Review

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# Plant Foods as Healthy Sources of Dietary Fibre and Bioactive Compounds: Beyond Definitions. A Review

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## Abstract

Dietary fibre (DF) and bioactive compounds (BC) are essential components of a healthy diet and are abundant in plant-rich dietary patterns. Increasing evidence demonstrates that their combined and synergistic actions significantly influence human health, largely through their effects on the gut microbiota. This review highlights the need for more precise terminology regarding DF and BC, as inconsistent use of these terms can create confusion among both consumers and researchers. The DF complex encompasses all non-digestible food components that have a positive effect on human health, together with the BC associated with them, recognising that DF often serves as a carrier for these compounds throughout the digestive tract. Although recommended intakes for BC have not been established, intake levels observed in populations adhering to healthy dietary patterns may serve as useful reference points. Updated data on the intake and estimated bioavailability of polyphenolic compounds in the contemporary Spanish diet are presented.

**Keywords:** DF complex; bioactive compounds; polyphenolic bioavailability; healthy diet; gut microbiota

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## 1. Introduction

The scientific connection between diet and health has been well documented for many decades, with substantial and increasingly robust evidence showing that a healthy lifestyle (including adherence to a healthy dietary pattern) can help individuals achieve and maintain good health and reduce the risk of chronic diseases at all stages of life. The core elements of a healthy dietary pattern are remarkably consistent across the lifespan and influence all health outcomes. The Dietary Guidelines for Americans 2020–2025 (2020) focus their recommendations on consuming a healthy dietary pattern and emphasise the importance of the overall pattern, rather than isolated nutrients, foods, or food groups. A dietary pattern is defined as the usual combination of foods and beverages that constitute an individual's complete daily intake within a social group. It is therefore all the components of food consumed that act synergistically in the body and affect the health of the consumer.

All foods in the diet obviously contribute to this synergistic effect, but some food groups such as plant-based foods, and especially fruits and vegetables, are widely recognized as healthy. This suggests that changes in dietary behaviour and lifestyle, such as increasing the consumption of fruits and vegetables and engaging in regular physical activity, are practical strategies for reducing the incidence of chronic diseases (Liu, 2013).

FAO and WHO (2020) jointly held an international expert consultation on sustainable and healthy diets. The consultation agreed on guiding principles for "Sustainable Healthy Diets", based on foods, nutrient-based dietary guidelines, and environmental, social, cultural, and economic sustainability. The guiding principles of this health-related consultation define the characteristics of a healthy pattern, highlighting that a varied diet with significant amounts of different plant food

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groups and a limited number of processed foods provides the components (nutrients and non-nutrients) necessary for good health and the enjoyment of well-being.

Environmental risk factors include tobacco use, insufficient physical activity, excessive alcohol consumption, and diets high in salt, sugar, and fats, particularly saturated fats. Unhealthy diets and lack of physical activity may manifest in individuals as raised blood pressure, elevated blood glucose, increased blood lipids, and obesity, among other harmful effects. These are termed metabolic risk factors and can lead to cardiovascular disease, the leading non-communicable disease in terms of premature deaths.

Conversely, several healthy dietary patterns exist around the world, such as the DASH diet (Dietary Approaches to Stop Hypertension), the Mediterranean diet, the MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay), the traditional Asian diet, and the Nordic diet. All of these share common characteristics: a higher content of plant-based foods (fruits, vegetables, whole grains, legumes, seeds, nuts) and a lower content of foods of animal origin (fatty and processed meats) (Global Burden, 2020).

Public health guidelines from various organisations recommend a minimum daily intake of five servings of fruits and vegetables. The Eurodiet core report, the World Cancer Research Fund, and the WHO/FAO recommend at least 400 g/day; Denmark recommends 600 g/day, and the USA 640–800 g/day (Olaya et al., 2019). The health benefits of regular fruit and vegetable consumption are evident. However, a significant gap remains between recommended and actual consumption. According to Lee et al. (2022), only one in ten adults meets daily fruit and vegetable intake recommendations.

There is increasing evidence to suggest that the health benefits of plant-derived foods are attributed to the synergy or interactions between bioactive compounds (BC) and nutrients in whole foods. Therefore, consumers should obtain nutrients and BC from a balanced diet with a wide variety of plant foods for optimal nutrition, health, and well-being, rather than from dietary supplements (Liu, 2013).

Traditionally, macronutrients and micronutrients have been considered the main components of food. However, in plant-derived foods, the presence of other minor components that are not essential for life but possess properties with a high impact on health is widely recognised. Plant foods are generally high in water, vitamins, minerals, and contain significant amounts of dietary fibre (DF) and bioactive compounds (BC) with important biological properties, including antioxidant activity.

Therefore, discussing nutritional content alone to define a healthy dietary pattern is insufficient, as scientific evidence suggests that BC actively contribute to reducing the risk of chronic diseases (Kris-Etherton et al., 2002). In this regard, and continuing the line of reasoning outlined above, the health benefits of fruits and vegetables are attributed to the synergistic effects within a complete diet between nutrients and the BC of foods.

Despite numerous high-quality publications offering valuable insights on this topic, the authors of this work have perceived the need to standardise definitions and concepts to facilitate the exchange of high-value information and contribute to advancing knowledge in the scientific fields involved. In this sense, it is useful to clarify, among other terms, the concepts of DF and BC, which are highly relevant to the main objective of this work.

Recently, Kussmann et al. (2023) described four categories of food components: (1) macronutrients; (2) micronutrients; (3) phytonutrients; and (4) microbiota regulators. It is a bold classification and may be improved, but it provides a solid structural basis for relating dietary components to disease prevalence and healthy dietary patterns.

Kussmann et al. (2023) did not explicitly mention DF among the food components. However, they introduced the concept of microbiota regulators, a category that should include all non-digestible compounds reaching the colon. This more physiological criterion opens a new space to reconsider what has traditionally been termed DF. These considerations will be developed later.

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## 2. Bioactive Compounds: Clarifying Definitions and Related Concepts

Foods are products of various origins that contain nutrients, compounds responsible for organoleptic characteristics, and other substances that exert a positive effect on health independently of their nutritional value. In some cases, foods may also contain substances with negative health effects. Bioactivity, therefore, can be understood in either a positive or negative sense.

In the scientific literature, terms such as BC, phytochemicals, phytonutrients, nutraceuticals, and functional foods frequently appear. The indiscriminate use of these terms can confuse both consumers and researchers. Consequently, these concepts need to be standardised or redefined, ideally within an appropriate legal framework. Vettorazzi et al. (2020) noted that the boundaries between these terms are unclear and are often used interchangeably or with subtle differences in meaning. Although commonly employed worldwide, there is no international consensus on their definitions.

According to Frank et al. (2020), the most basic dictionary definition of a bioactive compound is "having or producing an effect on a living organism". However, this broad definition is insufficient for contemporary safety assessment frameworks and regulatory standards regarding human health. These authors define bioactive food components as constituents of foods or dietary supplements, other than those required to meet basic nutritional needs, which are responsible for changes in health status. Another definition describes BC as extra-nutritional substances that are biologically active and naturally occurring (Chaudhary & Garg, 2023). Câmara et al. (2021) describe food bioactive compounds as all compounds, mostly without nutritional value and naturally present in food, that exert a bioactive effect on the human body.

Xavier et al. (2024) noted that BC are present in both natural and processed foods and have potential health benefits. Their roles include mitigating inflammation, providing antioxidant effects, reducing lipid levels, and regulating gene expression, which are crucial for preventing chronic diseases such as type 2 diabetes and cancer.

As previously mentioned, Kussmann et al. (2023) proposed a clear classification that we consider particularly relevant. The authors observed that nature offers a virtually unlimited source of compounds with positive effects on human health, termed natural bioactives. These components are classified into four groups: macronutrients (carbohydrates, lipids, proteins), micronutrients (vitamins and minerals), phytonutrients/phytochemicals (terpenes, alkaloids, phenolics, organosulfur compounds), and gut microbiome regulators (probiotics, prebiotics, symbiotics, postbiotics). This classification encompasses all natural components of food.

The origin of BC is also important. They derive from a variety of natural sources, including plants, microorganisms, animals, and marine organisms (Jha & Sit, 2022; Swamy & Akhtar, 2019), and may interact with one or more components of living tissue, producing a wide range of potential effects (Guaadaoui et al., 2014; Shetty & Sarkar, 2020). Guaadaoui et al. (2014) highlighted that these substances can be natural (terrestrial or aquatic; plant, animal, or other sources such as microorganisms) or synthetic, either partially or fully.

Generally, BC are understood to have positive effects on health, but some authors emphasise that effects can be positive or negative. Frank et al. (2020) argue that a bioactive compound is defined by its biological activity and refers to phytochemicals or animal-derived components with demonstrated activity in biological systems, usually animals and/or humans, without specifying whether the activity is beneficial or harmful. Biesalski et al. (2009) noted that biological activity is recognised in compounds with positive effects, but this classification is narrow, as negative effects, such as toxicity, allergenicity, or mutagenicity, often dose- and bioavailability-dependent, also represent bioactivity.

In our view, a bioactive compound is one that exerts a positive effect on human health, beyond its nutritional role. For instance, the antioxidant activity of ascorbic acid is independent of its function as a vitamin.

In plants, BC are commonly referred to as phytochemicals. Phytochemicals constitute a heterogeneous group of minor food components, including terpenes, alkaloids, phenolics, and organosulfur compounds. Although not essential nutrients, they are associated with numerous proven and potential health benefits. They can also be defined as non-nutritive plant chemicals with protective or disease-preventive properties (Kussmann et al., 2023). Hoang & Kim (2021) and Probst et al. (2017) define phytonutrients as BC commonly present in plant-based foods such as fruits, vegetables, grains, and teas.

Other authors present a broader but less precise concept, describing phytochemicals as chemical compounds produced by plants through primary and secondary metabolism, which possess biological activities (Banwo et al., 2021; Schmidt et al., 2019; Verma & Thakur, 2021). Lalitha (2024) categorises phytochemicals into primary and secondary metabolites according to their role in plant metabolism. Primary metabolites (e.g., carbohydrates, amino acids, proteins, lipids, nucleic acids) are essential for plant growth and basic functions. Secondary metabolites, including alkaloids, glucosinolates, cyanogenic glycosides, phenylpropanoids, flavonoids, and terpenes, protect plants from pathogens, UV light, and herbivores.

Delgado et al. (2021) note that primary metabolites (proteins, lipids, carbohydrates) are directly involved in intrinsic metabolic processes, such as growth, development, and reproduction. Secondary metabolites (phytochemicals) confer selective advantages to the plant, despite not being part of its main metabolic pathways.

Phytochemicals thus originate from secondary plant metabolism, but in some studies, the term phytonutrient is used interchangeably, which can cause confusion. According to Delgado et al. (2021), phytonutrients may be considered as whole-plant extracts containing one or more phytochemicals. Examples include turmeric, garlic, cinnamon, graviola, and oregano. Phytonutrients are plant foods containing multiple natural BC (phytochemicals) that elicit specific biological activities. Kussmann et al. (2023) similarly define phytonutrients as natural compounds in plant foods such as vegetables, fruits, whole grains, nuts, and legumes, including phenolic compounds, alkaloids, terpenes, and other secondary metabolites. These compounds can act synergistically with other nutrients to exert beneficial effects, including antioxidant, anti-inflammatory, and neuroprotective properties. Thus, the terms phytonutrient and phytochemical are largely equivalent. Lalitha (2024) also confirms that phytochemicals, also known as phytonutrients, are abundant in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, and spices. Therefore, a phytonutrient can be defined as a plant-derived bioactive compound (e.g., resveratrol) associated with positive health effects (Frank et al., 2020).

Phytochemicals are naturally occurring BC abundant in vegetables, fruits, whole grains, nuts, seeds, legumes, tea, and dark chocolate (Cao et al., 2017; Singh & Chaudhuri, 2018; Xiao & Bai, 2019).

The term “functional food” was introduced in Japanese literature in 1984 to distinguish a tertiary function of foods, distinct from primary (nutrition) and secondary (preference) functions (Aronson, 2017). Functional foods contain biologically and physiologically active compounds that provide health benefits beyond basic nutrition (Daliri & Lee, 2015; IFT, 2020; Konstantinidi & Koutelidakis, 2019; Ramakrishna et al., 2020). The constituents responsible for functionality are generally termed “bioactive compounds” (Banwo et al., 2021).

Functional foods are also defined by the Functional Food Center/Functional Food Institute (Martirosyan & Ekblad, 2022) as “natural or processed foods that contain biologically active compounds which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit using specific biomarkers, to promote optimal health, reduce the risk of chronic or viral diseases, and manage their symptoms.” There is no universal regulatory definition, and meanings vary among organisations.

The term “nutraceutical” was coined by Stephen De Felice in 1989, derived from “nutrition” and “pharmaceutical”. Nutraceuticals are foods or components of foods that confer health benefits, including the prevention and/or treatment of disease (Puri et al., 2022). Aronson (2017) notes there is no internationally agreed definition of nutraceuticals, functional foods, or similar terms such as “health foods”. These terms are vague and non-discriminatory; evidence suggests they should be replaced with more precise terminology. Vettorazzi et al. (2020) similarly highlight that “bioactive compounds", "nutraceuticals", and "functional foods" are widely used in industry and by consumers, yet the boundaries between them are unclear.

Lupton et al. (2014) point out that, unlike traditional nutrients (vitamins, minerals, proteins, essential fatty acids, amino acids) which have dietary reference intake (DRI) values, there is no such evaluative framework for bioactives. DF is one exception.

In conclusion, BC in plant foods are often called phytochemicals, while some authors use the term phytonutrients. Epidemiological evidence shows that higher intake of BC, such as in the Mediterranean diet, is associated with lower prevalence of cardiovascular disease, cancer, diabetes, and neurodegenerative diseases. Consequently, functional foods containing health-protective components are increasingly popular (Cámara et al., 2021).

The spectrum of food BC exhibits substantial diversity in origin, structure, and bioactive effects (Câmara et al., 2021). Prominent examples in fruits and vegetables include phenolic compounds (flavonoids, tannins, phenolic acids, stilbenes, lignans), terpenes and terpenoids (carotenoids, phytosterols), glucosinolates, and alkaloids (Banwo et al., 2021; Câmara et al., 2021; Chaudhary & Garg, 2023; Delgado et al., 2021; Kussmann et al., 2023). Lalitha (2024) also considers saponins, polysaccharides, and DF as plant-derived bioactive components. Cámara et al. (2021) support including polysaccharides such as cellulose.

Phenolic compounds in plants occur predominantly in soluble or bound forms. Soluble phenolics are synthesised mainly in the endoplasmic reticulum and accumulate in vegetative (leaves, stems, roots) and reproductive (fruits) organs. Bound phenolics form when soluble compounds translocate to the cell wall and conjugate with macromolecules such as cellulose and proteins via ester and glycosidic bonds (Agati et al., 2012; Kováčová & Malinová, 2007; Malenčić et al., 2007).

BC from animal sources should also be considered, as they are not exclusive to plants. Examples include bioactive peptides (e.g., carnosine in red meat) and polyunsaturated fatty acids (PUFAs, e.g., docosahexaenoic acid—DHA in fish) (Cámara et al., 2021). Chaudhary & Garg (2023) additionally include omega-3 fatty acids, conjugated linolenic acid, L-carnitine, chitin, chitosan, choline, and glucosamine.

Kussmann et al. (2023) suggest that bioactive food compounds should also encompass gut microbiome regulators, including probiotics, prebiotics, symbiotics, and postbiotics.

Based on the reviewed literature, we propose the following definition: "Bioactive compounds are chemicals found naturally, mainly in plant-based foods (fruits, vegetables, nuts, oils, whole grains), which exert demonstrable beneficial effects on human health and contribute to reducing the risk of chronic diseases. Some may be essential nutrients (e.g., certain vitamins), others non-essential (e.g., carotenoids, polyphenols). They modulate biological functions to promote optimal health, rather than to prevent a deficiency."

## 3. Dietary Fibre: Definition and Components. Towards a Physiological Concept

The scientific community unanimously recognises DF as an essential component for human health. Disagreements often arise, however, when attempting to relate the concept of DF to its physiological properties, as the term encompasses a wide variety of chemical structures.

DF is not a single entity; it is far more complex than it initially appears. This complexity partially explains the apparent inconsistencies sometimes described in the literature, as well as the wide range of physicochemical properties and physiological effects associated with fibre intake. The chemical components comprising the fibre complex vary according to the food source, and consequently, the physicochemical and physiological properties and associated health effects depend on the source of fibre (Fardet, 2017). Dietary fibre encompasses a diverse set of plant-derived compounds, including but not limited to carbohydrates, that resist digestion by human enzymes and are associated with health-promoting effects.

This work does not aim to provide an exhaustive review of the evolution of the fibre concept, but rather to emphasise and deepen understanding that DF does not correspond to a single type of compound. Rather, it is a heterogeneous mixture of substances with diverse physical, chemical, and physiological properties. The common feature of the compounds included in the fibre complex is that they are non-digestible in the small intestine. As such, they cannot be straightforwardly addressed from either an analytical or nutritional perspective. Furthermore, the complexity of these structures is compounded by the wide variety of physiological effects derived from consuming fibre-containing foods.

As a starting point, we refer to the Codex Alimentarius definition, which is particularly important because Codex sets global standards for food. This definition underpins analytical methods, food labelling, nutrient reference values, and health claims (Smith et al., 2009). Codex defines DF as: "Carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by endogenous enzymes in the human small intestine and belong to the following categories: (1) Edible carbohydrate polymers naturally occurring in the food as consumed; (2) Carbohydrate polymers obtained from food raw materials by physical, enzymatic, or chemical means, which have been shown to have a physiological effect beneficial to health as demonstrated by generally accepted scientific evidence to competent authorities; (3) Synthetic carbohydrate polymers which have been shown to have a physiological effect beneficial to health, as demonstrated by generally accepted scientific evidence to competent authorities."

Stephen et al. (2017) support this definition, describing DF as a mixture of qualitatively and quantitatively diverse chemical structures, dependent on the fibre source. Physiological properties depend on the type and quantity of these structures and their physicochemical properties in the intestinal environment, including viscosity, water-holding capacity, and interactions with minerals, fats, and sugars. On this basis, a classification of DF materials according to the characteristics of their main components could be proposed.

Therefore, the concept of DF can be understood as the association of different chemical structures, all non-digestible. Depending on the plant source, the composition of this mixture varies (Figure 1). In some fibres, polysaccharides predominate, as in citrus fibre. Others, such as fibres from legumes or red fruits, contain a high number of associated polyphenols. In some plant materials, phytosterols predominate, such as in nuts or plant-derived fats, while others, like green leafy vegetables, are rich in carotenoids. Each fibre type is structurally distinct, with different physicochemical properties and physiological behaviours, yet all consist of non-digestible components in the small intestine and require specific chemical methods for characterisation.

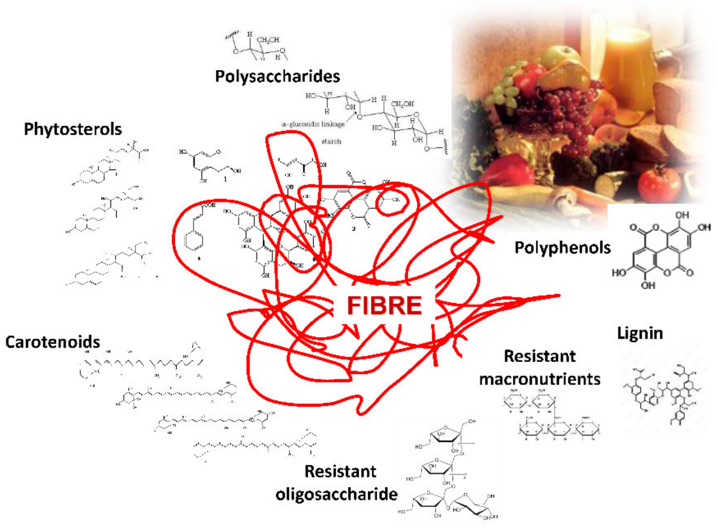

**Figure 1.** Free representation of dietary fibre complex.

Non-digestible components of foods may include carbohydrates, such as polysaccharides and resistant oligosaccharides. Polysaccharides comprise both non-starch polysaccharides (NSP) and resistant starch. The NSP fraction includes cellulose, hemicelluloses, β-glucans, pectins, gums, mucilage, galactomannans, alginates, carrageenans, and arabinoxylans. Other non-digestible components not classified as carbohydrates include lignin, undigested macronutrients, and phytochemical compounds such as polyphenols, carotenoids, and phytosterols (Figure 2).

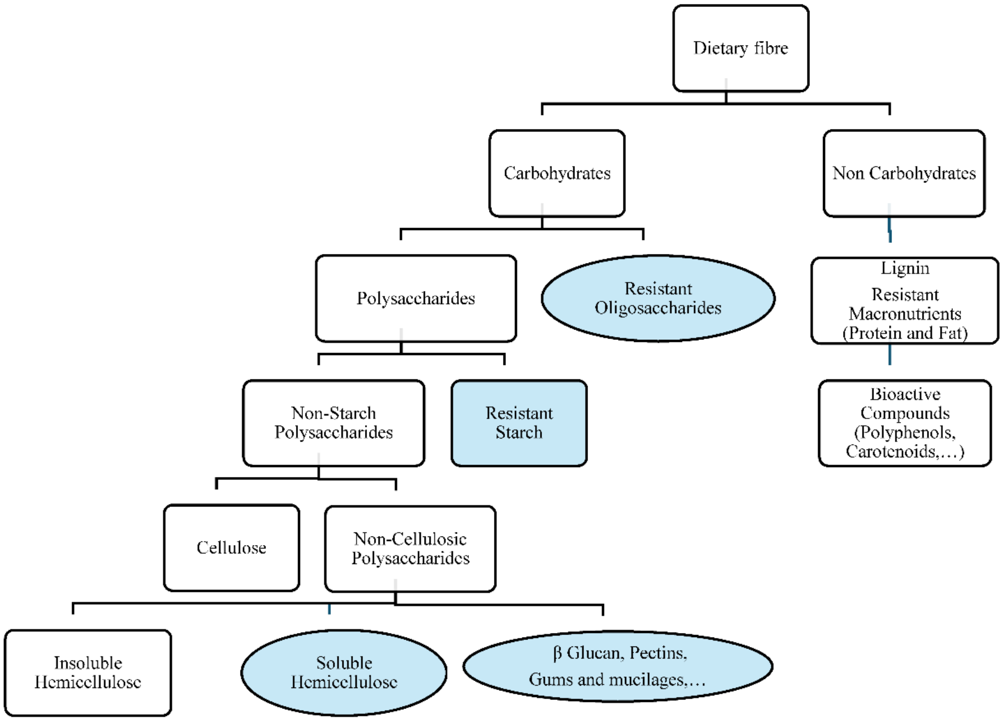

**Figure 2.** Composition of dietary fibre. Rectangular shapes: Insoluble components; Oval shapes: Soluble components; Shaded: Fermentable components.

Both carbohydrate and non-carbohydrate fractions contain soluble and insoluble components. Regardless of solubility in the intestinal environment, these non-digestible compounds are potentially fermentable by the gut microbiota. Generally, soluble compounds are more readily fermented than insoluble ones.

Saura-Calixto et al. (2000) coined the term "indigestible food fraction" for the set of non-digestible components of foods and proposed an analytical method based on physiological conditions in the intestinal tract. This method has been applied to all plant-based foods in the Spanish diet, allowing for the individual characterisation of all components.

An important effect of non-digestible dietary components is their role in maintaining a healthy gut microbiota. DFs are recognised as critical determinants of gut microbiota composition and function. Fermentable fibres have been shown to result in distinct differences in microbiome composition and in the production of beneficial metabolites.

## 4. The Role of DF in Transporting Phenolic Compounds and Others Bioactive Compounds

DF does not constitute a single, well-defined chemical group, but rather a combination of chemically heterogeneous substances, as previously discussed. The physiological and physicochemical effects of DF are determined by the relative amounts of individual non-digestible components, their interactions with each other, and their integration within the food matrix. DF is capable of transporting phytochemicals bound to the fibre matrix through the human gastrointestinal tract (Goñi et al., 2006; Hervert-Hernández & Goñi, 2011; Saura-Calixto et al., 2007). Consequently, the presence of phytochemicals associated with DF may significantly influence its physiological properties and health effects.

DF and antioxidants are both recognised as beneficial food constituents and functional ingredients, yet they are often studied separately in chemical and nutritional research. However, a substantial proportion of antioxidant, such as polyphenols (Saura-Calixto et al., 2007) and carotenoids (Goñi et al., 2006; Serrano et al., 2005), are bound to DF in plant foods and constitute a major part of the total antioxidants in the diet. It is therefore reasonable to assume that the association between antioxidants and DF contributes significantly to health benefits, and that some of the effects attributed to DF intake may be due to the DF–antioxidant complex. Accordingly, DF and antioxidants could be considered jointly in nutrition and health studies, combining the properties of both in a single material. This approach has led to the concept of antioxidant DF (ADF), defined as a material containing significant amounts of natural antioxidants associated with the fibre matrix. ADF meets the requirements for DF content and intrinsic antioxidant activity derived from the natural constituents of the material, rather than from added antioxidants or compounds released by prior chemical or enzymatic treatments (Goñi & Saura-Calixto, 2009). However, not all fibres exhibit antioxidant activity; only those containing sufficient antioxidante BC associated with the main fibre matrix confer measurable antioxidant properties. In this paper, we refer to this set as the DF complex. The dietary fibre complex is proposed as a conceptual and physiological framework intended to complement, rather than replace, existing regulatory definitions such as Codex

The concept of ADF is useful for distinguishing DF-rich materials with substantial antioxidant capacity from those with negligible activity. Furthermore, the antioxidant capacity of the whole material reflects the cumulative, synergistic activity of polyphenols and other antioxidant constituents. This parameter provides an integrated measure of the antioxidant units potentially carried by DF through the human gut, highlighting the fibre matrix's role as a carrier of BC —an essential physiological function for certain materials.

Phenolic compounds, key bioactives in healthy dietary patterns, can be classified according to intestinal bioaccessibility, which depends partly on the composition of the DF complex and its physicochemical characteristics. Two major groups can be distinguished: 1) Phenolic compounds potentially bioaccessible in the small intestine: These include polyphenols that are solubilised in the stomach and small intestine or released from the food matrix by digestive enzymes. These low-molecular-weight polyphenols (LP) could be absorbed, at least partially, through the small intestinal mucosa, followed by metabolism and systemic effects. Only 5–10% of polyphenols bioaccessible in the small intestine are absorbed (Clifford, 2004); the remainder may reach the colon and are considered part of the second group. 2) Phenolic compounds potentially bioaccessible in the large intestine: A large proportion of polyphenols remain unabsorbed along the gastrointestinal tract, accumulating in the large intestine, where they could be extensively metabolised by the gut microbiota (Garcia-Alonso et al., 2022; Wan et al., 2021). These macromolecular polyphenols (MP) include proanthocyanidins and polymeric flavonoids, often associated with other non-digestible food components. The microbiota hydrolyses, reduces, or decarboxylates polyphenols, producing metabolites (e.g., dihydroxyphenyl acids, urolithins, equol) with significant biological activity. The microbiota's capacity to metabolise non-digestible polyphenols exceeds that of physicochemical or biotechnological treatments, reflecting its complexity and genomic diversity.

In chemical terms, group 1 polyphenols are also called extractable polyphenols, comprising low-molecular-weight compounds (monomers to decamers) soluble in aqueous organic solvents (methanol, acetone, ethanol, ethyl acetate, etc.). These include flavonoids (flavanols, anthocyanins, flavonols), benzoic and hydroxycinnamic acids, stilbenes, extractable proanthocyanidins, hydrolysable tannins, and others. Non-extractable polyphenols, corresponding to group 2, are macromolecular and include proanthocyanidins, polymeric flavonoids, and low-molecular-weight polyphenols bound to other food components or entrapped within the food matrix, along with minor

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amounts of carotenoids and other bioactives. These compounds reach the colon, where they interact with the microbiota during colonic fermentation, which has significant health implications for the host. This process likely contributes to a substantial proportion of the biological activity associated with the consumption of fruits, vegetables, and other plant-derived foods and beverages (Williamson & Clifford, 2010).

To our knowledge, only the intestinal microbiota can disrupt the complexes formed by non-digestible food components under physiological conditions, releasing associated compounds that become bioaccessible in the large intestine (Saura-Calixto et al., 2010). Microbial catabolites are often better absorbed than their parent compounds due to the colon's large absorptive area, high luminal concentrations, and specific absorption mechanisms (Williamson & Manach, 2005).

In summary, only a small fraction of dietary polyphenols are bioaccessible in the small intestine (LP), which are soluble and extractable using organic solvents. The majority (MP) remain associated with other non-digestible components in the food matrix, often overlooked in bioavailability and metabolism studies, yet they exert substantial health effects (Saura-Calixto et al., 2007).

## 5. Gut Microbiota: The Unknown Organ That Maintains Our Health

### *5.1. Gut Microbiota: A Brief Introduction*

The human microbiota comprises all the microorganisms in our body, which can be categorised as commensals, mutualists and pathogens according to their behaviour. Scientific research on the gut microbiota is booming, with experts from different disciplines collaborating to expand our understanding of this vital organ. Our knowledge of the human microbiota has increased considerably since the introduction of 16S rRNA next-generation sequencing (16S rDNA gene). This technological breakthrough has revolutionised our understanding of microbiota composition and its implications for human health.

The most densely populated human organ is the colon ($10^{11}$–$10^{12}$ cells $g^{-1}$). It houses more than 70 per cent of all microbes in the human body, including between 500 and 1000 different species. Humans are born essentially sterile and acquire intestinal microorganisms from their mother and the external environment. Microbial colonisation of the gastrointestinal tract is influenced by various factors, such as mode of delivery, feeding regimen and antibiotic therapy (Ross et al., 2010).

Interestingly, most of the references consulted indicate that the microbiota genome comprises more than 3.3 million genes, 150 times more than the human genome. Furthermore, each individual microbiome is unique and differs from that of other humans (Gilbert et al., 2018). While the human genome encodes only approximately 25,000 genes, human microbiomes are estimated to encompass between 2 and 20 million genes, representing up to 99.9 per cent of the human body's genetic capacity (Del Chierico et al., 2014).

These figures regarding the bacterial population and its proportion relative to the number of human cells have been critically examined using information published by Sender et al. (2016). Recently, Dey (2025) concluded that the number of gut bacteria is ten times lower than previously predicted based on traditional data, and that the ratio between bacterial cells and human cells is approximately 1:1, constituting a total bacterial mass of 0.2 kg for a typical 70 kg man. This is clearly a very interesting and topical subject for discussion.

The human colonic microbiota can be considered a closely co-evolved microbial partner of the human genome, extending host-encoded functions and enabling the host to obtain energy and other biologically active compounds from food components that would otherwise remain inaccessible and be excreted as waste (Gilbert et al., 2018; Van Hul et al., 2024).

Microbial communities change dynamically within and between each stage of life, from birth to death (Hou et al., 2022; Van Hul et al., 2024), and respond to the host environment. Most intestinal phylotypes belong to a restricted set of phyla, such as Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Verrucomicrobia (Del Chierico et al., 2014), but the relative abundance of bacterial phyla and species usually varies in response to external factors, especially diet. Diet is one of the most significant determinants of microbial diversity in the gastrointestinal tract, and dietary components influence both microbial populations and their distribution (Del Chierico et al., 2014; Ross et al., 2010; Turnbaugh et al., 2007).

The entire colonic bacterial population has been described as a “new organ” that performs specific, key functions for maintaining optimal health. The gut microbiota is equivalent to an internal organ in itself, but it is prone to adaptation and alteration, with far-reaching effects throughout the human body (Ross et al., 2010). This includes effects on the liver, brain, pancreas and immune system (Hou et al., 2022). These functional effects are undoubtedly due to the ability of the microbiota to produce various metabolic compounds with bioactive properties, which are transported throughout the body via the circulatory system. There is now no doubt that the composition of the microbiota exerts a major influence on human health (Hou et al., 2022).

The gut microbiota supports a wide variety of physiological functions and possesses enzymatic and metabolic activities that influence host nutrition and health (Heavey & Rowland, 2004).

The physiological effects of the microbiota result from a three-way interaction between non-digestible food components (substrates), microbiota and epithelial cells (colonocytes), interactions that occur within the intestinal ecosystem continuously throughout life. Bacteria, original substrates, preformed metabolites, metabolic residues and epithelial cells interact in the intestinal environment, producing numerous effects on the health of both the intestinal ecosystem and the host (Hou et al., 2022).

Once non-digestible dietary components reach the colon, they become available for the fermentative activities of the colonic microbiota. These compounds appear to modulate both species composition within the gut microbiota and the metabolite profile in the colon; some metabolites are absorbed, while others remain within the intestinal ecosystem. Interactions, competition and synergistic effects between bacterial enzymes, dietary substrates and metabolites can be expected (Freilich et al., 2011).

Del Chierico et al. (2014) proposed a map of the individual gut microbiota based on meta-omics studies of the relationship between consumption of a healthy diet, such as the Mediterranean diet, and the prevalence of disease. Their conclusions are consistent with the comments in previous paragraphs, although most reviewed trials showed considerable variability due to small sample sizes and the wide range of pathologies studied. These are common limitations in many studies exploring the relationship between diet and healthy microbiota balance.

Whether effects are beneficial or detrimental depends on the balance between bacterial populations. When this balance is altered, dysbiosis occurs and risk factors for disease increase (Compare et al., 2012). Dysbiosis refers to an imbalance in the microbial community in the gut and represents a precursor to diseases such as hepatic steatosis, metabolic syndrome, behavioural abnormalities, metabolic disorders and inflammatory conditions (Del Chierico et al., 2014; Freilich et al., 2011). Such imbalance may involve reduced microbial diversity, shifts in the relative abundance of different species, or overgrowth of potentially harmful microorganisms. The appearance of dysbiosis depends largely on the type of substrates used by the microbiota—and it must be remembered that these substrates derive primarily from the diet. Zhang et al. (2010) reported that dietary alterations account for 57 per cent of total variation in gut microbiota, whereas genetic background accounts for only 12 per cent.

To date, interactions affecting metabolic pathways and numerous metabolites have been widely documented (Freilich et al., 2011; Chu et al., 2024; Mao et al., 2024). However, the effects of some of these on the modulation of intestinal ecology and the growth of specific microbial species remain poorly understood. Undigestible dietary compounds may confer health benefits by modulating the gut microbiota. In this regard, substrates may exert a dual positive effect: inhibiting pathogenic bacteria while stimulating beneficial ones. The effects of the intestinal microbiota on host health depend mainly on the type of substrate and, more specifically, on the type of fermentable substrate, since both substrates and their metabolites contribute to gastrointestinal health by modulating microbial balance, inhibiting pathogens and stimulating beneficial bacteria.

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In summary, the set of non-digestible dietary compounds corresponds to a broader concept than DF, since it includes not only classic fibre components but also all food components and/or digestion residues that share a common characteristic: they are not digested in the small intestine. This group constitutes a heterogeneous set of constituents that are key in preventing various chronic diseases. The DF complex can be fermented by the gut microbiome and thus modulate the ecology and metabolism of the bacterial population, as well as interact with epithelial cells. Both metabolites and parent compounds can affect numerous metabolic pathways and influence host health.

The type of compounds reaching the colon also determines the amount of material not degraded by bacteria and the flow of non-fermented material excreted. Increased bacterial proliferation, metabolite production (some of which are gases) and water retention by certain fibre components increase the volume of intestinal contents, thereby reducing transit time. The DF complex increases faecal weight and reduces transit time, which decreases both the concentration of toxic metabolites and the exposure of the colonic mucosa to toxins. These factors may reduce the incidence of tumoural foci (Binns, 2013).

Our understanding of the gut microbiome remains limited. There is no agreed definition of what a "healthy" gut microbiome should look like. This hampers our ability to assess the healthiness of gut microbiota profiles based on metagenomic data and makes it difficult to determine what a health-promoting microbiota should look like according to diet and life stage. However, increases in pathological strains may indicate dysbiosis. Current approaches therefore tend to focus on reducing these strains while increasing the abundance of health-promoting bacteria such as *Bifidobacterium* and *Lactobacillus* spp. (Van Hul et al., 2024).

The increase in certain probiotic bacteria has shown encouraging results in numerous preclinical studies, sparking scientific, industrial and public interest in probiotics and prebiotics (see below) as potential tools for managing and modulating the gut microbiome in consumers and patients. Genomics, bioinformatics and artificial intelligence are facilitating the establishment of mechanistic links within the intestinal microbial ecosystem and their effects on host health (Malwe & Sharma, 2023; O'Grady et al., 2019; Saxena et al., 2024; Wu et al., 2023).

### *5.2. DF and Bioactive Compounds: Food for the Gut Microbiota*

Despite the enormous relevance of the intestinal microbiota in human health, its study is not the main objective of this work. Therefore, we will focus solely on the study of the non-digestible components of the diet which, due to their indigestible nature, constitute food for the microbiota, or, as Kussmann et al. (2023) described them, microbiota regulators.

The set of non-digestible compounds (dietary fibre complex) mentioned in the previous sections provides the source of nutrients and fuel for the growth and development of the intestinal microbiota. As indicated above, the composition of these non-digestible compounds can be highly varied, and therefore the metabolites formed and the physiological effects expected will also be diverse. Hence, many studies indicate that DFs may influence the occurrence of specific bacterial taxa, with this effect varying between individuals. Among the consequences associated with health benefits is microbial diversity, a topic still under debate; nonetheless, human gut microbial diversity generally responds to diet and can influence health (Delzenne et al., 2025; Van Hul et al., 2024; Yong, 2012).

It is important to emphasise both the quantity and type of substrates reaching the colon, as these will constitute the "primary food" for the microbiota and are the principal determinants of bacterial proliferation rates. Energy and bioactive metabolites are produced as a result of interactions between substrates and bacteria in the intestinal environment.

The host's diet exerts a major influence on the composition of the gut microbiota (Ross et al., 2010). Both microbiota and host maintain a nutritionally symbiotic relationship, and as a result, the gut microbiota exerts major effects on various host processes, ranging from gastrointestinal immunity to pathophysiological conditions such as inflammatory bowel disease, colorectal cancer, energy metabolism and obesity. Moreover, the gut microbiota plays a dominant role in intestinal mucosal immune responses, barrier reinforcement, and nutrient metabolism (Ross et al., 2010; Van Hul et al., 2024).

The intricate mechanisms underlying the truly symbiotic relationships between the complex array of gut microbes and their mammalian host may not only be associated with the entire bacterial population but, in some cases, specifically with the production of bioactive molecules (Ross et al., 2010). Various dietary components are metabolised by the gut microbiota to produce bioactive molecules with recognised beneficial effects on host health, including roles in preventing colon cancer, cardiovascular disease, and immune-mediated disorders. Furthermore, host–microbiota interactions are also implicated in the pathogenesis of chronic disorders such as Crohn's disease, irritable bowel syndrome and ulcerative colitis (Elson and Cong, 2012; Shen et al., 2025; Zheng et al., 2020).

The relative proportions of individual non-digestible components, and how they interact with one another and with the food matrix, determine the physiological and physicochemical effects of the mixture of chemical entities which we will continue to refer to as dietary fibre complex or the set of non-digestible compounds, as it does not correspond to the traditional concept of DF, as explained earlier. The complexity of fibre matrices—determined by the chemical nature and degree of polymerisation of certain components, and by the presence of oligosaccharides, polysaccharides and other associated compounds such as phytochemicals—is particularly significant in some plant materials. Clearly, there are numerous types of indigestible, fibre-like compounds, and interactions among them naturally occur within foods. Regulatory components of the microbiota include non-starch polysaccharides, lignin, resistant oligosaccharides, resistant starch, the undigested fractions of other nutrients (fat and protein), and endogenous waste found in the colon. Additionally, associated BC—such as polyphenols, carotenoids, terpenes, terpenoids and organosulphur compounds—should be included; these are grouped as phytonutrients in the classification proposed by Kussmann et al. (2023).

In conclusion, a variety of substrates (DF complex), depending on the diet consumed, become available for fermentation and influence the metabolic processes occurring within the colon. Some components of complex fibre may exert prebiotic effects, others may have strong antioxidant activity, and yet others may form viscous solutions that modify glycaemic responses. Therefore, each DF type needs individual evaluation, and specific health benefits must be demonstrated before a commercial product can be offered.

Once non-digestible dietary components reach the colon, they become available to the fermentative activities of the human colonic microbiota. These compounds appear to modulate both species composition within the gut microbiota and the metabolite profile in the colon. Some metabolites will be absorbed, while others remain within the intestinal ecosystem. Interactions, competition and synergistic effects between bacterial enzymes and dietary substrates are expected.

Colonic fermentation is an anaerobic breakdown of substrates by microbial metabolic activity. In addition to processing diet-derived material, the gut microbiota can perform a range of biotransformations on colonic substrates, affecting their absorption and bioavailability. Moreover, the presence of substrates may modulate bacterial populations, and the composition and activity of the intestinal microbiota can influence health and disease through their involvement in nutrition, physiological processes and the pathogenesis of certain conditions.

The treasure of natural bioactives must be exploited more efficiently and sustainably for the benefit of human and planetary health. Natural BC can make a significant and sustainable contribution to improving human and animal health and to promoting a healthier, more sustainable food system. Both contribute to a more efficient and affordable healthcare system and a more careful use of terrestrial and marine food resources.

Microbiome regulators include health-promoting bacterial strains known as probiotics, as well as edible food components that can influence the composition and activity of the gut flora, referred to as prebiotics. These prebiotic compounds include some components of the non-digestible set—among them phenolic compounds—overlapping with macronutrient and phytonutrient categories. Additionally, optimised combinations of prebiotics and probiotics, known as symbiotics, as well as postbiotic products (including microbially derived short-chain fatty acids, enzymes and vitamins), are also considered part of the microbiome regulator class. Advances in bioanalytical techniques and systems nutrition science provide new opportunities to study these compounds, combining traditional empirical knowledge with modern scientific approaches to elucidate the role of non-digestible food components in human and planetary health (Liu, 2022).

In the past, the concept of prebiotics was limited to non-digestible carbohydrates, but recent evidence strongly suggests that polyphenols also possess prebiotic activity. Indeed, the prebiotic effect may be enhanced when substantial amounts of dietary fibre complex are present. Therefore, regular consumption of a diet rich in plant-based foods may beneficially balance gut microbial ecology, helping prevent gastrointestinal disorders and enhancing host health.

It is increasingly evident that, in order to interpret the role of dietary components in population health, in addition to the necessary epidemiological data, it is essential to establish a new descriptive definition of the set of non-digestible components reaching the colon. Furthermore, analytical methods must be developed to accurately quantify and characterise this set of substrates. Such information would advance our knowledge of the underlying mechanisms of action responsible for their beneficial effects.

## 6. Intake, Bioavailability and Prudent Reference Values of Phenolic Compounds

Several systematic reviews and meta-analyses of both observational and interventional studies have demonstrated a relationship between a diet rich in BC and a reduced risk of numerous chronic diseases. This evidence justifies and encourages further research efforts aimed at establishing a robust methodological process for defining dietary recommendations specific to BC (Biesalski et al. 2013; Del Bo et al. 2019).

As with essential nutrients, some non-essential food components cannot be synthesised by the body. However, because they provide health benefits and are considered part of a healthy dietary pattern, they may be regarded as bioactive food components.

In contrast to essential nutrients, non-essential bioactive food components do not typically produce clearly described clinical deficiency symptoms when their intake is inadequate. Moreover, unlike essential nutrients, it may not be possible to carry out experiments that unequivocally demonstrate cause–effect relationships for non-essential food components, whether provided as pure substances, within foods, or from food extracts (Biesalski et al. 2013).

Therefore, there is increasing interest in developing dietary recommendations for BC. Such recommendations require detailed information on the long-term association between habitual intake and health at the population level, which can only be obtained through large-scale observational studies. Nutritional epidemiology relies heavily on accurate intake estimation. However, current methods involve two steps: (1) quantifying food consumption and (2) converting this information into data on nutrients, BC and kilocalories using food composition tables or databases. Both approaches present significant limitations and fail to estimate the systemic presence of BC (Biesalski et al. 2013; Del Bo et al. 2019; Ottaviani et al. 2023; Xu et al. 2021).

Both objectives face considerable challenges. Firstly, estimating the consumption of foods that are sources of BC is prone to methodological errors; secondly, existing databases describing the content and composition of dietary polyphenols are incomplete and insufficient for accurately determining dietary intake. Furthermore, data estimating the intake of these compounds in whole diets are scarce and often controversial for several reasons that will be outlined below. Together, these limitations make estimating BC intake using population-level epidemiological data a difficult—if not nearly impossible—task. Moreover, to clarify the importance of BC in human health, it is essential to know not only the amount consumed in the diet but also their bioavailability within the body.

In summary, despite the relevance of understanding BC intake for population health, no established reference values exist for prudent intake of these compounds. Therefore, in this section, we address three main challenges: (1) determining the intake of polyphenolic compounds in the population, as they represent the major dietary BC; (2) determining their bioavailability in the body; and (3) establishing reference and/or prudent intake values.

### *6.1. Intake of Phenolic Compounds in Populations*

Bioactive intake is typically estimated using a two-step process, both of which are subject to considerable bias, insufficient specificity and precision, and, consequently, certain errors.

First of all, estimating polyphenol intake requires secure and reliable data on food consumption within each population. Dietary and supplement data are usually obtained from 24-hour dietary recalls and/or food frequency questionnaires. However, a homogeneous protocol for data collection would be necessary, as many uncontrolled factors influence the final estimation of food consumption data.

On the other hand, the choice of databases is another critical factor when estimating polyphenol intake. The most commonly used are the USDA (2024) and Phenol-Explorer (Rothwell et al., 2013).

These databases have improved considerably in recent years and increasingly provide more precise and detailed information on polyphenol composition. However, their use still presents important limitations. Although they provide information on a wide range of foods, the list does not include all food and polyphenol sources. In addition, the effects of seasonality, storage, and food processing must be taken into account, as these undoubtedly represent critical points (Xu et al., 2021).

Experimental variability in food consumption estimates is often assumed. However, food composition data are rarely questioned. Most databases provide information based only on average food composition, not actual composition (Del Bo et al., 2019). Moreover, literature data on the content and composition of dietary polyphenols are partial and insufficient for determining true dietary intake. While the content of certain phenolic compounds in many common foods is reported, comprehensive data on their levels in complete diets are lacking (Xu et al., 2021).

Another important limitation in the case of polyphenolic compounds is that databases do not include MP, which contributes to a general underestimation of intake (Arranz et al., 2010; Macagnan et al., 2016). Data on dietary polyphenols generally correspond to several categories of compounds that we have included in the group referred to as LP in previous sections of this work. These are quantified in aqueous–organic extracts of foods, while the remaining polyphenols present in extraction residues are usually ignored. This represents a significant error in intake estimation, as these unextracted polyphenols are quantitatively higher than LP. They are potentially degraded by the enzymatic activity of the gut microbiota and are partially absorbed systemically (Palafox-Carlos et al., 2011; Saura-Calixto et al., 2010). This is relevant since these compounds appear to exert potential protective effects through the production of microbiota-derived metabolites (González-Sarrías et al., 2017). Significant amounts of MP have been reported in specific plant foods (Goñi et al., 2009; Saura-Calixto, 2011) and in complete diets (Goñi and Hernández-Galiot, 2019).

There is a large body of literature addressing specific types of polyphenols in individual foods; however, studies examining total polyphenols in whole diets are scarce. Therefore, the use of terms such as “total phenolic content” can create confusion when comparing results, as this term may be incomplete if it refers only to LP. In the present work, total polyphenol content refers to the sum of LP and MP.

In a systematic review by Del Bo et al. (2019), which included studies conducted in Europe, North America and Asia, the average total polyphenol intake was estimated at 1500 mg/day, 900 mg/day and 800 mg/day in Japan, European countries and North and South America respectively. These values are significantly lower than those reported by other authors who included MP (Saura-Calixto, Serrano & Goñi, 2007; Goñi & Hernández-Galiot, 2019).

The intake of polyphenols (LP plus MP) was determined several years ago in the complete Spanish diet. The mean total polyphenol intake in 2007 was around 3000 mg/person/day (Saura-Calixto, Serrano & Goñi, 2007). Data updated for 2023, prepared for this document and presented below, indicate that the current estimated intake is 2065 mg/person/day. Although intake has decreased in recent years, both values remain significantly higher than those estimated for other populations mentioned above. Using the same methodology as Saura-Calixto, Serrano & Goñi (2007), the intake of total polyphenolic compounds (LP plus MP, 2196 mg/day) was assessed in an elderly population from a Spanish region (Goñi & Hernández-Galiot, 2019). Again, these values are notably higher than population intakes published by other authors (Del Bo et al., 2019), owing to the inclusion of MP.

### *6.2. Intestinal Bioaccessibility and Bioavailability of Phenolic Compounds*

Bioactive compounds are metabolised by digestive enzymes in the small intestine or by the enzymatic activity of the microbiota in the large intestine. The metabolites produced can be absorbed, enter the bloodstream and be distributed systemically throughout the body, or remain within the intestinal environment. Biomarkers of bioactive intake are usually based on the presence of these compounds or their metabolites in blood, urine, or other biospecimens. This process is influenced by the specific absorption, distribution, metabolism, and excretion of each bioactive compound; therefore, their use requires careful validation. This aspect will be discussed in the following section.

Moreover, the bioaccessibility and bioavailability of each bioactive compound vary considerably depending on its type and origin. For example, the most abundant compounds in consumed fruit are not necessarily those that produce the highest concentrations of active metabolites in target tissues (Manach et al., 2005). Consequently, to study the role of BC in human health, it is essential to understand the bioavailability of each compound within the food matrix. Many researchers are investigating these aspects, but they are still not fully understood (Barba et al., 2017; Kamiloglu et al., 2021; Manach et al., 2005), and the topic remains an open field of research.

To exert their biological properties, polyphenols must be available to some extent in the target tissue. Food polyphenols must therefore be bioavailable in some form to exert biological effects. The enzymatic release of each compound from the food matrix is one of the key steps in the bioavailability of polyphenols in the gastrointestinal tract. This release may occur in the small intestine and/or the large intestine or it may not occur at all. Only small polyphenol molecules (LP) originally present in the food, along with those released from the matrix by digestive enzymes (in the small intestine) or by microbial enzymes (in the colon), may be bioaccessible and therefore potentially bioavailable. Understanding these mechanisms is essential to relate dietary intake to disease prevalence and to safely estimate reference intake levels for these compounds.

Additionally, the quantity of bioaccessible food polyphenols may differ both qualitatively and quantitatively from the polyphenols recorded in food databases, as bioaccessibility is not taken into account during database compilation. Furthermore, most studies on polyphenol bioavailability rely predominantly on pure single molecules (either isolated from foods or chemically synthesised), even though their bioavailability from whole foods may differ substantially. Certain beverages and individual foods have also been examined (Manach et al., 2005), but their relevance to health may be limited because their contribution to total dietary polyphenol intake is normally low.

As noted above, the estimated content of MP in the complete diet is nearly double that of LP, and the physiological effects of both groups depend closely on their degree of intestinal bioaccessibility. LP released from the food matrix during digestion—referred to as bioaccessible polyphenols in the small intestine—are soluble in the digesta and susceptible to absorption across the gut barrier. However, most polyphenols are transported through the gut within the non-digestible fraction of food, mainly the insoluble fraction (Goñi et al., 2009; Saura-Calixto, 2011). This aspect has been discussed in a previous section. These compounds include condensed tannins and hydrolysable phenolic compounds, as well as a substantial number of small polyphenol molecules (LP). All of them are resistant to digestion in the small intestine and reach the colon, where they interact with the colonic microbiota. The indigestible soluble fraction may also transport polyphenols to the colon, as these molecules do not cross the intestinal barrier and instead act as fermentation substrates for the microbiota together with other non-digestible dietary constituents (Goñi et al., 2009).

The abundant colonic microflora plays a fundamental role in polyphenol metabolism. Colonic fermentation generates bioactive metabolites that may follow two pathways: some pass into the

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bloodstream, whereas others remain in the colonic environment together with the material not catabolised by the microbiota.

Saura-Calixto, Serrano & Goñi (2007) quantified the bioaccessibility of total dietary polyphenolic compounds (LP and MP) in the Spanish population. Using the same methodology, the intestinal bioaccessibility of LP and MP in the current Spanish diet (2023) has been estimated and is presented in Figure 3.

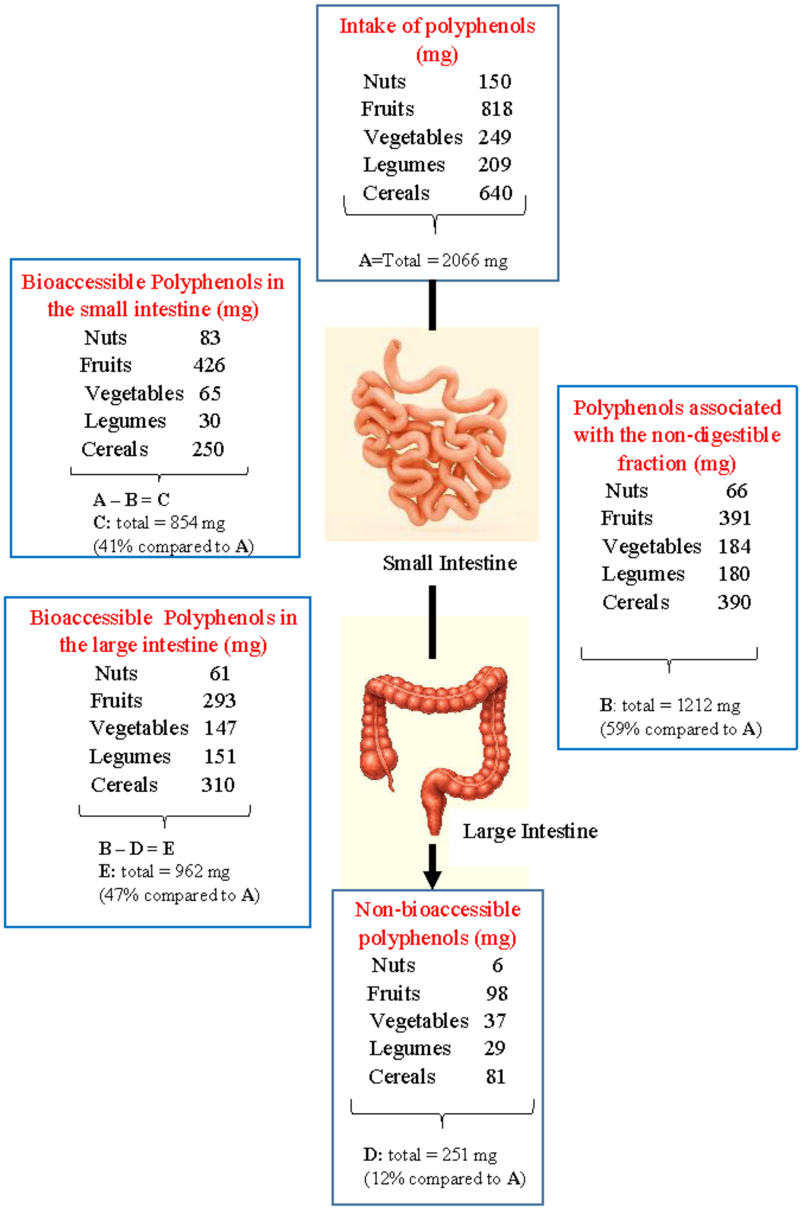

**Figure 3**. Estimation of the intestinal bioaccessibility of polyphenols (LP plus MP) consumed from solid plant foods in the Spanish diet, 2023 (mg/person/day).

An *in vitro* experimental model was used, and extrapolation to healthy humans has limitations. Nonetheless, information on the potential bioaccessibility of different compounds in the small and large intestine is valuable, as the foods analysed were prepared as consumed in a typical Spanish diet, and the experimental conditions simulated digestive and microbial processes occurring throughout the gastrointestinal tract. Moreover, this methodological protocol allows for a more detailed analysis of the metabolites produced in both digestion phases from whole foods (Saura-Calixto, Serrano & Goñi, 2007).

It is estimated that approximately 41% of total dietary polyphenols from solid plant foods are bioaccessible in the small intestine. Figure 3 does not include polyphenols from liquid foods, although it must be assumed that polyphenols from beverages are fully bioaccessible in the small intestine, as they pass directly into the intestinal fluids. These would constitute the main dietary contributors of bioaccessible LP in the small intestine. Therefore, the values shown in Figure 3 are underestimated.

Other studies have reported very low bioavailability of bioaccessible polyphenols in the small intestine, with values ranging from 5–10% (Clifford, 2004). Thus, it seems reasonable to consider that, together with the polyphenols associated with the non-digestible fraction that reach the colon, a substantial proportion of polyphenols potentially bioaccessible in the small intestine are also found in the colon due to their low bioavailability. As shown in Figure 3, 59% of polyphenols ingested from solid foods are potential substrates for colonic fermentation (fraction B in Figure 3).

The current average daily polyphenol intake in the Spanish diet is estimated at around 2066 mg/day (fraction A in Figure 3). Of this amount (LP and MP combined), 41% is bioaccessible in the small intestine (fraction C in Figure 3), while 47% is bioaccessible in the colon (fraction E in Figure 3). Only 12% of polyphenols are not bioaccessible in the gastrointestinal tract (fraction D). These data may be useful for the design and interpretation of epidemiological and intervention studies addressing the health effects of polyphenols and plant foods.

Assessing polyphenol bioavailability is of physiological and economic importance, as these ingredients are widely used in the food industry. However, despite the growing body of available data, it remains difficult to draw definitive conclusions about the bioavailability of most dietary polyphenols. Further research is needed on the identification and quantification of their true biological activity, as well as on the development of strategies to improve their bioavailability. Advancing knowledge in this field will be essential for understanding the role of polyphenols in human health and for optimising dietary recommendations for the population.

### *6.3. Recommended Intakes or Prudent Reference Values for Phenolic Compounds*

Recommended nutrient intakes are established on the basis of health problems identified when a dietary deficiency occurs. The adverse physiological or metabolic effects produced by such deficiencies are reversed when the missing nutrient is incorporated into the diet. These dietary standards are thus based on scientific evidence regarding the nutritional needs of virtually all healthy individuals in the population—consistent with the definition of a nutrient. However, this work focuses on dietary BC, which are not classified as nutrients, although the same procedures may serve as a foundation for attempting to establish recommended intakes of BC.

Whereas RDA/DRI committees have defined numerical standards for all known essential nutrients, this has not been the case for BC. Some countries, such as China and South Korea, have established numerical recommendations for specific bioactives (Lupton et al., 2014). Erdman (2023) conducted a comprehensive review outlining the development of Recommended Dietary Intakes (RDIs) and Dietary Reference Intakes for BC. More recently, Yates et al. (2017) published a useful framework for developing evidence-based guidelines which have been rigorously reviewed by qualified experts for both efficacy and safety. Their recommendations aim to communicate the intake levels of specific dietary BC associated with identified health benefits. They proposed a structured, sequential four-step decision-making process and emphasised that "the translation of evidence into recommendations should occur through a structured and transparent process, managed by credible health organisations with expertise and responsibility for developing food and nutrition recommendations". Yates et al. (2017) further highlighted the need to quantify causal relationships between bioactive markers and accepted markers of health or normal function, as well as to determine the safety and toxicity of each bioactive substance, to ultimately translate scientific evidence into quantified intake recommendations.

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To date, no standardized process exists for establishing RDI-like recommendations for BC. This represents a considerable challenge, as demonstrating a relationship between reduced chronic disease risk and intake of a bioactive compound is far more complex than identifying disease prevention attributable to deficiency of an essential nutrient (Lupton et al., 2014).

Determining which specific component(s) of a food account for reduced chronic disease risk is equally challenging. Such risk reduction may result from a single bioactive compound, from multiple compounds within the same food, or from synergistic interactions between bioactives. Most health-promoting foods contain complex mixtures of bioactive components, rarely dominated by a single compound. Establishing causality for an individual bioactive—or a related group—regarding chronic disease reduction is essential for producing meaningful public health recommendations.

In his proposal for establishing RDIs, Erdman (2023) underscored the need for rigorous scientific evaluation of both the efficacy and safety of a BC before issuing recommendations for health professionals and consumers. He also stressed that such processes must be conducted by health authorities. At present, sufficient scientific evidence is available to begin advancing in this field and to establish an appropriate framework for evaluating BC.

Developing dietary guidelines for the intake of food bioactives within a healthy dietary pattern is an important objective. This process clearly requires at least two types of knowledge: (1) reliable data on food composition and intake to estimate exposure to dietary bioactives, and (2) the ability to assess the intake levels required to exert protective effects.

However, given the methodological errors that inevitably occur when estimating BC intake, such estimates become merely indicators of dietary exposure and do not necessarily reflect actual bioactive intake. This makes it difficult to associate estimates with specific health effects, and can lead to misleading conclusions in which positive or negative associations between intake and health are incorrectly attributed to BC themselves rather than to the foods providing them. An alternative approach is the use of nutritional biomarkers to establish reference intakes. Biomarkers rely on the systemic presence of a bioactive compound and are therefore not affected by variability in food composition or gaps in food composition databases.

Intake assessments based on nutritional biomarkers represent an advanced alternative, but there are multiple challenges that must be addressed in order to obtain reliable data. Recent reviews have discussed these challenges and highlighted limitations in estimating polyphenol intake and establishing prudent reference values (Del Bo et al., 2019; Ottaviani et al., 2023).

Biomarkers of bioactive intake are typically based on the presence of the compounds or their metabolites in blood, urine, or other biospecimens. Their interpretation, however, depends on compound-specific absorption, distribution, metabolism, and excretion; their development thus requires careful validation. According to Ottaviani et al. (2019), nutritional biomarkers are currently the only reliable molecular tools for estimating intake of food bioactives. This field, although already advancing, requires considerable further effort from researchers and institutions. These authors describe the steps involved in identifying and evaluating intake biomarkers for bioactives and highlight common pitfalls and potential solutions.

Although observational studies are essential for identifying potential roles of diet-related compounds, well-controlled and targeted dietary intervention studies—particularly those assessing dose-response relationships—are crucial for identifying a reference or prudent intake (e.g., for health-promoting properties) of food bioactives such as polyphenols, either for the general population or specific vulnerable groups (e.g., the elderly). One advantage of establishing science-based recommendations for selective bioactives is that consumers may be encouraged to increase their intake of plant-based foods if authoritative statements and recommended intake levels are available for bioactives associated with health benefits.

What appears increasingly clear, based on available data, is that dietary patterns rich in BC are associated with better health outcomes. On this premise, a dietary pattern high in foods rich in BC may be proposed as a means of deriving specific recommendations. In this context, Saura-Calixto and Goñi (2009) examined the evolution of food consumption in the Spanish diet (Mediterranean diet pattern) between 1964 and 2005 and concluded that this healthy dietary pattern could be defined on the basis of four essential dietary indicators. They provided the ranges of their values considered healthy: 1) monounsaturated to saturated fatty acid ratio (range: 1.6 to 2.0); 2) intake of the DF complex (41 to 62 g/person/day); 3) antioxidant capacity of the whole diet (3,500 to 5,300 Trolox equivalents/person/day); 4) phytosterol intake (370 to 555 mg/person/day). Indicators 2, 3, and 4 are based on the intake of BC.

While there is insufficient evidence to recommend precise intake levels, efficacy, or safety for these substances, particularly when used as isolated supplements, it is generally agreed that, when consumed as part of a balanced diet, their benefits are substantial.

Most BC are found in plant-based foods such as fruits, vegetables, and whole grains, the consumption of which is associated with reduced risk of numerous chronic diseases. The decline in polyphenol intake observed in the U.S. population since 2007 (Del Bo et al., 2019) coincides with reduced consumption of fruits, vegetables, and whole grains, reinforcing the need to increase intake of these food groups. Similar trends have been observed in other populations, including Spain. Figure 4 shows the evolution of plant-based food consumption (2000 – 2023) in the Spanish diet. A decrease in the intake of fruits, vegetables, and grains is apparent, similar to trends reported in the U.S. population. Nonetheless, an increase in nut and legume consumption, foods particularly rich in polyphenolic compounds, has also been observed.

Table 1 presents the current estimated intake of polyphenolic compounds (LP and MP) in the Spanish population. These values are quite similar to those assessed in the 2005 diet of the same population. Small differences in food consumption (Figure 4) are not reflected in the intake of total polyphenolic compounds (Table 1): LP intake remains unchanged, while MP intake has decreased slightly.

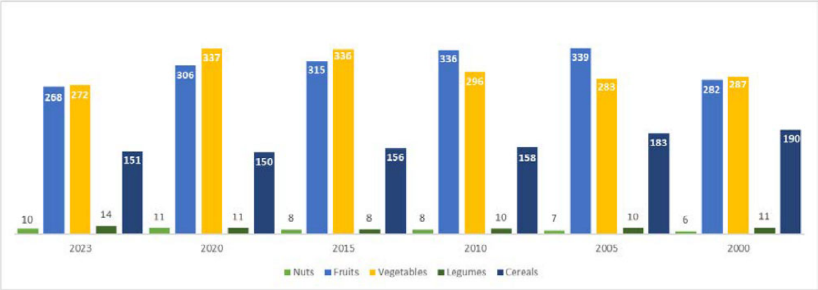

**Figure 4.** Evolution of household food consumption in the Spanish diet (g/p/d).

**Table 1.** Estimation of the intake of polyphenolic compounds in plant foods in the Spanish diet (mg/p/d).

| | **Low Molecular Polyphenols (LP)** | **Macromolecular Polyphenols (MP)** | |
|---|---|---|---|
| | | **Macromolecular Polymeric Polyphenols (MPP)** | **Molecular Hydrolysable Polyphenols (MHP)** |
| **Nuts** | 68,34 | 15,29 | 65,82 |
| **Fruits** | 178,28 | 408,60 | 230,97 |
| **Vegetables** | 95,86 | --- | 152,84 |
| **Legumes** | 21,30 | 105,97 | 82,03 |
| **Cereals** | 118,33 | -- | 521,99 |
| **TOTAL** | 482,11 | 529,86 | 1053,65 |

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Clearly, much work remains to be done to advance this field—both by researchers and public authorities. Priorities include: 1)Improving and standardising dietary assessment methods; 2) Standardising analytical procedures for BC analysis; 3) Updating and expanding food composition databases; 4) Establishing validated biomarkers specific to the intake of defined BC, such as polyphenolic compounds

## 7. Conclusions

The health benefits of plant-based foods are attributed to the synergistic effects that arise within a complete diet through interactions between nutrients and non-nutrient components. Among the components responsible for these effects are DF and BC. These two terms are closely related and sometimes controversial, although both are essential for a healthy diet.

The term DF is generic and non-specific, primarily because it does not refer to a single type of compound but rather to a heterogeneous mixture of chemical components whose composition varies depending on the source. They all share one common property: they are not digested in the small intestine. Based on this idea, the term DF complex has been defined as: “a group of chemical compounds present in plant-based foods that are not digested by endogenous enzymes in the human digestive tract, and which exert a beneficial effect on human health”. With regard to BC, the indiscriminate use of different terminology creates confusion among both consumers and researchers. In this work, the most commonly used terms have been integrated into the following definition: “BC are naturally occurring chemicals, mainly in plant-based foods such as fruits, vegetables, nuts, oils, and whole grains, which exert demonstrable beneficial effects on human health and contribute to reducing the risk of chronic diseases. Some are essential nutrients (e.g., certain vitamins), while others are non-essential and non-nutrients (e.g., carotenoids and polyphenols). Their role is to modulate biological functions to promote an optimal state of health, rather than to prevent a deficiency”. Most BC are chemically associated with remaining non-digestible food components, traditionally grouped under the term DF. Fibre compounds therefore act as carriers of BC, and both act synergistically in maintaining the health of the gut microbiota, since all non-digestible components present in the large intestine serve as substrates for microbial fermentation. It is important to emphasise the relevance of the quantity and type of substrates that reach the colon, as these constitute the “main food” for the microbiota and are a key determinant of microbial proliferation rates and overall gut health. Although desirable, there are currently no established reference values for a prudent intake of these compounds, and data on bioactive compound intake remain scarce. However, estimating bioactive compound intake within healthy dietary patterns could provide indicative figures for correlation with disease prevalence. For this reason, Low Molecular Polyphenols and Macromolecular Polyphenols intakes and their intestinal bioaccessibility within the current Spanish diet have been assessed.

The current average daily intake of polyphenols in the Spanish diet is estimated at approximately 2066 mg/day, including both of them. This value does not include polyphenols from liquid foods, although most polyphenols from beverages can be assumed to be bioaccessible in the small intestine, as they pass directly into intestinal fluids. These would likely constitute the major dietary contributors of Low Molecular Polyphenols bioaccessible in the small intestine. Therefore, the values presented in this work may be underestimated. The estimated Macromolecular Polyphenols content of the complete diet is almost double that of Low Molecular Polyphenols, and the physiological effects of both groups of polyphenols are closely linked to their degree of intestinal bioaccessibility. Forty-one per cent of total dietary polyphenols from solid plant foods were bioaccessible in the small intestine, while 47 per cent were bioaccessible in the large intestine. Only 12 per cent were not bioaccessible anywhere in the gastrointestinal tract.

Although these estimations inevitably involve limitations, they represent a valuable contribution to the design and interpretation of studies on the health effects of polyphenols. The analyses were performed using plant-based foods processed according to typical Spanish dietary habits, replicating common patterns of consumption. The experimental conditions for digestive enzymes and the enzymatic activity of colonic microbiota simulated realistic conditions throughout the gastrointestinal tract. Clearly, much remains to be done to advance this field, both by researchers and by public health authorities.

## References

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location & functional significance. In *Plant Science* (Vol. 196, pp. 67–76). https://doi.org/10.1016/j.plantsci.2012.07.014

Aronson, J. K. (2017). Defining 'nutraceuticals': neither nutritious nor pharmaceutical. In *British Journal of Clinical Pharmacology* (Vol. 83, Issue 1, pp. 8–19). Blackwell Publishing Ltd. https://doi.org/10.1111/bcp.12935

Arranz, S., Silván, J. M., & Saura-Calixto, F. (2010). Nonextractable polyphenols, usually ignored, are the major part of dietary polyphenols: A study on the Spanish diet. *Molecular Nutrition & Food Research*, *54*(11), 1646–1658. https://doi.org/10.1002/mnfr.200900580

Banwo, K., Olojede, A. O., Adesulu-Dahunsi, A. T., Verma, D. K., Thakur, M., Tripathy, S., Singh, S., Patel, A. R., Gupta, A. K., Aguilar, C. N., & Utama, G. L. (2021a). Functional importance of BC of foods with Potential Health Benefits: A review on recent trends. In *Food Bioscience* (Vol. 43). Elsevier Ltd. https://doi.org/10.1016/j.fbio.2021.101320

Barba, F. J., Mariutti, L. R. B., Bragagnolo, N., Mercadante, A. Z., Barbosa-Cánovas, G. V., & Orlien, V. (2017). Bioaccessibility of BC from fruits & vegetables after thermal & nonthermal processing. In *Trends in Food Science & Technology* (Vol. 67, pp. 195–206). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2017.07.006

Biesalski, H. K., Dragsted, L. O., ElmaDietary fibrea, I., Grossklaus, R., Müller, M., Schrenk, D., Walter, P., & Weber, P. (2009). BC: Definition & assessment of activity. *Nutrition*, *25*(11–12), 1202–1205. https://doi.org/10.1016/j.nut.2009.04.023

Biesalski, H. K., Erdman, J. W., Hathcock, J., Ellwood, K., Beatty, S., Johnson, E., Marchioli, R., Lauritzen, L., Rice, H. B., Shao, A., & Griffiths, J. C. (2013). Nutrient reference values for bioactives: New approaches needed? A conference report. *European Journal of Nutrition*, *52*(1 SUPPL.), 1–9. https://doi.org/10.1007/s00394-013-0503-0

Binns, N. (2013). Probiotics, prebiotics & the gut microbiota. ILSI Europe ISBN: 9789078637394.

Câmara, J. S., Albuquerque, B. R., Aguiar, J., Corrêa, R. C. G., Gonçalves, J. L., Granato, D., Pereira, J. A. M., Barros, L., & Ferreira, I. C. F. R. (2021). Food BC & emerging techniques for their extraction: Polyphenols as a case study. In *Foods* (Vol. 10, Issue 1). MDPI AG. https://doi.org/10.3390/foods10010037

Cao, H., Chai, T. T., Wang, X., Morais-Braga, M. F. B., Yang, J. H., Wong, F. C., Wang, R., Yao, H., Cao, J., Cornara, L., Burl&o, B., Wang, Y., Xiao, J., & Coutinho, H. D. M. (2017). Phytochemicals from fern species: potential for medicine applications. *Phytochemistry Reviews*, *16*(3), 379–440. https://doi.org/10.1007/s11101-016-9488-7

Chaudhary, M., & Garg, A. P. (2023). BC from plants & animals: A review. *International Journal of Advanced Biochemistry Research*, *7*(1), 105–117. https://doi.org/10.33545/26174693.2023.v7.i1b.175

Chu, C., Liu, S., Nie, L., Hu, H., Liu, Y., & Yang, J. (2024). The interactions & biological pathways among metabolomics products of patients with coronary heart disease. In *Biomedicine & Pharmacotherapy* (Vol. 173). Elsevier Masson s.r.l. https://doi.org/10.1016/j.biopha.2024.116305

Clifford, M. N. (2004). Diet-derived phenols in plasma & tissues & their implications for health. In *Planta Medica* (Vol. 70, Issue 12, pp. 1103–1114). https://doi.org/10.1055/s-2004-835835

Daliri, E. B. M., & Lee, B. H. (2015). Current trends & future perspectives on functional foods & nutraceuticals. In Beneficial microorganisms in food & nutraceuticals (pp. 221–244). Cham: Springer.

Compare, D., Coccoli, P., Rocco, A., Nardone, O. M., De Maria, S., Cartenì, M., & Nardone, G. (2012). Gut-liver axis: The impact of gut microbiota on non alcoholic fatty liver disease. In *Nutrition, Metabolism & Cardiovascular Diseases* (Vol. 22, Issue 6, pp. 471–476). https://doi.org/10.1016/j.numecd.2012.02.007

Del Bo, C., Bernardi, S., Marino, M., Porrini, M., Tucci, M., Guglielmetti, S., Cherubini, A., Carrieri, B., Kirkup, B., Kroon, P., Zamora-Ros, R., Liberona, N. H., &res-Lacueva, C., & Riso, P. (2019). Systematic Review on Polyphenol Intake & Health Outcomes: Is there Sufficient Evidence to Define a Health-Promoting Polyphenol-Rich Dietary Pattern? *Nutrients*, *11*(6). https://doi.org/10.3390/nu11061355

Del Chierico, F., Vernocchi, P., Dallapiccola, B., & Putignani, L. (2014). Mediterranean diet & health: Food effects on gut microbiota & disease control. In *International Journal of Molecular Sciences* (Vol. 15, Issue 7, pp. 11678–11699). MDPI AG. https://doi.org/10.3390/ijms150711678

Delgado, Y., Cassé, C., Ferrer-Acosta, Y., Suárez-Arroyo, I. J., Rodríguez-Zayas, J., Torres, A., Torres-Martínez, Z., Pérez, D., González, M., Velázquez-Aponte, R. A., &ino, J., Correa-Rodríguez, C., Franco, J. C., Milán, W., Rosario, G., Velázquez, E., Vega, J., Colón, J., & Batista, C. (2021). *Biomedical Effects of Popular Phytonutrients & Their Phytochemicals: A Comprehensive Review*. https://doi.org/10.20944/preprints202106.0371.v1

Delzenne NM, Bindels LB, Neyrinck AM, Walter J. The gut microbiome & dietary fibres: implications in obesity, cardiometabolic diseases & cancer. Nat Rev Microbiol. 2025 Apr; 23(4):225-238. doi: 10.1038/s41579-024-01108-z. Epub 2024 Oct 10. PMID: 39390291

Dey, P. (2025). All That Glitters Ain't Gold: The Myths & Scientific Realities About the Gut Microbiota. Nutrients, 17, 3121. https://doi.org/10.3390/nu17193121

Dietary Guidelines Advisory Committee. 2024. Scientific Report of the 2025 Dietary Guidelines Advisory Committee: Advisory Report to the Secretary of Health & Human Services & Secretary of Agriculture. U.S. Department of Health & Human Services. https://doi.org/10.52570/DGAC2025

Elson, C. O., & Cong, Y. (2012). Host-microbiota interactions in inflammatory bowel disease. In *Gut Microbes* (Vol. 3, Issue 4). https://doi.org/10.4161/gmic.20228

Erdman, J. W. (2023). Health & nutrition beyond essential nutrients: The evolution of the bioactives concept for human health. In *Molecular Aspects of Medicine* (Vol. 89). Elsevier Ltd. https://doi.org/10.1016/j.mam.2022.101116

FAO & WHO, 2020. Dietas saludables sostenibles - Principios rectores. Roma. https://doi.org/10.4060/ca6640es.

Fardet, A. (2017). Do the Physical Structure & Physicochemical Characteristics of Dietary Fibres Influence their Health Effects? Dietary Fibre Functionality in Food & Nutraceuticals: From Plant to Gut, First Edition. Edited by Farah Hosseinian, B. Dave Oomah & Rocio Campos-Vega. © 2017 JohnWiley & Sons Ltd. Published 2017 by JohnWiley & Sons Ltd.

Frank, J., Fukagawa, N. K., Bilia, A. R., Johnson, E. J., Kwon, O., Prakash, V., Miyazawa, T., Clifford, M. N., Kay, C. D., Crozier, A., Erdman, J. W., Shao, A., & Williamson, G. (2020). Terms & nomenclature used for plant-derived components in nutrition & related research: Efforts toward harmonization. *Nutrition Reviews*, *78*(6), 451–458. https://doi.org/10.1093/nutrit/nuz081

Freilich, S., Zarecki, R., Eilam, O., Shtifman Segal, E., Henry, C.S., Kupiec, M., Gophna, U., Sharan, R. & Ruppin, E. (2011) Competitive & cooperative metabolic interactions in bacterial communities. Nat. Commun. 2:589 doi: 10.1038 / ncomms1597

Garcia-Alonso, A., Sánchez-Paniagua López, M., Manzanares-Palenzuela, C.L, Redondo-Cuenca, A., & López-Ruíz B. (2022). Edible plant by-products as source of polyphenols: prebiotic effect & analytical methods. Critical Reviews in Food Science & Nutrition, 63(31), 10814-10835, DOI: 10.1080/10408398.2022.2084028

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., & Knight, R. (2018). Current underst&ing of the human microbiome. *Nature Medicine*, *24*(4), 392–400. https://doi.org/10.1038/nm.4517

GBD 2019 Diseases & Injuries Collaborators. (2020). Global burden of 369 diseases & injuries in 204 countries & territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*, 396: 1204–22

Goñi, I., Serrano, J., & Saura-Calixto, F. (2006). Bioaccessibility of β-carotene, lutein, & lycopene from fruits & vegetables. Journal of Agricultural & Food Chemistry, 54(15), 5382–5387. https://doi.org/10.1021/jf0609835

Goñi & Hernández-Galiot. (2019). Dietary intake & type 2 diabetes. In *Nutrients* (Vol. 11, Issue 9). MDPI AG. https://doi.org/10.3390/nu11092165

Goñi, I. & Saura-Calixto, F. (2009). Antioxidant DF. Concept & properties. Supplement to AgroFood industry hi-tech - May/June 2009 - vol 20 n 3, 21-22.

Goñi, I., Díaz-Rubio, M. E., Pérez-Jiménez, J., & Saura-Calixto, F. (2009). Towards an updated methodology for measurement of dietary fibre, including associated polyphenols, in food & beverages. *Food Research International*, *42*(7), 840–846. https://doi.org/10.1016/j.foodres.2009.03.010

González-Sarrías, A., Espín, J. C., & Tomás-Barberán, F. A. (2017). Non-extractable polyphenols produce gut microbiota metabolites that persist in circulation & show anti-inflammatory & free radical-scavenging effects. In *Trends in Food Science & Technology* (Vol. 69, pp. 281–288). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2017.07.010

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, A. (2014). What is a bioactive compound? A combined definition for a preliminary consensus. *International Journal of Food Sciences & Nutrition*, *3*(3), 17–179. https://doi.org/10.11648/j.ijnfs.20140303.16

Heavey, P. M., & Ian Rowl&, D. R. (2004). 8 Gastrointestinal cancer. *Best Practice & Research Clinical Gastroenterology*, *18*(2), 323–336. https://doi.org/10.1053/ybega.2004.440

Hervert-Hernández, D. & Goñi, I. (2011) Dietary Polyphenols & Human Gut Microbiota: a Review.

Hervert-Hernández, D. & Goñi, I.(2011) 'Dietary Polyphenols & Human Gut Microbiota: a Review', *Food Reviews International*, 27: 2, 154 — 169, doi: 10.1080/87559129.2010.535233.

Hoang, T., & Kim, J. (2021). Phytonutrient supplements & metabolic biomarkers of cardiovascular disease: An umbrella review of meta-analyses of clinical trials. In *Phytotherapy Research* (Vol. 35, Issue 8, pp. 4171–4182). John Wiley & Sons Ltd. https://doi.org/10.1002/ptr.7079

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., & Chen, Z. S. (2022). Microbiota in health & diseases. In *Signal Transduction & Targeted Therapy* (Vol. 7, Issue 1). Springer Nature. https://doi.org/10.1038/s41392-022-00974-4

IFT (Institute of Food Technologists). (2020). Functional foods: Opportunities & challenges. In J. MacAulay, B. Petersen, & F. Shank (Eds.), IFT expert report Accessed on 15 Sepember 2020 https://www.foodprocessing.com/assets/Media/Medi aManager /IFTFunctionalFoodsReport.pdf.

Jha, A. K., & Sit, N. (2022). Extraction of BC from plant materials using combination of various novel methods: A review. In *Trends in Food Science & Technology* (Vol. 119, pp. 579–591). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2021.11.019

Kamiloglu, S., Tomas, M., Ozdal, T., & Capanoglu, E. (2021). Effect of food matrix on the content & bioavailability of flavonoids. In *Trends in Food Science & Technology* (Vol. 117, pp. 15–33). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.10.030

Konstantinidi, M., & Koutelidakis, A. E. (2019). Functional Foods & BC: A Review of Its Possible Role on Weight Management & Obesity's Metabolic Consequences. *Medicines*, *6*(3), 94. https://doi.org/10.3390/medicines6030094

Kováčová, M., & Malinová, E. (2007). Ferulic & Coumaric Acids, Total Phenolic Compounds & their Correlation in Selected Oat Genotypes. In *Czech J. Food Sci* (Vol. 25, Issue 6).

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., & Etherton, T. D. (2002). *BC in Foods: Their Role in the Prevention of Cardiovascular Disease & Cancer*.

Kussmann, M., Abe Cunha, D. H., & Berciano, S. (2023). BC for human & planetary health. In *Frontiers in Nutrition* (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fnut.2023.1193848

Lalitha, A. (2024). Phytochemicals & health benefits. *Annals of Geriatric Education & Medical Sciences*, *11*(1), 29–31. https://doi.org/10.18231/j.agems.2024.007

Lee, S. H., Latetia, ;, Moore, V., Park, S., Harris, D. M., & Blanck, H. M. (2022). Morbidity & Mortality Weekly Report Adults Meeting Fruit & Vegetable Intake Recommendations-United States, 2019. https://www.myplate.gov/eat-healthy/fruits;

Liu, R. H. (2013). Dietary BC & their health implications. *Journal of Food Science*, *78*(SUPPL.1). https://doi.org/10.1111/1750-3841.12101

Exploring lnnovation with Scientific Thinking

Liu, D. (2022). Exploring lnnovation with Scientific Thinking. *Chin Med Sci J*, 37(2): 87-90 doi: 1O.24920/004123

Lupton, J. R., Atkinson, S. A., Chang, N., Fraga, C. G., Levy, J., Messina, M., Richardson, D. P., Van Ommen, B., Yang, Y., Griffiths, J. C., & Hathcock, J. (2014). Exploring the benefits & challenges of establishing a DRI-like process for bioactives. *European Journal of Nutrition*, *53*(SUPPL. 1). https://doi.org/10.1007/s00394-014-0666-3

Macagnan, F. T., da Silva, L. P., & Hecktheuer, L. H. (2016). DF: The scientific search for an ideal definition & methodology of analysis, & its physiological importance as a carrier of BC. In *Food Research International* (Vol. 85, pp. 144–154). Elsevier Ltd. https://doi.org/10.1016/j.foodres.2016.04.032

Malenčić, D., Popović, M., & Miladinović, J. (2007). molecules Phenolic Content & Antioxidant Properties of Soybean (Glycine max (L.) Merr.) Seeds. In *Molecules* (Vol. 12). http://www.mdpi.org

Malwe, A. S., & Sharma, V. K. (2023). Application of artificial intelligence approaches to predict the metabolism of xenobiotic molecules by human gut microbiome. In *Frontiers in Microbiology* (Vol. 14). Frontiers Media SA. https://doi.org/10.3389/fmicb.2023.1254073

Manach, C., Williamson, G., Mor&, C., Scalbert, A., & Rémésy, C. (2005). *Bioavailability & bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies 1-3*.

Mao, L., Gao, B., Chang, H., & Shen, H. (2024). Interaction & Metabolic Pathways: Elucidating the Role of Gut Microbiota in Gestational Diabetes Mellitus Pathogenesis. In *Metabolites* (Vol. 14, Issue 1). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/metabo14010043

Martirosyan, D. & Ekblad, M. (2022). Functional foods classification system: exemplifying through analysis of BC. In *Functional Foods Science* (Vol. 2, Issue 4). https://www.doi.org/ffs.v2i4.919

O'Grady, J., O'Connor, E. M., & Shanahan, F. (2019). Review article: DF in the era of microbiome science. In *Alimentary Pharmacology & Therapeutics* (Vol. 49, Issue 5, pp. 506–515). Blackwell Publishing Ltd. https://doi.org/10.1111/apt.15129

Olaya, B., Essau, C. A., Moneta, M. V., Lara, E., Miret, M., Martín-María, N., Moreno-Agostino, D., Ayuso-Mateos, J. L., Abduljabbar, A. S., & Haro, J. M. (2019). Fruit & vegetable consumption & potential moderators associated with all-cause mortality in a representative sample of spanish older adults. *Nutrients*, *11*(8). https://doi.org/10.3390/nu11081794

Ottaviani, J. I., Fong, R., Kimball, J., Ensunsa, J. L., Gray, N., Vogiatzoglou, A., Britten, A., Lucarelli, D., Luben, R., Grace, P. B., Mawson, D. H., Tym, A., Wierzbicki, A., Smith, A. D., Wareham, N. J., Forouhi, N. G., Khaw, K. T., Schroeter, H., & Kuhnle, G. G. C. (2019). Evaluation of (−)-epicatechin metabolites as recovery biomarker of dietary flavan-3-ol intake. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-49702-z

Ottaviani, J. I., Schroeter, H., & Kuhnle, G. G. C. (2023). Measuring the intake of dietary bioactives: Pitfalls & how to avoid them. In *Molecular Aspects of Medicine* (Vol. 89). Elsevier Ltd. https://doi.org/10.1016/j.mam.2022.101139

Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The Role of Dietary Fibre in the Bioaccessibility & Bioavailability of Fruit & Vegetable Antioxidants. In *Journal of Food Science* (Vol. 76, Issue 1). https://doi.org/10.1111/j.1750-3841.2010.01957.x

Puri, V., Nagpal, M., Singh, I., Singh, M., Dhingra, G. A., Huanbutta, K., Dheer, D., Sharma, A., & Sangnim, T. (2022). A Comprehensive Review on Nutraceuticals: Therapy Support & Formulation Challenges. In *Nutrients* (Vol. 14, Issue 21). MDPI. https://doi.org/10.3390/nu14214637

Probst, Y. C., Guan, V. X., & Kent, K. (2017). Dietary phytochemical intake from foods & health
outcomes: A systematic review protocol & preliminary scoping. BMJ Open, 7(2), e013337. https://doi.org/10.1136/bmjopen-2016-013337

Ramakrishna, R., Sarkar, D., & Shetty, K. (2020). Functional bioactives from barley for human health benefits. In K. Shetty, & D. Sarkar (Eds.), Functional foods & biotechnology: Biotransformation & analysis of functional foods & ingredients (1st ed., p. 454). United Kingdom: CRC Press.

Rothwell, J.A., Perez-Jimenez, J., Neveu,V., et al. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. (2013) Vol. 2013: article ID bat070; doi:10.1093/database/bat070.

Ross, R. P., Mills, S., Hill, C., Fitzgerald, G. F., & Stanton, C. (2010). Specific metabolite production by gut microbiota as a basis for probiotic function. In *International Dairy Journal* (Vol. 20, Issue 4, pp. 269–276). https://doi.org/10.1016/j.idairyj.2009.12.003

Saura-Calixto, F. (2011). Dietary fibre as a carrier of dietary antioxidants: An essential physiological function. In *Journal of Agricultural & Food Chemistry* (Vol. 59, Issue 1, pp. 43–49). https://doi.org/10.1021/jf1036596

Saura-Calixto, F., & Goñi, I. (2009). Definition of the Mediterranean Diet Based on BC. Critical Reviews in Food Science and Nutrition,49 (2),145 - 152. http://dx.doi.org/10.1080/10408390701764732

Saura-Calixto, F., Garcia-Alonso, A., Goni, I., & Bravo, L. (2000). In vitro determination of the indigestible fraction in foods: An alternative to dietary fibre analysis. Journal of Agricultural & Food Chemistry, 48(8), 3342–3347. https://doi.org/10.1021/jf0000373

Saura-Calixto, F., Pérez-Jiménez, J., Touriño, S., Serrano, J., Fuguet, E., Torres, J. L., & Goñi, I. (2010). Proanthocyanidin metabolites associated with DF from in vitro colonic fermentation & proanthocyanidin metabolites in human plasma. *Molecular Nutrition & Food Research*, *54*(7), 939–946. https://doi.org/10.1002/mnfr.200900276

Saura-Calixto, F., Serrano, J., & Goñi, I. (2007). Intake & bioaccessibility of total polyphenols in a whole diet. *Food Chemistry*, *101*(2), 492–501. https://doi.org/10.1016/j.foodchem.2006.02.006

Saxena, R., Sharma, V., Saxena, A. R., & Patel, A. (2024). Harnessing AI & Gut Microbiome Research for Precision Health Background-Underst&ing the Human Gut Microbiota. *Issue 1 Journal of Artificial Intelligence General Science (JAIGS)Online)*, *2*(1), 3006–4023.

Schmidt, H. de O., Rockett, F. C., Pagno, C. H., Possa, J., Assis, R. Q., de Oliveira, V. R., da Silva, V. L., Flôres, S. H., & Rios, A. de O. (2019). Vitamin & bioactive compound diversity of seven fruit species from south Brazil. *Journal of the Science of Food & Agriculture*, *99*(7), 3307–3317. https://doi.org/10.1002/jsfa.9544

Sender R, Fuchs S, Milo R (2016) Revised Estimates for the Number of Human & Bacteria

Cells in the Body. PLoS Biol 14(8): e1002533.doi:10.1371/journal.pbio.1002533

Serrano, J., Goñi, I. & Saura-calixto, F. (2005). Determination of â-Carotene & Lutein Available from Green Leafy Vegetables by an in Vitro Digestion & Colonic Fermentation Method. *J. Agric. Food Chem*. 53, 2936-2940.

Shen, Y., Fan, N., Ma, S. xia, Cheng, X., Yang, X., & Wang, G. (2025). Gut Microbiota Dysbiosis: Pathogenesis, Diseases, Prevention, & Therapy. In *MedComm* (Vol. 6, Issue 5). John Wiley & Sons Inc. https://doi.org/10.1002/mco2.70168

Shetty, K., & Sarkar, D. (2020). Introduction: Metabolic-Driven ecological rationale to advance biotechnological approaches for functional foods. In K. Shetty, & D. Sarkar (Eds.), *Functional foods & biotechnology: Biotransformation & analysis of functional foods & ingredients* (1st ed., p. 454). United Kingdom: CRC Press.

Singh, D., & Chaudhuri, P. K. (2018). A review on phytochemical & pharmacological properties of Holy basil (Ocimum sanctum L.). In *Industrial Crops & Products* (Vol. 118, pp. 367–382). Elsevier B.V. https://doi.org/10.1016/j.indcrop.2018.03.048

Smith, R. D., Correa, C., & Oh, C. (2009). Trade, TRIPS, & pharmaceuticals. In The Lancet (Vol. 373, Issue 9664, pp. 684–691). Elsevier B.V. https://doi.org/10.1016/S0140-6736(08)61779-1

Stephen, A. M., Champ, M. M. J., Cloran, S. J., Fleith, M., Van Lieshout, L., Mejborn, H., & Burley, V. J. (2017). DF in Europe: Current state of knowledge on definitions, sources, recommendations, intakes & relationships to health. *Nutrition Research Reviews*, *30*(2), 149–190. https://doi.org/10.1017/S095442241700004X

Swamy, M. K., & Akhtar, M. S. (2019). Natural Bio-active compounds. Volume 2:chemistry, Pharmacology & health Care Practices. Springer Nature Singapore Pte Ltd. https:// doi.org/10.1007/978-981-13-7205-6 (eBook).

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The Human Microbiome Project. In *Nature* (Vol. 449, Issue 7164, pp. 804–810). Nature Publishing Group. https://doi.org/10.1038/nature06244

U.S. Department of Agriculture, Agricultural Research Service. (2024). FoodData Central [Database]. https://fdc.nal.usda.gov/

Van Hul, M., Cani, P. D., Petitfils, C., De Vos, W. M., Tilg, H., & El-Omar, E. M. (2024). What defines a healthy gut microbiome? In *Gut* (Vol. 73, Issue 11, pp. 1893–1908). BMJ Publishing Group. https://doi.org/10.1136/gutjnl-2024-333378

Verma, D. K., & Thakur, M. (2021). Phytochemicals in food & health: Perspectives for research & technological development. USA: Apple Academic Press. ISBN: 9781771889124.

Vettorazzi, A., de Cerain, A. L., Sanz-Serrano, J., Gil, A. G., & Azqueta, A. (2020). European regulatory framework & safety assessment of food-related BC. In *Nutrients* (Vol. 12, Issue 3). MDPI AG. https://doi.org/10.3390/nu12030613

Wan, M. L. Y., Co, V. A., & El-Nezami, H. (2021). Dietary polyphenol impact on gut health & microbiota. In *Critical Reviews in Food Science & Nutrition* (Vol. 61, Issue 4, pp. 690–711). Taylor & Francis Ltd. https://doi.org/10.1080/10408398.2020.1744512

Williamson, G., & Clifford, M. N. (2010). Colonic metabolites of berry polyphenols: The missing link to biological activity? In *British Journal of Nutrition* (Vol. 104, Issue SUPPL.3). https://doi.org/10.1017/S0007114510003946

Williamson,G., & Manach, C. (2005). Bioavailability & bioefficacy of polyphenols in humans. II. Review Bioavailability & bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr , 81(suppl):243S–55S

Wu, J., Singleton, S. S., Bhuiyan, U., Krammer, L., & Mazumder, R. (2023). Multi-omics approaches to studying gastrointestinal microbiome in the context of precision medicine & machine learning. In *Frontiers in Molecular Biosciences* (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fmolb.2023.1337373

Xavier, J. R., Sanjay, B. S., Gupta, D., Mehta, S., & Chauhan, O. P. (2024). BC of foods: Phytochemicals & peptides. In *Food & Humanity* (Vol. 3). Elsevier B.V. https://doi.org/10.1016/j.foohum.2024.100354

Xiao, J., & Bai, W. (2019). Bioactive phytochemicals. In *Critical Reviews in Food Science & Nutrition* (Vol. 59, Issue 6, pp. 827–829). Taylor & Francis Inc. https://doi.org/10.1080/10408398.2019.1601848

Xu, Y., Le Sayec, M., Roberts, C., Hein, S., Rodriguez-Mateos, A., & Gibson, R. (2021). Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review. In *Advances in Nutrition* (Vol. 12, Issue 5, pp. 1781–1801). Oxford University Press. https://doi.org/10.1093/advances/nmab017

Yates, A. A., Erdman, J. W., Shao, A., Dolan, L. C., & Griffiths, J. C. (2017). Bioactive nutrients - Time for tolerable upper intake levels to address safety. *Regulatory Toxicology & Pharmacology*, *84*, 94–101. https://doi.org/10.1016/j.yrtph.2017.01.002

Yong, E. (2012). Gut microbial "enterotypes" become less clear-cut. *Nature*. https://doi.org/10.1038/nature.2012.10276

Zhang, C., Zhang, M., Wang, S., Han, R., Cao, Y., Hua, W., Mao, Y., Zhang, X., Pang, X., Wei, C., Zhao, G., Chen, Y., & Zhao, L. (2010). Interactions between gut microbiota, host genetics & diet relevant to development of metabolic syndromes in mice. *ISME Journal*, *4*(2), 232–241. https://doi.org/10.1038/ismej.2009.112

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota & immunity in health & disease. In *Cell Research* (Vol. 30, Issue 6, pp. 492–506). Springer Nature. https://doi.org/10.1038/s41422-020-0332-7