologias como essas devem ser amplamente desenvolvidas em relação a sistematização da análise de dados resultantes, ainda pouco explorada na área dos alimentos. A construção de bibliotecas, hoje praticamente inexistentes, bem como o uso de *softwares* para auxílio na identificação, como abordado neste trabalho, são etapas importantes que devem ser consideradas.

No estudo experimental, o conteúdo de CFNE foi afetado pela concentração de HCl e pela temperatura. O uso de concentrações de 1,0 e 8,0 % de ácido clorídrico (*v/v*), consideradas baixas, bem como o uso de menores temperaturas (65 e 80 °C) e tempos de hidrólise reduzidos

(5 e 20 min), foram capazes de obter maiores concentrações e um conjunto maior de compostos, em relação as condições mais agressivas testadas e encontradas na literatura. Essas condições promovem um equilíbrio entre a liberação/extração e degradação, maximizando o conteúdo de CFNE nos extratos. Nessas condições, foi extraído o maior conteúdo de NEPC, que representa 39 e 22 % (*m/m*) do PC total para casca e semente de uva em pó, respectivamente.

Avaliando cada classe de CFNE separadamente para as duas matrizes, observou-se que essas condições amenas foram capazes de extrair grandes concentrações de taninos condensados, que é a principal classe desses compostos em ambas as matrizes. Além disso, os resultados indicam que as condições de hidrólise podem ser moduladas para a obtenção de extratos ricos em uma classe específica. Taninos hidrolisáveis e ácidos hidroxibenzóicos, por exemplo, foram melhor extraídos usando concentrações de ácido mais altas e temperaturas mais altas. Já os taninos condensados apresentaram maiores concentrações quando se aplicaram os valores intermediários de temperatura e concentração de ácido.

Para a casca da uva em pó, 8,0 % da concentração de HCl, 65 °C e 20 min foram as melhores condições de hidrólise. Para o pó de semente de uva, as melhores condições foram 1,0 % da concentração de HCL a 80 °C por 5 min. Os resultados deste trabalho demonstram que a fração CFNE pode ser obtida a partir das matrizes estudadas com a aplicação de condições de hidrólise mais brandas, com menor degradação. Os resultados permitiram concluir que o método de hidrólise ácida deve ser otimizado de acordo com o objetivo do estudo, como a classe de CFNE de interesse, e a composição da matriz alimentar. A falta de otimização e padronização pode levar a resultados diferentes. A melhor condição será alcançada quando ocorrer um equilíbrio entre a liberação/extração e a degradação, que é dependente das condições de hidrólise.

Diante do exposto, algumas perspectivas foram pensadas ao longo do desenvolvimento desta pesquisa, como a aplicação de metodologias alternativas de extração dos CFNE, como o uso de tecnologias enzimáticas e emergentes. Também, entender a bioacessibilidade *in vitro* e *in vivo* dos CFNE, efeitos na microbiota intestinal, determinação do percentual de absorção e o comportamento durante a digestão gastrointestinal, é fundamental para completa compreensão destes compostos para a saúde humana.

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APÊNDICE A - Curvas Analíticas e Parâmetros MS

O método de HPLC utilizado para a quantificação dos compostos fenólicos apresentou boa linearidade ($R^2 > 0,98$) nas faixas de concentração estudadas e baixos valores limites de detecção e quantificação (Tabela S1, Figuras S1-S4).

Tabela S1. Dados obtidos das curvas analíticas de padrões fenólicos obtidos HPLC.

Padrão	Inclinação	Intercepto	LD[a] (mg L^{-1})	LQ[b] (mg L^{-1})	$R^{2,c}$
Ácido Gálico	97266	3887,9	0,05	0,15	0,99
Ácido Clorogênico	89881	32854	0,13	0,40	0,99
Catequina	21559	1931,3	0,07	0,20	0,99
Epicatequina	22728	2363,1	0,16	0,49	0,99
Ácido Vanílico	64384	11603	0,03	0,08	0,99
Ácido Cafeico	167223	9699,33	0,05	0,14	0,99
Ácido *p*-cumárico	201000	22899	0,05	0,15	0,99
Ácido Elágico	143636	30872	0,30	0,90	0,99
Ácido Ferúlico	168216	3071,3	0,06	0,19	0,99
Quercetina	113536,24	157893,10	0,64	1,95	0,99
Naringenina	100180,50	6235,49	0,05	0,15	0,99
Apigenina	143975	63815	0,60	1,82	0,99
Cianidina 3-O-glicosídeo	117148	21745	0,09	0,28	0,99

[a]Limite de detecção (LD), [b]Limite de quantificação (LQ), [c]Coeficiente de determinação.

Entre os parâmetros testados no MS, a fragmentação foi avaliada, permitindo a construção da Tabela S2. A energia de colisão e fragmentos dos padrões de compostos fenólicos utilizados como referência foram avaliados no modo negativo e no modo positivo. Na Tabela S2, os resultados da otimização destes compostos estão apresentados no modo negativo, com exceção da cianidina (modo positivo). Além disso, a otimização foi realizada com os padrões em 20 mg L^{-1} em MeOH, utilizando a estratégia de 20 % de íons percursores.

Tabela S2. Otimização da fragmentação dos padrões.

Composto	Colisão	Fragmentos MS^2 (-) (*m/z*)
Ácido Gálico	26,2	125,0253
Ácido Clorogênico	26,2	191,0558
Catequina	29,2	109,0294/203,0701
Epicatequina	29,2	109,0294/203,0699
Ácido Vanílico	23,2	108,0214/152,0117
Ácido Cafeico	26,2	135,0455
Ácido p-cumárico	23,2	119,0504
Ácido Elágico	59,7	117,0362/145,0299
Ácido Ferúlico	23,2	134,0375/178,0269
Quercetina	32,2	121,0301/151,0042
Naringenina	29,2	119,0460/151,0037
Apigenina	38,4	117,0346/151,0037
Cianidina 3-O-glicosídeo	26,3	287,0638

Além disso, a calibração interna também foi avaliada para diminuir o erro entre a massa teórica e experimental. Na Tabela S3 estão apresentados estes resultados para os padrões de compostos fenólicos utilizados como referência na identificação dos compostos fenólicos presentes nos extratos de casca e semente de uva em pó.

Tabela S3. Erros obtidos após a calibração interna HPLC-MS.

Composto	t_R (min)	λ_{max} (nm)	$[M-H]^-$ teórica	$[M-H]^-$ experimental	Erro (ppm)
Ácido Gálico	11.7	270	169,0142	169,0142	-0,28
Catequina	20.3	279	289,0718	289,0713	-1,60
Cianidina 3-O-glicosídeo	20.4	279, 517	449,1084	-	-
Ácido Clorogênico	20.4	325	353,0878	353,0869	-2,57
Epicatequina	23.0	279	289,0718	289,0711	-2,29
Ácido Cafeico	23.1	288, 324	179,0350	179,0350	0,09
Ácido Vanílico	23.2	260, 292	167,0350	167,0352	1,30
Ácido *p*-cumárico	28.3	309	163,0401	163,0399	-1,03
Ácido Elágico	28.8	253	300,9990	300,9988	-0,63
Ácido Ferúlico	30.1	322	193,0506	193,0502	-2,24
Quercetina	40.1	370	301,0354	301,0353	-0,26
Naringenina	44.2	288	271,0612	271,0613	0,38
Apigenina	45.2	267, 336	269,0455	269,0455	-0,17

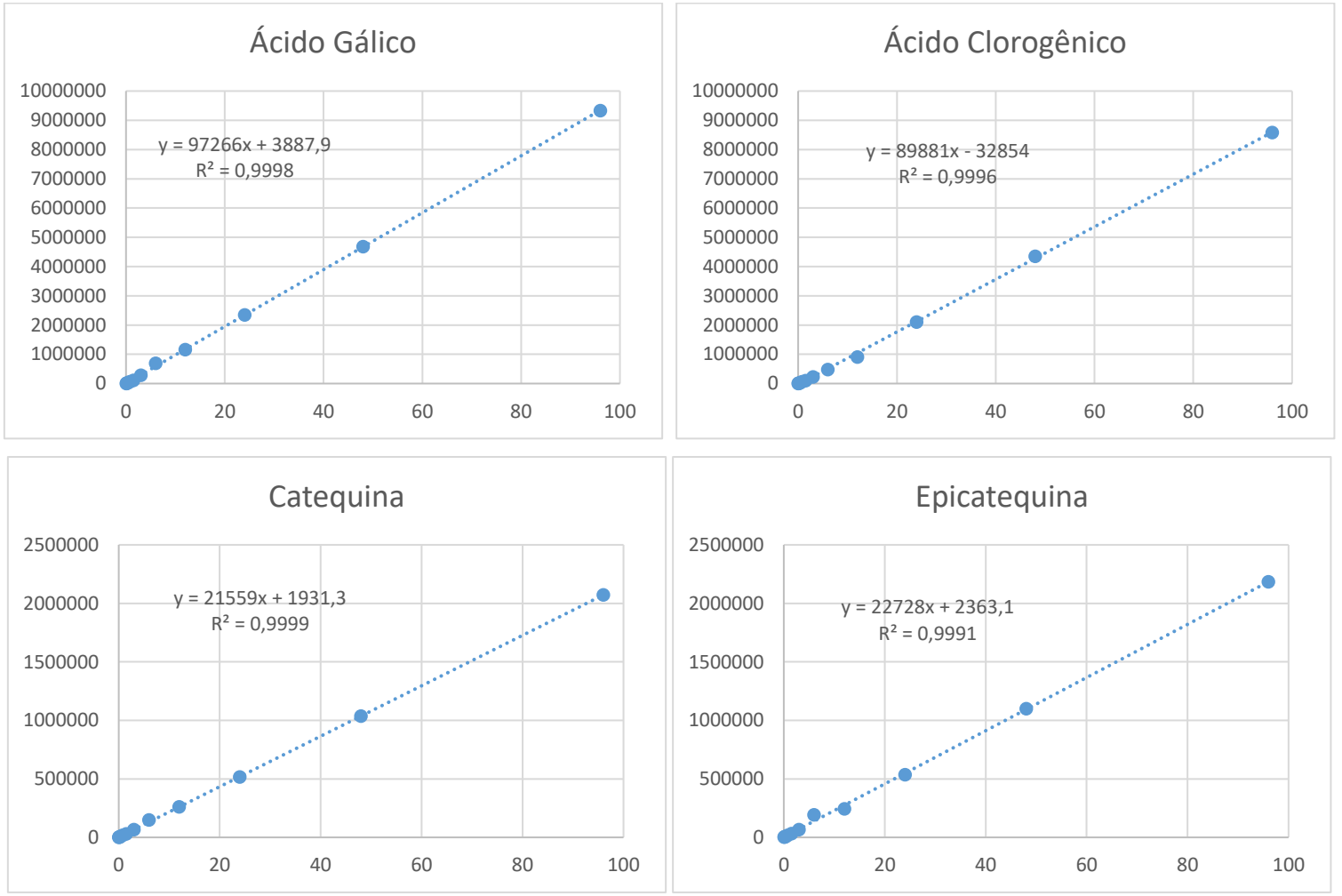

Figura S1. Curvas analíticas dos padrões dos compostos fenólicos ácido gálico, ácido clorogênico, catequina e epicatequina.

Fonte: autora.

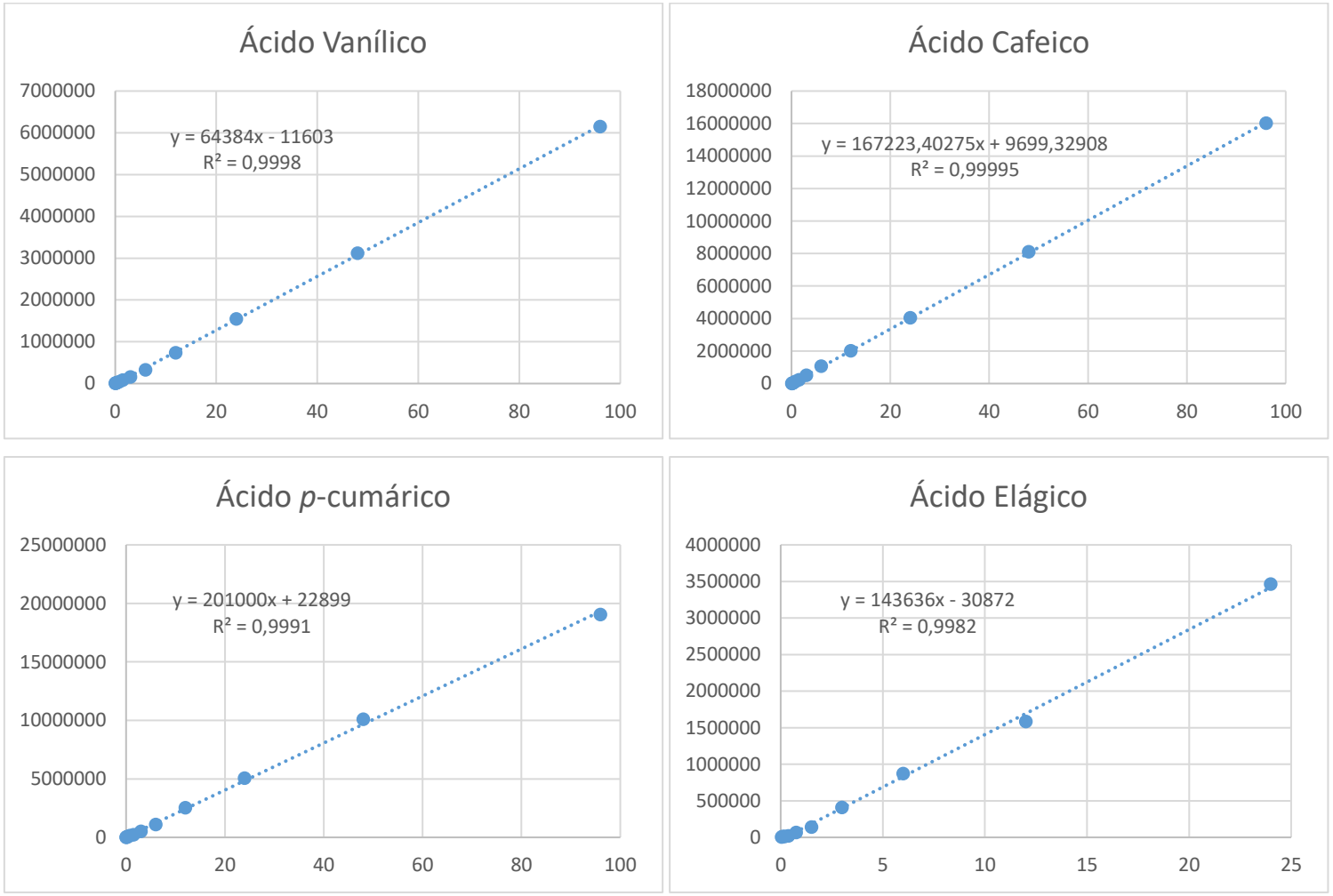

Figura S2. Curvas analíticas dos padrões dos compostos fenólicos ácido vanílico, ácido cafeico, ácido *p*-cumárico e ácido elágico.

Fonte: autora.

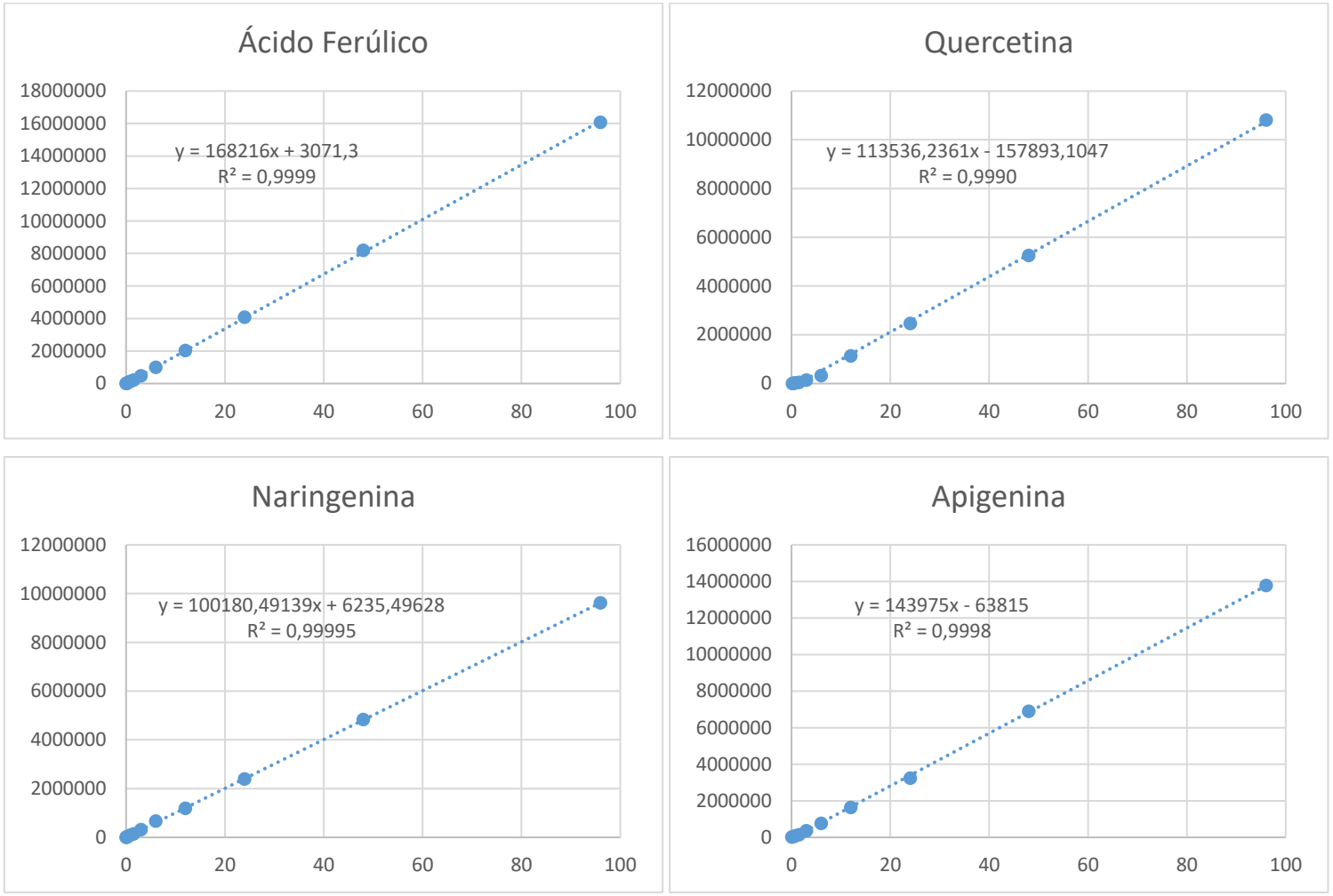

Figura S3. Curvas analíticas dos padrões dos compostos fenólicos ácido ferúlico, quercetina, naringenina e apigenina.

Fonte: autora.

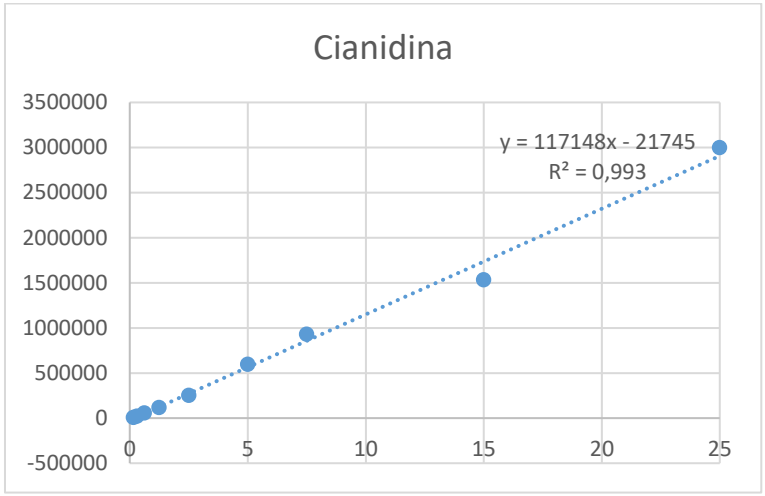

Figura S4. Curva analítica do padrão do composto fenólico antociânico cianidina 3-O-glicosídeo.

Fonte: autora.