Chapter 4
Natural Colorants

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4.1 Introduction

Natural colorants are an alternative to substitute synthetic ones in food products. The use of natural colorants in food formulations reduce the possible toxicity and allergenic characteristic associated with the use of synthetic colorants. Furthermore, the use of natural colorants can increase food acceptance, since consumers demand healthier food products with a clean labels, over the recent years [1, 2].

Several natural pigments can be used as food colorants and/or preservative, the most common natural pigments are anthocyanins, betalains, carotenoids, annatto, β-carotene, lycopene, lutein, paprika, carminic acid, chlorophylls, and curcumin [1, 2]. These pigments can be obtained from algae, fruit, vegetables, and other comestible plants, fruits, and vegetables. Furthermore, most of these natural pigments also have antioxidant, antimicrobials, anti-obesity, antidiabetic, anticarcinogenic, cardiovascular protection, and neuroprotective properties and have been used extensively in pharmacology, and recently in the food industry to produce functional foods. However, the colorimetric and functional properties of natural colorants can be modified due the oxygen presence, as well as with increase of the temperature. Other factors such as light and change of the pH also can modify the colorants properties, reducing their effectiveness and limiting their application in food industry [3–5]. This review aims compressively to analyze the state of the art related to the use of natural colorants in foods, focusing especially on their sources, properties, and potential application in the food sector. Furthermore, it was discussed their approbation by international regulatory agencies.

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4.2 Main Natural Colorants

4.2.1 Anthocyanins

Anthocyanins are one of the major studied natural pigments belonging to the flavonoid family, having more than 700 derivatives of different anthocyanins. It is soluble in water, has low or no toxicity, and has different shades of color, ranging from red to blue [1, 2]. They are found in many leaves, flowers (hibiscus), vegetables (cabbage, eggplant), tubers (purple potatoes), and fruits (grapes, raspberries, blueberries, cherries, and pomegranates) which are colored red, purple, or blue [3, 4].

Chemically, anthocyanins have a glycosidic structure (sugars and active acids) linked to an aglycone (anthocyanidin). Anthocyanidins are flavyl ion structures (2-phenylbenzopyrilium) with the keto oxygen at the C1 position and are characterized by the power to produce different types of anthocyanins through glycosylation or acylation of varying sugar and phenolic or aliphatic portions [4, 5]. The main anthocyanidins that represent about 90% of all anthocyanins identified are cyanidin, pelargonidin, petunidin, peonidin, malvidin, and malvidin delphinidin [2]. The structure of the main anthocyanins can be seen in Table 4.1.

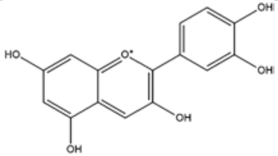
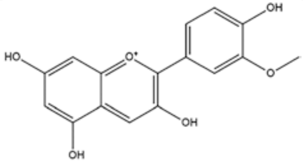
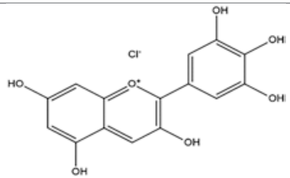
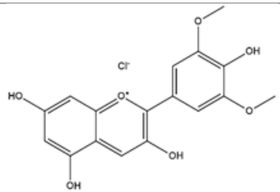
Food processing alters the accessibility and content of anthocyanins in the raw material. The bioaccessibility of anthocyanins is dependent on the food matrix and the anthocyanin structure [26]. Anthocyanins in nature are stabilized by co-pigmentation, self-association, and metal complexation [5]. As isolated anthocyanins, their stability is highly affected by temperature, oxygen, light intensity, and pH during processing, whereas they are generally stable in refrigerated storage and at acidic pH [27–29]. As it is a reactive compound, its color easily degrades because the flavyl cation reacts due to the lack of electrons. The red flavyl cation goes to a colorless carbinol alkali and eventually becomes a chalcone [30].

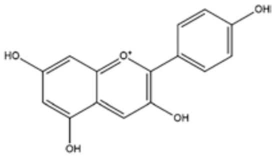
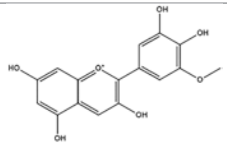
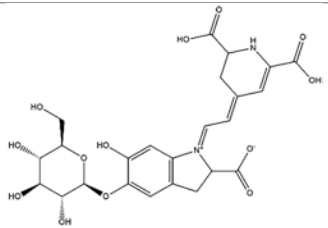
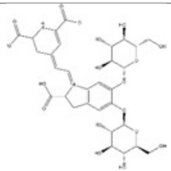
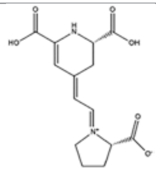
Anthocyanins are considered food additives (FAs) by the Codex Alimentarius Commission [31, 32], which has the definition of adding or restoring the color food. The standard number is 163 and varies according to the raw source, for example, grape skin (163 (ii)), extract from blackcurrant (163 (iii)), purple color from corn (163 (iv)), among others.

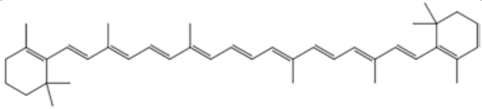
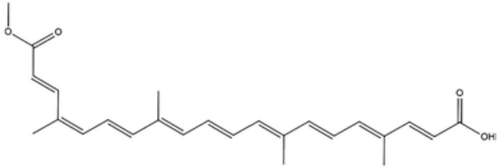
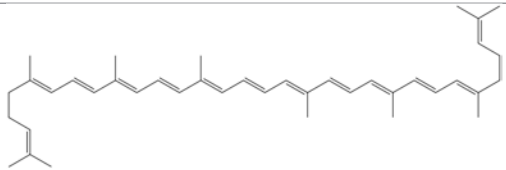
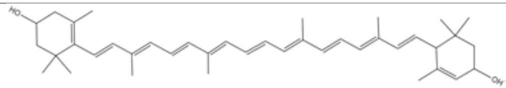
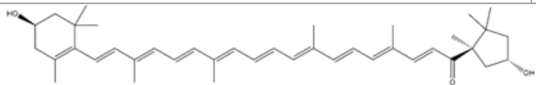
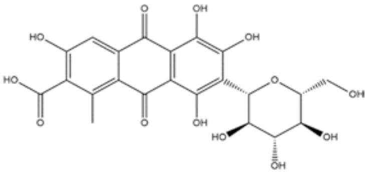
The anthocyanin color depends on several aspects such as its structure, pH, UV radiation, co-pigmentation, the concentration of the compound, presence, or absence of oxygen. Each anthocyanidin reflects a different color due to its chemical structure (Table 4.1). Cyanidin and peonidin remember reddish-purple, delphinidin, and malvidin appearing reddish-blue or purple pigment, pelargonidin, and petunidin red gives an orange hue to flowers and red to some fruits [4].

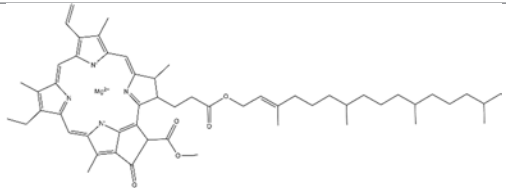
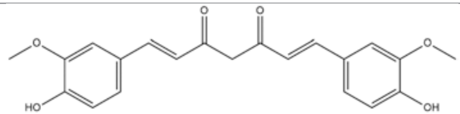
At different pH values, the ionic molecular structure of anthocyanins changes and there is a change in the tone and stability of this pigment. For example, in acidic forms, anthocyanins are found in red color. They are purple in neutral pH, and basic pH anthocyanins change to blue, green, and yellow [33–35]. Also, the anthocyanins concentration is an important factor that influence in the intensity of the extract color.

Table 4.1 Natural colorants, structure, color, primary sources, and degradation factors

Chemical classification	Pigment	Structure	Color	Primary source	Main degradation factors	Reference
Anthocyanins	Cyanidin		Red	Blackberries (*Rubus fruticosus* L.)	Heat, light, and oxygen	[6]
	Peonidin		Purple	Cranberry (*Vaccinium oxycoccus*)	Heat, light, and oxygen	[7]
	Delphinidin		Red	Uva (*Vitis vinifera*)	Heat, light, and oxygen	[8]
	Malvidin		Red-blue	Blueberry (*Vaccinium myrtillus*)	Heat, light, and oxygen	[9]

	Pelargonidin		Reddish-Orange	Strawberry (*Fragaria x ananassa*)	Heat, light, and oxygen	[10]
	Petunidin		Red	Black goji (*Lycium ruthenicum* Murr.)	Heat, light, and oxygen	[11]
Betalains	Betanin		Red-Violet	Beet (*Beta vulgaris*)	Heat, light, and oxygen	[12]
	Gomphrein		Purple	Purple Flowers (*Gomphrena globose* L.)	Heat, light, and oxygen	[13]
	Indicaxanthin		Red	Red prickly pears	Heat, light, and oxygen	[14]

Carotenoids	β-carotene		Orange-yellow	Carrot (*Daucus carota* L.)	Oxygen and light	[15–17]
	Bixin, norbixin		Red, yellow	Annatto (*Bixa orellana* L.)	Oxygen, heat, and light	[16, 18]
	Lycopene		Red	Tomato (*Solanum lypopersicum*)	Heat, oxygen, and light	[17]
	Lutein		Yellow	Marigold (*Tagetes erecta* L.)	Heat, oxygen, and light	[16, 19]
	Paprika (capsanthin)		Red-orange	Red pepper (*Capsicum annum L.*)	Heat and oxygen	[16, 20]
Carminic Acid	Carminic Acid		Red	Insects (cochonilla)	pH, light, temperature variation	[21]

Chlorophyll	Chlorophyll-a		Green	Green vegetables and microalgae	pH, heat-stable, and enzymatic reaction	[22, 23]
Curcumin	Curcumin		Yellow	*Curcuma longa* L.	Heat, light, and oxygen	[24, 25]

The color variation among anthocyanins is due to the change in hue results from a bathochromic shift, in which the light absorption band in the visible spectrum range changes from a shorter to a longer wavelength, changing the color from red to purple at acidic pH. This variation is also influenced by the presence of methoxy groups, in which the greater the presence of a methoxy group in the anthocyanin molecule, the greater the increase in blue/purple colors, such as malvidin, and the lower the concentration, the greater the increase in red color, as in pelargonidin [36].

In the literature, studies shows that anthocyanins have an high potential for application, as a natural colorant, mainly in food products and packaging. However, also have been applied in other products, such as fabrics, human hair, and sensitized solar cells [37]. In addition to dyes, the compounds may have beneficial health effects with antioxidant, anticancer, and anti-inflammatory properties [5]. As a natural food coloring and functional ingredient, the use of anthocyanins has been limited due to the low stability and interaction with other compounds in the food matrix. Therefore, alternatives such as encapsulation and adsorption have been studied to deal with these limitations [38].

4.2.2 Betalains

Betalains are nitrogenous pigments of plant origin belonging to the order Caryophyllales, formed by a central structure that is betalamic acid and substitute radicals conjugated with cyclo-Dopa (cyclo-3,4-dihydroxyphenylalanine) derived from glucosyl and amines. There are more than seventy known betalains, and they all have the same basic structure, where they are differentiated only by the modification of the radicals R1 and R2. These conjugates determine betalain classifications: red-violet betacyanins, betanidine derivatives (addition of betalamic acid and cyclo-DOPA), and yellow-orange betacyanins, resulting in the resulting from the condensation of a-amino acids or amines with betalamic acid [5, 39]. The betacyanins reported are betanin, gomphrenin, bougainvillea, and amaranthine, and betaxanthines are divided into two groups: those that are derived from amino acids and those that are derived from amines [22].

An example that for a long time was the only source of betalain, red beet (*Beta vulgaris*), but today, other sources also such as flowers, fruits (red pitaya), vegetables (chard), stems (cacti), roots, leaves, seeds, grains, and some fungi (*Amanita muscaria*, *Hygrocybe*, *and Hygrophorus*) [22–24].

Betalains are classified as FAs under code E-162 in the European Union or 73.40 by the Food and Drug Administration (FDA). The fluorescent pigmentation of betacyanins are weaker compared to betaxanthines, but their intensity is high by the carboxyl groups and low by the aromatic ring and hydroxyl groups; betaxanthines, on the other hand, have a maximum wavelength between 320 and 475 nm, which corresponds to blue light, and emission maximums between 500 and 660 nm, which corresponds to green light [25, 40].

Betalains have intrinsic and extrinsic factors that affect their stability, for both during the extraction and food processing. Its water-soluble pigments are stable from pH 3–7 and versatile for foods with low acidity and neutral content. The advantages of betalains are that colors are pH-independent and are more stable than anthocyanins in that pH range [41]. At pH values lower than 3, the anionic betalain (red color) structure is converted to cationic form (violet), showing a visible color change from red to blue violet. At pH higher 7 occurs the color change from yellow to brown due the hydrolysis of the aldimine bond and generating betalamic acid and cyclo-dopa-5-O-glycoside [42].

The bioactive compound‘s stability is affected by oxygen, light, metal ions, high water activity (aw), and temperature, limiting their use in the food industry, such as in frozen foods or in food with short shelf life. Due the biosynthesis of the compounds, betalain solutions’ must storage under low oxygen levels, low temperatures, and dark environment [40, 43, 44]. As well as others bioactive compound, several techniques to stabilize the betalain are being proposed such as encapsulation by drying methods, ionic gelling, and emulsions [45, 46], using several wall materials.

4.2.3 Carotenoids

Carotenoids are a class of lipophilic natural colorants widely distributed in nature, ranging from yellow, orange, and red. Carotenoids are tetraterpenoid pigments whose basic structure consists of 8 isoprene groups, with two C_{20} geranylgeranyl pyrophosphate molecules (composed of 4 isoprene units) linked head to head to form a C_{40} polyene chain with conjugated double bonds. The variable number of conjugated double bonds imparts carotenoids the property to absorb visible light (between 400 and 500 nm), resulting in their characteristic coloration in the yellow to red range [47, 48].

Currently, the carotenoids can be divided into two groups due to their chemical composition and structure: carotene; formed exclusively by carbons and hydrogen atoms (β-carotene, α-carotene, and lycopene); xanthophyll, in addition to carbon and hydrogen, they also contain oxygen in the structure (lutein, zeaxanthin, astaxanthin, and β-cryptoxanthin). Besides, carotenoids with a shorter carbon chain are apocarotenoids, such as bixin (annatto pigment) and crocetin (a component in saffron) [48, 49].

Carotenoids are synthesized by all photosynthetic organisms (including plants, algae, and cyanobacteria) and by some fungi, bacteria, and animals non-photosynthetic. In photosynthetic systems, carotenoids participate in light-harvesting, essential for photoprotection. In non-photosynthetic tissues and organisms, carotenoids play a role as pigments yellow, to red range. So, animals, including humans, cannot synthesize carotenoids, thus, this compound must be ingested through the diet. In foods, these compounds are present in leafy vegetables, e.g., broccoli and spinach (yellow color, unmasked when chlorophylls are degraded);

in non-leafy vegetables like carrots and pumpkin (orange color) and red color of peppers; and in fruits, e.g., watermelon and tomatoes (red colors) and orange color of mango and papaya. In addition, the red color of some fish (e.g., salmon) and crustaceans (e.g., cooked lobster, crab, and shrimp), and the yellow color of egg yolk are due to carotenoid accumulation in animal tissues. The most frequent carotenoids in food are β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene [48, 50].

Natural carotenoids from all sources food generally exist in their stable *trans* configuration, with smaller fractions in the *cis* form exhibiting less stability [48, 51]. However, the conjugated double bond system is susceptible to oxidation and isomerization during processing and storage. Oxygen, light, metal ions, and enzymes are factors that stimulate the oxidation process. The degradation product results in the formation of low molecular weight colorless compounds (volatiles) devoid of any biological activity [52].

As a natural food colorant, carotenoids can produce a range of pigments, including yellow, orange, and red, depending on the source, and are recognized as GRAS (Generally Recognized as Safe) by several regulatory agencies such as the Food and Drug Administration and the European Food Safety Authority (EFSA). Some applications of carotenoids include meat products (sausages), vegetable oils, and butter [53]. However, their use as a colorant and functional ingredient is challenging due to their water insolubility, instability, and low bioavailability. So encapsulation is a successful strategy that enhances their solubility and provides resistance against stresses during processing and digestion [47].

In addition to using carotenoids as a natural colorant, these compounds present various health benefits. The main health benefit of the carotenoids is their enzymatic conversion to retinol (Vitamin A), which is an essential micronutrient related to growth, development, immunity, epithelial barrier integrity, reproduction, and vision. Also, act as an antioxidant, anti-obesity, antidiabetic, anticarcinogenic, cardiovascular protection, and neuroprotective [47, 54].

4.2.3.1 Annatto

Annatto is the name given to the red crude extract obtained from the waxy arils that cover the seeds of the achiote tree (*Bixa Orellana L.* seeds). The achiote is a small tree or shrub native from the tropical regions including Brazil, Peru, and Mexico. However, it also grows in South and Central America [55].

Bixin and norbixin are main compounds from annatto extract, a liposoluble (bixin) and other, hydro soluble (norbixin), besides others compounds such as isobixin, beta-carotene, and lutein have been found in extract of the seeds annatto [56]. However, the bixin, is the main color compound, accounting for 80% of the total annatto pigments. Bixin ($C_{25}H_{30}O_4$) is a carotenoid of 9-cis configuration (structure present in an oxygenated carotenoid, e.g., lutein, which belongs to the xanthophyll group) and 2 carboxyl groups [55, 57].

Bixin and norbixin (E 160b) pigments exhibit high biodegradability, low toxicity, besides are stable to thermal processing, and are approved by the FDA for use in food and drinks [58]. In food industries, annatto extract is the main colorant used in the manufacture of cheese and butter, composed mainly the norbixin (a water-soluble component); this pigment confers the yellow/orange color to cheddar cheese. Also, it is applied in bakery products, snacks, and soft drinks. In general, lipid-soluble bixin is used in fatty food, whereas norbixin has been applied in food content high protein [51, 57]. However, the presence of highly conjugated π-bond structures in bixin and norbixin molecules makes them susceptible to oxidation and reduction reactions [59]. In addition, annatto being a carotenoid confers many health benefits. It acts as an antioxidant, anti-cancer, hypoglycemic, antibiotic, and anti-inflammatory [60].

4.2.3.2 β-carotene

β-carotene is the most familiar carotenoid with orange-yellow color [(E 160a (ii))] [61], obtained mainly from carrots and fungus. β-carotene has a core structure of 40 carbon atoms with 9 conjugated double bonds in the polyene chain and 2 β-ionone rings at both ends of the molecule ($C_{40}H_{56}$), providing it a lipophilic character [15, 62]. This compound can be found in various fruits and vegetables with a wide range of colors, from red to yellow, for example, in dehydrated red peppers (42.9 mg/100 g), dehydrated or raw carrot (33.95 and 8.28 mg/100 g, respectively), and raw grape leaves (16.19 mg/100 g) [63].

β-carotene naturally occurring in raw fruits and vegetables in the trans-isomers chemical form [15]. However, cis- β-carotene such as 9 cis-, 13 cis-, and 15 cis-β-carotene were found in marine microalgae species [64]. In particular, the natural 9 cis- β-carotene has been showed good results for the diseases, such as atherosclerosis, psoriasis, and inhibiting atherogenesis and retinitis pigmentosa [65].

In addition, β-carotene is the main precursor of vitamin A, resulting from the two β-ionone rings, and plays a significant role in human health [66]. It acts as an antioxidant and inhibits lipid peroxidation, shows a protective effect against cancer, cardiovascular diseases, and slows down the process of aging [51, 64].

Currently, β-carotene is one of the most exploited carotenoids used to develop functional foods, cosmetics and health-related products, and medicine [15]. However, the highly unsaturated chemical composition makes the pigment prone to oxidation in the presence of light, temperature, and metal ions, resulting in significant loss of pigment and reduction in bioactivity [51]. Encapsulation is a solution to address these limiting factors because nano or microcapsules delivery systems can improve the stability, dispersity, and bioavailability of bioactive compounds within the target food matrix [67].

4.2.3.3 Lycopene

Lycopene is a red-colored carotenoid (E 160d) with a molecular structure of $C_{40}H_{56}$, responsible for the red color of some fruits like pink grapefruit, red guava, watermelon, papaya, and is mainly present in tomatoes (*Solanum lycopersicum*) [68, 69]. Found predominantly as an all-trans isomer, but the isomers of 5-cis, 9-cis, 13-cis, and 15-cis can also was identified. The lycopene cis isomer is more absorved in human orgor body anism. This cis-trans isomerization occurs due to acidity, oxygen, heat, and light [70]. When humans ingest high lycopene content brings several health benefits due the antioxidant effect: cardioprotective, antihypercholesterolemic, antidiabetic, and anticancer. However, as nutraceutical or in a food matrix, some difficulties must be overcome, high lipophilicity and solubility in aqueous solvents, problems with stability, and thermal degradation [68].

4.2.3.4 Lutein

Lutein ($C_{40}H_{56}O_2$) is a naturally occurring fat-soluble carotenoid classified as functional xanthophyll hydroxy. This compound is abundant in vegetable and animal sources such as dark-green leafy vegetables, flowers, fruits, and egg yolks. Due to its colorant power, lutein is classified as a natural food colorant (INS 161b or E-161b) and has important biological activities such as antioxidant, anti-inflammatory, and anticancer activities. Its structure is characterized by a long carbon chain with alternating single and double carbon–carbon bonds with attached methyl side groups, according to Table 4.1 [71–75].

Its technological application has limitations, such as sensitivity to environmental factors processing and storage conditions such as heat, light, oxygen, pH, temperature, water activity, water peroxides, and lipoxygenase [76]. Different stabilization application methods improve lutein bioavailability and promote various technological applications, such as spray drying encapsulation, freeze-drying, nanoemulsions, liposomes, electrostatic complexation, and assembly, among others [38, 76–81].

Lutein esters can be applied as colorants in baked goods, dairy products, beverages, instant cereals, frozen drinks, condiments, and sweets. However, lutein esters are easily degraded due to multiple unsaturated double bonds during processing and storage [82].

4.2.3.5 Other Carotenoids

The carotenoids: capsanthin and capsorubin are presents in paprika (red pepper) of the genus *Capsicum annum L.*, Solanaceae family. Paprika, is widely used as a food ingredient, mainly as a pigment (red color) (E 160c), associated with the presence of carotenoids [83]. Paprika is native to the tropical and humid regions of Central and South America. It is widely cultivated in Brazil, Mexico, Peru, and Bolivia. South Korea and Japan have high daily food consumption of paprika [84]. The red-orange color of paprika (*Capsicum annuum L.*) is due to the presence of the carotenoids: capsanthin and capsorubin. In this sense, capsanthin is mainly responsible for the red color, representing 40–60% of the total carotenoids in different varieties. Other carotenoids in red and orange bell peppers are β-carotene, β-cryptoxanthin, and zeaxanthin. In mature pepper fruits, the total carotenoid contents showed great variability ranging from 0.69 to 30 $mg.g^{-1}$ dry weight or 15 to 320 mg. 100 g^{-1} fresh weight, found in the pericarp and placenta [18, 84].

Paprika is traditionally used to impart pungency, color, and taste attributes in meat products, soups, sauces, and snacks. In meat products, color is improved due to the intense red color. Other characteristics are currently considered in the food industries, such as antimicrobial or antioxidant activities [18].

4.2.4 Carminic Acid

Carminic acid (7-α-$_{D}$-glycopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2 anthracenecarboxylic acid) is a water-soluble colorant extracted from insects, females of the species *Dactylopius coccus* Costa (cochonilla). These insects are found in Peru, Mexico, and the Canary Islands [85]. Carminic acid has a molecular structure composed of an anthraquinone chromophore linked to glucose, and a carboxyl group, resulting in light stability and low toxicity [86]. Its color varies according to the pH of the medium; at acidic pH, its color is orange; at slightly acidic to neutral pH, it is red, and at basic pH, the color is violet [87]. Despite having good stability in the presence of light, there is still vulnerability to photodegradation [88], thermal variations, and acidic pH [89] when used in its pure form. The extraction and purification of carminic acid are dependent on several steps, making the process complex, laborious, expensive, and dependent on several variables [90].

Carminic acid is widely used in food, medicine, and cosmetics. In Parma, Italy, the EFSA, FDA, and the USA require the presence of the information "cochineal extract" on the food label if the content is >1.8% or the carmine coloring has >50% carminic acid content. Its code as a food additive is E-120 [86, 91, 92].

4.2.5 Chlorophylls

Chlorophylls, the pigments responsible for green coloration in nature, are cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring with a function during photosynthesis [19]. Its structure is composed of a magnesium (Mg^{2+}) molecule linked to the center of a structure containing the porphyrin macrocycle that consists of four pyrrole rings. The phytol chain's side chain is strongly hydrophobic and attached to the porphyrin ring [93]. This component is fat-soluble and mainly extracted in a non-polar or organic solvent. The various organic solvent used to extract chlorophyll-a, such as acetone, methanol, ethanol, and chloroform [94].

Nowadays, solvents and green extraction techniques have been used to extract chlorophyll. Green solvent-based extraction using 2,3-butanediol demonstrated high yield and antioxidant activity with reduced specific energy consumption. Extraction yield. 2,3- butanediol and isopropyl alcohol exhibited the highest chlorophyll extraction yields, that is, more than 70%. The extraction yields of chlorophyll-a using ethanol, ethyl lactate, and methanol were 49%, 48%, and 36%, respectively. Acetone, 1,3-butanediol and 1,3-propanediol extracted 32%, 37%, and 16% of the chlorophyll a, respectively [95]. Other techniques such as supercritical extraction, ultrasound-assisted extraction, and extraction with ionic liquids also showed extraction yields above 70%, in addition to maintaining the stability and biological activity of chlorophyll [96–98].

Chlorophyll is defined as a food additive that adds or restores color in a food [32]. Currently, this pigment is classified according to the international numbering system (INS) or the standard number in European Commission (E). In both cases, their numbering is the same, being 141 for chlorophylls and chlorophyllins, copper complexes (INS-141 or E-141), 141 (i) for chlorophylls, copper complexes; 141 (ii) chlorophyllins, copper complexes, potassium, and sodium salts [32, 99].

Copper salts are added to preserve the green color to form a chelated and more stable version of chlorophyll. Copper chlorophyllin is produced by the manufacturing process, including replacing the magnesium ligand with Cu2+, yielding a more stable product than the parent chlorophyll [100]. In addition to increasing the chemical stability of the component, this practice also improves its thermal stability, which helps in its application as a food colorant [19]. Among the green food colorants, E-141ii (also known as copper complexes of chlorophyllins) is the most used in food technologies due to its hydrophilic character and high green color stability [101]. Table 4.1 shows the general structure of the chlorophyll, chlorophyllins, and copper complexes.

4.2.6 Curcumin

Curcumin is a water-insoluble polyphenol with antioxidant, anti-inflammatory, antimutagenic, anti-Alzheimer, anticancer, antimicrobial, neuroprotective, cardio-protective activities. In addition to its nutraceutical benefits, curcumin (E100 – INS 100) is a natural yellow colorant that can replace artificial colorants such as tartrazine (E102) [16, 20, 102, 103].

Curcumin shows a low molecular weight (368.38 g/mol), a melting point of approximately 183 °C, and low water solubility (0.6 μg/mL). Its chemical structure comprises two methoxyphenyl rings, which are symmetrically linked in conjugation through the β-diketone portion, which confers exciting properties. The β-diketone structure is responsible for the intramolecular transfer of the hydrogen atom that leads this molecule to keto-enol tautomerism (Table 4.1). At pH 3–7, the

Table 4.2 Natural colorants added to food matrix

Source	Natural pigment	Food product	Main results	Reference
Figs, blackthorns	Anthocyanin: Cyanidin	Donuts, Dairy pastry	Anthocyanin extracts from fig and blackthorn were incorporated into donut icing and dairy pastry as colorants. Antioxidant and antimicrobial activities significantly increased in both extracts. The donuts topping presented less firmness and consistency and the "beijinho" presented greater softness, in both. Nutritionally there were no significant differences, but there were in the rheological properties. In 24 h, the blackthorn extract topping donuts lost color considerably, while the fig extract was stable	[113]
Blue-corn	Anthocyanin: Cyanidin	Polvorones	Anthocyanins were used to enrich commercial wheat flour with polvorones. The addition of blue corn flour did not change bromatological aspects but increased the content of phenolic compounds (6.1 times) and antioxidants (27.9 times) compared to control samples. The flour enriched with anthocyanins showed greater softness and general acceptability and color and flavor	[114]
Jabuticaba, cochineal	Anthocyanins, Carminic acid	Fresh sausage	Microencapsulated jabuticaba and cochineal carmine were added to fresh sausage as natural dyes. Jabuticaba reduced the lipid oxidation of sausages during 15 days of storage at 1 °C compared to the control sample and to carmine. The color intensity of carminic acid was higher than that of sausage with jabuticaba extract	[115]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Red pitaya	Betalains: Betacyanins	Intelligent packaging	Betacyanins were added to films with different polysaccharides (chitosan, k-carrageenan and locust bean gum) and polyvinyl alcohol (PVA). Betacyanins increased the antioxidant capacity and pH/ammonia sensitivity of the films. The type of polysaccharide influenced the intensity of color, intermolecular interactions, antioxidant capacity and sensitivity to ammonia and pH of betacyanins. The film with locust bean gum/PVA/red pitaya pulp extract showed the highest color and antioxidant stability, being the most suitable for monitoring shrimp freshness	[116]
Red beet	Betalains: Betanin	Intelligent packaging	The addition of betanin dye considerably reduced the transparency and hydrophilicity of the surface of the gelatin films. The higher the dye concentration, the greater the solubility of the films. Films containing betalains showed better color retention capacity, regardless of concentration, compared to films containing curcumin and anthocyanins. The color of the films varied from reddish-purple to violet. Film with promising capabilities for food freshness monitoring application	[117]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
β-carotene commercial	Carotenoids: β-carotene	Mayonnaise	β-carotene loaded lipid particles were used as a colorant in mayonnaise. Sample with 5 mg of β-carotene/25 g of commercial mayonnaise shows the best color, resembling homemade mayonnaise. A significant difference was obtained between the control and the colored sample. However, during 15 days of storage, the parameters L* and b* did not show statistically significant differences with time. In contrast, parameter a* showed an increase of the red color intensity	[118]
Carrot	Carotenoid	Tapioca pancakes	Cassava gum fortified with carrot carotenoid microparticles was used to prepare tapioca pancakes. Due to the heat process, low levels of carotenoid loss (22%) were observed upon preparing tapioca "pancakes" using the fortified cassava gum. ΔE value of 8.79 for the cassava gum fortified by microparticles suggests that the color change was classified as very distinct after storage 30 days at 10 °C in dark conditions	[119]
Mandarin epicarp (*Citrus reticulata*)	Carotenoids	Cakes and bread	Carotenoids extract from mandarin epicarp were used as a natural coloring additive with the potential to reduce the use of tartrazine in bakery products such as cakes and bread. The cake had a lower concentration of carotenoids regarding the bread, possibly because of the temperatures used. The overall color change (ΔE) was greater in the crust than in the crumb in the two products. For the ΔE in the crumb, it was observed that the values were >2	[120]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Annatto seeds (*Bixa orellana L.*)	Carotenoids: norbixin	Isotonic drinks	Norbixin microcapsules were added to isotonic tangerine soft drinks. Isotonic drinks with microcapsules presented a more intense orange color and lower color loss during storage under accelerated conditions (heat and light) than the control sample (added with non-encapsulated norbixin)	[121]
Marigold (*Tagetes erecta* L.)	Lutein	Sheep milk yogurt	Lutein was added to yogurt manufactured from sheep milk. Lutein did not influence fermentation patterns, but post acidification was observed, mainly in groups with the highest lutein concentrations. The yogurt color obtained a yellow color due to the addition of the natural colorant lutein, showing Hue Angle values around 90.51° ± 0.42. At 7.8 mg of LT per portion (200 mL), which has reached the minimum daily intake recommended by many researchers for health benefits	[122]
Lyophilized biomass of *Muriellopsis sp*	Lutein	Mayonnaise	Mayonnaise has been enriched with lutein. From the addition of the natural colorant, it was possible to obtain the color of traditional commercial mayonnaise. In 632 grams of the lutein-enriched mayonnaise, a polyphenol content of 3.63 mg GAE and an ORAC of 33.40 μmol/TE allowed for a polyphenol intake of 0.09 mg EAG/day and an antioxidant capacity of 0.79 μmol ET/day	[123]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Tomato	Carotenoids: lycopene	Bread and muffin	Bread and muffins supplemented with tomato pomace (35% and 40%) have enhanced nutritional properties such as dietary fiber, vitamin C, antioxidant activity, and minerals. In addition, they presented acceptable color and sensory properties and a softer crumb texture than the control bakery products. There was an increase in a* and b* for the color parameters, while the L* values decreased	[124]
Stinging nettle (*Urtica dioica L.*)	Carotenoids: lutein and β-carotene	Egg pasta	The use of stinging nettle as a functional ingredient for enriching egg pasta provides for 11% more lutein and 55% more β-carotene than non-enriched pasta even though the cooking process produces loss phytocompounds due to temperature degradation and water boiling	[125]
Tumeric rhizomes	Curcumin	Buffer solution as simulating food and yogurt	Nano encapsulated curcumin presented the highest antioxidant potential by OxHLIA and TBARS. At pH 3.0, curcumin showed a yellow color favoring the keto-form formation. This approach encourages their application as health-promoting compounds to substitute artificial food coloring additives	[126]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Tumeric rhizomes	Curcumin	Gelatin films	Curcumin has been applied as a natural colorant on fish gelatin-based films. The addition of colorants altered the color, light barrier, and wettability of the films. The addition of colorants provided films with the capacity to sense pH changes before and after immersion in a fatty food simulant. These properties establish the suitability of the films for intelligent fatty food packaging applications	[117]
Tumeric residue	Curcumin	Hydrogel coating for sausages	Turmeric residue was used to prepare light-activated antimicrobial hydrogel coatings. The coatings were applied to the surface of cooked sausages and evaluated for their ability to prevent bacterial cross-contamination. It was observed that UV-A light-exposed hydrogels coatings could inactivate more than 5 log CFU/mL of *L. innocua* after light treatments as short as 5 min. In addition, the light-activated antimicrobial activity of the hydrogel coatings was not affected by the incubation temperature	[127]
Microalgae (*Arthrospira platensis* F&M-C256, *Chlorella vulgaris* Allma, *Tetraselmis suecica* F&M-M33 and *Phaeodactylum tricornutum* F&M-M40)	Chlorophyll	Cookies	The addition of chlorophyll-rich microalgae provided innovative and stable green shades that varied, depending on the microalgae used, from a bluish-green (*A. platensis*) to a brownish-green (*P. tricornutum*). Increasing the microalgae content from 2% to 6% resulted in a significant increase ($p < 0.05$) in the total phenolic content and the cookies' antioxidant capacity	[128]

ketone form predominates, while at pH above 8, the enol form is a majority. Its industrial use is difficult to achieve due to the low water affinity, pH, and thermal instability [20, 38]. These limitations can be overcome by applying stabilization methods such as spray drying, ionic gelation, liposomes, among others [38, 104–106].

4.2.7 Other Natural Colorants

The main natural colorants already used for the food industry are anthocyanins, betalains, carotenoids, carminic acid, chlorophylls, and curcumin. Despite the noticeable advances regarding the replacement of artificial colors by natural colors, the natural blue color is still an industry challenge. The blue natural sources are limited, and this pigment is also further limited by its poor light stability and high sensitivity to heat, losing color at temperatures ≥45 °C. In addition, none of the existing natural blue colorants reach the versatility, low cost, and intensity of synthetic blues. Phycocyanin is a precursor of the blue colorant, and recently, FDA approved phycocyanin as a spirulina extract [16, 107]. *Spirulina* sp. synthesizes C-phycocyanin (phycocyanin from cyanobacteria), a blue and water-soluble pigment [108]. Examples of natural blue dye applications are blue cheese, ice cream, and dairy beverages [16, 109, 110].

4.3 Applications of Natural Food Colorants

FAs are molecules introduced in foodstuffs to carry out specific technological functions such as preservative food, improving color, taste, sweetening, and texturizing; they are added during food manufacture, complying with the regulatory criteria of each country [111].

The colorants are widely employed among the FAs because color is one of the most important sensory aspects and an essential criterion for consumer choice. During the processing and storage of food, losses of the natural color of the product are observed such as juice, cream, feed solutions, thus colorants additives are added to give it a more attractive appearance. Synthetic food additives (SFAs) are the principal colorants used by food industries. However, they are progressively substituted by a natural source, due mainly to the consumer preferences and the numerous health effects such as allergic reactions and the toxicity of the SFAs [112].

Natural colorants, derivatives from anthocyanin, betalains, carotenoids, carminic acid, chlorophylls, and curcumin, provide a wide variety of colors to use in food products. Also, play a health beneficial (antioxidant, anticancer, and anti-inflammatory). So, this topic presents the primary studies that used natural pigments to apply in food products, according to described in Table 4.2.

Anthocyanins show promising results in replacing synthetic dyes (red, pink, and orange) in dairy products. Tereucan et al. [129] added an extract from purple potato to milk and yogurt and showed high dye stability in cold storage, resembling commercial storage time using synthetic dyes. Swer et al. [130] applied anthocyanins extracted from Sohiong (*Prunus nepalensis* L.) in the processing of yogurt, syrup, and candies and observed that higher concentrations of the pigment were more acceptable in the sensory evaluation when compared to the control sample, being at the same level organoleptically. In addition, higher pigment concentrations also favored color stability in all products. Other examples of the application of anthocyanins as dyes in yogurts are Byamukama et al. [131], using anthocyanins from blackberry (*Morus rubra*) and Pires et al. [132] who incorporated natural dyes obtained from edible flowers such as *Centaurea cyanus* L. (cornflower) and *Dahlia mignon* (dahlia).

Anthocyanins also were applied in other products; for example, Sampaio et al. [133] developed a soft drink formulation with anthocyanin extract from purple (cv. *Purple*, *Violetta,* and *Kefermarkter Blaue*) and red (cv. *Rosemary*, *Red Emmalie,* and *Red Cardinal*) potatoes. Soft drinks with extracts presented good sensory and shelf life profiles than the commercial control dye E-163. Montibeller et al. [134] evaluated the stability of grape skin anthocyanins in kefir and carbonated water as a dye. They observed that the stability of the compound followed the first-order reaction kinetics and was different in the different matrices, indicating the use of dark packaging to avoid the degradation of anthocyanin. Albuquerque et al. [135] applied anthocyanin extract from jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg.) in macarons and obtained a more stable color than the commercial dye E-163 within 6-day shelf life.

In food packaging, anthocyanins are applied to active and intelligent packaging to monitor the freshness of various products due to their ability to change color at different pHs. Several works have different matrices, carbohydrates (starch, pectin), proteins (chitosan, gelatin), and other sources, with different raw materials containing the compound. Zheng et al. [136] produced two colorimetric films based on chitin and sodium alginate/gelatin containing anthocyanins from goji berries to monitor pork freshness and obtained good accuracy in colorimetric response to amine gases and good durability. Sani et al. [137] developed methylcellulose and chitin nanofiber films with anthocyanins from barberry (*Berberis vulgaris* L.) to monitor freshness in fish. The indicator changed color from red to pink and then yellow with increasing pH and consequent ammonia vapor concentration. In addition to its properties as a dye, it also acted significantly as an antioxidant and antimicrobial. An example of the combination of active/intelligent packaging is the study carried out by Wu et al. [138], who developed a film based on gellan gum and *Clitoria ternatea* extract to monitor shrimp freshness. The application had a satisfactory result in releasing the compound and acting as an antimicrobial, as in the colorimetric alteration, suggesting that this package can be used in foods. Other examples of anthocyanin sources used in intelligent packaging are butterfly pea [139], purple potato [140, 141], red cabbage [37], turmeric (*Crocus sativus* L.), and red barberry [142].

Betalains are also used as a coloring agent in food products or active and intelligent packaging; however, they are less studied than anthocyanins. Otálora et al. [143] developed gummy bears containing encapsulated betalains from (*Opuntia ficus-indica*). Betalain stability was investigated, and there was no significant loss at 4 °C for 30 days, indicating good stability under these conditions. The gummy color was bright red-purple. Moghaddas Kia et al. [144] developed gelatin/gellan-based gummy bears with red beetroot extract as a dye and observed an increase in gum surface gloss with the combined use of gellan gum and red beetroot extract. The color of the gummy was satisfactory, with low concentrations of the extract (0.3% and 0.1%) demonstrating the potential of betalain as a food coloring. Kharrat et al. [145] studied the stability of betalains from prickly pear (*Opuntia stricta*) extract in salami. The extract showed positive results in the sensory analysis of salami, a promising substitute for carminic acid or other synthetic dyes, also obtained good results as an antimicrobial and antioxidant agent. Roriz et al. [13] incorporated beetroot extract (*Gomphrena globosa* L.) into ice cream. The dye remained stable during storage (−22 °C, 60 days) and obtained similar results to commercial betalain, indicating its use as a substitute. Yang et al. [146] compared anthocyanin from grape (*Vitis vinifera*) and betalains from red beet (*Beta vulgaris*) as colorants in white currant juice with regard to storage time, color, and sensory stability. They observed that anthocyanins are more stable than betalains during storage at room temperature and 4 °C. The color of the juice became more yellow and clear, which indicates the degradation of the compound. The mixture of the two compounds did not result in greater stability, and this option is not favorable.

In packaging, we have the study carried out by Yao et al. [147] on developing antioxidant, antimicrobial, and ammonia-sensitive films using betalain-rich forage palm extract (*Opuntia ficus-indica*). They obtained good responses to the ammonia concentration (color ranging from purple to orange) containing only 2% and 3% by weight of extract and improving the functional properties. Qin et al. [148] developed an active/smart packaging incorporating betalains from red pitaya (*Hylocereus polyrhizus*) bark in starch/polyvinyl alcohol films, which 1% by weight of extract was efficient to monitoring the freshness of shrimp. The film was very sensitive to ammonia, changing from pink to yellow during 48 h in contact with the shrimp. In addition to being an indicator, betalain extract resulted in a greater light barrier antioxidant and antimicrobial properties.

Another natural colorant used in food are the carotenoids that confer colors from yellow to red. These pigments are unstable due to environmental factors such as pH, temperature, oxygen, and light. Thus, the studies used encapsulation to protect these compounds. Carotenoids from yellow bell pepper pigments were encapsulated with β-cyclodextrin, and their stability in isotonic beverages (pH: 2.9; 0.02, 0.05, and 0.06% of pigment addition) were evaluated. Lutein, zeaxanthin, α-cryptoxanthin, α-carotene, and β-carotene were the main carotenoids found. Extract added in beverages exhibited dose-dependent luminosity and redness increase but decrease in yellowness. Good results for the color stability indices were demonstrated for isotonic drinks stained with complex obtained by inclusion method compared to those stained with crude yellow pepper extract (storage 21 days) [149].

β-carotene encapsulated in a beeswax-based solid lipid particle was tested concerning their colorant power by selecting a food matrix widely appreciated and consumed (mayonnaise). The best formulation presented 5 mg of β-carotene per 25 g of mayonnaise, resembling as much as possible the appearance of homemade mayonnaise, as it might be more attractive for consumers. The color parameters were evaluated for 15 days at 6 °C, showing color stability and nutritional value maintenance after 15 days under storage [118].

Lutein was applied as a colorant to replace urucum, in the Prato cheese formulation, at 16 and 32 mg L^{-1} concentrations and did not show differences in the quality attributes such as color, pH, texture, as well as did not affect the maturation profile and sensory acceptance. Also, lutein kept stable in cheese for 60 days, thus maintaining its antioxidant capacity [150]. Lutein was also applied in feed supplementation, raw milk, and mozzarella cheese. The lutein content in raw milk increased approximately three-fold after 2 months of dietary supplementation with lutein. Most of the lutein remained in the mozzarella cheese during the cheese-making process, but part was lost to the whey, hot water, and brine. Approximately 20% of lutein was lost during the 8 weeks storage period of the mozzarella cheese [151].

The addition of lutein colorant was evaluated on the oxidative stability in yogurt for 35 days stored at 5 °C under presence and absence of light. Yogurts (120 g) with the addition of 0.5, 1.5, and 2.5 mg of lutein colorant were evaluated for the sensory acceptance, showing no differences between aroma and flavor attributes among samples. The addition of lutein also conferred oxidative stability to the yogurts. The lutein content remained stable during exposure to light, meaning that the lutein added in the yogurt was present in the product during the storage [152]. The stability of the natural colorant lutein, obtained from the biomass of the microalgae *Muriellopsis sp*, and its antioxidant activity were evaluated during the storage of mayonnaise at 5 °C for 3 months. It was observed that the addition of natural colorant maintained the commercial color of mayonnaise for 3 months and the storage period [123].

4.3.1 Colorants from Agroindustry Waste

Adding value to fruit and vegetable by-products would satisfy global demand for NFAs and reduce environmental impacts. In this context, carotenoid obtained from waste was employed in bakery products. Mehta et al. [124] investigated the effect of the carotenoids from tomato pomace, in physicochemical characteristics, and shelf-life stability of the bread and muffin products. Bread and muffin supplemented with tomato pomace showed enhance nutritional properties like dietary fiber, vitamin C, antioxidant activity, minerals, and acceptable color and sensory properties. Furthermore, increase in the shelf-life compared to control bakery products with or without preservatives. Another study evaluated ultrasound-assisted carotenoid extraction from mandarin epicarp for use as a natural coloring in two baked goods: cakes and breads to reduce tartrazine preservative used in these product [120].

Carminic acid is mainly applied to meat products during their curing with salts to give the meat color. It is already allowed in some countries and international legislation, but few studies involve the compound [153]. Recently Ongaratto et al. [154] studied the incorporation of carminic acid adsorbed into zinc hydroxide salt in mortadella to improve stability and color, resulting in the pinkish, slightly reddish color characteristic of mortadella, indicating a promising potential in the substitution of curing salts.

Chlorophyll is used to add green color to food. The natural colorant was incorporated into fresh gluten-free pasta. The addition of a platensis biomass as an ingredient resulted in doughs with an attractive appearance. The gluten-free supplemented pasta showed higher antioxidant activity than the control, good mechanical properties, and high in vitro digestibility without affecting the cooking properties of the pasta [155]. Freeze-dried *Chlorella sorokiniana* biomass rich in chlorophyll dye was incorporated into pasta. Replacing 5% flour increased protein and lipid content to 15.7 ± 0.50% and 4.1 ± 0.06%, respectively. Meanwhile, adding the microalgae Chlorella to the pasta helped increase the polyunsaturated fatty acids, chlorophyll, and carotenoids necessary for preventing foodborne diseases [156]. Emulsifiers Sucrose fatty acid ester and quillaja saponin were applied to protect the chlorophyll of green tea and vegetable juices from the bleaching process. The formation of chlorophyll nanoparticles (100 nm) caused a self-stacking to form many aggregates soluble in the aqueous emulsifier solution, suppressing the discoloration of Chl. In this way, applying nano encapsulated chlorophyll by emulsions in green drinks becomes possible [157].

Chlorophyll natural colorant can be applied as a pH-sensitive natural indicator in intelligent films to monitor the quality of foods such as fish and minimally processed green peppers [136, 158]. Despite being a colorant legally used to add green tones to foods, chlorophyll derivatives are prohibited in Europe and America for fats and oils. One of the main frauds is the application of the green dye derived from chlorophyll in olives and olive oil to return or intensify the green color of these products that are degraded due to processing.

Curcumin is a hydrophobic colorant, so it needs to undergo a modification/compatibilization process with the aqueous medium to improve stability and enable its application in hydrophilic food matrices. Curcumin nano encapsulated by solid-phase dispersion was incorporated as a coloring in yogurt. Curcumin maintained the color of the yogurt during the entire storage period (7 days at 4 °C) without causing relevant changes in the nutritional composition and fatty acid profiles. In addition, it was also possible to observe the antioxidant, anti-inflammatory, cytotoxic, and antibacterial activity [103]. An essential application of curcumin can be observed in yellowish foods such as cheese. Curcumin was used in Minas Frescal cheese as a photosensitizing agent in antimicrobial photodynamic therapy for the inactivation of pseudomonas. Inactivation of 7 log CFU/mL of *P. fluorescens* was observed at a 62.50 μg/mL concentration of curcumin solubilized in ethanol [159]. Curcumin solutions were infused into cooked oysters by vacuum to inhibit growth of total mesophilic and total psychrophilic bacteria in oysters during storage. A positive antimicrobial effect was observed through increased shelf life of cooked oysters [160].

Curcumin-loaded nanoemulsions were incorporated into a commercial salad dressing. To stabilize the emulsions, whey protein and quillaja saponin were used. The salad dressings produced were light or yellow, characteristic of classic production ingredients such as mustard, egg yolk, and riboflavin. The viscosity of the salad dressing mixtures decreased as the nanoemulsion concentration in the mixtures was increased due to the fitting into the spaces between the larger particles in the salad dressings. It was also observed greater ease of mixing the nano drops with the salad dressing, indicating that this technology can be used as a vehicle for curcumin dye and other hydrophobic bioactive compounds in commercial products [161].

Curcumin can be used with natural coloring in active packaging and intelligent packaging. Curcumin was incorporated into chitosan and polyethylene oxide films by electrospinning to monitor chicken breast freshness. The packages were stored at 4 °C for 8 days, showing the color change of the nanofiber film from bright yellow to reddish, which allowed the detection of color changes even with the naked eye of the untrained consumer [162]. Tosati et al. [163] incorporated curcumin in an edible coating based on starch and bovine gelatin, replacing the synthetic casing used in the coating of frankfurter sausage. It was possible to observe the antimicrobial effect of the natural colorant applied as active packaging in sausages stored at 5 °C for 20 days, while the uncoated sausages had a shelf life of 10 days.

Natural pigments were applied in several types of food, beverages, cereals, milk, meats, and bakery and were used in intelligent packaging. Most studies evaluate the changes in physical and chemical characteristics with the addition of these ingredients. However, few studies focus on sensory evaluation, which is essential for the consumer acceptance and purchase of food. In addition, it is essential to highlight that natural colorants are unstable due to environmental factors such as pH, temperature, oxygen, and light [38]. Thus, many studies used encapsulation to protect these compounds and used them as food ingredients.

4.4 Conclusions

This chapter summarized the importance of using natural additives in foods, their main sources, and their properties. Based on the literature, pigments such as anthocyanins, betalains, carotenoids, annatto, β-carotene, lycopene, lutein, carminic acid, chlorophylls, and curcumin can be isolated from natural sources, being used as food colorants.

Based on the reviewed literature, anthocyanins have been the most used colorants, followed by lutein, carotenoids, betalains, chlorophyll, and carminic acid. Several food products such as milk, yogurt, cheese, candies, soft drink, kefir, macarons, ice cream, mayonnaise, salami, beverages, and pasta can be produced by incorporating natural colorants. Furthermore, the use of these natural pigments can reduce the oxidation of foods during storage, increasing their shelf life. In addition, anthocyanins, betalains, and chlorophyll have been the main natural colorants used to develop active and intelligent food packaging. The stabilization of natural colorants must be investigated aiming to increase their stability when applied in foods.

Conflicts of Interest The author declares no conflict of interest.

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