

Variation of Carotenoid Content in *Agastache rugosa* and *Agastache foeniculum*

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(Received: 27 April 2012;

Accepted: 11 February 2013)

AJC-12965

The present study describes the variation in carotenoid content between different organs (leaf, flower, stem and root) of *Agastache rugosa* and *Agastache foeniculum* species. Analysis of distinct parts of the two *Agastache* spp revealed the presence of 6 carotenoids-violaxanthin, antheraxanthin, lutein, zeaxanthin, α -carotene and β -carotene. However, the levels of these carotenoids varied significantly among the different plant organs. All 6 carotenoids were observed in the leaves of both species. Moreover, the amount of carotenoids in the leaves was much higher than in the stem, flower or root. The carotenoid content was higher in all parts of *A. rugosa* compared with *A. foeniculum*. In particular, the levels of α -carotene was 9.6-fold higher in the leaves of *A. rugosa* than that in the leaves of *A. foeniculum*. The levels of lutein and β -carotene in the stem of *A. rugosa* were 2.8 and 2.1-fold higher, respectively, compared with those in the stem of *A. foeniculum*. Thus, the amount of carotenoids varied widely between the different parts of *Agastache* spp. The variation in carotenoid content between the two species was significant.

Key Words: *Agastache* spp, Carotenoids, Leaf, Stem, Flower, Root.

INTRODUCTION

Carotenoids are the second most abundant pigment and a diverse group of more than 750 naturally occurring red, orange and yellow pigments¹ that accumulate in the plastids of leaves, flowers and fruits, thereby attracting pollinators and seed dispersal agents². In plant, carotenoids play vital roles in photosynthesis, photomorphogenesis and photoprotection^{3,4}. Carotenoids are a group of phytonutrients that can lower the bad cholesterol and triglyceride levels in the blood stream. Moreover, carotenoids are the precursors of an important plant hormone, abscisic acid⁵, which is involved in regulation of the plant stress response.

A number of carotenoids serve as precursors of vitamin A and act as antioxidants that could protect the human body from the harmful functions of the free radicals may prevent degenerative diseases⁶. A diet with carotenoid-rich vegetables and fruit has been shown to protect against some cancers, heart diseases, cataracts and UV-induced skin damage⁷.

Agastache rugosa Kuntze is a perennial herb of the mint family (Labiatae). It is widely distributed in East- and South-east Asian countries. Since a long time this herb is traditionally

used in Chinese medicine for the treatment of cholera, vomiting and miasma and has been reported to possess antitumor, HIV integrase inhibitory, antifungal and cytotoxic activities. It is mentionable that the leaves of *A. rugosa* are used in cooking to season fish-based foods and its flowers are a prime source of honey⁸⁻¹⁰.

Another species of *Agastache foeniculum* (Pursh) Kuntze is also a perennial herb belongs to the same family. The origin of this herb is around north-central and northern regions of North America and was traditionally used by Native Americans as a medicine to treat cough, fevers, wounds and diarrhea. The special feature of this herb has anise-scented leaves that are used as a seasoning, to prepare tea and in potpourri. Moreover, the characteristics purple flower spike attracts bees, which make a fragrant honey from the nectar^{11,12}. Therefore, in the present paper, we analyzed the carotenoid content in different organs (*i.e.*, the leaf, stem, flower and root) of *A. rugosa* and *A. foeniculum*.

EXPERIMENTAL

Plant materials: *A. rugosa* and *A. foeniculum* were grown in a greenhouse at an experimental farm of Kongju National

University (Yesan, Korea). Plant materials were excised from mature plants and dissected into flowers, leaves, stems and roots. The samples were immediately frozen in liquid nitrogen and stored at -80 °C until further use for carotenoid analysis.

Extraction and HPLC analysis of carotenoids: Carotenoids were extracted from *Agastache* spp. samples (0.1 g) with 3 mL of ethanol containing 0.1 % ascorbic acid (w/v). This mixture was vortexed for 20 s and incubated in a water bath at 85 °C for 5 min. Subsequently, 120 µL of potassium hydroxide (80 % w/v) was added to saponify any potentially interfering oils. After vortexing and incubating at 85 °C for 10 min, the samples were placed on ice and 1.5 mL of cold deionized water and 0.05 mL of β-apo-8-carotenol (12.5 µg mL⁻¹; an internal standard) were added. The carotenoids were subsequently extracted twice with 1.5 mL of hexane and centrifuged at 1200 g following each extraction in order to separate the layers. Finally, the extracts were freeze dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol. For HPLC analysis, the carotenoids were separated on an agilent 1100 HPLC system using a C₃₀ YMC column (250 mm × 4.6 mm, 3 µm; Waters Corporation, Milford, MA) and detected using a photodiode array (PDA) detector at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mm ammonium acetate, whereas solvent B comprised 100 % methyl *tert*-butyl ether (MTBE). The flow rate was maintained at 1 mL min⁻¹ and samples were eluted with the following gradient: 0 min, 83 % A/17 % B; 23 min, 70 % A/30 % B; 29 min, 59 % A/41 % B; 35 min, 30 % A/70 % B; 40 min, 30 % A/70 % B; 44 min, 83 % A/17 % B; and 55 min, 83 % A/17 % B.

RESULTS AND DISCUSSION

The variations in carotenoid content between different parts of *Agastache* spp. (*A. rugosa* and *A. foeniculum*) are shown in Table-1. Analysis of the different parts of *Agastache* spp., revealed the presence of 6 different carotenoids *i.e.*, violaxanthin, antheraxanthin, lutein, zeaxanthin, α-carotene and β-carotene. However, the levels of these carotenoids varied significantly between the different organs of *Agastache* spp. In addition, while all 6 carotenoids were found in the flowers of *A. rugosa*, violaxanthin was absent from the flowers of *A. foeniculum*. Moreover, the levels of β-carotene, antheraxanthin, zeaxanthin and lutein were 1.97, 1.7, 1.6 and 1.5-fold higher, respectively, in the flowers of *A. rugosa* than in those of *A. foeniculum*. The carotenoid content in the leaves of both species of *Agastache* was much higher than in any other part of the plant. Furthermore, while all 6 carotenoids were

observed in the leaves of both species of *Agastache*, the content of all carotenoids was greater in the leaves of *A. rugosa*. In particular, the amount of α-carotene in *A. rugosa* leaves was 9.6-fold higher than that in *A. foeniculum* leaves. The levels of zeaxanthin, violaxanthin, β-carotene, lutein and antheraxanthin were 2.3, 2.1, 1.9, 1.5 and 1.4-fold higher, respectively, in the leaves of *A. rugosa* than those in *A. foeniculum* leaves. Among the 6 carotenoids, 3 carotenoids *i.e.*, lutein, zeaxanthin and β-carotene were found in the stem of *A. rugosa*, whereas only 2 carotenoids *i.e.*, lutein and β-carotene were observed in the stem of *A. foeniculum*. Moreover, the overall level of carotenoids was higher in the stem of *A. rugosa*. Specially, the levels of lutein and β-carotene were 2.8 and 2.1-fold higher, respectively, in the stem of *A. rugosa* than those in the stem of *A. foeniculum*. Furthermore, the carotenoid content was higher in the stem than in the leaf for both species of *Agastache*. The amount of carotenoids in the roots of both *Agastache* spp. was much lower than in other parts of the plants. Among the 6 carotenoids identified, only 2 carotenoids *i.e.*, lutein and β-carotene were found in the roots of both species of *Agastache*. Similar to the carotenoid content in the stem, the overall level of carotenoids was higher in the roots of *A. rugosa*. In particular, the amount of lutein was 1.7-fold higher in the roots of *A. rugosa* compared with those of *A. foeniculum*. It was previously reported that there was significant variation in oil content of the different lines of *Agastache* spp., ranging from 0.07 to 2.73 (per cent volume/dry weight) for leaves and from 0.10 to 3.00 (per cent volume/dry weight) for flowers¹³. Variation in the essential oil composition was high among lines of *A. foeniculum* but low among lines of *A. rugosa*¹³. *Calendula officinalis* L. is a medicinal plant that accumulates large amounts of carotenoids in its inflorescences. The yellow-to-orange colour of inflorescences is mostly due to carotenoids and the shade is dependent on pigments content and profile¹⁴⁻¹⁶. Some works refer to qualitative aspects (separation and identification of carotenoids) and others to quantitative determination^{17,18} (total carotenoid content). It was found the variation of carotenoid contents in four varieties of *Calendula officinalis* L. flowers¹⁹. The carotenoids composition of petals, pollens, leaves and stems of calendula was investigated by HPLC and found difference among the organs²⁰. In present study we also found a difference among the organs that agree with the results of Bako *et al.*²⁰.

The carotenoid content in the different organs of *Agastache* spp. varied widely. However, the leaves were found to possess the highest levels of all 6 carotenoids analyzed. In addition, the species also influenced the carotenoid content

TABLE- 1
CAROTENOID CONTENT IN *Agastache rugosa* AND *Agastache foeniculum*

| <i>Agastache</i> spp. | Organs | Carotenoids (µg g ⁻¹) | | | | | |
|-----------------------------|--------|-----------------------------------|----------------|--------------|-------------|------------|--------------|
| | | Violaxanthin | Antheraxanthin | Lutein | Zeaxanthin | α-Carotene | β-Carotene |
| <i>Agastache rugosa</i> | Flower | 0.9 ± 0.0 | 0.5 ± 0.0 | 33.3 ± 2.4 | 3.1 ± 0.2 | 0.4 ± 0.0 | 36.1 ± 1.4 |
| | Leaf | 11.7 ± 3.4 | 5.5 ± 0.7 | 277.1 ± 61.2 | 29.4 ± 4.1 | 4.8 ± 0.1 | 499.2 ± 22.2 |
| | Stem | 0 | 0 | 94.3 ± 11.7 | 8.0 ± 0.0 | 0 | 65.0 ± 7.3 |
| | Root | 0 | 0 | 2.7 ± 0.6 | 0 | 0 | 1.1 ± 0.5 |
| <i>Agastache foeniculum</i> | Flower | 0 | 0.3 ± 0.0 | 22.1 ± 2.42 | 1.9 ± 0.3 | 0.4 ± 0.0 | 18.3 ± 1.4 |
| | Leaf | 5.6 ± 3.4 | 3.8 ± 0.7 | 189.7 ± 61.2 | 13.04 ± 4.1 | 0.5 ± 0.1 | 260.9 ± 22.2 |
| | Stem | 0 | 0 | 33.7 ± 11.7 | 0 | 0 | 31.1 ± 7.3 |
| | Root | 0 | 0 | 1.6 ± 0.6 | 0 | 0 | 1.1 ± 0.6 |

with *A. rugosa* containing higher amounts of carotenoids in all its organs. Therefore, the leaves of *A. rugosa* may have commercial or medicinal applications, particularly in the production of carotenoids.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110010231).

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