Ciencia Nicolaita 93:26-34; Abril de 2025 doi:10.35830/cn.vi93.827

Carmine red from cochineal (*Dactylopius coccus*), a natural dye: a review

Gabriela Monserrat Ochoa Manzo[1], Héctor Eduardo Martínez Flores[2]*, José Octavio Rodiles López[2], Liberato Portillo[3]

[1] Programa Institucional de Doctorado en Ciencias de la Salud y Farmacéuticas. Universidad Michoacana de San Nicolás de Hidalgo.
[2] Facultad de Químico Farmacobiología. Universidad Michoacana de San Nicolás de Hidalgo.
[3] Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara.

Historial
Manuscrito recibido: 1 de septiembre de 2024
Manuscrito aceptado: 18 de octubre de 2024
Manuscrito publicado: abril de 2025

***Autor para correspondencia**
Héctor Eduardo Martínez-Flores
hector.martinez.flores@umich.mx
Orcid:0000-0002-0044-9399

Abstract

The present review focuses on the natural carmine red dye extracted from insect *Dactylopius coccus*. It describes the insect, its infestation and development in the cactus plant, the insect collection, the dye extraction process, and the derived products that contain carminic acid as a natural red dye. Information about newly developed technologies such as microwave and ultrasound extraction are summarized, which may be useful for the recovery of bioactive compounds from cochineal grana. These new methods offer significant advantages in terms of efficiency and sustainability, as they have proven to be more environmentally friendly while increasing the extraction yield of carmine, promising a healthier and more sustainable future for the natural color industry.

Keywords: *Dactylopiidae*, carminic acid, natural pigment, extraction processes, emerging technologies

Introduction

In the food industry, the use of additives is essential to ensure the quality, safety, and shelf life of products (Erkan, 2010; Albuquerque *et al*., 2021). Based on the Codex Alimentarius and the official Mexican regulations, through the Federal Commission for the Protection against Sanitary Risks (COFEPRIS), the definition of an additive is: "A substance that is neither normally consumed as a food itself, nor used as an ingredient. It is added to products for technological purposes at the manufacturing, transformation, preparation, treatment and packaging stages". Additives can be classified according to their function as preservatives, texturizers, flavorings, colorants, acidulants, antioxidants, sweeteners, among others (Carocho *et al*., 2014; Vega, 2021).

Colorants are responsible for adding color to foods to improve their appearance, as this plays a crucial role in the choice and acceptance of a product, as the consumer's first impression is based on visual perception (Herrera *et al*., 2023). Colors are divided into synthetic (produced by chemical synthesis) and natural (derived from vegetables, insects, minerals, and microorganisms). The colorants market is growing at an annual rate of 4.6% and its reach in the global market is estimated at $2.3 billion US dollars per year (Rushikesh and Girirajsinh, 2022). Mordor Intelligence analyzed the global natural colors market and determined its value to be $1.6 billion US dollar in 2020, with Compound Annual Growth Rate (CAGR) of 8.5% during the forecast period from 2021 to 2026, mainly due to the better acceptance of healthy foods that do not contain synthetic additives, as well as their functional properties, thus this trend has prompted manufacturers to replace artificial colors with natural ones (Landim *et al.,* 2021). The use of artificial colors generates too much controversy and concern regarding safety, health, and toxicity issues (Gebhardt *et al.,* 2020).

Carmine is a natural dye widely used in the food industry composed mainly of carminic acid, which is obtained by aqueous extraction of the female cochineal (*Dactylopius coccus*), an insect that lives on the cladodes of cactus plants it parasitizes by feeding on their sap (Pérez and Becerra, 2001). The objective of this review is to deepen the knowledge of "cochineal carmine", including its origin, characteristics, extraction methods, applications, and its future in food technology in the context of the growing trend towards the use of natural colors in food.

Cochineal and carmine red

The cochineal is an insect (**Figure 1**) belonging to the family *Dactylopiidae* and the genus *Dactylopius*, of which about ten species have been identified; these are of great economic importance due to their carminic acid content, which is used to produce a red dye. They are also important as biological control agents; however, some can be invasive and pose a threat to commercial cactus crops (Chavez-Moreno *et al.,* 2009; Van Dam and May, 2012).

The most notable *Dactylopius* species are the fine cochineal (*D. coccus*) and the wild cochineal (*D. opuntiae*) (**Figure 2**). The latter grows like a pest in cactus orchards and its body is covered with a white wax that resembles “milkweed” (algodoncillo) and contains a low concentration of carminic acid. On the other hand, the fine cochineal is covered with a wax that resembles a “white powder” and contains a higher concentration of carminic acid (Domínguez, 2012).

Fine cochineal grana (*Dactylopius coccus*)

The cochineal is native to tropical and subtropical regions of the Americas (Müller-Maatsch and Gras; 2016), particularly Mexico (Van Dam and May, 2012). The females of this insect synthesize an anthraquinone glycoside called carminic acid as a defense mechanism (Zacarias-Alvarado *et al.,* 2020). From this compound, several dye-like products are obtained, which begins with harvesting and marketing the females of the dried insect (Arroyo and Medina, 2021). The post-harvest activities include detaching the insect from the stem, cleaning, sieving to remove wax, sacrificing, drying, and selecting the insect, all of which affect the percentage of carminic acid and therefore the quality of the cochineal (Inglese *et al.,* 2018).

The quality of the grana is classified into three groups based on its carminic acid content: low, between 18 and 19.97%; medium, between 20 and 20.75%; and high, when the carminic acid content is greater than 21% (Herrera and Llanderal-Cázares, 2011). These quality standards have been established by ITINTEC (Institute of Technological Research, Industries, and Technical Standards) of Peru. The following products can be obtained and marketed from grana cochineal: broodstock (live cochineal), dried cochineal, cochineal

Figure 1. Dehydrated cochineal grana insects (*Dactylopius coccus*) that have undergone a sacrificial process followed by dehydration. The average size is typically between 2 and 6 mm in length, depending on the growth stage and the dehydration process applied to the insects.

Figure 2. *Dactylopius opuntiae* growing on a cactus pad hanging in a nopaloteca used for the reproduction of cochineal. The white powder visible on the cactus pad is wax, a coating produced by the cochineal to protect itself and adhere to the cactus as it feeds. At this stage, the cochineal insects are alive.

powder, aqueous or alcoholic cochineal extract, carmine, and carminic acid in its pure form (**Figure 3**). Internationally, the price of the grain and its products is constantly fluctuating, as its value is directly related to the amount of carminic acid it contains (Flores-Alatorre *et al.,* 2014). Peru is the largest producer of dried cochineal grain, accounting for 85% of world production, followed by Mexico and the Canary Islands. To obtain the dye, insects are sacrificed and dehydrated, and more than 150,000 insects are needed to produce 1 kg of dried cochineal (Attokaran, 2017).

Carminic acid

Carminic acid is one of the oldest known natural dyes (Szadkowski *et al.,* 2022), and chemically, it is a β-C-glucopyranose derived from an anthraquinone and consists of an anthraquinone nucleus attached to a carboxyl group, one methyl group, four hydroxyls, and one hexose (Liu *et al.,* 2021). **Figure 4** shows the chemical structure of carminic acid and **Figure 5** shows the carminic acid powder.

Carminic acid was first synthesized in 1998 by Allevi *et al.* (1998). Its IUPAC name is 7-α-D-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxoanthracenecarboxylic acid (Alizadeh *et al.,* 2022), with a condensed molecular formula of $C_{22}H_{20}O_{13}$ and a molecular weight of 492.4 Da. Carminic acid is soluble in water, alcohol, alkaline and acidic solutions, has good light stability, and its color depends on pH (Dufossé, 2014). It changes from orange to purple as pH increases from 2 to 12 (Favaro *et al.,* 2002) and is red at pH 6. Therefore, carminic acid, carmine, and cochineal extract are among the most widely used pigments and dyes in the food industry. The consumption of carminic acid is strictly regulated, with a daily intake of 2.5 mg of carminic acid per kg of body weight per day (EFSA, 2015). Therefore, the content of carminic acid in foods should be controlled (Kavieva and Ziyatdinova, 2022). Based on the safety data sheet provided by Sigma-Aldrich, carminic acid does not present significant toxicological concerns. It is not classified as a hazardous substance, and no acute toxicity data, or carcinogenicity has been reported. Mutagenicity tests, such as the Ames test, have shown negative results. Therefore, carminic acid appears to be safe, the acceptable daily intake of 2.5 mg/kg body weight.

Figure 3. Cochineal powder, obtained after the insect is collected from the cladode, is washed, the wax removed and ground to produce the raw dye.

Cochineal and carmine extract

Cochineal extract and carmine are naturally derived food colors that have excellent heat, light, and acid stability and offer a wide range of shades from bright pinks and reds to oranges and lavenders. The FDA in its Code of Federal Regulation Title 21 (21CFR73.100) defines cochineal extract as a concentrated solution obtained after removal of alcohol from the aqueous-alcoholic extract of cochineal grana and its specifications are pH between 5.0 and 5.5 at 25 °C. Its chemical composition is protein ≤ 2.2%; total solids range from 5.7 to 6.3%; methanol should not exceed 150 ppm; and carminic acid should not be lesser than 1.8% (21CFR73.100; Favaro et al., 2002).

Carmine (**Figure 6**) is a natural red pigment derived from the cochineal insect. It is produced through the aqueous extraction of carminic acid and then combined with aluminum or calcium-aluminum to form a lacquer pigment. Carmine is a coordinate complex with a six-membered chelate ring structure formed when the

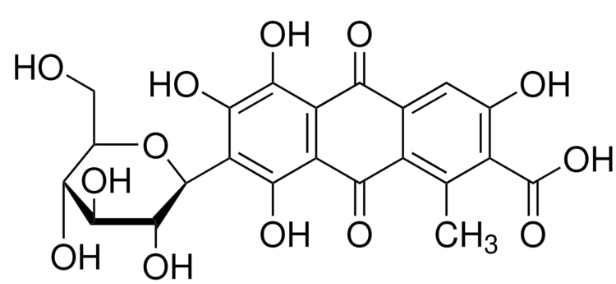

Figure 4. Chemical structure of carminic acid. Carminic acid, a natural anthraquinone compound produced by cochineal insects, owes its red color to alternating single and double bonds.

Figure 5. Carminic acid powder.

Figure 6. Carmine or carmine lacquer.

aluminum atom chelates with two carminic acid molecules through the 5-hydroxyl group and the ortho-carbonyl oxygen (Favaro *et al.,* 2002).

In addition, carmine color or carmine lake is a complex in which aluminum is bound to carminic acid, to which maltodextrin is usually added to ensure a final carminic acid content of 48.3 to 51.9%, according to the specifications established by the FDA and EFSA (European Food Safety Authority), which require that it contain not less than 50% carminic acid in chelates, not more than 3% in relation to carminic acid for 4-aminocarminic acid and not more than 1% of matter insoluble in dilute ammonia. In the European Union, carmine lakes are regulated, and some are not allowed in certain food applications because 4-aminocarminic acid (4.ACA), a natural ammonium from cochineal protein, is under regulatory concern in Europe owing its allergic effects (Gómez, 2023).

4-aminocarminic acid

The process for the preparation of 4-aminocarminic acid was presented in a United States patent. The protocol consists of heating a mixture of carminic acid, citric acid, ammonia and water, in an oil bath at 115-120 °C for 40 min, followed by evaporation and drying (Schul, 1992). Its molecular formula is $C_{22}H_{21}NO_{12}$ and its molecular weight is 491.10 Da (Sabatino *et al*., 2012). Its chemical structure is 7-β-D-glucopyranosyl-9,10-dihydro-5-amino-3,6,8-trihydroxy-1-methyl-9,10-dioxoanthracene-2-carboxylic acid (Cooksey, 2019). The use of this dye is regulated due to possible allergic reactions, but despite being banned in the EU, it was found in Italy during a 12-month quality control study conducted by the Italian Ministry of Agriculture, Food, Sovereignty and Forests in 2011 and 2012, so that 50% of beverages and food additives were labeled as E120 (Sabatino *et al*., 2012; Schweiggert, 2018). There are concerns about the misuse of this colorant and the lack of transparent labeling to inform consumers.

4-carminic acid is a derivative of carminic acid that imparts and retains a deep red color to acidic foods that is not possible with carmine. Its existence was revealed by Kawasaki *et al.* (2002) upon purification and identification of an unknown red pigment in an apple syrup. This dye was developed in response to the need to obtain a brighter red color in acidic foods (pH < 3), but it is not approved for use in foods, although it is intentionally or unintentionally introduced into the marketplace and is called "acid-stable carmine" and is produced by the reaction of carminic acid with ammonia (Dapson, 2005, 2007).

Challenges of using carmine

One of the most used dyes in food technology is the synthetic red FD&C Red #40, but the trend is to use natural dyes such as cochineal carmine. The most used carmine option in the food industry is in the form of aluminum lacquer (carmine), and it is the natural and effective alternative to synthetic dyes (Dufossé, 2014). Despite this, there are three major challenges with carmine dye: first, it cannot be Kosher or Halal certified because it is derived from an insect, and on the other hand, manufacturers are facing pressure from the vegan market (Watson, 2013). Second, its possible health effects due to the presence of aluminum (Ding *et al*., 2019). Finally, its consumption can cause allergies due to the presence of proteins or protein residues (Gultekin and Doduc, 2013). Currently, there have been cases of occupational asthma and rhinoconjunctivitis in male butchers who inhaled constant amounts of carmine during work, dermatitis from lipstick application, and anaphylactic reactions in a woman who consumed yogurt and another who drank Campari during dinner (Beaudouin *et al*., 1995; Baldwin *et al*., 1997; Acero *et al*., 1998; Lizaso *et al*., 2000; Müller-Maatsch and Gras, 2016). A modern preparation of carmine has been proposed by Schmidt-Jacobsen and Frandsen (2011), using pure carminic acid, aluminum and calcium salts, and whey protein to replace the natural allergenic cochineal protein (Ohgiya *et al*., 2009). In fact, extraction of carminic acid from cochineal has long been considered a difficult and complicated process with low yields (Cooksey, 2019).

Methods of extraction of natural dyes

Natural color extraction is a process involving the use of solvents. Its main objective is to separate the desired pigment found in the matrix; usually found in small amounts along with some components such as fibers, carbohydrates, and proteins, among others. The purpose of the extraction process is to transfer as much of the pigment as possible from the matrix to the solvent. The material containing the pigment must be reduced in particle size and then screened to improve extraction efficiency. Extraction becomes a key step to achieve higher dye efficiency and yield (Adeel *et al*., 2018; Mansour, 2018; Rehman *et al*., 2018). Extraction methods are classified into two classes: first, conventional methods such as Soxhlet, boiling, and use of solvents; second, methods with emerging technologies, which include techniques such as microwave, ultrasound, supercritical extraction, pressure liquid extraction, and others. These advanced methods are being studied to replace conventional extraction processes, as they allow more efficient extraction in short times and with higher dye recovery (Sankar, 2015; Da Silva *et al*., 2020; Bota and Indrie, 2021).

Conventional methods

Conventional methods have been used since ancient times and play a fundamental role in obtaining natural colorants from various plant and animal sources. These substances can be extracted using organic solvents such as acetone, ether, chloroform, ethanol, methanol, water or aqueous mixtures with non-polar solvents (Rehman *et al*., 2018; Mansour, 2018).

The extraction method using a mixture of water and alcohol is more efficient than the aqueous method because it can extract both soluble and insoluble substances in water, and especially because aqueous extraction involves soaking the material for a long time, boiling it, and filtering it; similarly, acids or alkalis can be added to alcoholic solvents to release a greater amount of colorant (Mansour, 2018; Rehman *et al.*, 2018). These methods are easy to perform and, in some cases, economical, but the disadvantages are long extraction times, higher energy consumption, and the presence of toxic residual solvents that can be harmful to humans and the environment, since they are flammable, volatile, and explosive; the extraction yields are also low, and the dyes are less resistant to heat and have lower active ingredient content in the extracts (Zannou *et al.*, 2022).

Emerging technologies

The rising demand for high-quality natural products has encouraged the use of technologies that optimize the yield and performance of the active ingredient, reduce the use of solvents, extraction times, and energy consumption and are environmentally friendly.

Among the methods with the most research is the so-called "Supercritical Extraction", which uses a gas, for example carbon dioxide, under conditions of high temperature and pressure, where the so-called supercritical state is reached and physicochemically there is no difference between a liquid-gas state, and components can be extracted from natural materials (Padma and Nisha, 2021). On the other hand, the so-called "high pressure liquid extraction" also uses high temperature and pressure conditions, but the solvent remains in a liquid state, which improves solvent diffusion and mass transfer due to the decrease in surface tension between the components and thus facilitates the rupture of the cells from the solid matrix (Leonarski *et al.*, 2023).

Microwave extraction is based on the uniform heating of the matrix using electromagnetic radiation with a frequency of 0.3 to 300 GHz, causing the polarized molecules to reorient themselves due to the heat generated and the friction between them, where the water within the plant matrix absorbs the energy microwaves and generates cell disruption, which is promoted by internal superheating and facilitates the exit of the compound from the matrix (Da Rocha and Noreña, 2020; Geow *et al.*, 2021).

Finally, ultrasonic extraction uses a probe that applies an intensity at a frequency of 20 kHz to a liquid medium to generate small bubbles that increase in size to a critical point that collapse, a phenomenon called cavitation, releasing large amounts of energy; such increase of temperature and pressure of the solvent, causes mass transfer, breaks the matrix, and achieves the extraction of the compound. The main parameters that affect this vibrational effect are the wave frequency and amplitude, the input power, and the ultrasonic distribution of the wave. This makes it an efficient and environmentally friendly method for the extraction of dyes (Geow *et al.*, 2021; Rutkowska *et al.*, 2017).

Conventional and emerging technologies to obtain cochineal and carmine extracts

The most used method to obtain this dye is described as follows: 1) The cochineal powder is subjected to an extraction process with organic solvents; in the past the extraction was carried out with water, and nowadays it is carried out using alcohol or an alkaline medium such as ammonia or sodium carbonate; 2) Solid-liquid separation, which involves flocculation and filtration; here we obtain the aqueous or alcoholic extract of cochineal; 3) For the preparation of carmine, alum (aluminum and potassium sulfate) is added to the soluble extract to precipitate the carminic acid in the form of a red precipitate (aluminum salt); 4) Finally, the precipitate is washed with water, dried, ground and sieved to obtain a fine and uniform powder that can be used as carmine dye (Attokaran, 2017; Borges *et al.,* 2012).

The extraction and purification of carminic acid from raw cochineal is a complex process that must be standardized to obtain the desired product. González *et al.* (2002) achieved efficient extraction of pigments from cochineal using a mixture of methanol and water (65:35), and the results suggest that the number of extractions is statistically the most significant factor. However, these extraction processes have several disadvantages due to their low efficiency, long extraction times, the use of toxic solvents, and the presence of allergenic proteins derived from the insect and possibly complexed with carminic acid, generating a negative impact on the health of the consumer. This has motivated the study of new extraction methods using emerging technologies to reduce the content of allergenic proteins, improve the stability of carminic acid and increase efficiency (Attokaran, 2017; Borges *et al.*, 2012).

Emerging technologies are a novel approach to address the challenges posed by industrial competitiveness to be more innovative, economical, and ecological (Chemat *et al.*, 2019). These include more efficient use of energy, greater mass and heat transfer, use of environmentally friendly solvents, and guarantees a safe and high-quality product (Pinela *et al.*, 2019). The main advantage of these technologies is the optimization of extraction through cell wall disruption (Manzoor *et al.*, 2021).

Borges *et al.* (2012) extracted carminic acid from cochineal using supercritical fluid extraction and pressurized liquid extraction, and then compared the results obtained with the conventional method. They found that both techniques directly produced a red precipitate of carminic acid, which is advantageous in industry due to its greater solid-state stability. The yields of carminic acid were significantly higher, 42.4% for pressurized solvent extraction and 39.4% for supercritical fluid extraction, compared to 18.5% for conventional solid-liquid extraction. The best results were obtained using methanol/water and ethanol/water solvents at 100 and 150 °C, respectively. These extraction methods provide an efficient alternative to obtain carminic acid with higher yields and stability in the solid state, which may be of interest in the food and dye industries (Adeel *et al.*, 2018).

The importance of studying new technologies to optimize the extraction of natural colorants is aimed at minimizing energy consumption, extraction times, and preserving the application of color in foods, thus achieving the optimization of process conditions. However, these methods face challenges such as the generation of free radicals due to high temperatures and pressures, degradation of pigments, and undesirable effects on organoleptic and nutritional aspects (Kutlu *et al.,* 2022).

Conclusions

Grana Cochineal is an insect that feeds on the cactus plant and produces a dye called carmine red, which is used in various industries, mainly in cosmetics and food. The demand for dyes derived from natural sources, especially of animal origin, such as cochineal, has been growing significantly, mainly due to the toxicity associated with artificial dyes. Although cochineal is a natural dye, traditional extraction methods involve the use of toxic solvents and can pose challenges in terms of both efficiency and food safety. This has led to the study of new extraction methods, emerging technologies such as supercritical fluid extraction, high pressure liquid extraction, ultrasound, and microwaves, which offer significant advantages in terms of efficiency and sustainability, as they have proven to be more environmentally friendly while increasing the extraction yield of carmine, promising a healthier and more sustainable future for the natural color industry.

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