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Perspective

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Concept and Health-Related Properties of Nonextractable Polyphenols: The Missing Dietary Polyphenols

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ABSTRACT: Most research studies in the field of dietary polyphenols or phenolic compounds use a chemical approach focusing exclusively on polyphenols extracted from plant foods with organic solvents. However, an appreciable part of polyphenols are not extracted with organic solvents and thus are ignored in biological, nutritional, and epidemiological studies. Recent studies have shown that these nonextractable polyphenols (NEPP) are a major part of total dietary polyphenols and that they exhibit a significant biological activity. A physiological approach is proposed on the basis that the bioavailability and health-related properties of polyphenols depend on their solubility in intestinal fluids, which is different from their solubility in organic solvents. This paper tries to clarify the concept of NEPP, distinguishing between chemical and physiological approaches and pointing out the main qualitative and quantitative differences between them. It is stressed that the literature and databases refer to only extractable polyphenols. Greater attention to NEPP may fill the current gap in the field of dietary polyphenols.

KEYWORDS: *polyphenols, nonextractable polyphenols, physiological approach, food and diet, health-related properties*

INTRODUCTION

The scope of research on polyphenols (PP) or phenolic compounds has been significantly extended from the traditional studies focused on their contribution to sensorial properties of foods and beverages to the predominant current studies on their biological and health-related properties.

Extensive knowledge of the PP content in foods and diets is essential for studies of nutrition and health, but only a fraction of the polyphenols that are ingested is considered in the current research because the literature and databases on food composition focus exclusively on extractable polyphenols (EPP), which are extracted from plant foods by using aqueous organic solvents.[1−3]

However, it has been reported that an appreciable amount of polyphenols, called nonextractable polyphenols (NEPP), can remain in the neglected residues of aqueous organic extraction[4−7] due to the ability of the aromatic rings and hydroxyl groups of polyphenols to form macromolecules (tannins) as well as to bind to polysaccharides, protein, and cell walls by covalent bonds (esters and ether), hydrogen bonding, and hydrophobic and hydrophilic interactions.

As a consequence, NEPP are discarded in studies on the bioavailability and metabolism of PP as well as in clinical trials and observational studies. However, NEPP are bioaccesible and bioavailable in the human gut, where they exhibit antioxidant activity and produce absorbable metabolites,[8−10] and they may have an important role in gastrointestinal health and contribute to the systemic effects associated with dietary antioxidants.

EPP may be only the tip of the iceberg (Figure 1). Notice that, in the field of polyphenols, over 30000 papers published in the past decade focus exclusively on EPP, whereas fewer than 30 papers can be found on NEPP (SCI Web of Knowledge, August 2012).

This paper has two objectives: (1) to state the chemical and physiological concept of NEPP, indicating their occurrence in plant foods and their physiological and health-related properties

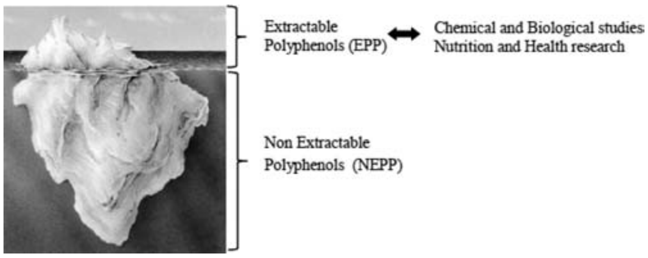

Figure 1. Known and missing dietary polyphenols.

and (2) to encourage research on NEPP to fill the current gap in dietary polyphenols research.

Origin of the NEPP Concept. The concept of NEPP emerged to elucidate a paradoxical result of the analysis of dietary fiber (DF) performed in carob pods.[11] The content of DF in this leguminous plant, determined by using the official AOAC method, was 40.4% (dw), and according to the traditional definition of DF, this amount must correspond to nonstarch polysaccharides and lignin. However, specific analysis of polysaccharides and lignin showed that the actual amount of these compounds accounted for only 18.9% (dw). That is to say, there was a gap of 21.5% that was identified as polymeric proanthocyanidins, a major type of NEPP. Similar paradoxical results were later observed in other plant foods.[4,12−14]

These results suggest two interesting facts: (1) Dietary fiber contains polyphenols, and these constituents may have a significant role in the DF properties that generally are attributed to polysaccharides, the major constituents of DF. Consequently, the definition of DF restricted to nondigestible polysaccharides and lignin could be extended to include PP. This new approach

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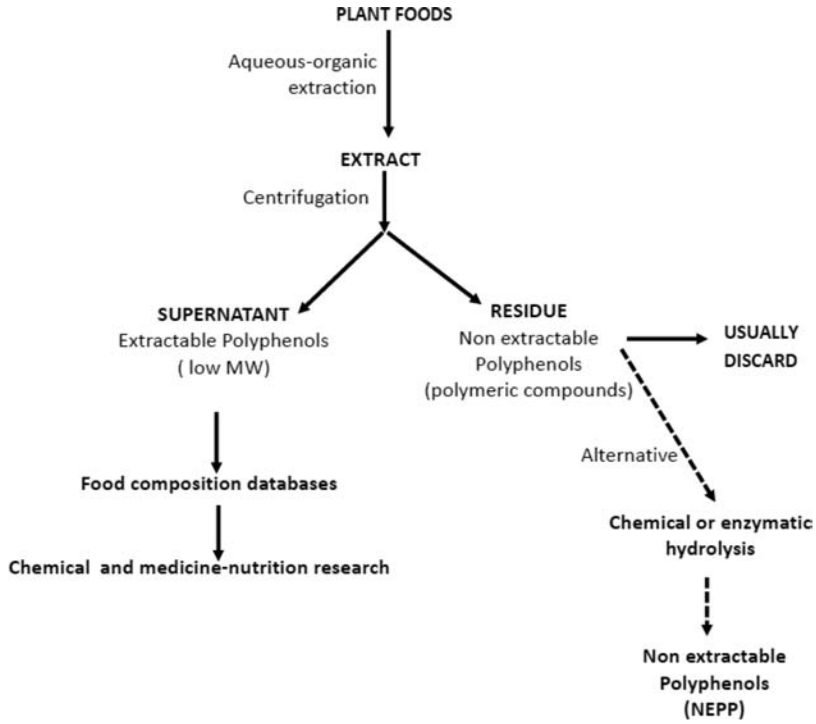

Figure 2. Usual procedure for the analysis of EPP in foods and suggested alternative procedure to determine NEPP.

of DF has been previously reviewed[15] and is outside the scope of this paper. (2) Plant foods may contain appreciable amounts of NEPP, which imply significant changes in the general assumption that total PP content in foods corresponds to EPP. This paper intends to show briefly the current knowledge on the emerging topic of nonextractable dietary polyphenols.

Why Have Nonextractable Polyphenols Been Ignored? The answer to this question is related to the common procedure used to obtain data on the content and composition of polyphenols in foods (Figure 2). Samples are treated with different aqueous organic solvents to obtain PP extracts that are used for HPLC analysis, isolation of specific compounds, and biological assays. These extracts contain low molecular weight compounds and are the unique source used for databases of PP in foods.

However, the neglected residues of these extractionsm as described below, may contain appreciable amounts of NEPP such as polymeric polyphenols or low molecular weight polyphenols bound to protein, polysaccharides, or cell walls. In fact, if these residues are treated with acids (HCl, H_2SO_4) or with enzymes (cellulases, proteases, digestive enzymes, bacterial enzymes), significant amounts of polyphenolic compounds release from the food matrix, which can be analyzed in the corresponding hydrolysates.[6,16,17] This shows that a complete determination of PP in foods requires analysis of both extracts and extraction residues.

DIETARY POLYPHENOLS: CHEMICAL AND PHYSIOLOGICAL CONCEPT

Chemical Approach. Dietary PP were divided first[4] on the basis of both solubility in organic solvents and physiological properties, into two groups:

Extractable Polyphenols. EPP comprise compounds with low molecular weight (mainly monomers to decamers) soluble in aqueous organic solvents (methanol, acetone, ethanol, ethyl acetate, etc). These compounds have a wide range of chemical structures, including flavonoids (flavanols, anthocyanins, flavonols, other subgroups), benzoic and hydroxycinnamic acids, stilbenes, extractable proanthocyanidins (EPA), hydrolyzable tannins, and others.

EPP are dissolved in the stomach and small intestine, where they can be absorbed at least partially through the small intestinal mucosa followed by metabolism and systemic effects.

Nonextractable Polyphenols. NEPP comprise compounds with high molecular weight (polymers) and polyphenols linked to cell wall macromolecules (proteins, polyssacharides) or trapped in the core of the food matrix that remain in the residue of aqueous organic extractions. Condensed tannins, or nonextractable proanthocyanidins (NEPA), and hydrolyzable polyphenols (HPP), including hydrolyzable tannins, or polyphenols released from macromolecules by hydrolysis are the major NEPP.

NEPP are not bioaccesible in the small intestine, and they travel through the gastrointestinal tract as insoluble substrates reaching the colon, where they release single PP and different bioavailable metabolites by the action of bacterial microbiota.

This classification of dietary polyphenols is a physiological or nutritional concept and not just a concept derived from chemical analysis. NEPP differ from the common terms " insoluble PP" used to report polyphenols complexes produced by food processing (thermal treatment of food, aging of beverages, tanning in leather processing) or precipitated in some analytical procedures.[18,19] NEPP is also a concept wider than the term "bound PP", which corresponds usually to hydroxycinnamic and benzoic acids linked to cell walls that release mainly from cereals by alkali hydrolysis.[20] The amount of these polyphenols bound to macromolecules by ester bonds may be only a part of NEPP, as indicated by the fact that the amount polyphenols found in the acidic hydrolysis of cereal products was higher than in alkali hydrolysis.[21]

The potential biological significance of NEPP was tested during the 1990s by in vitro assays and animal experiments addressing intestinal degradability, bacterial colonic fermentation, and effect on fat excretion. In vitro treatments of plant materials with digestive enzymes following dialysis, to simulate intestinal digestion and absorption, suggested the nonbioavailability of NEPP in the upper gut.[22] The resistance of these compounds to intestinal digestion was also shown by rat experiments;[4] in addition, interesting effects of NEPP in lipid digestibility such as an increase of fat excretion and a decreased absorption of bile acids and cholesterol were observed.[12,23] On the other hand, in vitro colonic fermentation assays showed that NEPP, similarly to insoluble DF, were resistant to degradation, whereas EPP such as catechin and tannic acid were extensively degraded, as occurs with soluble DF.[24]

From the year 2000, several authors have studied the health-related properties of NEPP, using either isolated NEPP or NEPP-rich matrices, combining in vitro models,[25,26] cell cultures,[27] animal models,[28−32] or humans trials.[33] These studies have shown that NEPP may have a beneficial role in gastrointestinal health, through an increase of intestinal antioxidant activity,[28] the modulation of *Lactobacillus* growth,[32] and a direct chemo-preventive effect, as shown by the reduction in the number and size of colonic crypts and the modulation of genes associated with tumor development.[29,31] The previously reported beneficial effects of NEPP on cardiovascular risk parameters have also been corroborated.[30,33] Also, the metabolic fate of NEPP has been explored by using in vitro[34] and animal models,[10,35] showing that NEPP, once subjected to colonic fermentation, are a source of absorbable and bioactive metabolites.

Studies on NEPP are still a minority among all of the research carried out in the polyphenols field, but a significant increase in NEPP research can be expected in the near future because of the increasing evidence of their nutritional relevance.

Ocurrence of NEPP in Foods and Diets. The determination of the food NEPP content requires different hydrolysis treatments (H_2SO_4, HCl−butanol, alkali, enzymes) in the extraction residues to release anthocyanins−cyanidins from proanthocyanidins and phenolic acids and flavonoids from HPP, following analysis of specific PP in the corresponding hydrolysates.[6,36−38]

Table 1 shows the content of the two major types of NEPP (proanthocyanidins and hydrolyzable phenolics, including hydrolyzable tannins) in specific foods. As can be observed, these foods contain appreciable amounts of NEPP, which are in many cases higher than the content of EPP.[1,3] Single polyphenols, including phenolic acids (gallic acid, ellagic acid, ferulic acid, sinapic acid) and flavonoids (epicatechin, delphinidin), were identified by HPLC-MS/MS in cereal products,[21] fruits,[37] nuts,[47] olives,[17] black currant pomace,[48] and other plant foods.[49]

Table 1. Nonextractable Polyphenol Content in Selected Foods

food group	food	NEPP class	content (mg/100 g fw[a])	ref
fruits and vegetables	açaí	proanthocyanidins	1240 ± 140[b]	39
	banana	proanthocyanidins	980−2300[b]	6, 40
	cranberry pomace	proanthocyanidins	1685	41
	peach	hydrolyzable phenolics	52.8 ± 2.9	37
	onion	hydrolyzable phenolics	410 ± 20[b]	42
cereals	whole-grain wheat flour	hydrolyzable phenolics	32	43
	whole-grain barley flour	hydrolyzable phenolics	60−135	44
nuts	walnut	hydrolyzable tannins	543	45
	Brazil nut	hydrolyzable tannins	210	7
others	cocoa powder	proanthocyanidins	602 ± 13	46

[a]fw, fresh weight. [b]Results expressed in dry weight.

Knowledge of the total intake of PP, including both EPP and NEPP, is essential for a better understanding of the nutritional properties of dietary PP. However, the current literature data on PP intake are limited to EPP.[50,51] A first report on dietary intake of PP, including both EPP and NEPP, in a whole diet (the Spanish diet) estimated a total PP intake of around 1800 mg/day/person, corresponding to 942 mg of NEPP and 880 mg of EPP.[8,21] Figures of the same order can be expected in other diets.

Physiological Approach. Obviously, from the physiological point of view the solubility of PP in biological fluids is more relevant than that in organic solvents, and consequently dietary PP can be defined as follows: (I) Extractable polyphenols include compounds solubilized in the upper gut, directly because of the physiological conditions (37 °C, pH 1−7, mobility, transit time) and compounds released from the food matrix by the action of digestive enzymes, which disrupt the macromolecular food structure (protein, carbohydrates, fat), favoring the release of bound or trapped PP. (II) Nonextractable polyphenols include compounds nonsoluble in intestinal fluids that travel through the gastrointestinal tract associated with indigestible macromolecules reaching the colon, where they release absorbable metabolites by the action of colonic bacteria.

A key feature of NEPP is its breakdown in the colon (Figure 3). These PP reach the colon along with major indigestible substrates (mainly carbohydrates and protein), and the microbiota disrupts macromolecules, producing mainly short-chain fatty acids (SCFA) (acetic, propionic, butyric) from carbohydrates along with nitrogen compounds from protein and low molecular weight PP and some phenolic metabolites (hydroxyphenyls acids, urolithins, etc.) from NEPP and also from EPP nonabsorbed in the small intestine.[34]

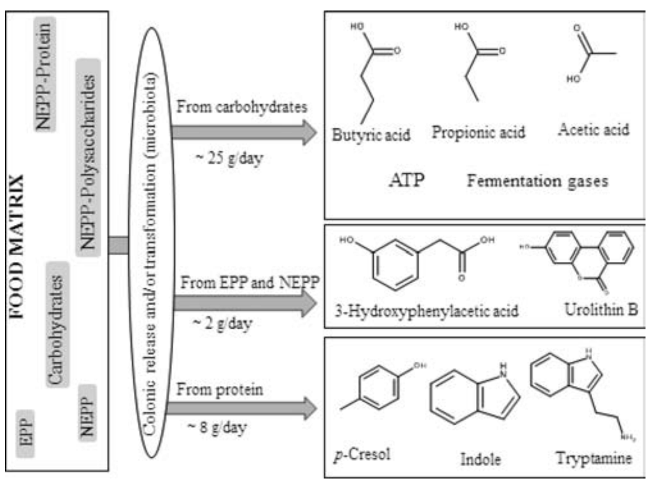

Figure 3. Major metabolites formed by colon microbiota from organic substrates.

This fermentation process enhances the intestinal antioxidant status, which may protect against dietary prooxidants and free radicals and also produces bioavailable metabolites with potential systemic effects.

Interactions, competition, and synergistic effects between bacterial enzymes and dietary substrates can be expected. The relative amount and type of polysaccharides and polyphenols and other compounds reaching the human colon may affect the type and concentration of metabolites in the colon. It must be noted that PP represent only about 5% of the total amount of substrates available for human colonic fermentation (estimated around 45 g/day).

Along this line, some studies of colonic fermentation of NEPP and EPP together with different amounts of DF or polysaccharides showed that DF may enhance the yield of PP metabolites, probably due to an increase of bacterial activity.[34,52,53] These results suggest that fermentation assays focusing on single polyphenols should be re-examined using physiological doses of PP along with other dietary substrates.

Chemical versus Physiological Approach. The total amount of food PP determined by using chemical or physiological approaches must be similar (EPP ch + NEPP ch = EPP phy + NEPP phy), but there are significant qualitative differences.

Figure 4 illustrates a typical example, corresponding to French bread.[54] As can be observed, similar total amounts of PP

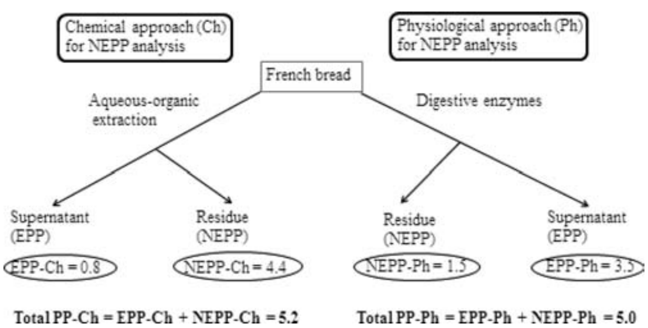

Figure 4. Polyphenol content in French bread: comparison between chemical and physiological approaches (results expressed as mg polyphenols/g dry weight).

were found by using chemical and physiological approaches (5.2 vs 5.0 mg/g dw, respectively), but what was especially remarkable was the fact that the EPP ch (0.8 mg/g dw) content was significantly lower that the EPP phy (1.5 mg/g dw). Similar results were obtained when chemical and physiological approaches were compared in different plant foods.[16]

These data appear to indicate an interesting fact: human digestive enzymes exhibit a capacity to solubilize food PP significantly higher than that of organic solvents. This suggests two possible weak points in the current research on PP: (1) The amount of PP considered in the field of dietary PP (EPP ch) may be significantly lower than the actual amount of PP daily present in the human small intestine (EPP phy). (2) NEPP are ignored, although they appear to be quantitative and qualitatively important in our daily diet.

These points support a physiological approach, including both EPP and NEPP, for a better understanding of the role of dietary PP in nutrition and health. The promising health-related properties of NEEP, summarized in Table 2, open attractive topics for research and also specific applications for new functional ingredients.

Table 2. Main Features and Potential Health Properties of NEPP

NEPP	ref
Main Features	
nonsoluble in aqueous organic solvents	6, 8
main classes: high molecular weight proanthocyanidins, hydrolyzable phenolics (including phenolic acids and hydrolyzable tannins)	49
nonbioaccesible neither bioavailable in the small intestine	16
bioaccessible and bioavailable in the large intestine	8, 16
time to reach maximum concentration in plasma ≥8 h	9, 10
Health-Related Properties	
enhancement of lipid excretion	12, 30
increase of intestinal antioxidant activity	28
modulation of *Lactobacillus* growth in rat cecum	32
chemopreventive effect: reduction of the number and size of colonic crypts	29
modulation of gene expression: down-regulation of genes associated with tumor development and proto-oncogenes, up-regulation of tumor suppressor genes	31
production of phenolic metabolite with potential systemic effects	34, 35

CONCLUDING REMARKS

(1) The amount of NEPP in plant foods, made up of polymeric and HPP, is substantially higher than EPP.
(2) NEPP constitute a major part of dietary PP.
(3) NEPP and their bioavailable metabolites exhibit a significant biological activity associated with gastrointestinal health and potential systemic effects.
(4) Further studies to determine contents of NEPP in foods and diets as well as their contribution to the health-related properties of dietary polyphenols and antioxidants are needed.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

PP, polyphenols; DF, dietary fiber; EPP, extractable polyphenols; NEPP, nonextractable polyphenols; EPA, extractable proanthocyanidins; HPP, hydrolyzable polyphenols; EPP ch, extractable polyphenols by chemical approach; NEPP ch, nonextractable polyphenols by chemical approach; EPP phy, extractable polyphenols by physiological approach; NEPP phy, nonextractable polyphenols by physiological approach; SCFA, short-chain fatty acids.

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