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Short communication

Free radical scavenging capacity and antioxidant activity of cochineal (*Dactylopius coccus* C.) extracts

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ABSTRACT

Carminic acid (CA) is a natural red pigment produced by *Dactylopius coccus* C. insects. It is widely used in the food industry to replace synthetic colourants. Despite being known for a long time, its antioxidant properties have not been studied so far. The aim of this study was to determine CA activities by different methods namely, free radical scavenging capacity against DPPH and ABTS radicals as well as its inhibition ability of β-carotene bleaching enzymatically induced by lipoxygenase (LOX). CA exhibited a remarkable antiradical activity similar to that of known antioxidants such as quercetin, ascorbic acid and trolox. Effectiveness of CA to protect β-carotene in the co-oxidation with linoleic acid is attributed to enzymatic inhibition of LOX rather than peroxyl radical trapping.

1. Introduction

Colour is one of the most important external qualities of foods that determines their acceptance by consumers. Food industry adds colourants to manufactured products in order to intensify that feature. However, the use of synthetic colourants has increasingly been questioned by consumers; this has renewed the interest in natural substances able to replace them. As an alternative to those pigments obtained from plant, some of them produced by insects can be taken into account. *Dactylopius coccus* insects produce a dye, commonly known as "grana cochinilla" or cochineal carmine. The main constituent of this dye is carminic acid (CA), an anthraquinone derivative shown in Fig. 1.

CA is one of the best natural dyes from the technological point of view because it is fully soluble in water, stable against oxidation, light and high temperatures. It is highly appreciated by its good tinctorial qualities, and the colour hues can vary from red to yellow according to the pH value of the medium. It is extensively used in food, cosmetic and pharmaceutical industries, as well as in dyed handmade textile products (Lloyd, 1980). As an internationally allowed food colourant, CA is used in many products such as jams, ice cream, dairy products, canned food, meat products and beverages (Yamada, Noda, Mikami, & Hayakawa, 1993).

Although CA has been used all over the world since ancient times as a colourant, to the best of our knowledge, there are no reports about its antioxidant activity. The main objective of this work was to determine the free radical scavenging capacity of CA against two different radicals and the protective ability in the β-carotene-linoleic acid coupled oxidation enzymatically induced of cochineal extracts.

2. Materials and methods

2.1. Materials

D. coccus samples were obtained from Santiago del Estero (Argentina) of greenhouse reared populations with the system of hanging *Opuntia ficus-indica* cladodes. The insects were dried at 70 °C in an oven until reaching a constant weight. They were finely ground to a particle size ~250 μm (100 mesh) and stored until use.

Sixty milligrams of dried cochineal powder were mixed with methanol and filtered. The solution was adjusted to a final concentration of $1.87 \cdot 10^{-4}$ M, determined by UV–Vis spectrophotometry at 494 nm by using CA absorption coefficient value of 6800 M^{-1} cm^{-1} (Budavari, O'Neil, Smith, Heckleman & Kinneart, 1996). Cochineal CA content was determined according to Alvarez-Valdés, Diodato, and Nazareno (2006).

2.2. Chemicals

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), Tween-20 (polyoxyethylene-sorbitan monolaurate) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased

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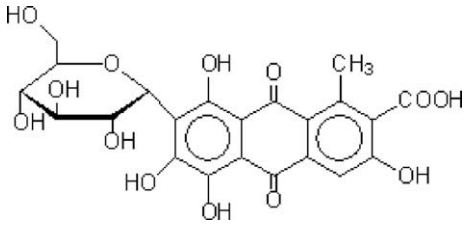

Fig. 1. Chemical structure of carminic acid (CA).

from Aldrich (Buenos Aires, Argentina). Soybean lipoxygenase type I-B, CA and β-carotene (purified before being used by chromatography in a 15 cm silica-gel open column eluted with light petroleum ether), were provided by Sigma (Buenos Aires, Argentina), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was from Fluka (Buenos Aires, Argentina), linoleic acid (99%) was from Riedel de Haën (Buenos Aires, Argentina) and Quercetin (98%) was from Parafarm (Buenos Aires, Argentina). All other reagents (potassium persulphate, borate sodium, methanol, acetic acid, hydrochloric acid and chloroform) were supplied by Ciccarelli (Buenos Aires, Argentina).

2.3. DPPH· scavenging capacity assay

Radical consumption by the action of extracts was determined according to Brand-Williams, Cuvelier, and Berset (1995). Typical procedure consisted of adding an aliquot of the extract to a cuvette containing 3 ml of c.a. 80 μM DPPH· solution. Reaction progress was followed by UV–Vis spectrophotometry and measuring the absorbance at 515 nm in cycles for 10 min. Radical consumption was expressed as percentage of antiradical activity (ARA) as proposed by Burda and Oleszek (2001) and calculated according to the following Eq. (1):

$$\%\mathrm{ARA} = 100 \times \left[1 - \frac{A_{SS}}{A_0}\right] \quad (1)$$

where A_0 is the absorbance of DPPH· solution before adding the antioxidant and A_{SS} is the absorbance at the steady state estimated by mathematical fitting of the kinetic curves.

Percentages of radical consumption for different antioxidant concentrations were measured. EC_{50} value corresponds to the concentration that scavenged 50% of the radicals, expressed as the antioxidant/DPPH· mole ratio. Other parameters such as antiradical power (ARP), defined as the inverse of EC_{50} (Brand-Williams et al., 1995) and the stoichiometric factor (n), corresponding to the number of radical moles consumed per mole of antioxidant added were calculated (Perez, Leigthon, Aspee, Aliaga, & Lissi, 2000). Vitamin C equivalent antioxidant capacity (VCEAC) was also calculated (Kim, Lee, Lee, & Lee, 2002), by using ascorbic acid as a reference compound to prepare the standard curve.

2.4. ABTS·+ scavenging capacity assay

An ABTS stock solution (7 mM) was mixed with 2.45 mM potassium persulphate solution and incubated at room temperature in the dark for 16 h (Rice-Evans, Miller & Paganga, 1996). After that time, the solution was diluted with water to an absorbance value of 0.7 ± 0.1 AU at 734 nm.

An aliquot of extract was added to a cuvette containing 3 ml of ABTS·+ solution. Radical consumption was monitored by UV–Vis spectrophotometry at 734 nm. Results were expressed as trolox equivalent antioxidant capacity (TEAC) (Ozgen et al., 2006) by using trolox as a reference compound for calibration purposes.

2.5. Antioxidant activity in the β-carotene-linoleic acid co-oxidation enzymatically induced by soybean lipoxygenase

The experiment was carried out according to Chaillou and Nazareno (2006) with minor modifications. Linoleic acid solution was prepared by mixing this compound with Tween-20 and diluting with 0.01 M borate buffer pH 9 up to a 330 μg/ml concentration. An aliquot of 500 μL a saturated solution of β-carotene in chloroform was mixed with the same amount of Tween-20. Chloroform was removed using a nitrogen stream. β-Carotene solutions were prepared by adding pH 9 buffer to a final carotene absorbance equal to 1.00. β-Carotene and linoleic acid solutions were mixed in a 3 ml cuvette; then, an aliquot of 50 μl of the cochineal extract corresponding to a CA concentration of 893.6 μM were added. Finally, 200 μl of 1000 μg/ml LOX solution were added to initiate the reaction, which was measured by monitoring the absorbance at 464 nm during 10 min. The same procedure excluding extract addition was done for control. All assays were carried out in triplicate at room temperature (25 ± 1 °C). Antioxidant activity (AOA) was calculated as suggested by Burda and Oleszek (2001) as the percentage of inhibition of β-carotene bleaching of the samples compared to that of the control using the Eq. (2):

$$\%\mathrm{AOA} = 100 \times \left[1 - \frac{(A_s^0 - A_s^t)}{(A_c^0 - A_c^t)}\right] \quad (2)$$

Where A_s^0 is the absorbance of the sample at 0 min, A_c^0 is the absorbance of the control at 0 min. A_c^t and A_s^t are the absorbances at t = 10 min of control and sample, respectively. Quercetin was used as a reference compound. All determinations were performed by triplicate.

3. Results

3.1. Free radical scavenging capacity of cochineal extracts

3.1.1. Antiradical activity towards DPPH·

Fig. 2A and B shows the kinetic profiles for DPPH· disappearance by addition of cochineal extracts as a function of CA concentration. CA behaves as a dose-dependent antioxidant.

From these results, the effective CA concentration to reduce the 50% of the radical corresponds to a CA/DPPH· mole ratio of 0.27. Calculated value for CA antiradical power (ARP) and its stoichiometric factor (n) are shown in Table 1. Values obtained are similar to those reported by Brand-Williams et al. (1995) for ascorbic acid, a well-known powerful free radical scavenger.

Another expression form of ARA results is the vitamin C equivalent antioxidant capacity (VCEAC) which represents the amount of ascorbic acid that presents the same capacity of the sample (Kim et al., 2002). For cochineal extract, VCEAC value is 345 μg vitamin C/mg of CA. Taking into account that CA content in these cochineal samples is 23%, VCEAC value corresponds to 79 μg vitamin C/mg dried cochineal sample.

3.1.2. Antiradical activity towards ABTS·+

Fig. 3A shows the kinetic profiles for radical disappearance for different concentration CA additions. According to these results and using the calibration curve done for trolox (R = 0.99919 for a range of 5–20 μM), antiradical activity determined corresponds to 1.42 mol TEAC/mole CA.

3.2. Antioxidant activity in β-carotene-linoleic acid co-oxidation reaction induced by LOX

In this system, β-carotene is involved in a co-oxidation with linoleic acid induced by the enzyme lipoxygenase. Oxidation reaction