Carotenoid Pigments in *Rosa mosqueta* Hips, an Alternative Carotenoid Source for Foods

D. Hornero-Méndez and M. I. Mínguez-Mosquera*

Departamento de Biotecnología de Alimentos, Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012 Sevilla, Spain

Carotenoid composition has been investigated in *Rosa mosqueta* hips (*Rosa rubiginosa*, *Rosa eglanteria*). Six major carotenoids were identified (*β*-carotene, lycopene, rubixanthin, gazaniaxanthin, *β*-cryptoxanthin, and zeaxanthin) together with other minor carotenoids (violaxanthin, antheraxanthin, and *γ*-carotene). An average composition has been estimated as follows: *β*-carotene (497.6 mg/kg of dry wt), lycopene (391.9 mg/kg of dry wt), rubixanthin (703.7 mg/kg of dry wt), gazaniaxanthin (289.2 mg/kg of dry wt), *β*-cryptoxanthin (183.5 mg/kg of dry wt), zeaxanthin (266.6 mg/kg of dry wt), and minor carotenoids (67.1 mg/kg of dry wt). Possible uses in food technology are outlined and discussed including the preparation of highly colored oleoresins as natural colorants of food and beverages and as provitamin A sources.

Keywords: *Rosa mosqueta; carotenoids; pigments; food*

INTRODUCTION

Carotenoid pigments have attracted the attention of research for many years. They are basically C40 tetraterpenoids with an extended conjugated double-bond system, which gives them red to yellow coloration. Carotenoids, together with chlorophylls and anthocyanins, are the most important pigments for providing attractive colors in fruit and vegetables and may be found in all parts of the plant: roots, leaves, flowers, fruit, and seeds. More than 600 different carotenoids have been isolated and identified from natural sources; many of them (>100) have been found in fruits and vegetables (Isler, 1971).

Carotenoid pigments have been studied in many rose species in the past (Kuhn and Grundmann, 1934; Karrer and Jucker, 1948), not only because of their participation in petal coloring but also for the intense color (from yellow to red) of the rose hips after blooming. However, such studies were carried out from the viewpoint of the flowers' ornamental value and mainly by classical genetic studies, crossing rose species and varieties to correlate petal coloration with carotenogenic gene alleles. Therefore, although qualitative data are available (Märki-Fischer et al., 1983, 1984; Hodisan et al., 1997), there is a lack of data on quantitative composition (Razungles et al., 1989).

Rosa mosqueta (*Rosa rubiginosa*, *Rosa eglanteria*) is a wild rose, widespread throughout the world—in Europe, Asia, and South America (Chile and Argentina). In New Zealand and Australia it has been considered a pest since its introduction in the 1800s. Its fruits (hips) have long been used extensively for pharmacological purposes (skin care and antiulcer properties); the oil extracted from the seeds is included in many cosmetic preparations for its high content of linolenic (45−50%) and linoleic (40%) fatty acids (Marchini et al., 1988; Moreno-Giménez et al., 1990). Moreover, these fruits have been used as food, mainly for preparing jam, tea, and alcoholic beverages after fermentation. It is noteworthy for its high vitamin C content, up to 15 times higher than in citrus fruits. Rose hips have a characteristic intense reddish color due to carotenoid pigments, and this fact makes *R. mosqueta* a highly interesting and profitable source of carotenoid pigments. In the present work, we present qualitative and quantitative data on the carotenoid content of *R. mosqueta*, and we outline possible uses of this natural source of carotenoids in food technology.

MATERIALS AND METHODS

Samples. *R. mosqueta* (*R. rubiginosa*, *R. eglanteria*) hips were collected at the Sierra de Grazalema natural park (Cádiz, Spain). Fruits were harvested in winter in their fully ripe stage from bushes of ∼10 plants growing in the same area. Hips were subsequently taken to the laboratory and frozen at −30 °C until analysis.

Pigment Extraction. Five grams of hips (previously cleaned of seeds) were extracted with acetone, until complete exhaustion of color. All extracts were pooled and transferred to diethyl ether. This phase containing carotenes and xanthophylls was saponified with 50 mL of 20% KOH/methanol during 2 h. Pigments were subsequently extracted with diethyl ether, taken to dryness in a rotary evaporator, and dissolved in 10 mL of acetone. A 1 mL aliquot was kept at −30 °C for subsequent HPLC analysis.

Pigment Isolation and Identification. Pigments were isolated by TLC using silica gel 60GF plates (20 × 20 cm) and two solvent systems: System A (light petroleum ether 40−60 °C) was used for separation and isolation of *β*-carotene and lycopene, and system B (petroleum ether 65−95 °C/acetone/diethylamine, 10:4:1) was used for the rest of the pigments. For the isolation of rubixanthin and gazaniaxanthin semipreparative RP-HPLC was used under the same conditions for separation and quantification described below, with a flow rate of 5 mL/min.

Routine procedures for the identification of carotenoids have been used, which consisted of separation of pigment by TLC and cochromatography with purified pigments, observation of pigment color on TLC plates under white and UV light,

* Author to whom correspondence should be addressed (e-mail hornero@cica.es; fax +34-954691262).

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Table 1. Identification Data for the Main Carotenoid Pigments in *R. mosqueta* Hips

pigment	TLC R_f	TLC color in plate	UV−visible (λ_{max}) in acetone			chemical test for 5,6-epoxide	FT-IR >C=O	FT-IR −OH	EI-MS [*m/z*, (relative intensity, %)] molecular ion $[M]^+$	EI-MS characteristic fragments
β-carotene	0.26[a]	yellow-orange	(424)	452	476	−	−	−	536 (100)	444 (15), 430 (6)
lycopene	0.15[a]	deep red	448	474	506	−	−	−	536 (54)	467 (16), 444 (10), 430 (12), 69 (100)
β-cryptoxanthin	0.57[b]	yellow	425	449	476	−	−	+	552 (100)	534 (30), 460 (9), 446 (5)
zeaxanthin	0.42[b]	orange	424	449	476	−	−	+	568 (100)	550 (19), 532 (10), 476 (45), 462 (6)
rubixanthin	0.37[b]	orange	440	464	494	−	−	+	552 (100)	534 (8), 483 (6), 465 (1), 460 (15), 69 (85)
gazaniaxanthin	0.37[b]	orange	440	464	494	−	−	+	552 (100)	534 (7), 483 (6), 465 (3), 460 (15), 69 (82)

[a] Elution system A: light petroleum ether 40−60 °C. [b] Elution system B: petroleum ether 65−95 °C/acetone/diethylamine, 10:4:1.

recording of UV−visible spectra (Hewlett-Packard UV−vis diode array spectrophotometer model 8452A) in different solvents and comparison with the values reported in the literature (Britton, 1985; Davies, 1976; Davies and Köst, 1988; Foppen, 1971), and examination of 5,6-epoxide, carbonyl, and hydroxyl groups by chemical derivatization and FT-IR spectroscopy (Bio-Rad FTS-7 IR spectrophotometer). Electron impact mass spectra (EI-MS) were obtained with a VG model Quattro instrument with a direct insertion probe system at a 70 eV ionizing voltage and an ion source temperature 230−240 °C.

Authentic purified samples of β-carotene and lycopene were kindly provided by Hoffmann-La Roche (Basel, Switzerland). Authentic samples of β-cryptoxanthin, zeaxanthin, violaxanthin, and antheraxanthin were isolated and purified from natural sources (*Capsicum annuum* and *Menta arvensis*) as described by Mínguez-Mosquera and Hornero-Méndez (1993). Rubixanthin and gazaniaxanthin were isolated from rose hips as described by Arpin and Liaaen-Jensen (1969).

HPLC Separation and Quantification of Carotenoids. Monitoring and quantification of the carotenoid pigments were carried out by RP-HPLC using a method previously developed by us (Mínguez-Mosquera and Hornero-Méndez, 1993). The method uses a C18 reverse phase column (Spherisorb ODS-2; 5 μm, 0.4 cm × 25 cm; Hewlett-Packard, Las Rozas, Madrid, Spain) and a binary gradient elution system of acetone/H_2O at a flow rate of 1.5 mL/min. Injection volume was 5 μL, and detection was carried out 450 nm. Quantification was accomplished using *all-trans*-β-apo-8′-carotenal as internal standard, which was added at the saponification step (1 mL of a 100 μg/mL stock solution). HPLC analyses were performed with a Waters quaternary pump system model W600E and Waters photodiode array detector model M996, both controlled by a Millenium 2010 acquisition data station.

RESULTS AND DISCUSSION

Six main carotenoids were identified from *R. mosqueta* hips by their chromatographic behavior and UV−visible spectra: β-carotene, lycopene, rubixanthin, gazaniaxanthin, β-cryptoxanthin, and zeaxanthin, which is in accordance with other studies on carotenoids in the *Rosa* genus (Arpin and Liaaen-Jensen, 1969; Märki-Fischer et al., 1983, 1984; Razungles et al., 1989; Hodisan et al., 1997). Identification was confirmed by cochromatography and mass spectrometry. Table 1 shows the identification data.

β-Carotene (β,β-carotene) was isolated from the TLC plate at R_f 0.26 under system A developing conditions, showing a UV−visible spectrum with λ_{max} at (425), 452, and 476 nm in acetone, which indicates the presence of nine double conjugated bonds and two β-rings in the molecule. The mass spectrum of β-carotene showed a molecular ion at *m/z* 536, which is consistent with the $C_{40}H_{56}$ formula.

Lycopene (ψ,ψ-carotene) was isolated at R_f 0.15 under the same chromatographic conditions as for β-carotene. Properties such as low polarity, red color, and UV−visible spectrum with λ_{max} at 448, 474, and 506 nm in acetone made easy the identification. This was confirmed by the mass spectrum with a molecular ion at *m/z* 536 ($C_{40}H_{56}$) and the characteristic fragment $[M - 69]^+$ due to the presence of ψ end groups.

β-Cryptoxanthin (β,β-caroten-3-ol), with R_f 0.57 under system B conditions, showed a UV−visible spectrum with λ_{max} at 425, 449, and 476 nm in acetone, which agree with the same chromophore as for β-carotene, that is, nine double conjugated bonds and two β-rings. The mass spectrum showed a molecular ion at *m/z* 552 ($C_{40}H_{56}O$) with a fragment at *m/z* 534 $[M - 18]^+$ due to the loss of one H_2O, which agrees with the presence of a hydroxy group, which was confirmed by the FT-IR spectrum.

Zeaxanthin (β,β-carotene-3,3′-diol) exhibited R_f 0.42 under the chromatographic conditions of system B and a UV−visible absorption spectrum with λ_{max} at 424, 449, and 476 nm in acetone. The FT-IR spectrum clearly revealed the presence of one or more hydroxy groups, which was confirmed by the mass spectrum with a molecular ion at *m/z* 568 consistent with a formula of $C_{40}H_{56}O_2$. Fragments at *m/z* 550 $[M - 18]^+$ and 532 $[M - 18 - 18]^+$ demonstrated the presence of two hydroxy groups, and the high value 5.2 for the ratio $[M]^+/[M - 18]^+$ indicates that the hydroxy group was not allylic.

Isolation of rubixanthin (β,ψ-caroten-3-ol) and its 5′-Z isomer, gazaniaxanthin, was achieved by semipreparative HPLC by using the method of Mínguez-Mosquera and Hornero-Méndez (1993). Both pigments showed the same UV−visible spectra with λ_{max} at 440, 464, and 494 nm in acetone and only slight differences in polarity (retention time at 11.9 and 12.2 min, respectively). Mass spectra showed a molecular ion at *m/z* 552 ($C_{40}H_{56}O$), and the fragments $[M - 18]^+$ and $[M - 69]^+$ confirmed the presence of one hydroxy group and a ψ-end group in the molecule. Both pigments, rubixanthin and gazaniaxanthin are commonly found in rose hips. In fact, rubixanthin is the main pigment in rose hips and was first isolated from *R. rubiginosa* (Kuhn and Grundmann, 1934). Gazaniaxanthin, the 5′-Z isomer of rubixanthin, was first isolated from petals of *Gazania rigens* flowers (Schön, 1938). Initially, both pigments were considered to be identical, although their nature was unequivocally demonstrated later (Arpin and Liaaen-Jensen, 1969).

Other minor carotenoids, namely, violaxanthin, antheraxanthin, and γ-carotene, were identified only on the basis of UV−visible spectroscopy properties, chromatographic behavior, and cochromatography with standards.

Qualitatively, *R. mosqueta* hips have a wide range of carotenoid types, from carotenes (lycopene and β-carotene) to xanthophylls (zeaxanthin, β-cryptoxanthin, rubixanthin, and gazaniaxanthin). Only β-carotene and

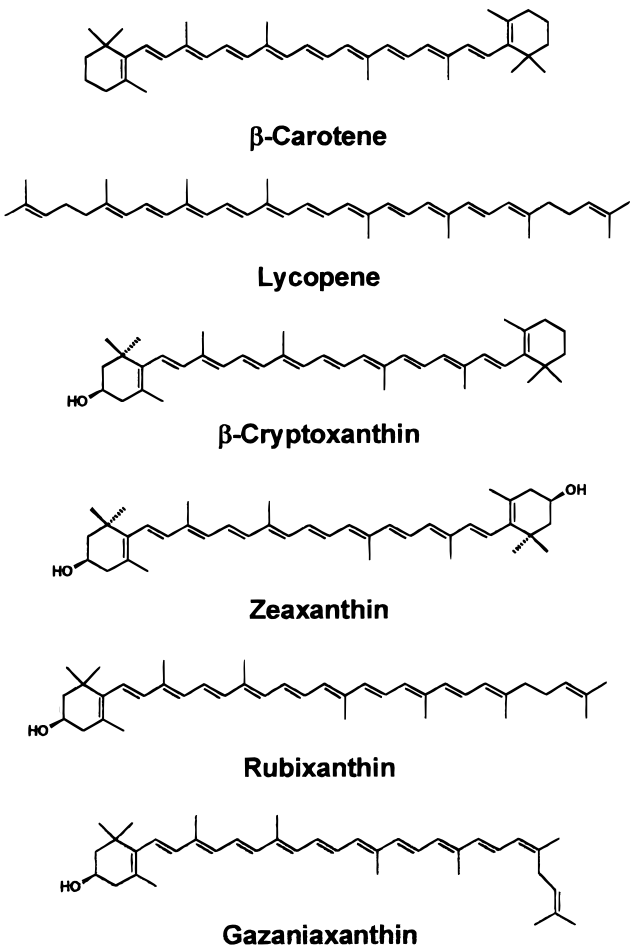

Figure 1. Chemical structures of the main carotenoids from *R. mosqueta* hips.

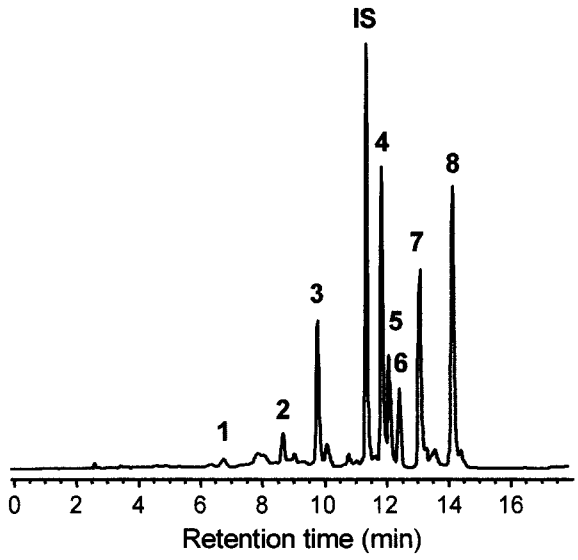

Figure 2. Reverse phase HPLC chromatogram of a carotenoid extract from *R. mosqueta* hips. Peaks: (1) violaxanthin; (2) antheraxanthin; (3) zeaxanthin; (4) rubixanthin; (5) gazaniaxanthin; (6) β-cryptoxanthin; (7) lycopene; (8) β-carotene; (IS) internal standard (*all*-trans-β-apo-8′-carotenal).

β-cryptoxanthin have provitamin A activity. Figure 1 shows the chemical structures of these pigments.

Carotenoid composition was quantified by reversed phase HPLC. Figure 2 shows a typical HPLC chromatogram for a carotenoid extract from *R. mosqueta*. Table 2 summarizes the average carotenoid composition in *R. mosqueta* hips. The total carotenoid content (~2400 mg/kg of dry wt) makes *R. mosqueta* a good natural source of these pigments, comparable with other common sources (Table 3) such as tomatoes, carrots, and red pepper (Gross, 1991). The β-carotene content (497.6 mg/kg of dry wt), plus β-cryptoxanthin (183.5 mg/kg of dry wt), and therefore the provitamin A value, is close to that of some varieties of red pepper (*Capsicum annuum*, with 700−950 mg/kg of dry wt) (Mínguez-Mosquera and Hornero-Méndez, 1994) and much higher than that of most green vegetables and fruits (Gross, 1991). Lycopene levels (391.9 mg/kg of dry wt) are close to those in some tomato varieties (600−1900 mg/kg of dry wt). This fact is of special interest because recent nutritional and epidemiological studies have probed the importance of lycopene in preventing certain cancers (Olson, 1989; Ziegler, 1989; Sengupta and Das, 1999). The major pigment, rubixanthin, and its 5′-cis isomer, gazaniaxanthin, are found in almost all rose species. These two pigments have no provitamin A activity but, together with other pigments such as zeaxanthin and lycopene, may have the beneficial properties of carotenoid pigments in being good antioxidants as well as natural colorants (Britton, 1995).

Table 2. Main Carotenoid Composition in *R. mosqueta* Hips

pigment	concn[a] (mg/kg of dry wt)
β-carotene	497.6 ± 32.1
lycopene	391.9 ± 28.3
rubixanthin	703.7 ± 40.5
gazaniaxanthin	289.2 ± 21.1
β-cryptoxanthin	183.5 ± 12.6
zeaxanthin	266.6 ± 15.3
other minor	67.1 ± 4.0
total	2399.6 ± 153.9

[a] Average composition of 10 analyses (mean ± SD).

Table 3. Total Carotenoid Content in *R. mosqueta* and Other Common Fruits and Vegetables (Gross, 1991)

	carotenoid content	
source	mg/kg of fresh wt	mg/kg of dry wt
R. mosqueta (*Rosa rubiginosa* L.)	360	2400
tomato (*Lycopersicon esculentum* L.)	70−190	950−1700
red pepper (*Capsicum annuum* L.)	900−1500	3000−8000
carrot (*Daucus carota* L.)	124−430	
maize (*Zea mays* L.)	6−57	
pumpkin (*Cucurbita maxima* L.)	70−110	2300−3250
spinach (*Spinacia oleracea* L.)	60−150	
sweet potato (*Ipomoea batatas* L.)	21−70	1000−1200

Therefore, *R. mosqueta* hips are a good potential source of carotenoids for the food industry. The process used by the pharmaceutical industry to obtain oils rich in linoleic and linolenic acids from hip seeds produces a waste containing peel and pulp, which would easily yield a carotenoid concentrate. Rose hips could be processed to give various products, such as a colorant powder, similar to paprika, (after dehydration and grinding), or a concentrated oleoresin (by extracting hips with organic solvents such as *n*-hexane). Possibly, the oleoresin would be the more interesting product, because it allows easy storage, handling, and transportation and can be added directly and in small quantities to many kinds of food (including drinks), pharmaceuticals, and animal feed products. In summary, *R. mosqueta* could be used as an alternative source of carotenoid pigments for the food industry in areas where climate

and other environmental factors make other traditional crops uneconomic.

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