Chapter

Flavonoids: A Group of Potential Food Additives with Beneficial Health Effects

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Abstract

Recently, there has been an increasing interest in health-promoting products which are also natural and safe for consumption because the consumer market has been searching for a healthy lifestyle. This global market trend has driven the food industry to invest in developing innovative products containing bioactive components. Flavonoids are a group of phenolic compounds of low molecular weight, consisting of 15 carbon atoms. Their alterations in the heterocyclic ring's substitution pattern generate six subclasses: flavonols, flavanols, flavones, flavanones, isoflavones and anthocyanins. Also, different studies have reported that diets rich in flavonoids provide numerous benefits associated with health-promoting effects by reducing the risk of development of chronic diseases such as cardiovascular diseases, diabetes type II and some types of cancers. These effects have been related to their biological properties which also include other activities such as colorant effects (*e.g.,* anthocyanins), transforming them into potential food additives with desirable capacities. Therefore, this review aims to revise the classes of flavonoids and their main biological properties as well as the most used extraction techniques applied for obtaining these compounds, their bioavailability and the application to formulate new natural food additives.

Keywords: flavonoids, health benefits, extraction techniques, bioavailability, food additives

1. Introduction

The growing interest in a healthy lifestyle has led the food industry to establish an alliance with the scientific community to create a viable and effective alternative for the consumer, carrying out several studies about the bioactive potential of various compounds present in natural matrices [1].

The recently discovered properties of phenolic compounds have been exploited, and the food industry has launched numerous new functional products whose health functionality is closely connected with their polyphenols content [2].

The scientific community have been developing several studies to determine the presence of phenolic compounds natural matrices, namely the presence of flavonoids. For example, in cereals, several kinds of flavonoids (principally glycosylated flavones) are distributed in these grass crops; in legumes, the presence of a total of 690 isoflavonoids have been reported; and in medicinal plants these molecules are a major constituent in lists of metabolites responsible for the bioactivities [3].

Flavonoids are a subdivision of polyphenols that are abundant in the human diet and can be found in several matrices; specifically, they are commonly found in fruits, vegetables, nuts, teas, dark chocolate, red wine and legumes [3].

These compounds are divided into principal subclasses of flavanols, including flavanol monomers (flavan-3-ols) and flavanol polymers (called proanthocyanidins), flavonols, flavanones, flavones, isoflavones, and anthocyanins (depending on the substitution at the heterocyclic ring (C-ring)) [4]. Regarding their physiological potential, flavonoids have a vast range of bioactivities, namely antioxidant, anti-inflammatory, vasorelaxant, anticoagulant, cardio-protective, anti-obesity and anti-diabetic, chemoprotective, neuroprotective, and antidepressant properties that are progressively being clarified [5]. These beneficial properties are strongly dependent on the polyphenols chemical structure [2].

1.1 Flavonols

Flavonols are the most important subgroup of flavonoids. Chemically, these compounds (as other flavonoids) have a characteristic 15-carbon skeleton (C6-C3-C6), two benzene rings constitute its structure (catechol B ring and resorcinol A ring) joined together by a 4-pyrone heterocyclic ring C (**Figure 1**) [6].

Some compounds of this subclass include quercetin, myricetin, kaempferol, galangin, and fisetin [7–9]. These molecules represent the most ubiquitous and abundant flavonoids in the plant kingdom (dicotyledonous plants, especially flowers and leaves of woody) [10, 11] and occur abundantly in fruits (*e.g.,* apples, bananas, several berries, pomegranate), vegetables (broccoli, red and white onion, tomato, spinach), cocoa and chocolate, and beverages (such as tea and red wine) [7, 12].

The scientific community has widely studied the positive effects of flavonols on human health. These molecules have been reported as important antioxidants due to their abilities to suppress free radical formation, scavenge free radicals, and upregulate or protect antioxidant systems. They also inhibit the enzymes associated with free radical production, reduce lipid peroxidation, and chelate metal ions in reducing free radical generation [10, 11]. In addition to the antioxidant potential, these molecules have shown other target biological activities such as antimicrobial, anti-viral (interruption of virus's entry and replication cycle) hepatoprotective,

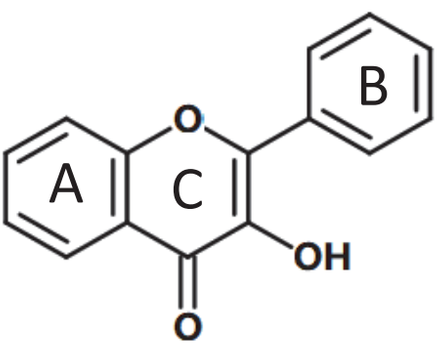

Figure 1.
Chemical structure of flavonols. Designed with eMolecules (https://www.emolecules.com/).

nephroprotective (effective for the treatment of chronic kidney disease), anti-inflammatory, vasodilatation effects, and cardiovascular protective effects (preventative role in coronary diseases). They also have been considered as potential anticancer agents [8, 13, 14].

However, despite all the flavonols have a broad spectrum of biological activities, kaempferol, myricetin, and quercetin are the main representatives and have been widely studied due to their health-promoting functions. Both kaempferol and quercetin have unique biological properties as anticarcinogenic, antimicrobial, antidiabetic, anti-viral, anti-allergic, antioxidant, and anti-inflammatory [7, 8, 11].

1.2 Flavanols (flavan-3-ols)

Flavanols or flavan-3-ols are another flavonoid subclass with a hydroxyl group at position 3 and a fully saturated carbon ring structure (**Figure 2**) [9].

The most common flavan-3-ol monomers are catechin, epicatechin, catechin gallate, epicatechin gallate, gallocatechin, epigallocatechin, gallocatechin gallate and epigallocatechin gallate [2]. These compounds are widely spread in nature and can be found in a wide range of natural matrices as apples, peaches, cocoa powder, nuts, dark chocolate, grapes, berries and beverages (such as red wine, tea, and cider) [9]. Furthermore, they can also be found in certain food plants, such as *Vitis vinifera*, *Camellia sinensis* and *Theobroma cacao* [15]. In these foods, flavanols can exist as monomers, such as epicatechin, or in oligomeric forms referred to as procyanidins or, more broadly, proanthocyanidins [16]. The presence of flavanols in food affects food quality parameters, principally the astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation [17].

Over time, the interest in flavanols has grown, and different studies have reported these compounds' health benefits. These compounds present several beneficial effects in consumers' health, acting as antioxidant (scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes), anticarcinogen, cardio-preventive (modulation of vascular homeostasis), antimicrobial, anti-viral, and neuro-protective agents [4, 18]. Besides, dietary intervention studies demonstrated that consuming certain flavanol-containing foods results in improved arterial function, a decrease in blood pressure, positive modulation of hemostasis, and improved insulin sensitivity [15]. In this sense, diets enriched in flavan-3-ol containing foodstuffs may provide beneficial health effects [17].

1.3 Flavones

Flavones are also a subgroup of the flavonoid class based on the backbone of 2-phenylchromen-4-one (2-phenyl-benzopyran-4-one). The molecular formula of the flavone molecule is $C_{15}H_{10}O_2$. It has a three-ring skeleton, C6-C3-C6, and the rings

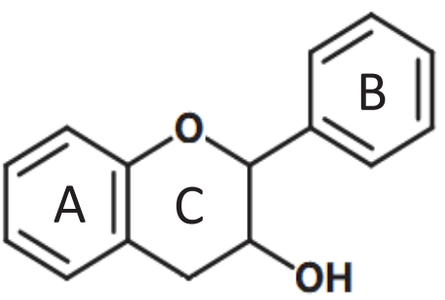

Figure 2.
Chemical structure of flavanol. Designed with eMolecules (https://www.emolecules.com/).

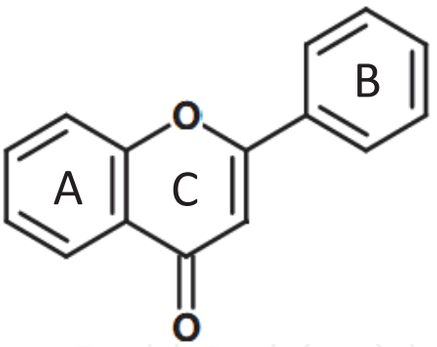

Figure 3.
Chemical structure of flavones. Designed with eMolecules (https://www.emolecules.com/).

are referred to as A-, C-, and B-rings, respectively (**Figure 3**). These compounds are also characterized by the presence of three functional groups, including hydroxy, carbonyl, and a conjugated double bond. Consequently, they exhibit characteristic reactions of all three functional groups [19].

The most abundant types of flavones are luteolin, apigenin and chrysin [20]. These compounds are commonly found in edible vegetables, fruits, nuts, seeds and plant-derived beverages and cereals, which are ingested inadvertently in our daily diet and positively impact consumers' health without significant side effects [3, 20].

The scientific community has carried out several studies to determine the biological potential of flavones. These molecules have received broad interest for their antioxidant potential [21] and their ability to modulate several enzyme systems involved in many diseases [22]. Also, these compounds have demonstrated to have other biological properties beneficial to health, namely anti-inflammatory activities [23], antibacterial [24], antifungal [25], antiviral [26] and anti-carcinogenic [27]. Furthermore, they also have immunomodulatory effects [28], and they intervene in the reduction of total cholesterol [29]. Recent studies in numerous disease areas (osteoporosis, prostate hyperplasia, endocrinology, and others) have shown that many disorders, specifically in the metabolic area, are multi-factorial and are better treated with combinations of drugs and natural products [19]. However, all these therapeutic actions depend and differ according to the different compounds belonging to the subclass of flavones [30].

1.4 Flavanones

Flavones are another subgroup of flavonoids and have a C6-C3-C6 skeleton composed of 3 rings, A-, C-, and B-, respectively, and a chiral carbon at the C-3 position (**Figure 4**) [31].

Formerly, flavanones were considered minor flavonoids, like chalcones, dihydrochalcones, dihydroflavonols and aurones; nevertheless, in the past 15 years, the total number of known flavanones has increased, and they are now considered a major flavonoid class like flavones, isoflavones, flavanols, flavonols and anthocyanidins. Nowadays, in nature, up to 350 flavanone aglycones and 100 flavanone glycosides have been identified [32].

Flavanones are mainly divided into naringenin, hesperetin and eriodicthiol [20]. They are characteristic compounds of citrus fruit, principally lemon, lime, mandarin (tangerine), sweet orange, grapefruit, sour (bitter) orange and tomato [33–35]. These compounds are also widely distributed in around 42 plant families (*Compositae, Leguminosae* and *Rutaceae*). They can be found in all plant parts, above

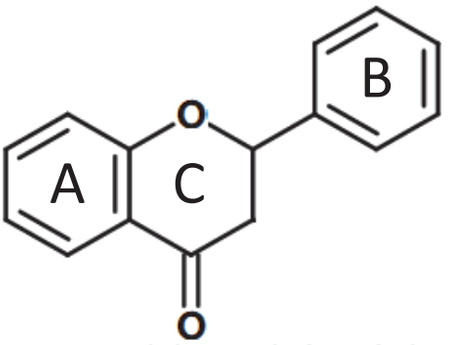

Figure 4.
Chemical structure of flavanones. Designed with eMolecules (https://www.emolecules.com/).

and below ground, from vegetative part to generative organs: stem, branches, bark, flowers, leaves, roots, rhizomes, seeds, fruits, peels, and others [32].

As in the other subgroups of flavonoids, flavanones also exhibit biological properties, which positively affect consumers' health. Properties associated with flavanone intake include antioxidant [34], anti-inflammatory [36], antitumor, antiviral [37] and antimicrobial activities [38]. Furthermore, flavanones are related to some beneficial effects, such as improved gastrointestinal function [39], decreased blood cholesterol level [38], cardioprotective effect [40] and reduction of inflammatory responses caused by SARS-CoV-2 infection [35].

1.5 Isoflavones

Some structural variation from flavones are presented in isoflavones, which differs from flavones in the location of the phenyl group's location at C3 rather than C2 position (**Figure 5**) [9].

Isoflavones are divided into genistein, daidzein and glycitein [20]. These compounds are naturally-occurring plant compounds and are usually found in legumes from the *Fabaceae* family (chickpeas, beans, lupine and soybean) and red clover (*Trifolium pratense*), as well as small amounts of isoflavones are also contained in other plant products, fruits, vegetables (broccoli and cauliflower), barley and nuts [20, 41, 42].

Isoflavones have also demonstrated bioactive properties that intervene beneficially in human health. The therapeutic effects of isoflavones are anti-inflammatory, antioxidant [43], anti-obesity [44] and antitumor activities [45]. Besides, several benefits are associated with isoflavones, such as relieving menopausal symptoms [46], hepatoprotective [47], cardiovascular protection [48], therapeutic potential in the control of diabetes [49], osteoporosis prevention and treatment [50], modulatory effect of the intestinal microbiota [51] and studies in rats have reported an improvement in kidney function in obese rats [52].

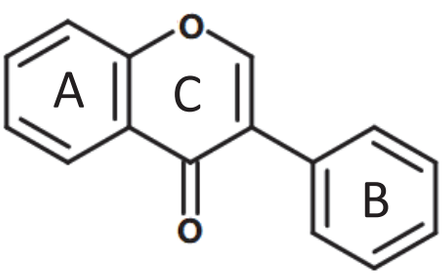

Figure 5.
Chemical structure of isoflavones. Designed with eMolecules (https://www.emolecules.com/).

1.6 Anthocyanins

Anthocyanins belong to the large group of flavonoids, being considered the most revealing water-soluble pigments for extraction from natural matrices [53]. Regarding their chemical characterization, anthocyanins come from a basic structure of 3 rings of the C6-C3-C6 shape, defined as an aglycone portion, called the flavylic cation (**Figure 6**). When associated with chemical groups in the R positions, it is called anthocyanidin [53, 54].

The most common types of anthocyanins are cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin [55]. The anthocyanin compounds are present in a composition of a wide range of vegetables (red onion, radish, red cabbage, red lettuce, eggplant, red-skinned potato and purple sweet potato), flowers (red hibiscus, red rose, red pineapple sage, red clover, and pink blossom) and several red fruits, such as: cherries, plums, strawberries, raspberries, blackberries, grapes, and many others [56, 57].

Anthocyanins are involved in many biological activities that positively impact human health. The use of these molecules for medicinal purposes has been long supported by epidemiological evidence. Still, just in recent years, some of the specific, measurable pharmacological properties of isolated anthocyanin pigments have been proven by controlled *in vitro*, *in vivo* or clinical research studies [57]. According to several authors, the health benefits are associated with the increase of sight acuteness, anti-carcinogenic activity, antioxidant capacity, antiulcer activity and the maintenance of normal vascular permeability (vitamin C_2), as well as acting in the prevention of various diseases, such as coronary and degenerative diseases, diabetes, inflammation or reduction of the risk of obesity, among others [58–60].

2. Biological properties of flavonoids

The bioactive properties of flavonoids are directly linked to the functions they exert. Flavonoids, present in higher plants' cells, have a protective role against parasites and other pathogens (participating in allelopathy processes), herbivores, and ultraviolet (UV) radiation [61]. They have a regulatory function like most lipid-soluble vitamins and act as pollinating agents. The varied colors they can have attract pollinators, thus contributing to plant seeds' dispersion [62]. The following sections display a set of functions attributed to these compounds, seeking to relate their bioactivity to their chemical features and/or possible mechanism of action.

2.1 Antioxidant activity

The antioxidant properties of flavonoids have been recognized over the years. Given the wide presence of flavonoids in various fruits, vegetables, legumes, grains

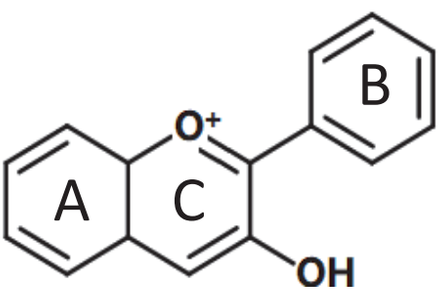

Figure 6.
Chemical structure of anthocyanins. Designed with eMolecules (https://www.emolecules.com/).

and nuts, these compounds represent approximately two-thirds of the phenols consumed in the diet, being the class predominantly described [63, 64]. The mechanisms underlying the antioxidant properties of flavonols include eliminating free radicals and the chelating activity of transition metal ions, being the preventive action and chain-breaking mechanisms responsible for the high bioactivity of flavonoids [65, 66]. In fact, flavonoids can eliminate free radicals and reduce their formation and/or their effects. As expected, the chemical structure plays a key role in the antioxidant activity of flavonoids. That is, due to the reducing capacity of the phenolic hydroxyl groups (presence of hydrogen-/electron-donating substituents), flavonoids can donate hydrogen; thanks to the ability to delocalize the unpaired electron leading to the formation of a stable phenoxyl radical, flavonoids can protect against damage caused by reacting oxygen species (ROS), and flavonoids can chelate transition metals capable of promoting the formation of hydroxyl radicals in reduced forms through the Fenton reaction under abnormal conditions. This property is strongly dependent on the arrangement of hydroxyls and carbonyl group around the molecule [66]. Considering these characteristics that underlie the antioxidant potential of flavonoids, studies carried out over the years have shown that the flavonoids with greater antioxidant activity have the following structure features: (i) a certain hydroxylation pattern, particularly in the ring B, namely 3′,4′-dihydroxyl group (*e.g.*, 3′, 4′-catechol group); (ii) the 3 − OH moiety in the C ring; (iii) the C2 = C3 double bond in the C ring conjugated with a C4-carbonyl group in the ring, causing electron delocalization from the B ring; and (iv) both 3 − OH group in C ring and 5 − OH group in A ring combined with a 4-carbonyl group and C2 = C3 double bond [65–68]. The influence of the chemical structure on the bioactivity of flavonoids was also demonstrated by Novaes et al. [69]. The authors tested the antioxidant potential of 11 flavonols extracted from the leaves of *Annona coriacea* Mart. using four approaches, and attributed the differences obtained in the assays to the B-ring substitution pattern of flavonols. Recent studies also suggest that the C − H bonds may contribute to the antioxidant activity of flavonols since they evaluated the antioxidant properties of 13 flavonoids with the hydroperoxyl radical (HOO) and concluded that the C − H bonds (C3 − H of the flavonoid backbone structures) play a fundamental role in the antioxidant properties of flavonoids containing 4-carbonyl and/or 3-hydroxyl groups. These groups release the single electron on the C3 radical (the C3 − H bond) into the O − C3 − C4 − O system and form intermolecular bonds to stabilize the radicals, yielding reduced bond dissociation energy (C3 − H) and increased the antioxidant activity of the flavonoids. This study also showed that the hydrogen atom transfer (HAT) mechanism is the main pathway for flavonoids' antioxidant activity [68].

If the abovementioned features favor the antioxidant potential of flavonoids, on the other hand, the presence of saccharide groups seems to reduce the antioxidant properties of these molecules. Still, some studies showed that *C*-glycosyl flavonoids have greater potential than *O*-glycosides [70, 71]. It should be highlighted that although the antioxidant activity of glycosides is weaker than the corresponding aglycone, the bioavailability is reasonably increased *in vivo* due to the cleavage of glycosidic bonds that frequently occur. Therefore, under these conditions, the antioxidant activity is increased [66].

However, the abundant consumption of flavonoids, as polyphenols in general, through the daily diet does not always correspond to obtaining the effects observed *in vitro* in the natural matrix of origin. Nevertheless, there are already some studies carried out in this direction. Some works proved that even given the sensitivity of these biomolecules (to food processes and storage), it is possible to obtain positive results when inserted in foods, namely as ingredients/additives with preservative capacity, and bring potential health benefits, as described in the following sections.

2.2 Antimicrobial activity

The antimicrobial properties of natural products rich in flavonoids have been reported and recognized since antiquity. Of the best-known products, propolis can be highlighted, whose healing properties have been mentioned for thousands of years and used to treat wounds and ulcers. In fact, propolis's antimicrobial properties have been attributed to its high content of flavonoids, particularly galangin and pinocembrin [72]. As previously mentioned, flavonoids' bioactivity is related to their function in nature, namely protecting plants against pathogens. In this way, plant-derived flavonoids have different antibacterial mechanisms of action than conventional drugs, and generally, their bioactivity does not confer resistance. In fact, to the best of our knowledge, no report claims to have observed bacteria developing resistance to plant-based antimicrobials. In this way, antibacterial agents based on natural extracts rich in flavonoids represent an important alternative in developing new antibacterial formulations, both from a clinical perspective, as in any other application such as the food sector [73]. The possible mechanisms of antimicrobial action of flavonoids are briefly: (i) cell envelop synthesis inhibition (*e.g.,* quercetin, myricetin, luteolin); (ii) inhibition of nucleic acid synthesis (*e.g.,* quercetin, kaempferol, apigenin); (iii) bacterial motility inhibition (*e.g.,* sinensetin, luteolin, epigallocatechin gallate); (iv) inhibition of ATP synthesis on electron transport chain (*e.g.,* baicalein, silibinin, silymarin); (v) bacterial toxins inhibition (*e.g.,* naringenin, kaempferol, quercetin 6-hydroxyflavone); (vi) biofilm formation inhibition (*e.g.,* genistein, apigenin, naringenin); (vii) inhibition of bacterial enzyme-dependent virulence (*e.g.,* amoradicin, kaempferol-3-rutinoside, baicalin); (viii) membrane disruption (*e.g.,* apigenin, catechin, quercetin); (ix) inhibition of bacterial efflux pumps (*e.g.,* luteolin, morin, rutin); and (x) inhibition of bacterial quorum sensing (*e.g.,* naringin, taxifolin, chrysin) [74]. Therefore, when relating the chemical structure of flavonoids with their antimicrobial activity through various mechanisms, quercetin, apigenin, kaempferol, fisetin, myricetin, luteolin, taxifolin, or naringenin features can be highlighted, among others.

2.3 Prebiotic activity

Polyphenols' prebiotic effects have also been explored, with available reports from pre-clinical and clinical studies. Flavonoids have been the most investigated phenolic compounds in terms of their effects on the composition of the intestinal microbiota and the health benefits of the host [75]. It must be highlighted that the current definition of prebiotic recognizes that, in addition to the stimulation of *Bifidobacterium* and *Lactobacillus* bacteria, prebiotic targets include other microorganisms, such as *Roseburia*, *Eubacterium* and *Faecalibacterium* spp. However, they are not limited to these genera [76]. The health benefits associated with prebiotics are well known and comprise immunomodulation, increased mineral absorption, improved intestinal function and positive effects on glucose homeostasis, inflammation, blood lipid profile, satiety and defense against pathogens [77]. The main studied flavonoids that revealed potential prebiotic effects proven in pre-clinical studies include anthocyanins (bilberry extract, grape pomace extract, and Arctic berry extracts), proanthocyanidins (Arctic berry extracts, and cranberry extract), proanthocyanidin A (cinnamon bark extract), catechins and caffeine (green and black tea extracts, decaffeinated green and black tea polyphenol extracts, oolong tea water extracts, aqueous, raw and ripe Pu-erh tea extract), isoflavones (soy extract), naringenin (*S. chinensis* pollen extract), polymeric and oligomeric procyanidin (apple), genistein and hydroxysafflor yellow A [75]. In addition to the beneficial effects on the intestinal microbiota, authors also found other beneficial health

effects such as the decrease in: (i) digestive enzymes activity; (ii) fat mass gain; (iii) liver steatosis; (iv) adiposity; (v) body weight gain and metabolic endotoxemia; (vi) serum lipid profile, glucose and insulin; (vii) serum triglycerides; (viii) oxidative damage and inflammation; and (ix) fasting blood glucose. On the other hand, they found an increase in insulin sensitivity, expression of hepatic lipid metabolism genes, glucose tolerance, and glycolysis.

In clinical trials, anthocyanins consumed in a wild blueberry drink have been studied, in a dose of 25 g/250 mL of water. The study included 20 healthy male individuals, with a 6-week consumption of the drink. After this period, an increase of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the human gut was observed [78]. In another study, they inserted punicalagins and ellagic acid (from pomegranate extract) in capsules with 0.45 g or 1.8 g of extract. These capsules were administered to 49 overweight-obese individuals (mild hyperlipidemia) for 9 weeks. After this time, the authors verified an increase of *Faecalibacterium* and decreased lipopolysaccharide-binding protein in the gut microbiota [79].

2.4 Colorant activity

Color is the most important sensory perception that defines consumer expectations about foods' organoleptic properties [80]. Thus, adding or improving food color has been one of the food industry's commitments to make products more appealing. Although each coloring agent used by the food industry in the European Union is subjected to a rigorous safety assessment, some problems of intolerance and/or allergies or hyperactivity have been related to its consumption [81], which may justify the consumer's preference for natural additives to the detriment of the artificial counterparts. In this way, the scientific community has been looking for natural alternatives to the artificial colors widely used in the industry. The major natural pigments obtained from nature include chlorophylls, carotenoids, betalains and flavonoids. Among the main classes of flavonoids used as coloring agents, the flavonols and anthocyanins stand out, obtaining a range of colors between cream, yellow, pink, red, blue and black [82]. There is already a natural coloring agent, based on flavonoids, approved by the regulatory authorities for its use in the food sector, namely anthocyanins (E163). The approved anthocyanin extract is obtained from the natural strains of vegetables and edible fruits, including blackcurrant pomace and grape skin [83]. The pH strongly influences the color of anthocyanins. In acidic conditions, anthocyanins are red, while in basic pH, they appear blue, being purple in solutions with neutral pH. Hence, grapes are one of the best sources of this natural red pigment, since their anthocyanins are largely methylated, leading to an increase in color intensity and higher stability [84, 85]. After consulting the literature, we found that most studies on natural food colors based on flavonoids are strongly focused on anthocyanins. Other classes of flavonoids have been studied as copigments. Given the sensitivity of anthocyanins to various factors, not only pH but also temperature, light, oxygen, among others. Copigments or other co-solutes can be added, even when colorless, since they trigger a hyperchromic effect [82]. Copigmentation may occur by forming (in the presence or absence of metal ions) noncovalent complexes involving an anthocyanin or anthocyanin-derived pigment (*e.g.,* a pyranoanthocyanin or anthocyanin-flavanol adduct) on the one hand and a copigment on the other; and by subsequent changes in optical properties of the pigment. Among these interactions, intramolecular and intermolecular copigmentation are the most important mechanisms of copigmentation [82, 86]. Also, the effects of flavonoid *C*-glycoside extracts from pigeon pea leaves and its main components vitexin (apigenin-8-*C*-glucoside) and orientin (luteolin-8-*C*-glucoside) on the color and anthocyanins stability of blueberry juice were evaluated.

Authors verified that the addition of flavonoid *C*-glycoside markedly extended the half-life of anthocyanin and enhanced the juice quality. The need to explore other target flavonoids to obtain additional coloring agents to be applied in food formulations seems apparent [87].

2.5 Other activities

Besides the properties previously described, some other bioactivities have been assigned to flavonoids, such as anti-diabetic, anti-inflammatory or anticancer activities. For instance, a new approach to treat diabetes with enhanced antidiabetic activity from a flavonoid nanoparticulate system has been proposed. This system with new biodegradable releasers would increase the solubility of flavonoids and consequently their bioavailability, preventing flavonoid from first-pass metabolism and intestinal absorption in the form of a flavonoid nanoparticulate system. Flavonoids exert their antidiabetic properties by enhancing insulin secretion via regeneration of pancreatic β-cells, enhancing insulin-mediated glucose uptake by target cells, inhibiting aldose reductase and increasing Ca^{2+} uptake [88]. Antidiabetic activity of flavonoids depends on the chemical criterion (C-2-C-3 double bond and ketonic group at C-4 position on ring B) which is fundamental for the bioactivity of polyphenols [89]. The antioxidant and anti-inflammatory activity of the flavonol fisetin (7, 3′, 4′-flavon-3-ol) has been evaluated, showing that it could improve the plasma insulin and antioxidant levels in diabetic rats and significantly decrease the levels of blood glucose. Therefore, the authors suggested that fisetin could be considered as an adjunct for the treatment of diabetes [90]. Regarding anthocyanins, in addition to their coloring capacity, other studies suggest that these flavonoids also have bioactivity with a potential impact on human health, namely antioxidant activity, chemopreventive potential, anti-inflammatory and immunomodulatory properties [91]. Some of the bioactivities attributed to flavonoids have been specifically pointed to flavonoid glycosides. For example, the *C*-glycosidation improved the intracellular antioxidation performance of apigenin [92]. Moreover, many *in vitro* assays reported the positive role of flavonoid glycosides on the immune response (*e.g.,* quercetin 3-*O*-xyloside). Other flavonoid glycosides have shown anticancer properties (quercetin 6-*C*-glucoside), anti-inflammatory and analgesic effects (luteolin 6-*O*-rhamnoside) and anti-parasitic activity (acyl flavonoid glycosides) [92]. Furthermore, it has been suggested that some isoflavonoids may exert antiophidic potential against *Bothrops jararacussu* snake venom [93].

3. Extraction and production techniques for flavonoids recovery

According to the current and continuously increasing demand for new healthy products for a better lifestyle of the population, an increase in the number of techniques used to extract bioactive compounds has occurred. Choose the best extraction technique for each sample is essential in terms of the quality and quantity of the target molecules obtained, that is, flavonoids. Nowadays, there is many extraction techniques that can be employed. These techniques are selected depending on the characteristics of the raw material, which are, in general, plants, food or liquid samples such as wine, tea or olive oil [94]. Independently of the source, the samples must be homogenized. Hence, the most used methods are grinding, milling, filtration, pulverizing and mechanical stirring. Depending on the raw material, before or after homogenization, a pretreatment could be used to facilitate or improve the homogenization and the extraction process. The pretreatments usually used are freezing (in a freezer or by liquid nitrogen), different drying

process and freeze-drying [95]. However, the use of these pretreatments can affect the extract characteristics, limiting the optimization of the extraction process [96–98]. There is no standard for every source of raw material, so selected pretreatments must be chosen depending on the physical and chemical characteristics of the samples. Moreover, depending on the pretreatment and homogenization method, the sample must be stored in the appropriate conditions or perform the extraction immediately before the homogenization and the pretreatment [99, 100]. The extraction techniques can be divided into two groups, conventional and novel approaches.

3.1 Conventional extraction techniques

Conventional extraction techniques are characterized using conventional solvents, with or without heat and usually under agitation. In a standard conventional extraction, the sample is homogenized and submerged in a solvent or a mix of solvents. Using this extraction methodology, flavonoids are obtained through the diffusion and mass-transfer phenomena [101, 102]. The most used methods are maceration and Soxhlet. Nevertheless, other techniques like percolation, hydrodistillation, boiling, reflux and soaking can be used [103, 104]. The advantages of using these techniques are their simplicity and low cost, so they are preferred by companies [102]. On the other hand, these methods have several disadvantages: high volumes of solvent, low extraction yields and long times. Furthermore, due to the sensitivity of flavonoids to high temperature, in extraction assisted by heat, the compounds' biological properties could be affected [105, 106].

For its simplicity and low cost, extraction by Soxhlet is the most used [106]. The main advantages of this method are three. The first one is that through repeated cycles, the sample is in contact with fresh solvent almost all the time, helping the displacement of the mass transfer equilibrium. In the second place, after the extraction, it is not necessary to filter the sample. Lastly, the amount of sample extracted can be improved easily by simultaneous parallel extraction, which needs very little investment. However, Soxhlet extraction has some disadvantages concerning other conventional extractions. The main disadvantages of this technique are its duration (*i.e.,* between 6 and 12 h or higher) and the large amount of solvent used. In addition, after extraction, the high amount of solvent in the sample is usually needed to evaporate. Moreover, the high operating temperature could produce the thermal decomposition of some compounds. Despite the weaknesses, nowadays, Soxhlet extraction could be combined with other novel techniques to improve extraction efficiency [107].

Significant differences in the extraction yields between different conventional extractions can be observed. There are also differences between the number of compounds obtained and their bioactivity within the same method. These variations are caused by the parameters directly implicated in the extraction process like temperature, time, number of extractions (cycles), the ratio of solvent to raw material or type of solvent [108]. **Table 1** shows diverse examples of extractions carried out under different conditions and different methods to compare the conventional extraction methods.

3.2 Novel techniques

Non-conventional extraction techniques put their effort in concentrate the energy to extract the bioactive compounds in a more efficient and/or selective way than in conventional extractions. Nowadays, methods that employ microwaves, ultrasounds, high pressure, supercritical fluids or digestive enzymes can extract

Type (cycles)	Substrate	Solvent (%)	Temperature (°C)	Time (min)	Yields	References
Batch	*A. marmelos* (fruit)	Ethanol	50	50	2.04 mg/g dw	[109]
HRE	*G. affine*	Eth:W (80:20)	—	150	1.16% (Flavonoid extraction yield)	[110]
Mac	*P. oleracea* L.	Eth:W (70:20)	25	2880	5.6 mg/g dw	[111]
Reflux	*P. oleracea* L.	Eth:W (70:20)	—	150	6.8 mg/g dw	[111]
SAE	*Moringa oleifera* L. (leaves)	Eth:W (52:48)	30	30	12.77 ± 0.65 mg/g dw	[112]
SBE	*A. marmelos* (fruit)	Ethanol	50	450	48.15 mg RE/g	[109]
Soxhlet	*A. marmelos (*fruit)	Ethanol	60	460	67.85 mg RE/g	[109]
Soxhlet	*G. affine*	Eth:W (80:20)	—	300	1.48% (Flavonoid extraction yield)	[110]
Soxhlet	*M. oleifera* L. (leaves)	Ethanol	90	180	10.67 ± 0.27 mg/g dw	[112]
Soxhlet	*P. oleracea* L.	Eth:W (70:30)	—	300	7.0 mg/g dw	[111]
Soxhlet	*Pleurotus florida*	Methanol	—	300	0.40 ± 0.03 mg QE/g dw	[113]
Soxhlet	*Humulus lupulus* (hops)	Ethyl acetate	77	480	11.4 ± 0.6 (wt% extract)	[114]
Soxhlet	*H. lupulus* (hops)	Metanhol	65	480	25.8 ± 0.7 (wt% extract)	[114]
Soxhlet	*H. lupulus* (hops)	N-hexane	69	480	6.7 ± 0.2 (wt% extract)	[114]
Soxhlet	*H. lupulus* (hops)	Ethanol	78	480	25.8 ± 0.9 (wt% extract)	[114]
Soxhlet	*Mentha spicata* L. (leaves)	Methanol	40	360	267.33 ± 3.12 mg/g dw	[115]
Soxhlet	*M. spicata* L. (leaves)	Ethanol		360	218 ± 4.24 mg/ dw	[115]
Soxhlet	*M. spicata* L. (leaves)	Petroleum ether		360	30.47 ± 2.34 mg/g dw	[115]
Soxhlet	*M. spicata* L. (leaves)	Eth:W (70:30)		360	257 ± 3.47 mg/g dw	[115]
Soxhlet	*Populus temula*	Methanol	—	2880	11.5 mg/g dw	[116]
ASE	*Rheum palmatum* L	Met:W (80:20)	80	10	3.9 mg/g dw	[117]
ASE (3)	*Passiflora* species (leaves)	Eth:W (40:60)	40	30	49.22 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Eth:W (40:60)	80	30	41.46 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Ethanol	40	30	110.89 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Ethanol	80	30	101.61 mg GAE/g dw	[118]
ASE (1)	*Passiflora* species (leaves)	Eth:W (70:30)	40	10	72.60 mg GAE/g dw	[118]

Type (cycles)	Substrate	Solvent (%)	Temperature (°C)	Time (min)	Yields	References
ASE (1)	*Passiflora* species (leaves)	Eth:W (70:30)	80	10	79.43 mg GAE/g dw	[118]
ASE (5)	*Passiflora* species (leaves)	Eth:W (70:30)	40	50	65.78 mg GAE/g dw	[118]
ASE (5)	*Passiflora* species (leaves)	Eth:W (70:30)	80	50	84.53 mg GAE/g dw	[118]
ASE (1)	*Passiflora* species (leaves)	Eth:W (40:60)	60	10	58.54 mg GAE/g dw	[118]
ASE (1)	*Passiflora* species (leaves)	Ethanol	60	10	85.83 mg GAE/g dw	[118]
ASE (5)	*Passiflora* species (leaves)	Eth:W (40:60)	60	50	60.21 mg GAE/g dw	[118]
ASE (5)	*Passiflora* species (leaves)	Ethanol	60	50	80.31 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Eth:W (70:30)	60	30	60.57 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Eth:W (70:30)	60	30	63.70 mg GAE/g dw	[118]
ASE (3)	*Passiflora* species (leaves)	Eth:W (70:30)	60	30	60.89 mg GAE/g dw	[118]
ASE	*Durio zibethinus* M. (leaves)	N-hexane	200	13	839.2 ± 232.3 QE/ 100 mg dw	[119]
ASE (2)	*Morus. atropurpurea* Roxb.	Water	100	20	4.83 ± 1.52 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	Methanol	100	20	15.3 ± 0.6 mg/ GAE g dw	[120]
ASE (2)	*Morus. Atropurpurea* Roxb.	Met:W (50:50)	100	20	4.28 ± 0.24 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	Met:AA (99.5:0.5)	100	20	11.58 ± 0.5 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	Acetone	100	20	13.8 ± 0.6 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	A:W (50:50)	100	20	13.2 ± 0.3 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	A:AA (99.5:0.5)	100	20	13.5 ± 0.8 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	Met:W:AA (50:49.5:0.5)	100	20	15.1 ± 0.1 mg/ GAE g dw	[120]
ASE (2)	*Morus. atropurpurea* Roxb.	A:W:AA (50:49.5:0.5)	100	20	14 ± 1 mg/GAE g dw	[120]
Mac	*Cassia alata*	Ethanol	60	120	70.13 ± 4.43 mg QE/g dw	[121]
Mac (2)	*Brassica oleracea* L var. *botrytis* L subvar *cymosa* (flower)	A:W (70:30) Methanol	4	1440	0.32 ± 0.05 mg GAE/g fw	[122]
Mac (2)	*B. oleracea* L var. *botrytis* L (flower)	A:W (70:30) Methanol	4	1440	0.18 ± 0.01 mg GAE/g fw	[122]

Type (cycles)	Substrate	Solvent (%)	Temperature (°C)	Time (min)	Yields	References
Mac (2)	*B. oleracea* L var. *capitata* L (leaves)	A:W (70:30) Methanol	4	1440	0.1 ± 0.01 mg GAE/g fw	[122]
Mac (2)	*Lactuca sativa* L (leaves)	A:W (70:30) Methanol	4	1440	0.1 ± 0.01 mg GAE/g fw	[122]
Mac (2)	*Brassica chinensis* L (leaves)	A:W (70:30) Methanol	4	1440	0.94 ± 0.7 mg GAE/g fw	[122]
Mac (2)	*Artemisia vulgaris* Cantley (leaves)	A:W (70:30) Methanol	4	1440	0.44 ± 0.07 mg GAE/g fw	[122]
Mac (2)	*Daucus carot* L subsp. *sativus* (Hoffm) *Arcang* (root)	A:W (70:30) Methanol	4	1440	0.045 ± 0.002 mg GAE/g fw	[122]
Mac (2)	*Allium cepa* L (bulb)	A:W (70:30) Methanol	4	1440	0.51 ± 0.04 mg GAE/g fw	[122]
Mac (2)	*Lycopersicon esculentum* Mill (fruit)	A:W (70:30) Methanol	4	1440	0.08 ± 0.01 mg GAE/g fw	[122]
Mac (2)	*Capsicum annum* L (fruit)	A:W (70:30) Methanol	4	1440	0.32 ± 0.02 mg GAE/g fw	[122]

SBE: sequential batch extraction; HRE: heat reflux extraction; SAE: stirring-assisted extraction; QE: quercetin equivalent; RE: rutin equivalent; Mac: maceration; AA: acetic acid; A: acetone; MA: maceration with agitation; GAE: gallic acid equivalent; ASE: accelerated solvent extraction; dw: dry weight; and fw: fresh weight.

Table 1.
Summary of studies of extraction of flavonoids from different sources.

more compounds of interest at a lower cost. Moreover, these novel techniques decrease the extraction time, increase the compounds' selectivity and reduce the amount of solvent per extraction. In addition to solvent reduction, some of these techniques allow the use of solvents less harmful to the environment and human health. Therefore, some of these techniques are green methods that can be used with green solvents. This fact has prompted companies to optimize these techniques for subsequent implementation on an industrial scale [101, 123].

3.2.1 Ultrasound-assisted extraction (UAE)

The parameter most characteristic of ultrasound is the frequency. The frequency of ultrasound is between 20 kHz and 10 MHz, while the frequency of sound is between 16 Hz and 20 kHz. The ultrasound-assisted extraction (UAE) uses one of the two types of ultrasound, power ultrasound (low frequency and high intensity), to extract different compounds from a wide variety of sources [124]. The mechanism of action of UAE consists in the formation of cavitation bubbles in the medium or solvent used. The appearance of voids by the compression and rarefaction cycle, which subjects the liquid to points above its critical molecular distance, creates cavitation bubbles in the medium. The compression and rarefaction cycle produce the enlargement of the bubbles until the bubbles collapse. This collapsing liberates a considerable amount of energy and subject the medium to high temperatures (4726.85°C) and pressures (2000 atm) now of the collapse. This extreme condition produces microjets that can break solid surfaces like vegetable cells favoring

Type (cycles)	Substrate	Solvent	Temperature (°C)	Time (min)	Yield	References
UAE	*Celastrus hindsii* (leaves)	Eth:W (65:35)	40	29	23.60 ± 0.31 mg QE/g dw	[72]
UADESE	*Lycium barbarum* (fruit)	CC:1,2-Propanediol (33:67)	Rt	90	4.9 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:Glycerol (33:67)	Rt	90	2.9 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:EG (33:67)	Rt	90	8.7 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:Malic a. (50:50)	Rt	90	11.1 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:Malonic a. (50:50)	Rt	90	8 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:p-Ta (33:67)	Rt	90	88.9 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:La. (33:67)	Rt	90	16.5 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC: Oxalic a. (33:67)	Rt	90	11 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:Resorcinol (25:75)	Rt	90	6.5 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC:Xylitol (50:50)	Rt	90	3.2 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	CC: Urea (33:67)	Rt	90	5.5 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	Water	Rt	90	3.6 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	Methanol	Rt	90	3.2 mg/g fw	[35]
UADESE	*L. barbarum* (fruit)	Ethanol	Rt	90	4.2 mg/g fw	[35]
UAE	*Citrus* sp. (peel)	Water	40	30	19.595 ± 2.114 mg GAE/g dw	[73]
UAE	*Crataegus* sp. (seeds)	Eth:W (72:28)	65	37	16.45 ± 0.2 mg/g dw	[74]
UAE	*Crinum asiaticum*	Eth:W (60:40)	64	47	16.4 mg/g dw	[75]
UAE	*Prunella vulgaris* L. (fruit)	Eth:W (41:59)	79	30.5	36.2 mg/g dw	[76]
HPAE	*Momordica cochinchinensis* (leaves)	Eth:Water (50:50)	25	3	5.8 ± 0.1 mg QE/g dw	[77]
UAE	*M. cochinchinensis* (leaves)	Eth:Water (50:50)	25	20	5.9 ± 0.2 mg QE g dw	[77]
HHPE	*Solanum lycopersicum* (pulp)	Hexane:W (60:40)	20	10	21.5 ± 0.1 mg QE g dw	[78]
HPAE	*Agave americana* (leaves)	Methanol	150	240	15.5 mg/g dw	[79]

Type (cycles)	Substrate	Solvent	Temperature (°C)	Time (min)	Yield	References
HPAE	*Flos sphorae*	Eth:W (75:25)	80	120	200 ± 8.63 mg/g dw	[80]
MAE	*Aegle marmelos*	Eth:W (80:20)	50	1	77.26 mg RE/g dw	[16]
MAE	*Trigonella foenum-graecum* (seeds)	Eth:W (80:20)	80	4	37.18 mg QE/g dw	[81]
MAE	*Cassia alata*	Ethanol	Rt	4	135 ± 3 mg QE/g dw	[82]
MAE	*Epimedium sagittatum* (leaves)	Eth:E (60:20)	65	25	97.19 mg/g dw	[83]
MAE	*Astragalus mongolicus* (root)	Eth:W (90:10)	110	25	1.19 ± 0.04 mg dw	[84]
SFE (CO_2)	*Ziziphus jujuba* Mill.(leaves)	Eth:W (90:10)	52.5	113	29.011 mg/g dw	[85]
SFE (CO_2)	*Odontonema strictum* (leaves)	Eth:W (95:05)	65	270	18.92 mg QE g dw	[86]
SFE (CO_2)	*Maydis stigma* (flowers)	Eth:W (20:80)	51	120	4.24 mg/d dw	[87]
SFE (CO_2)	*Pueraria lobata* (Roots)	Ethanol	50	90	16.95 ± 0.43 mg/g dw	[88]
SFE (CO_2)	*Vaccinium myrtillus* (fruit)	Eth:W (10:80)	Rt	30	72.18 ± 1.13 mg/g dw	[89]
SFE (CO_2)	*Lepidium sativum*	Eth:W (96:4)	50	70	58 mg RuE/g dw	[90]

UAE: ultrasound-assisted extraction; CC: choline chloride; EG: Ethylene glicol; a.: acid; p-Ta: p-toluenesulfonic acid; La.: levulinic acid; UADESE: ultrasound-assist deep eutectic solvent extraction; HPAE: high pressure assisted extraction; HHPE: high hydrostatic, pressure extraction; Rt: room temperature; SFC-CO_2: supercritical CO_2 fluid extraction; dw: dry weight; and fw: fresh weight.

Table 2.
Parameters that affect novel extraction techniques of flavonoids from different sources.

intracellular compounds' extraction. Moreover, microjets also benefit the solvent-substrate interaction by reducing the particle size [124–126].

This method has been used in the food and pharmaceutical industries for several purposes [125]. The yield of the flavonoid extraction depends on diverse parameters: frequency, solvent, solid-solvent ratio. **Table 2** shows numerous studies about the optimization of flavonoid extraction from different raw materials. Although UAE is considered a green extraction technique for their reduction of energy and time consuming, the use of green solvents with UAE is currently a new trend. In the case of flavonoids and other phenolic compounds, deep eutectic solvents (DES) are becoming a viable alternative to traditional polluting solvents (**Table 2**) [127–131]. The use of UAE has numerous benefits compared with other conventional and novel techniques. UAE obtains higher yields and productivity with lower extraction times and solvent consumption than the conventional techniques. Moreover, it is more ecofriendly and a wider variety of solvents can be used. Furthermore, the extraction can be carried out at low temperatures reducing the risk of thermal degradation of flavonoids. Nevertheless, UAE has some drawbacks. Before the extraction, a filtration step is required, and the unstable compounds are not suitable for this method [132].

3.2.2 High pressure assisted extraction (HPAE)

Le Chatelier's principle states that if a system in equilibrium is perturbed, it restores the balance changing other parameters [133]. Therefore, if a system (solvent – raw material) is subjected to an increase in pressure, it will suffer a decrease in volume that will result in a more efficient extraction [134]. The volume changes produce variations in the cellular membrane and other big molecules that can cause the cell membrane and organelles' rupture, thus facilitating the transfer of bioactive compounds to the solvent [135]. The process of high pressure-assisted extraction (HPAE) has three stages. Firstly, the sample is mixed with the solvent in the pressure vessel at ambient pressure. The sample is subjected to a sudden pressure change up to 100–1000 MPa. At this point, the plant cell wall, the cell membrane, or any other barriers are subjected to a large differential pressure between the inside and outside of the barrier producing deformations and ruptures. The solvent penetrates the barriers through the ruptures and deformations, accessing the cell interior. Once the solvent is in the cell interior, the mass transfer of soluble compounds is favored. Moreover, the differential pressure could exceed the cell's deformation limit (cell wall and/or membrane). This will collapse, resulting in the liberation of all the compounds which will flow to the outside and dissolved in the solvent. Finally, all pressure is quickly released to atmospheric pressure, which produces cell expansion deforming the cell wall and membrane again [136]. The parameters that are usually considered at the time of the extraction are: temperature, pressure, type of solvent and concentration, holding pressure time, the ratio of solvent to raw material and the number of cycles [137]. **Table 2** shows how some of these parameters affect the extraction of flavonoids.

Regarding their advantages, the use of HPAE has demonstrated that the extraction could be performed at low temperatures without damaging heat-sensitive compounds or other compounds. Moreover, HPAE is considered an environment-friendly process, so it is a suitable alternative [138]. Other advantages are: the possible combination of more than one solvent to extract more than one type of compound, short periods of extraction, low use of energy or high cell penetration, resulting in higher mass transfer and extraction performance [137]. Nevertheless, in most cases, this technique uses some contaminant solvents, and after the extraction process, a filtration step is mandatory [139].

3.2.3 Microwave-assisted extraction (MAE)

The microwave-assisted extraction (MAE) consists of applying electromagnetic waves to produce changes in the cell wall and membrane. Microwaves have a frequency between 300 MHz and 300 GHz and belong to the electromagnetic field [140]. The MAE process's main advantage is the synergetic combination of heat and mass gradients flowing in the same direction [141]. The electromagnetic waves interact with the polar components inside the cells producing heat through ionic conduction and dipole rotation only in the compounds with an adequate dielectric constant [142]. Depending on the interaction between the compounds and the microwaves the compounds can be classified into three categories: opaque, transparent and absorbing materials. Microwaves heat only absorbing materials by the absorption of the energy of the electromagnetic waves. The mass transfer of flavonoids is produced because of the capacity to heat the cell's intracellular volume, causing an increase of the intracellular pressure producing the collapse of the cell wall and membrane. Then, the compounds can flow out of the cell and the gradient of heat flows [143].

The yield of the flavonoid extraction will depend on the raw material and selected parameters, such as temperature and time of the extraction, composition of the

solvent, solvent-to-feed ratio, microwave power, the water content of the matrix and the number of cycles for optimal extraction of flavonoids [141]. **Table 2** shows the yields of some flavonoid extractions by MAE and how some parameters affect flavonoid recovery. In comparison with conventional extractions, MAE has demonstrated better time of the extraction, yield, selectivity and quality of the flavonoid extracted. Moreover, the amount of solvent required is lower than in other techniques [144]. Nevertheless, the solvent and target compounds must fulfill some characteristics, compounds must be polar, and solvent must be not too viscous and absorb microwave energy. However, thermally labile compounds cannot be extracted with this method and after the extraction, extract filtration is required [132].

3.2.4 Supercritical fluid extraction (SFE)

A supercritical fluid (SF) is a homogeneous liquid in which the liquid and gas state's demarcation surface disappears. This homogeneous state is caused by exceeding the critical point of temperature and pressure [145]. The diffusivity and density of a SF are between what is expected in a gas and a liquid. As the same as gases, SFs experience a change of density when temperature or pressure are altered, which can produce variations in the density affecting the solvating power [146]. Therefore, these phenomena can improve the solubility of the compounds in the SFs. Supercritical fluid extraction (SFE) is a complex process widely studied along the literature [145–148]. Nowadays, CO_2 is the most SF used for SFE. CO_2 has some very advantageous characteristics for SFE, low critical temperature (32°C) and pressure (704 MPa). Moreover, CO_2 in low concentrations is non-explosive, non-toxic, non-inflammable and is easy to purchase at a low price with a high degree of purity. Besides, CO_2 has more than double the diffusivity of other fluids with lower surface tension and viscosity. Nevertheless, CO_2 is more suitable for nonpolar compounds than for polar compounds [149, 150].

The main limiting parameters are temperature, pressure and time of extraction [151]. **Table 2** shows how these parameters affect the yield of flavonoid SFE. Moreover, other factors like flow rate, modifiers and fractionation can affect the yield of the extraction [146]. The main advantages of SFE are rapidity, low amount of solvent, high selectivity and yield. On the other hand, SFE is a complex process with many parameters to optimize. High investment is needed, and specific alterations such as adding modifiers when extracting polar compounds are necessary [132].

3.2.5 Enzyme assisted extraction (EAE)

The enzyme assisted extraction (EAE) consists of the disruption of the plant cell wall and membrane by the enzymatic digestion of the polysaccharides that conform these two barriers. The plant cell wall comprises a complex structural mixture of polysaccharides, such as hemicellulose, cellulose and pectin, together with other molecules such as structural proteins and lignin [152]. Pectin is composed of a chine of α-D-galacturonate and L-rhamnose units linked by glycosidic bonds in α-1,4 or 1,2 that create the structure called pectic elbows [153]. For the hydrolysis of pectin, several types of pectinases (protopectinases, esterases, depolymerases) are used in the juice industry but also in the extraction of polyphenols [154, 155]. Cellulose is a polymer consisting of glucose β-1,4, which linked to other molecules, gives protection and stability to the cell wall [156]. Cellulases catalyze the breakdown of cellulose. Although its mechanism of action is not fully established, the most accepted theory affirms that three different types of proteins work synergistically during cellulose catalysis. Endonucleases act first, followed by the cellobiohydrolases and, finally, exoglucanases, resulting in free glucose molecules [157]. Hemicellulose is a

Substrate	Enzymes	Solvent	Temperature (°C)	Time (min)	Compound	Yield	References
Ginko biloba (leaves)	Cellulase and pectinase	Eth:W (50:50)	60	1800	Flavonoids	28.3 mg/g dw	[91]
Grape skins	Lallzyme EX-V (commercial) cellulase and hemicellulose, polygalacturonase, pectin lyase, pectin methylesterase	Water	45	179	Flavonoid glycoside and flavan-3-oil	4 mg/g dw	[92]
Grape skins	Lallzyme HC (commercial) cellulase, polygalacturonase, pectin lyase, pectin methylesterase	Water	31	162	Flavonoid glycoside and flavan-3-oil	3.7 mg/g dw	[92]
Grape skins	Endozym Rouge (commercial) cellulase and hemicellulose, polygalacturonase, pectin lyase, pectin methylesterase	Water	39	85	Flavonoid glycoside and flavan-3-oil	3.7 mg/g dw	[92]
Grape skins	Endozym Contact Pelliculaire (commercial) cellulase and hemicellulose, polygalacturonase, pectin lyase, pectin methylesterase	Water	36	128	Flavonoid glycoside and flavan-3-oil	3.7 mg/g dw	[92]
Cajanus cajan (L.) Millsp.	Cellulase, beta-glucosidase, pectinase	Water	32.5	1080	Luteolin and apigenin	0.4 mg/g dw	[93]
Larix gmelina (Rupr) Rupr.	Cellulase, pectinase	Water	32	1080	Flavonoids	4.96 ± 0.29 mg/g dw	[94]
Citrus x *paradisi* (peel)	Cellulose ® MX, Kleerase ® AFP	Water	50	180	Phenolics	1.62 mg GAE/g fw	[95]

SBE: sequential batch extraction; HRE: heat reflux extraction; SAE: stirring-assisted extraction; QE: quercetin equivalent; RE: rutin equivalent; dw: dry weight; and fw: fresh weight.

Table 3.
Parameters that affect enzyme assisted extraction (EAE) of flavonoids.

heterogeneous mixture of carbohydrates homologous to cellulose, such as xyloglucans and mannans. Hemicellulases are a big group of enzymes with several enzymatic activities to break down all hemicellulose forms [158]. Lignins refer to aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenyl-propanoids [159]. Nowadays, enzymes kits for digestion of the cell wall are prepared to carry out the functions previously mentioned and thus liberate flavonoids in the cell interior and improve the solvent's mass transfer [160]. Besides, EAE could be used alone or combined with other techniques (MAE, UAE, SFE or HPAE) [161].

Parameters like temperature and pH are essential when working with enzymes. Moreover, selected enzymes, mode of action and time are other parameters to consider [161]. **Table 3** shows several studies of the extraction of flavonoids from different sources and the yield variation depending on some parameters that affect extraction efficiency. In terms of environmental pollution, this method is one of the most environmentally friendly. Besides, EAE could be performed at low temperature, valid for many different raw materials, and different enzymes can be selected depending on the targets of the extraction [160, 162–165].

4. Bioavailability of flavonoids

Bioavailability refers to the concentration of a molecule or related like-molecules that become absorbed and available for exerting their biological activity in the site of drug action of the target tissue, organ or system [166]. The term bioavailability is strongly related to the concept of bioaccessibility and to bioactivity. Bioaccessibility refers to the number of compounds that, after digestion, becomes available and absorbable through the intestinal epithelium. This definition is linked to bioactivity, which involves the physiological effects that biomolecules trigger in the organism and includes their transport through systemic circulation to the target receptor and their interaction with other biomolecules [167].

The bioavailability of polyphenolic compounds has been described to be poor since they hardly reach bioaccessibility rates higher than 30–50% [167]. Among the parameters involved in this low bioavailability, there are several physicochemical properties of flavonoids which include their chemical structure, polymerization degree, solubility, variability of attached saccharides or potential interactions they established with other compounds, or flavonoids stability, both during storage and along the digestion process [168]. Different approaches have been developed to enhance the accessibility to the final number of flavonoids or to blur their metabolism through digestion and improve and extend their chemical stability. These intend to maximize the bioavailability of flavonoids. To increase the available concentration of flavonoids in food, different treatments have been applied to food matrixes. The main purpose is to alter the matrix's structural organization in which biomolecules are embedded so they can get easily released. Both heating and freezing approaches have been tested and demonstrated to positively affect the bioaccessibility of polyphenols [167]. Nevertheless, other techniques requiring more technological development have demonstrated a better performance to improve flavonoids bioaccessibility (**Figure 7**). In fact, the pharmaceutical industry has established alternative approaches to improve the oral bioavailability of flavonoids with clinical applications. Some of the most utilized strategies are the use of absorption enhancers (nonionic surfactants, myo-inositol hexaphosphate, chitosan or pectin), the induction of structural transformations which include the introduction of functional groups with higher polarity (sulfuric acids, amino acids, carbamoyls, glycosides, etc.), or the complexation with a carrier (such as cyclodextrins, phospholipids or polymeric carriers) [169].

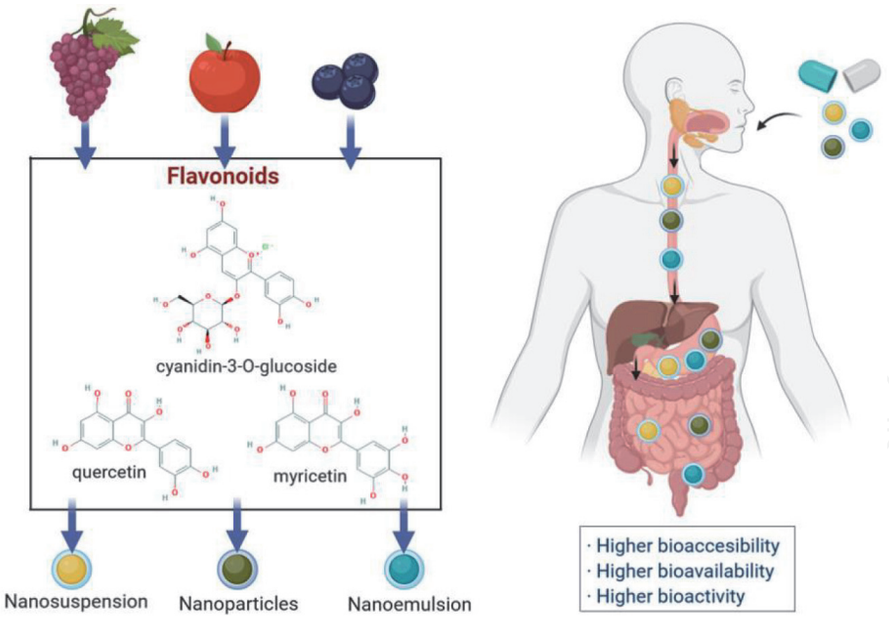

Figure 7.
Strategies to enhance flavonoids bioavailability. Nanosuspension, nanoencapsulation or nanoemulsions have been proved as successful approaches to improve flavonoids solubility and enhance their bioavailability, bioaccesibility and, bioactivity.

Among these approaches, nanosuspension, in which pure drug particles are combined with stabilizers, has been demonstrated as a promising strategy to enhance the bioavailability of flavonoids. This system facilitates the delivery of flavonoids using particles in the nanometers range, which allows reaching a higher concentration quickly by increasing solubility and dissolution rates. For instance, in a recently published work in which different nanosuspension formulae were applied, the solubility of myricetin was increased from 43 to nearly 75 times. This increment was accompanied by an improved bioavailability in the relative range of 161–357% [169]. Another flavonol, quercetin, was also submitted to nanosuspension. This strategy improved its saturation solubility about eleven times which also provided a much better bioaccessibility. The bioaccesibility increased slowly, reaching its maximum peak between 2 and 3 h, while the pure molecule reached this maximum at 1.5–2 h. The amount of quercetin released from the nanosuspension was higher than the pure, duplicating its bioaccessibility even at the last measured times [170]. The bioactivity of the orally administrated flavanone, naringenin, was also tested using rats. It was shown that the nanosuspension of naringenin was nearly 4 times higher when compared against the control [171].

A common methodology applied in both food and pharmacological industries for increasing flavonoids bioavailability and bioaccesibility, which ultimately enhances their potential bioactivity, relies on their encapsulation [169, 172]. Different techniques, with diverse complexity degrees, permit the encapsulation of a considerable variability of core ingredients using different shell materials for obtaining capsules with various physical properties. Some of the most used encapsulation methods include spray or freeze-drying, spray chilling and cooling, coacervation, fluidized bed coating, liposome entrapment, rotational suspension separation, extrusion and inclusion complexation, (micro)emulsions, etc. [173]. The main aim of the encapsulation process is to prevent biological and physicochemical degradation of bioactive ingredients. Encapsulation permits to extend the chemical stability of the target molecules and thus their bioactivities. Besides, encapsulation may also allow the controlled release of the compounds delivered using concentrations. Scientific literature provides several examples of flavonoids that have been

encapsulated. Among the benefits of encapsulation, bioaccesibility and bioavailability are two parameters that can be improved using this approach. Besides, encapsulation permits to embed flavonoids in the most appropriate matrices to reinforce their stability [172]. The flavonoid subclass of anthocyanins has been extensively used to evaluate the performance of different encapsulation techniques and materials. For instance, anthocyanins from grape peels have been submitted to encapsulation by emulsification/internal gelation using both spray and freeze-drying techniques. The former provided smaller microcapsules (0.6 μm) with higher encapsulation efficiency and better microcapsule and anthocyanins stability, which extended their release in simulated gastrointestinal digestion and improved their bioaccessibility [174]. Two major anthocyanins were identified in extracts from *Rubus idaeus* as cyanidin-3-*O*-glucoside and 3-*O*-sophoroside and encapsulated using β-lactoglobulin. This encapsulation strategy was demonstrated to increase the bioavailability of these anthocyanins after submitting them to simulated gastrointestinal digestion up to nearly double (19%) than its free presentation (11%) [175]. Different prototypes of nanoparticles were created for encapsulating commercial anthocyanins, containing as major representatives cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside. The improvement of free anthocyanins' bioavailability was determined to get increased from 17 to 27% when loaded in chitosan-based nanoparticles and to 40% when anthocyanins were encapsulated using a mixture of chitosan and β-lactoglobulin [176]. Other flavonoids have also been encapsulated with a demonstrated enhancement of their bioavailability. Quercetin, naringenin, and hesperetin were nanoencapsulated, thus preventing their degradation under the hydrolytic conditions of the small intestine. It also reinforced their bioaccesibility, especially in the case of quercetin which reached a relative bioaccesibility after simulated digestion of around 80% when encapsulated against the scarcely 10% when present as a free molecule. Similarly, free naringenin and hesperetin showed low bioaccesibility (about 15–20%) that reach about 70% when encapsulated [177]. The encapsulation of the glycoside flavonoid, naringin, prompted a progressive release in both gastric and intestinal environments when compared to the free molecule. In the intestine, the liberation was faster, probably due to the presence of bile salts and pancreatin. However, this poorer release at the gastric level and higher at the intestinal level is desirable. It would allow the arrival of higher concentrations of naringin to the intestine, and thus more amounts of this biocompound would be available for its adsorption [178].

Another technique tested for enhancing the bioavailability of flavonoids is based on their emulsion. This emulsion can be created by utilizing emulsifying agents or mixtures of oil-(co)surfactants-water, which permit the self-emulsion with a simple process, agitation. In fact, different alternatives of this approach have been proved successful for different kinds of flavonoids. Anthocyanins from blueberry fruits were micro-emulsified and their bioavailability and bioactivity were evaluated against the control (without vehicle). Non-purified and purified anthocyanins, especially malvidin-3-*O*-glucoside, showed hypoglycemic activity when emulsified while those without vehicle did not [179]. Quercetin was also self-emulsified in an oily matrix using Tween 20 as surfactant and ethanol as co-surfactant. The oral bioavailability of this specific emulsified flavonol was increased up to 5 times [180]. An even better bioavailability was obtained through the quercetin's nano-emulsion using an oil-water phase and different surfactants and co-surfactants. The optimized emulsified presentation of this flavonol was based on an equimolar mixture of Capryol and Labrafil as oily phase representing a 17% while water phase was around 33%, and remaining fractions corresponding to an equimolar combination of Labrasol and Tween 80 as surfactant and an equimolar mixture of Cremophor EL:polyethylene glycol 400 as co-surfactant. The emulsion obtained following this

protocol improved its gastrointestinal permeability, accompanied by enhanced bioactivity (higher mice weight reduction) and bioavailability in rats, 33 times higher than its aqueous dispersion [181]. Similarly, the flavonol, myricetin, was microemulsified using 6% of Tween 80, 12% of Cremophor RH40, 9% of Transcutol HP, 18% of WL 1349, and 55% of distilled water. This formula allowed the increment of its aqueous solubility more than 1000 times, increased its bioavailability 14 times and hence its bioactivities, both antioxidant and anti-proliferative [182]. From the sub-group of flavones, tangeretin was also emulsified using as part of the oily phase medium-chain triacylglycerol and lecithin to which aqueous phase was added following previously optimized temperature, pressure and stirring conditions. This emulsion doubled the bioavailability of tangeretin, which ultimately improved its tested bioactivities, especially the anti-proliferative [183, 184]. Also the flavone baicalein has been nano-emulsified using a composed oil (isopropyl myristate) and water, Cremophor EL35 as surfactant and propylene glycol as co-surfactant. This preparation improved intestinal absorption of baicalein along all the different parts of the intestine, especially in duodenum and jejunum. This kind of nanoemulsions protect the core ingredient from enzymatic damage and can prolong drug retention in the intestine. In fact, its pharmacokinetic behavior was improved 7 times, and its oral exposure increased nearly 15 times compared to the suspension of baicalein [185].

Therefore, very different techniques have been proved to improve the poor solubility of flavonoids and to enhance their bioavailability, bioaccesibility and, hence, their bioactivity. Among these techniques, the most successful ones are based on the application of nanosuspensions, encapsulations or emulsions of the flavonoids.

5. Development of flavonoids based natural additives: reported and future applications

Currently, consumers are increasingly aware of their healthier food choices, associating them with their health and well-being. In this way, there is a global demand on the part of the food industry to develop innovative natural products and health promoters that contain bioactive components [186].

Different bioactive ingredients, namely flavonoids, have been studied to adapt organoleptic, sensory and conservation properties. They have also been explored as functional ingredients with bioactive properties, such as antioxidant, anti-inflammatory and immunomodulatory referred to earlier in this manuscript [187–189]. Thus, bioactive compounds are considered valuable options to be explored in the design of innovative food formulations with health benefits.

Different flavonoids have been studied, and their bioactive properties have been proven by several authors, which has arisen the high interest of the food industry in their application in functional foods. Foods and beverages such as dairy products, bakery and confectionery products, meat products, juices and energy drinks, snacks, pasta, gums and sweets are some of the products explored the most in the addition of bioactive compounds [190].

In a recent study, the stability of anthocyanins from grape residues was evaluated when applied as a food coloring in carbonated water and proved that the degradation of the incorporated anthocyanins followed the kinetic behavior during storage, when exposed to light or dark [191]. The anthocyanin malvidin-3-glycoside showed the greatest stability when added to the water. Additionally, it was found that the light had adverse effects on the color of the carbonated water. Bakery and pastry products, recognized for providing consumers of all ages with pleasure and

fun, have been explored exponentially in an attempt to find functional natural ingredients and/or colors with potential for application in a highly competitive area [192]. A recent study intended to explore the bioaccesibility and bioavailability of phenolic compounds (namely flavonoids) obtained from green tea in wheat bread. The results showed an increase in the nutraceutical potential and the protection of lipids against oxidation. Also, *in vitro* studies showed that digestion induced the release of phenolic compounds in bread, proving bioaccesibility and bioavailability [193]. In its turn, another study optimized the obtaining of an anthocyanin-rich extract (33.58 mg anthocyanins per g of extract where cyanidin-3-*O*-glucoside appears as the major anthocyanin compound) from *Rubus ulmifolius* Schott. The authors described an extract with bioactive properties (antioxidant, anti-tumor and antimicrobial potential) with excellent coloring ability when applied to a pastry product, the donut, proving to be a great approach for replacing artificial colorants [194].

Dairy products have been extensively tested and explored due to the industry's high interest to supplement functional ingredients [195]. Aqueous extracts of *Foeniculum vulgare* Mill. and *Matricaria recutita* L. rich in phenolic compounds namely flavonoids (11.52 ± 0.11 mg/g and 17.89 ± 0.91 mg/g flavonoids, respectively) were explored as health-promoting natural ingredients in cottage cheese [196, 197]. The results obtained demonstrate that the tested natural extracts improved the natural conservation of the cottage cheese by increasing the shelf life and adding antioxidant properties to the product. Since yogurt is considered one of the most traditionally consumed dairy snacks globally, several studies explore functional ingredients with preservative and coloring properties. The coloring ability of different anthocyanin extracts from edible flowers (rose, cornflower and dahlia) was tested in yoghurts as a replacement for artificial colors (specifically E163, anthocyanin extract). The results showed the hydrophilic rose extract as the most appropriate natural ingredient to replace E163 since, in addition to not altering the nutritional composition of the product, it presented close scores in the color parameters achieved by the artificial colorant [198].

Some fruits have also been explored as natural ingredients. In a recent study, extracts from *Vaccinium myrtillus* L. fruits (blueberry) revealed a high concentration of anthocyanins (21.1 ± 0.2 mg/g), highlighting malvidin glycoside and delphinidin glycoside derivatives as the majority [199]. The high content of these compounds was responsible for their bioactive properties that arouse interest for incorporation. In the same study, the coloring potential of the natural ingredient in yoghurts was tested. The results showed that although the blueberry extract has a lower coloring capacity when compared to the artificial additive E163, it presented greater stability over the storage time.

However, incorporating these compounds in this type of food product has represented a challenge about the quality of the final product and the stability of bioactive compounds. The high-water content and low pH value of yogurt as well as the low solubility of polyphenols have represented a great challenge for the use of herbal extracts, especially hydrophobic extracts [200]. The use of bioactive compounds as natural ingredients in food products has been characterized in several studies as limited due to their stability and bioavailability. Storage conditions, thermal and non-thermal processes and extraction treatments are some of the parameters identified as responsible for affecting these compounds' effectiveness [201, 202]. Some bioactive compounds are susceptible to environmental factors, namely pH, temperature, oxygen, enzymes, light, metal ions, sulfur dioxide and ascorbic acid [203]. The molecular interactions between bioactive compounds with other food ingredients can also affect some properties of these compounds, such as bioavailability, bioactivity and organoleptic properties [204]. After oral consumption, the

chemical structure and bioactivities of the components are altered in intestinal metabolism. Thus, it is necessary to ensure that the bioactive compounds in the gastrointestinal tract are stable and allow controlled release at target points [205].

This type of limitations has been a concern for the food industry since it can hamper its industrial application. For example, quercetin is a flavonol recognized for its anti-diabetic properties; however, its low solubility and aqueous permeability limit its application. Anthocyanins, which are very attractive due to their ability to provide color and potential health benefits, have also represented a major industrial challenge in controlling their deterioration and increasing their bioavailability in food systems [206, 207].

For this reason, different microencapsulation and delivery systems have been explored to guarantee the production of functional foods with acceptable organoleptic characteristics and the controlled release of flavonoids, thus preventing interactions with other food components, and overcoming problems encountered during food processing and gastrointestinal transit [208, 209]. Some examples will be mentioned as follows. A blueberry-derived mixture of anthocyanins was encapsulated into chitosan nanoparticles, and its stability in a drink was evaluated. The results suggested that the chitosan nanoparticles delayed the anthocyanin degradation in the simulated gastrointestinal fluid and increased the anthocyanin storage stability in the drink [201]. In other work, an encapsulated polyphenolic extract (rich in anthocyanins) from Artemide black rice obtained through the atomization process with maltodextrins and gum arabic (50:50, w/w) was incorporated into biscuits. The results showed that the encapsulated ingredient emerged as the most stable during storage and cooking and with the most significant antioxidant capacity than the control biscuit [210].

Also, *in vitro* assays such as those presented above make it possible to understand the potential beneficial health effects these compounds may have after ingestion [211]. However, studies on *in vitro* release of microencapsulated phenolic compounds are still relatively scarce. A study demonstrated a better solubility of microencapsulated curcumin and quercetin in niosomes [212]. Also, an anthocyanin extract obtained from fruits of *V. myrtillus* L. was tested in a gastrointestinal model *in vitro*, and the results demonstrated an improvement in stability in adverse pH conditions during digestion, being released only in the intestinal mucosa [213].

The exploitation of natural ingredients with antioxidant and antimicrobial properties in combination with natural polymers has also been ceased by the scientific community in the development of edible films that allow to reduce the dependence on synthetic polymers and offer viable solutions for industrial application [214]. Polyamide, polyethylene terephthalate, ethylene vinyl alcohol, polyvinylidene chloride, polypropylene and polyethylene are some of the most widely used polymer materials in the food industry for food preservation. However, some authors report that polymers in direct contact with food allow the migration of the additives and other components to food, causing some adverse effects for consumers [215]. In this sense, studies on plastics and plasticizers and non-toxic bio-based food coatings to replace their synthetic counterparts have been increasing [216]. These coatings make it possible to coordinate natural polymers with bioactive ingredients from plant extracts with preservative and antimicrobial properties that improve the organoleptic and functional properties of food [217].

6. Concluding remarks and future research directions

Although many bioactive compounds are currently tested in different food matrices to improve their organoleptic properties, to fortify and functionalize these

same products, it is considered that the protection of such functional ingredients in the food matrix during processing, storage and passage the gastrointestinal tract has been little explored. Several flavonoids have been extensively studied and have shown to be highly promising bioactive compounds, capable of improving the physical-chemical, sensory and health properties of food products. However, studies on the effectiveness and interactions of these bioactive compounds for the development of new innovative products are still scarce and there are some gaps between digestion, metabolism and bioactive substance delivery approaches across biological barriers that must be explored. This type of studies' transition to a commercial scale is an essential future step in innovation to provide more practical information that can be transposed to industry. Thus, and to meet consumers preferences and requirements, there is a great need for new and more complete *in vivo* studies capable of verifying the appropriate dosage for different ingredients to be incorporated in different food matrices and, consequently, build the proper design of food products ensuring the desired safety and functionality. The production of functional foods is a current trend in the growing exploitation of the food industry. Therefore, the exploration of new bioactive compounds from different sources to deliver and modulate the properties of foods will be the objective of analysis in the scientific community to respond to the industry's needs.

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Conflict of interest

The authors declare no conflict of interest.

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