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CAROTENOIDS AS COLORANTS IN FOOD INDUSTRY

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Colorants in food industry

Colour is a significant characteristic of food and it is closely associated with expectations. Based on the colour of the food, we very often decide whether it can be expected to taste good or not. An undesirable colour in meat, vegetables or fruits warn consumers about a potential danger or at least of the presence of undesirable flavours. For this reason, several compounds are added to food matrix[1,2] in order to:

a) enhance colour already present
b) brighten otherwise un-coloured food
c) minimize batch-to-batch modifications
d) replace colour lost during food processing or storage
e) assure the colour uniformity to avoid variations in colour tone caused by seasonal variations
f) protect the flavour and light susceptible vitamins
g) help in the visual assignation of the food quality
h) supplement food with healthy compounds.

The use of colorants has a long history since they have been used in Japan already during the 8th century to colour soybean and adzuki-bean cakes. According to Mortensen *et al*. (2006)[2] and Aberoumand *et al.* (2011)[3] the use of colour in food was intensified in the late 19th century. The use of food colorants in the food industry is useful for both manufacturers and consumers in determining the acceptability of processed food[4]. The approved list of food colorants, including limit of maximum daily intake, differs along countries. From a regulatory standpoint, these food colorant lists are controlled by Food and Drug Administration (FDA) in the United States, and by European Food Safety Authority (EFSA) in European Union (EU). Other regulatory agencies are for example the Agency Pharmaceuticals and Medicinal Devices Agency (PMDA) in Japan, and the State Food and Drug Administration (SFDA) in China. Actually, in EU approximately forty substances are authorized as colorants and indicated with E followed by a number, whereas a much less number of additives are permitted in the USA. In USA these compounds are reported with the Code of Federal Regulation (CFR) number. In both cases, most of the listed colour additives are derived from natural sources[5].

Carotenoids as food colorant additives

When colorants are used as food additives, they can be divided following FDA indication in: certifiable and exempt from certification6. The first is subdivided in synthetic pigments and lakes while the other one consist of pigments derived from natural resources. Carotenoids belong to group of pigments from natural sources and are the most widely distributed ones. Carotenoids are responsible for many of the brilliant yellow and red

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colours of fruits, vegetables, fungi and shellfish[7,8]. These compounds are lipid-soluble and they are structurally divided into two major classes: carotenes (which include α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene) which are exclusively hydrocarbons, and xanthophylls (which include canthxanthin. neoxanthin, violaxanthin, and zeaxanthin) which are oxygenated containing hydroxyl-, methoxy-, carboxyl-, keto-, or epoxy- groups9. Several carotenoids are traditionally added to food directly as dried plant powered. They are also formulated with oil or aqueous media to make emulsions, and colloidal suspensions. These preparations have found applications to colour butter or margarine, canned soups, dairy products, desserts, egg products fruit juices and other type of beverages, confectionery, salad dressings, meat, and pasta[10]. Among the most frequently used carotenoids we found crocin, one if the most abundant constituents isolated from the dried stigmas and styles of the *Crocus sativus* L. (Iridaceae; common name saffron) (**Figure 1**). Commercial saffron production occurs in Spain, Italy, Greece, Turkey, and Morocco in the Mediterranean area, and in Iran in Asia. This spice is water soluble with Golden yellow or brown powder colour and has a characteristic odour of bittersweet, leathery and soft[11,12]. In fact, it energizes dishes with a pungent, earthy essence. In the EU, saffron is not viewed as a colorant, but it is considered an ingredient or a spice especially for rice and chicken. This carotenoid is particularly instable since it is sensible to light, heat, and pH[13].

The tropical tree *Bixa orellana* L. (Bixaceae), known as annatto, is also used as colorant. From the seeds, bixin and norbixin are obtained (**Figure 2**). This yellow to orange colorant has been traditionally used for colouring dairy products especially cheese such as Glouchester cheese, Chesire, Red Leicester, Cheddar, but also butter because it binds to the proteins. Beverages with neutral pH, cakes, custards, snack foods, breakfast cereals, smoked fish, sausages could be also coloured by using this products[14].

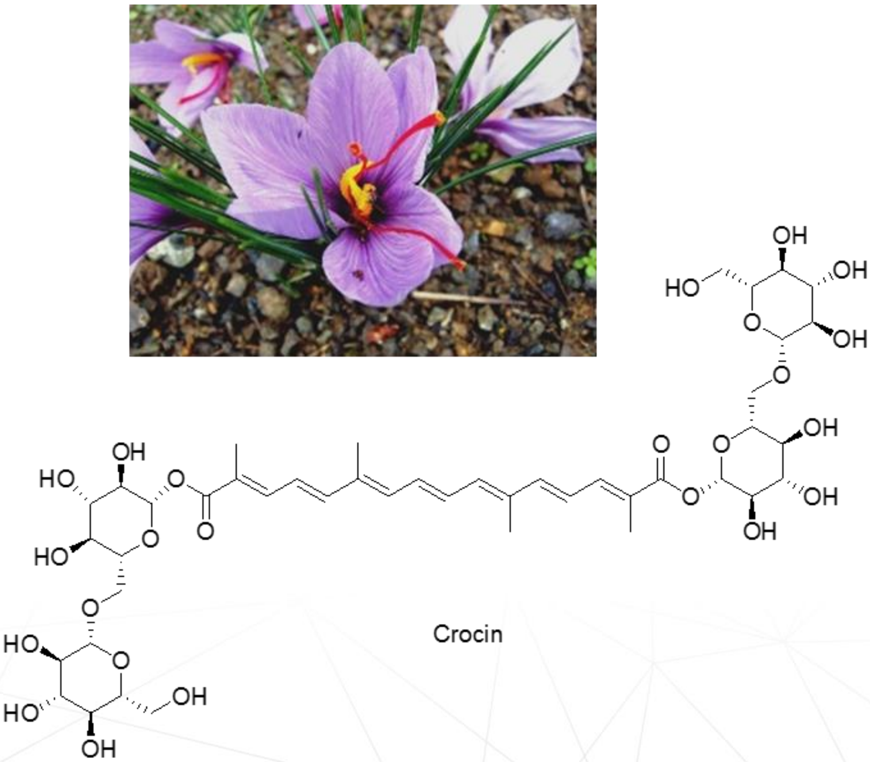


Figure 1. *Crocus sativus* and its main carotenoid, crocin.

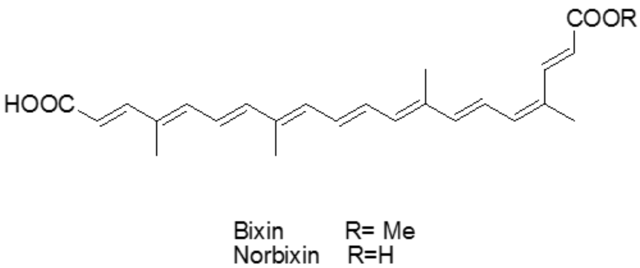
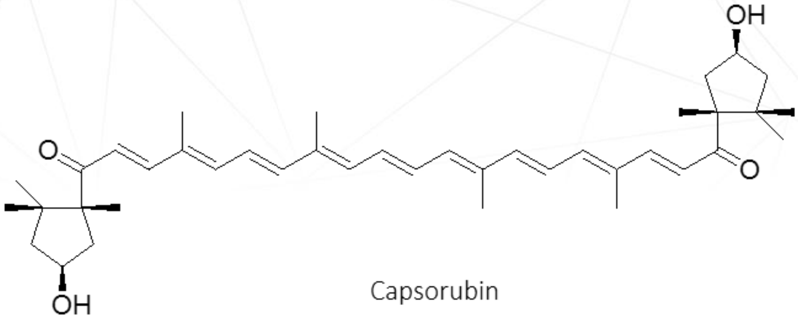


Figure 2. *Bixa orellana* and its main carotenoids bixin and norbixin. .

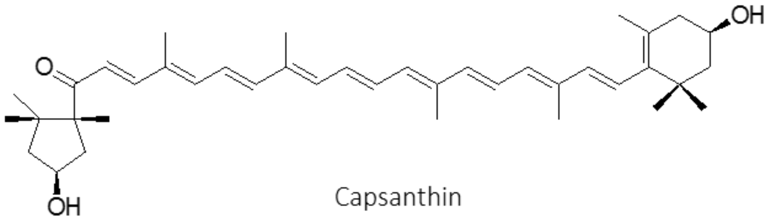
Several food colorants can be obtained also from *Capsicum annuum* (paprika) varieties. The fruits are characterized by low pungency and characteristics flavour16. Paprika contains yellow pigments such as antheraxanthin, β-carotene, β-cryptoxanthin, violaxanthin, and zeaxanthin and red-orange pigments such as canthaxanthin, capsanthin and capsorubin (**Figure 3**)[16]. Their combination is often used in order to obtain bright orange to red-orange colour in food matrix. Astaxanthin and canthaxanthin have found considerable application for salmonids (Salmo, Oncorhynchus and Salvelinus spp.), crustacean and egg pigmentation[17]. One of the main source of astaxanthin is the unicellular green alga Haematococcus pluvialis that produces this pigment as ester forms. More recently, lycopene has been allowed in the EU and USA as a food colorant. However, the only accepted source is tomatoes (Solanum lycopersicum). Lycopene is not largely used as a colorant because it is a expensive and susceptible to oxidative degradation e.g. when compared to β-carotene[2].

Healthy function of carotenoids that are used as colorant

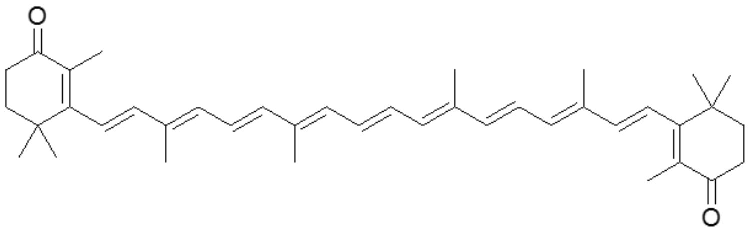
Dietary carotenoids are thought to provide health benefits while decreasing the risk of several diseases such as cancer and cardiovascular diseases. Their beneficial effects could be ascribed to their role as antioxidants. However, this antioxidant function is not completely clarified *in vivo*. Since carotenoids are an integral part of membranes they are able to react efficiently only with radicals generated inside this cellular compartment. Moreover, they possess pro-vitamin A activity. The major pro-vitamin A carotenoid is β-carotene. Apart from the above reported functions, carotenoids are shown to be of importance as immune response enhancers, photo-protection agents, and inducer of carcinogen-metabolizing enzymes[18]. However, it is important to consider how these phytochemicals are effectively assimilated, and how to reach the target tissue where they carry out their function (bioavailability). The section 15 of Regulation EC 1924/2006 on nutrition and health claims made on foods, establish that “in order to ensure that the claims are truthful, it is necessary that the substance that is the subject of the claim is present in the final product in quantities that are sufficient, or that the substance is absent or present in suitably reduced quantities, to produce the nutritional or physiological effect claimed”. Furthermore, “the substance should also be available to be used by the body. In addition, and where appropriate, a significant amount of the substance producing the claimed nutritional or physiological effect should be provided by a quantity of the food that can reasonably be expected to be consumed”.



Capsorubin



Capsanthin



Canthaxanthin

Figure 3. *Capsicum annuum* and its main carotenoids.

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The bioavailability and consequently the exertion of healthy properties of carotenoids is influenced by several factors such as nutrient status, genetic profile of the host, characteristics of food matrix, and properties of any co-ingested food[19]. Among carotenoids identified in plasma of humans, the most important ones are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin[20].

Toxicity, tolerance and allergies

Food allergy is a common disease, especially among children, with a cumulative prevalence of 3 to 6% and a worrying increase in its incidence[21]. Natural color additives are not included among the food groups identified as responsinble of food allergies by the Codex Alimentarius Commission of the World Health Organization[22]. However, Lucas et al. (1998)[23] demonstrated that reactions to natural colour additives such as carotenoids are rare but possible. An IgE-mediated reaction to saffron was observed in one case, but the high use of saffron as compared with this single report of an adverse reaction suggests that sensitivity to saffron is extremely rare. A possible adverse reaction in individuals with an uncommon hypersensitivity, and a possible worsening of the conditions of patients with urticarial can be observed with yellow food colorant called annatto, which is used as an additive in a number of foods and drinks. However, in its long history of use as food colorant, there has been only one reported case of anaphylaxis resulting after annatto ingestion. In general, the specific symptoms that can result after annatto ingestion can vary considerably amongst patients and may range from mild to severe.

Carotenoids are widely used as food colorant for culinary purposes. Thus, investigating their potential toxicity is of crucial importance. Several studies demonstrated that carotenoids are generally non-toxic. However, there are a few exceptions. An excessive intake of some of the most used carotenoids can cause a reversible yellowing of the skin known as carotenemia. Hueber et al. (2011)[24] reported that high doses of canthaxanthin could determine a reversible form of retinopathy. A high intake of β-carotene in smokers could increase the risk of stomach and lung cancer[25]. Crocin showed reversible and dose-dependent black pigmentation of the liver and acute hepatic damage associated with discoloration[26]. Successively, Hosseinzadeh et al. (2010)[27] demonstrated that crocin administered orally up to 3 g did not cause damage to any major organ in the body.

Concluding remarks

Carotenoids are widely used food colorant since they can confer yellow, orange, and red colour to different matrix. These compounds are widely distributed in nature and are used as food additives already for centuries. The most frequently used carotenoids are derived from Bixa orellana, Capsicum ssp., and Crocus sativus. These natural colorants are preferred to synthetic ones despite that their use is limited by instability to adverse pH, heat and/or light.

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