Food Chemistry 210 (2016) 541–550

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of free and esterified carotenoid composition in rose hip fruit by HPLC-DAD-APCI$^+$-MS

Lijie Zhong [a,*], Karl-Erik Gustavsson [a], Stina Oredsson [b], Bartosz Głąb [c], Jenny Lindberg Yilmaz [d], Marie E. Olsson [a]

[a] Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O. Box 101, SE-230 53 Alnarp, Sweden
[b] Department of Biology, Lund University, SE-223 62 Lund, Sweden
[c] Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, 80-822 Gdansk, Poland
[d] Scandinavian Biotechnology Research AB, P.O. Box 158, SE-230 53 Alnarp, Sweden

ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form 28 April 2016
Accepted 1 May 2016
Available online 2 May 2016

Chemical compounds studied in this article:
Violaxanthin (PubChem CID: 448438)
5,6-Epoxy-lutein (PubChem CID: 5281244)
Lutein (PubChem CID: 5281243)
Zeaxanthin (PubChem CID: 5280899)
Rubixanthin (PubChem CID: 5281252)
β-Cryptoxanthin (PubChem CID: 5281235)
Lycopene (PubChem CID: 446925)
β-Carotene (PubChem CID: 5280489)
Violaxanthin monolaurate (PubChem CID: 13932663)
Zeaxanthin laurate myristate (PubChem CID: 101997817)

Keywords:
Rose hip fruit
Carotenoids
Xanthophyll ester
HPLC-DAD-APCI$^+$-MS
Identification
Saponification
Fatty acid

ABSTRACT

Rose hip fruit, which contains high concentration of carotenoids is commonly used for different food products in Europe and it is considered to have medical properties. In this study, a simple, rapid and efficient HPLC-DAD-APCI$^+$-MS method was developed and applied to identify and quantify the carotenoids in rose hip fruit of four rose species, including both unsaponified and saponified extract. In the unsaponified extract 23 carotenoid esters were detected, in which either rubixanthin ester or violaxanthin ester was the dominant component of the ester composition. In the saponified extract 21 carotenoids, including 11 xanthophylls and 10 carotenes were detected. This is the first time the total carotenoid composition, including the carotenoid esters in rose hip fruit were identified and quantified. This work reveals the potential of rose hip fruit to be utilized as a healthy dietary material and give chemical information for the possible future development in the pharmacology field.

1. Introduction

The rose bush, is a woody perennial plant of the genus *Rosa*, within the family Rosaceae. It is widely spread in the world, mainly used as an ornamental plant for the showy and fragrant flower. The fruit of the rose plant, the rose hip, is typically orange to red in color, but dark purple to black color occurs in some special species. Depending on species, rose hip fruit ripens at the end of September or the beginning of October in Sweden (Uggla, 2004). The size and shape of rose hip fruits differ between species.

Rose hip fruit is commonly utilized as an edible material in the Eastern and Northern Europe. It can be used e.g. for herbal teas, jam, jelly, syrup, soup, and wine. For example, in Sweden the rose hip soup "nyponsoppa", is a very common and popular drink product in the supermarket. Palinka, a traditional Hungarian alcoholic beverage, is also made of rose hip fruit, and is popular in Hungary, Romania, and other countries sharing Austro-Hungarian history.

* Corresponding author.
E-mail address: lijie.zhong@slu.se (L. Zhong).

http://dx.doi.org/10.1016/j.foodchem.2016.05.002
0308-8146/

Rose hip fruit is known for its high vitamin C content, which can be up to 15 times higher than that of citrus fruits (Hornero-Méndez & Mínguez-Mosquera, 2000).

In recent decades, rose hip fruit has been more and more studied for its medical properties. It has been reported to be able to reduce osteoarthritis symptoms in clinical trials (Rein, Kharazmi, & Winther, 2004; Warholm, Skaar, Hedman, Mølmen, & Eik, 2003). Rose hip fruit extract has also been shown to have the ability to inhibit cell proliferation in breast cancer (MCF-7), colon cancer (HT-29) and cervical cancer (HeLa) cell lines (Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004; Tumbas et al., 2012). The medical properties of rose hip fruit derive from the rich phytochemical content, including compounds with health-promoting functions, such as vitamin C, flavonoids, phenolic acids, as well as carotenoids (Britton, Jensen, & Pfander, 2004; Olsson et al., 2004; Su et al., 2007).

The group carotenoids, also named tetraterpenoids, constitutes a branch of the terpenoid group, consisting of eight isoprene units. The carotenoids are natural pigments, responsible for the coloration of different plant parts, and commonly abundant in fruit and vegetables. The long continuous conjugated double bond in the chemical structure contributes to the chromophore, so that carotenoid compounds have a special absorbance range in the visible light spectrum. Free and esterified carotenoid compounds can be found in the natural world, and the free carotenoids can be divided as xanthophylls and carotenes. In matured fruits and vegetables, certain amount of xanthophyll is esterified with fatty acids, generating xanthophyll esters. There are about 750 natural carotenoids and the number is still rising (Britton et al., 2004). Plants can synthesize carotenoids, while humans are not capable of synthesizing these compounds, and must obtain them through diet. Some carotenoids are pre-cursors of vitamin A, while others may have more specific functions. Presently research on the carotenoid composition in rose hip fruit is still limited. A number of methods, using mainly high performance liquid chromatography–mass spectrometry (HPLC–MS) for the detection of carotenoids have been reported (Rivera, Christou, & Canela-Garayoa, 2014). However, a detailed and systematic characterization of carotenoids (including esters) in rose hip fruit has not been reported so far.

Therefore, the main objective of this study was to develop an applicable method for systematic determination and quantification of carotenoids in rose hip fruit. Previous investigations, including from our research group (Andersson, Rumpunen, Johansson, & Olsson, 2011) and others (Hornero-Méndez & Mínguez-Mosquera, 2000), have not identified individual carotenoid esters present in rose hip fruit, though a large part of carotenoids are present as esters. In this work, MS was applied to identify xanthophyll esters in rose hip fruit. The key point of the new method should be the compatibility of a good HPLC separation with informative DAD-MS signals. Therefore, the aim of this investigation was to develop a methodology capable to separate and characterize individual carotenoids in both saponified and unsaponified extracts in rose hip fruit exactly, rapidly and economically.

2. Materials and methods

2.1. Solvents and chemicals

All analytical grade organic solvents for extraction, saponification, LC–MS and GC analysis and 95–97% reagent grade sulfuric acid were purchased from Merck (Solna, Sweden). Reagent grade hydrochloric acid (37%) was purchased from Scharlab (Barcelona, Spain). Potassium hydroxide and potassium chloride were purchased from Duchefa Biochemie (Haarlem, Netherland). Butylated hydroxytoluene was purchased from Sigma-Aldrich (MO, USA). All-trans-lutein standard and all-trans-zeaxanthin standard were purchased from Extrasynthese (Genay, France). β-Carotene standard was purchased from Sigma-Aldrich (MO, USA). Fatty acid standards, including capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1, 9c), linoleic acid (C18:2, 9,12c) and linolenic acid (C18:3, 9,12,15c) were purchased from Larodan Fine Chemicals (Limhamn, Sweden). Deionized water was purified using a MilliQ-water system (Thermo Fisher Scientific, Lund, Sweden).

2.2. Plant material

Rose hip fruits were harvested in September 2014 in the park in Alnarp, (55°39′N, 13°04′E), Swedish University of Agricultural Sciences, Sweden. Fruits from four species were investigated in this study, *Rosa rubiginosa* L. (from Prettyhill Nurseries, UK, planted in 1977), *Rosa multiflora* Thunb. (from Alnarps Trädgårdar, Sweden, planted in 1977), *Rosa virginiana* P. Mill (from Prettyhill Nurseries, UK, planted in 1977), and *Rosa rugosa* Thunb. (from Löta trädskolor AB, Sweden, planted in 2004). Fruits of each species were collected from several adjacent bushes. As the carotenoid content generally increases during ripening, the rosehip samples were harvested at full ripening stage in the late harvest season according to a previous study (Andersson et al., 2011). The fruit width of the investigated species ranged from 0.5 cm to 3 cm. All samples were quickly transferred to −80 °C freezer for storage until analysis.

2.3. Carotenoid extraction

About 10–20 g fresh fruit of each species was freeze-dried. The freeze-dried material was then grounded for 20 s with a mill (Yellow line, A 10, IKA-Werke, Staufen, Germany) after removing the seeds. For each replicate, 10 mL ethanol/n-hexane (4:3, v/v) was used to extract 0.5 g freeze-dried rose hip powder. The samples were put in an ultrasonic bath (Bandelin Sonorex Digitec, Berlin, Germany) for 30 min, and then placed on an orbital shaker (Forma Scientific Inc., Marietta, OH, Sweden) at 4 °C for 20 h in darkness. The samples were centrifuged at 3000*g* at 4 °C for 10 min (Eppendorf Centrifuge 5702, Hamburg, Germany) and the supernatant was removed and kept at −80 °C until further analysis with HPLC. All analyses were performed in triplicates. The whole procedure was carried out fast to minimize exposure to light and oxygen.

2.4. Saponification

The whole procedure was based on modified methods from previous investigations (Browse, McCourt, & Somerville, 1986; Delgado-Pelayo & Hornero-Méndez, 2012; Vechpanich & Shotipruk, 2010). To 5 mL carotenoid extract, 500 μL 10% (w/v) potassium hydroxide in methanol: water (4:1, v/v) solution and about 200 mg butylated hydroxytoluene were added slowly under N_2. The mixture was vortexed. The reaction was allowed to take place for 45 min at 75 °C, during which the mixture was shaken periodically. An ice-water bath was used to stop the reaction. Five mL 2% (w/v) potassium chloride and 5 mL n-hexane/ethyl acetate (9:1, v/v) was then added for extraction. The sample was centrifuged at 3000*g* for 5 min (Eppendorf Centrifuge 5702, Hamburg, Germany), and the upper organic layer was collected. The extraction was repeated two times more. The total supernatant was evaporated to dryness and dissolved in 5 mL acetone.

2.5. HPLC-DAD-MS ($APCI^+$) analysis

An Agilent Technologies 1260 Infinity HPLC system (CA, USA) equipped with a diode array detector (DAD, G4212) was used.