Colorants of natural origin. Sources and applications. Review and general introduction.

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1. Introduction.

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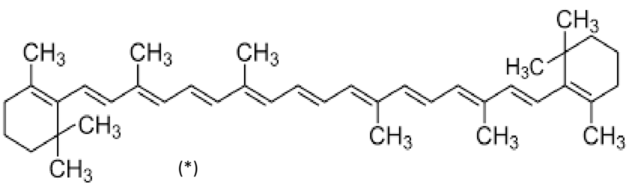
Colorants are substances that are added to foods to change their color and make them more attractive. The use of coloring substances is necessary since the consumer prefers colored products and associates the quality of the food with its visual appearance (freshness, quality, taste, etc). Normally, dyes are used to improve the appearance that the product would have naturally after processing, to correct the changes derived from it, or to ensure a good appearance during storage. They are also used to ensure a homogeneous color in those products that have changing colors in different production batches. These and many others are the reasons for the use of food dyes, but ultimately their function is always to improve the appearance of the food.

Throughout this introductory article we are going to talk indistinctly about food colouring or natural substances with colouring capacity, using these terms in a non-rigorous way, never from a strictly regulatory perspective. We will not try, throughout this article, the classification of an extract or product of natural origin as "coloured food" or as "food colouring". This question, on the other hand, of great relevance and implications, deserves a separate reflection and will be subject of revision in our next article, in which we will address it in depth.

In any case, normative questions apart, from the point of physics, the colour we see in a coloured substance, is a consequence of the interaction of light with the molecules that comprise it and more specifically with specific parts of them, called chromophores. The colour we observe is that of light that, within the visible spectrum, is not absorbed when it interacts with matter. When the light hits a dye, part of it is absorbed by the electrons of the atoms and molecules with which it collides. And with that, the electrons go from a lower energy orbital to a higher energy one. When this happens, the photons that have not been absorbed, are perceived by the human observer, as the colour complementary to the one corresponding to the frequency mostly absorbed.

In conjugated chromophores, the electrons jump between the energy levels of the nearest π orbitals, which means a lower energy jump than that between more energy bonds such as σ, so that the absorbed photons have wavelengths in the visible spectrum. The structure of the chromophore determines the wavelength absorbed by it. The more unsaturated bonds a molecule has, the absorbed wavelengths are larger, so, for example, beta-carotene has a large number of conjugated bonds which causes it to absorb light especially between 400 and 500 nm, hence its intense color yellow.



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Beta-carotene molecule

The use of colouring substances is a very old practice, used thousands of years ago. As today, dyes are used to meet the expectations of consumers. However, the widespread use of colouring substances has always been linked to human evolution and to the improvement of their quality of life. However, it was from the sixteenth century, in the era of discoveries and the first trade routes opened by Spaniards and Portuguese, when the importation of spices allowed these products to reach a Europe, increasingly rich and hungry for exotic products. Later, during the nineteenth and twentieth centuries, as a result of the industrial revolution, food processing and the use of food additives became more frequent. This growing demand for food colours and the scarce scientific knowledge of the time, sometimes led to unethical practices and the use of substances that were later identified as dangerous for people. Another issue was the numerous cases of fraudulent use of toxic substances. In addition, at this time began to proliferate the first synthetic dyes, which in general were cheaper because they were easier to produce. Fortunately, nowadays, both synthetic dyes and natural ones are reviewed by agencies such as the FDA and EFSA, which authorize or not, their food use, after having undergone strict toxicity and safety studies.

In relation to this issue, it must be said that the food safety of colouring substances has always been a cause of concern. Initially the use of dangerous minerals such as arsenic, chromium, lead and mercury, was banned. Later, the negative list principle was established in many countries, that is, substances not allowed for food use. In the twentieth century, this practice was gradually replaced by another that offered many more guarantees, the use of a positive list. Control model currently used in developed countries. The positive list implies that substances intended for human consumption have been subjected to safety tests and that they must comply with strict control criteria. And this must be so before its approval by the corresponding authorities. Today, all food colouring, like the rest of the food ingredients, are approved or rejected, after checking by the different official agencies around the world. In the European Union (EU), the E numbers are used for all additives, both synthetic and natural, that are approved in food applications. The colours of the food are known by the E numbers that begin with a 1. The safety of dyes and other food additives in the EU is evaluated by the European Food Safety Authority (EFSA), which, by specific regulations, defines the natural and artificial colours allowed with their applications and limitations.

2. Colours of natural origin

The list of colours of natural origin for food use is very extensive, and the laws that regulate their use change from one geographic region to another. Being among the strictest, those developed by the EFSA and the FDA. Without considering in detail the differences that exist between them, neither rigorous nor exhaustive with the applicable regulations, we will name some of the best known natural colouring substances:

Curcuma. Yellow. (E100). Curcumin Colour - Curcumin is the main curcuminoid of *curcuma longa*, a rhizome of the ginger family (*Zingiberaceae*). Curcuminoids are natural phenols. Curcumin can exist in several tautomeric forms, including a 1,3-diketo form and two equivalent enol forms. Curcumin is bright yellow and is often used to colour food. As a food additive, in Europe its code is E100. Curcumin is also commonly used as a flavouring in rice dishes and meats and is used in the preparation of curry. On the other hand, they are also attributed healthy properties (antioxidant, anti-inflammatory ...).

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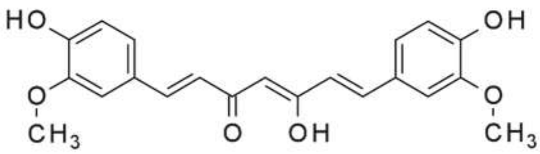

Curcumin (*)

Carmine color. Red. (E120). Cochineal, carminic acid, carmine. The carmine is in the form of red powder, insoluble in water at neutral pH, but soluble at basic pH. This substance is highly demanded for its high coloring power. It is obtained from the insect (*Dactylopius coccus*). To do this, the insects are first dried with hot air and then boiled in an ammoniacal solution, which is then filtered. Finally, alum is added to the filtrate to precipitate the colorant. It is believed that its use was already known in South America, specifically in the area of Peru, by pre-Inca civilizations. This coloring substance has many applications: it is used in the manufacture for example of paints, inks and cosmetics. In the pharmaceutical industry it is used in the elaboration of mouth rinses, toothpastes, in cosmetics it is used in the manufacture of lipsticks. In addition, it is used in the production of ice cream, some sausages, jellies, jams, yoghurts and juices.

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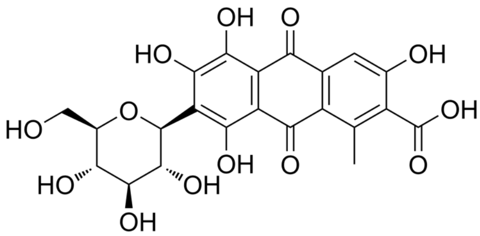

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Acid Carminic molecule

Chlorophylls. Green. (E140). Chlorophyll is a molecule of extreme importance in the metabolism of plants that allows them to absorb energy from light. Chlorophyll absorbs light with greater intensity in the blue portion of the electromagnetic spectrum, however, it does not do so in the area of green, hence the green colour of plants. These substances are not widely used because they are not very stable to heat, light and pH. During its degradation, compounds called pheophytins, of brownish and unpleasant appearance, are produced. Chlorophyll can be obtained from alfalfa (*Medicago sativa*) and other vegetables, algae and microalgae in the form of an oil-soluble extract. To avoid stability problems, copper compounds are usually prepared from chlorophyll extracts. Since the chlorophyll molecule contains an Mg atom, it is replaced by Cu by hydrolysis and an organometallic complex (chlorophyllin E141) is obtained. This complex has a greater colouring power and is more stable, besides being soluble in water. These dyes can be used in the food industry to colour dairy products, soft drinks, sauces, preserves, confectionery etc.

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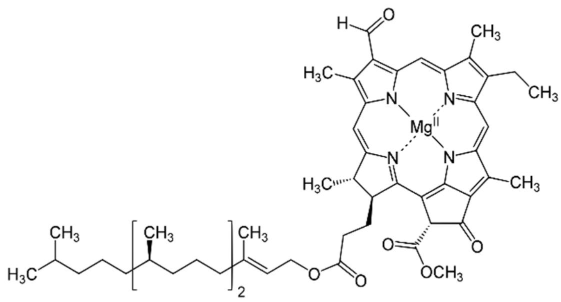

Molecular structure chlorophyll type (*)

Brown Caramel (E150). Caramel color is one of the oldest and most widely used food colorants, in fact, 80% by weight of the world consumption of food colors corresponds to this dye. It is also one of the main colorants that we elaborate in SECNA NATURAL INGREDIENTS GROUP. The caramel colour is soluble in water. It is manufactured by thermal treatment of carbohydrates in the presence of acids, bases or salts. Its colour varies from yellow to dark brown. The industrial use of this type of product began in the brewing industry in the 19th century, but nowadays it is found in many commercially produced foods and beverages. It is used in the elaboration of liqueurs, custards, milkshakes, bakery, chocolate, biscuits, in confectionery, in the preparation of fried potatoes, in desserts, in the elaboration of fish creams, in the production of ice cream, and in soft drinks and sodas. The caramel colour is widely approved for use in foods worldwide. For its preparation, it uses fructose

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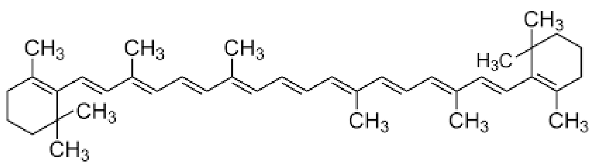
Photo property of SECNA NATURAL INGREDIENTS GROUP syrup, glucose syrup, invert sugar, sucrose, malt syrup, molasses, and starch hydrolysates. Sulfuric, phosphoric, acetic and citric acids can be used too; the alkalis used are ammonium, sodium, potassium and calcium hydroxides; and the salts are ammonium carbonate, sodium and potassium, bicarbonate, phosphate, sulphate and bisulphite. At the international level, the Committee of Experts on Food Additives of the Food and Agriculture Organization of the United Nations and the World Health Organization on Food Additives (JECFA) recognize four caramel-coloured classes, which are differentiated by the reactants necessary for its manufacture:

Código	Descripción	Características	Aplicaciones
E150a	Caustic caramel	Yellow-brown-reddish color. Stable in alcohol, tannins and rich in salt media	Spirits, cookies, cereals, bakery products and juice
E150b	Sulphite caramel	Yellow-brown-reddish color; stable in medium alcoholic beverages	Tea, wines, spirits, vinegars and pastries
E150c	Ammonia caramel	Dark reddish-brown color; Stable in rich in alcohol and salt media.	Beer, licorice, confectionery, sauces
E150d	Sulphite and ammonia caramel	Dark brown color; Stable in acid media.	Carbonated drinks, balsamic vinegar, coffee, syrups, sauces, soups and condiments.

The United States Food and Drug Administration (FDA) classifies colour caramel as an approved colour additive exempt from certification. The caramel colour is microbiologically stable since it is manufactured at very high temperatures and pressures and its water and pH activity are low.

Carotenoids (α-, β-, γ-) carotene. Yellow-Red-Orange E160a: The carotenes are tetraterpenes with 40 carbons, synthesized biochemically from eight isoprene units. Β-carotene is the most common form of carotene in plants, it has an intense orange colour and is, for example, the substance that colours carrots. Β-carotene is distinguished from the rest of carotenes by having beta rings at both ends of the molecule. In Europe, EFSA has classified it as a food colouring with the code E160a. In nature, β-carotene is a precursor of vitamin A. The separation of β-carotene from fruits rich in carotenoids is carried out by column chromatography. The separation of β-carotene from the mixture of other carotenoids is based on the different polarity of this compound versus the rest, since β-carotene is a non-polar compound. Being highly conjugated, it is deeply coloured and, since it has no functional groups, is very lipophilic.



Beta-carotene molecule (*)

Annatto, Roco, bixina or norbixina. Yellow Orange Red. (E160b). The bixina is a crystalline substance of reddish orange tonalities present in the seeds of the achiote trees of many tropical and subtropical regions of the world. It can be obtained in two ways: either as an extract, using organic solvents (acetone, for example) or alternatively by precipitation with KOH from its water-insoluble form norbixin. The active principles of this extract are mainly carotenoids. The bixina has been used historically in the elaboration of traditional dishes in the Aztec culture, as a coloring / flavoring ingredient. However, worldwide its main use is as a food dye in the form of powder and / or extract.

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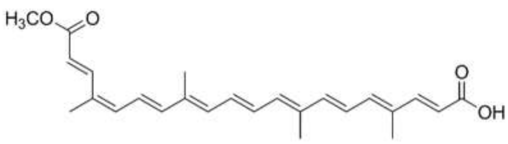
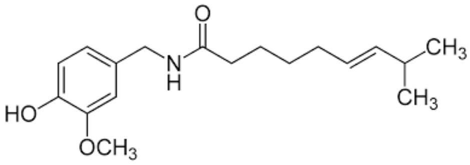


Bixine molecule (*)

Paprika, Paprika Oleoresin. Yellow Orange Red. (E160c). The paprika oleoresin (also known as paprika or pepper extract) is an oil-soluble extract of the fruits of *Capsicum Annum* (pepper) whose main use is as a coloring and / or flavoring in food. It is composed of different carotenoids (capsaicin, capsanthin and capsorrubin). The extraction can be performed by different nonpolar solvents, mainly hexane. Although the pepper is native to America, came to the old world thanks to the colonizers who took him first to Europe and then to Africa and Asia.



Capsaicin molecule (*)

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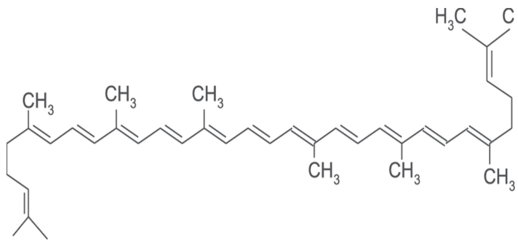
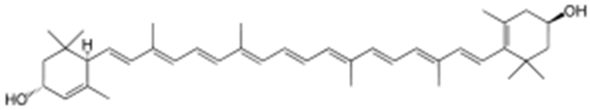
Lycopene Yellow Orange Red. (E160d). Lycopene is a natural fat-soluble substance responsible for the red color of some fruits and vegetables. It is found in tomatoes, watermelons, grapefruit and apricots, among others.



Lycopene molecule (*)

Lycopene is commercially available in the form of an oily extract, which is obtained by extraction with organic solvents. It is quite labile as it is degraded by light, presence of oxygen and pH and high temperatures. From the point of view of its chemical structure, lycopene, like the rest of the carotenes, is a tetraterpene, which in its carbon chain (typical of hydrocarbons) has eleven conjugated double bonds. Of all the carotenoids is the one that has a longer chain. This disposition is what gives it its intense red colour and its antioxidant and antiradical activity, characteristics for which lycopene is widely used as a colorant and food ingredient. In the metabolic pathways of carotenoids biosynthesis, lycopene is one of the first to appear, constituting the starting structure for the synthesis of the rest of carotenoids.

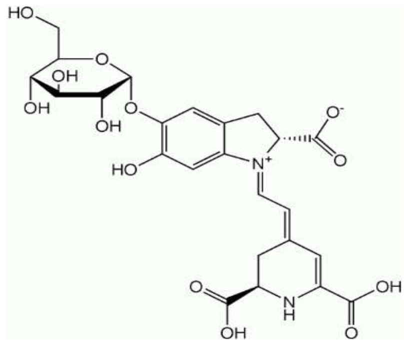
Xantófilas. Yellow-Orange-Red. (E161). Xanthophylls are pigmented compounds belonging to the group of carotenoids with one or more oxygen atoms in their structure (oxycarotenoids), being present in brown algae and diatoms, as well as in many terrestrial plants (they are those that provide the leaves dry its yellowish colour). They are more resistant to oxidation than chlorophyll and are believed to participate in photosynthetic processes. They are present, along with chlorophyll, throughout the whole life of the plant, but usually are not perceived to be masked by it. That is why when chlorophyll disappears during the autumn, the xanthophylls are exposed. They are soluble in organic solvents and, despite everything, easily oxidizable, due to their high number of double bonds, which leads to their instability in the presence of oxygen. They are also unstable against acids, light and heat. Xanthophylls are also found in egg yolk (lutein) in salmon meat and in some crustaceans (canthaxanthin). Some xanthophils are: E-161a Flavoxanthin, E-161b Lutein, E-161c Cryptoxanthin, E-161d Rubixanthin, E-161e Violaxanthin, E-161f Rodoxanthin, E-161g Cantaxanthin, E-161h Zeaxanthin, E-161j Astaxanthin



Luteine molecule. (*)

Most of these compounds do not have an extended use as food additives due to their instability, except for canthaxanthin. However, they are often used in the formulation of feed for fish farms (salmonids) and in the breeding of chickens. The objective is to make the meat of the fish or the yolk of the eggs have a more intense yellow colour.

Beet juice red, Betanin. Color Yellow-Orange-Red (E162), is a food colouring substance, chemically a glycoside, red, obtained from the beet in the form of an aqueous extract. This colouring substance is among the portfolio of products that we elaborate in SECNA NATURAL INGREDIENTS GROUP due to its natural origin, relative stability and broad applicability in the food industry. Betanin is quite stable at different pH levels, although it degrades when subjected to light, heat and oxygen. At present no harmful effect of this product is known and in general there is no limitation to its consumption, so it is frequently used in confectionery products, ice cream and dairy products, as well as in frozen products, in soft drinks, preserves vegetables, jams and fish preserves.



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(*) Betanin molecule

To obtain it, the beets are ground, pressed and the juice obtained is filtered, and then concentrated by evaporation. Sometimes it can also be obtained in the form of powder by atomization process.

Family of anthocyanins (E163). Anthocyanins are water-soluble pigments that change from red to purple and blue when the pH of the medium changes. Some plants rich in anthocyanins are blueberries, red raspberries and blackberries, blackcurrants, cherries, grapes and black carrots. Anthocyanins are also found in eggplant rind, black rice and red cabbage. However, in SECNA we obtain them mainly from grapes, black carrots and red cabbage. Anthocyanins belong to a primary class of molecules called flavonoids that are produced in all tissues of higher plants. In flowers, for example, the colouring aims to attract pollinators. On the other hand, in fruits, their colouring can help disperse the seeds by attracting herbivorous animals. Anthocyanins have a protective role in plants against extreme temperatures and are found predominantly in the outer cell layers. The most frequent anthocyanins are the glycosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. These compounds are present in approximately 10% of tree species in temperate regions. According to different authors, anthocyanins help reduce LDL cholesterol and protect the arteries against oxidation, among many other health benefits. Given the great diversity of anthocyanins, some of them are not yet approved for use as food colours. This is simply because the corresponding toxicological studies have not yet been carried out. However, other products rich in anthocyanins, such as red grape skin extract, carrot extract and black currant extract, have been authorized by the EFSA (E163) and have been consumed for a long time without any problem. Even in the US, where some anthocyanin extracts are not specifically included among the permitted colour additives, the use for other applications of grape juice, red grape skin and various fruit and vegetable juices rich in anthocyanins, has been common for a long time. In addition, there is a growing interest in this family of products and in the most abundant scientific literature that links some anthocyanins with beneficial effects for health, especially for their antioxidant capacity.

Anthocyanins Color of grape juice. Color Red, Blue or Violet (E163): The grape juice is obtained by shredding and mixing the grapes in a liquid medium. The juice is often sold fresh or fermented and turned into wine. In North America, the most common grape juice is purple and is made from Concord grapes, while white grape juice is commonly obtained from Niagara grapes, which are varieties of Native American grapes, a different species from the European wine grapes (Bobal, Tempranillo and Tintorera). In California, Sultana grapes (known there as Thompson Seedless) are also used to produce white juice. Grape extract is soluble in water and alcohol, but not in fats and oils. Because the color of the anthocyanins is affected by the pH changes, it is only used in products with pH less than 4. The anthocyanins of the grape in its different variants are one of the main references that are elaborated in SECNA NATURAL GROUP INGREDIENTS.

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Anthocyanins Black carrot extract. (E163). Black carrots are a natural colour source for foods that are produced mainly in Turkey and other regions of the Middle East and Asia. Carrots appeared in these areas about 5000 years ago, originally as a purple or yellow root, but then due to natural mutations, different colours and varieties emerged. In fact, the best-known varieties in Europe are relatively recent varieties, orange in colour, due to the selections made over the centuries by farmers for practical reasons and demand. The compounds that give colour to the black carrot are anthocyanins. Black carrot anthocyanins are more stable over a wider pH range than anthocyanins from other fruit or vegetable sources, which makes them ideal for use in food. The extracts of black carrot juice are sold by SECNA NATURAL INGREDIENTS GROUP in both liquid and powder form.

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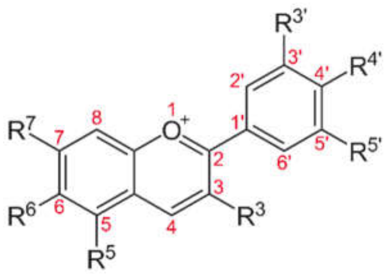

Type structure of anthocyanins (*)

But, in addition, the black carrot has a whole series of components very beneficial for health. For example, it has a high content of antioxidants such as vitamins C and E, as well as other phenolic compounds in addition to anthocyanins, such as hydroxycinnamates and caffeic acid, its antioxidant activity being much higher than that of other carrot varieties. Black carrots are more sensitive to low temperatures than other varieties, which is why they are not traditionally grown in the colder areas of Europe. In addition, black carrots have a very limited shelf life as an unprocessed product, so they are industrialized in situ in the form of juices and concentrates. Black carrot concentrate has multiple applications: alcoholic beverages, energy drinks, carbonated and non-carbonated beverages, confectionery, bakery, pastry, dairy, as well as cosmetics and pharmacy.

Anthocyanins Red cabbage juice (E163). The red cabbage (*Brassica oleracea var. Capitata f. Rubra*) is a species of cabbage, also known as red cabbage, purple cabbage or red kraut. Its leaves are dark red / purple. However, the plant changes color according to the pH value of the soil. In acid soils, the leaves grow more reddish, whereas if the soil has a basic pH the cabbages have a yellow color. In addition, red cabbage has a high content of vitamins C and K, and glucosinolates that according to recent studies are believed to stimulate the production of detoxifying enzymes which leads to potential health effects. To obtain it, due to its solubility in polar solvents, simple physicochemical extraction and purification processes are used to ensure its purity and stability. SECNA produces red cabbage juice in liquid and powder form for applications such as non-alcoholic drinks, wines and liquors, confectionery, confectionery and cookies. ice cream and dairy desserts, juices and jams.

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Anthocyanins Red radish juice (E163). The red radish (*Raphanus sativus*) is used as a raw material for its coloring capacity. Its color is usually red, although it turns yellow depending on the pH values of the medium. Red radish extract is soluble in water and insoluble in oils and is stable at acidic pH. To obtain it, the raw material is cleaned, cut, extracted by solid-liquid extraction, filtered, concentrated, sterilized and dehydrated. The most important anthocyanins of red radish are cyanidin (3-sphorioside-5-glucoside) and pelargonidin (3-sphorioside-5-glucoside). The red radish has an above-average stability in food and beverage applications as a natural coloring substance, being more stable in acidic conditions. This product can be widely used in fruit-flavored drinks, fruit and vegetable juice beverages, in soft drinks, in frozen foods, in sweets production, jams, sauces, among many other applications.

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Saffron. Yellow color. (E164). Saffron is a specie obtained from the flower of *Crocus sativus*, belonging to the Iridaceae family), whose main active ingredients are crocina (caroteonoid responsible for the yellow colour), pyrocrocin and safranal. Each saffron plant grows up to 20-30 cm and produces up to four flowers, each with three stigmas where the coloring compounds accumulate. These stigmas are dried and after used as seasoning and coloring. Saffron is one of the most expensive species in weight of the world because its cultivation, harvesting and elaboration is done by hand, and because to obtain a single gram of saffron, more than 200 flowers are needed. Saffron is native to Southwest Asia and was first cultivated more than 2000 years ago. Over the centuries, the zafran spread slowly throughout much of Europe and later reached Africa, North America and Australia. Saffron is currently grown in Iran, India, Morocco, Greece, Switzerland, England, USA, Spain and France. Iran is the first producer in the world with 90% of total production, followed a long way by Spain. The main use of saffron is as a flavouring and food colouring, used in small doses. It can be used in the preparation of many dishes based on rice, meat, etc. but it is also used in the preparation of sweets and creams.

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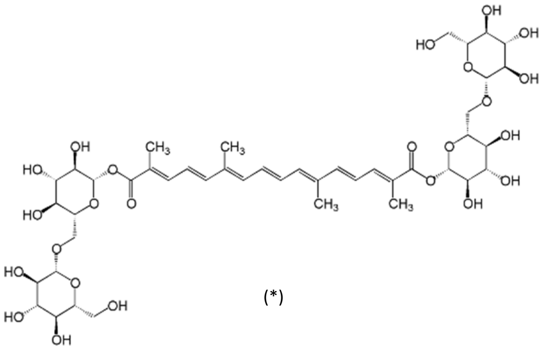

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Crocine molecule

Carbonate and calcium bicarbonate. White. (E170 i, ii). Calcium carbonate $CaCO_3$ is a white oxosal, very abundant in nature (calcite rocks, corals, egg shells) that has multiple industrial applications. In the food industry it is used as a neutralizer and as a hardener, since it improves the textural properties of vegetables. It is also used as an anti-caking and anti-wetting agent, as well as food coloring. Calcium carbonate is insoluble in water, but it can be emulsified to disperse in it. It is stable and does not require any special processing for its use. The EU has approved calcium carbonate as a food coloring, although in other countries its use as a color additive is not allowed. However, calcium carbonate is considered a harmless dye with no known side effects. In fact, calcium, in humans plays an essential role for the proper functioning of the body and its lack leads to serious diseases. We must remember that calcium is the most abundant mineral in our body and a fundamental component of bones. Commercially, calcium carbonate is presented as precipitated calcium carbonate (precipitation of calcium ions with carbonate ones) or as micronized calcium carbonate, the first of which is purer due to the technology used to obtain it.

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Iron oxides. Yellow Red. (E172 i, ii, iii): Both oxide and iron dioxide receive this name. These are yellow, red, orange or black pigments. They are insoluble in polar solvents and are obtained from the oxidation of iron ore. To do this, a sulphate or ferrous chloride solution is treated with a strong base producing a precipitate, which is subsequently oxidized by a stream of hot air. The iron oxides are in the form of a reddish powder, while the dioxides give almost yellow color. Iron oxides have been approved for use in sweets, hard and soft candies, mints and chewing gum. They are also used in pet food and sweets, in pastry and in the manufacture of chewing gum. Since iron is in the ferric form, its bioavailability is very low. It is considered toxic in very high doses.

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Spirulina blue: Spirulina is a blue-green algae that grows in fresh water and has been consumed for its nutritional properties (contains high levels of Fe, Zn, Mn, vitamins and polyunsaturated fatty acids) for centuries (there are references to its consumption since the time of the Aztec Empire). It is an easily digestible food and its nutrients have a high bioavailability. As a food coloring it has multiple applications, despite its characteristic smell and taste, since it is used in small doses in the coloring of food. Some of its most common applications are the production of ice cream, in confectionery in the elaboration of beverages, in dairy products, in the formulation of desserts, etc. It can be produced in raceways, tubular photobioreactors and plates, but it is most usual to be produced in outdoor ponds or protected inside greenhouses. The problem of strange flavors is significantly reduced if it is applied in the form of an extract. Although spirulina has a green coloration, the extraction and selective purification of phycocyanin gives rise to a very special blue extract, and of special interest, due to the limited natural sources of these tones.

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AlgaePARC in Wageningen (extracted from Wageningen UR web page)

These are not all the colouring substances of natural origin available, and probably in the future we are witnessing the appearance of new products with colouring capacities, obtained from different natural sources. However, if they are a good representation of the most common. As we have anticipated, in the next article we will discuss another issue of great importance to the sector, the classification of extracts and concentrates with colouring properties such as coloured foods or as food colours (JRC Technical Report. Regulation (EC) no 1333/2008) and the classification criteria that are proposed.

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