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Amino Acid Composition and Betaxanthin Formation in Fruits from *Opuntia ficus-indica**

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* Dedicated to Professor Dr. W. P. Hammes (Hohenheim) on the occasion of his 60 th birthday.

Abstract:

In contrast to earlier reports high levels of taurine (2-aminoethanesulfonic acid) were found in fruit juices of three cultivars of *Opuntia ficus-indica* (L.) Mill.. Whereas the occurrence of taurine in plant tissue was thought to be restricted to algae, fungi and the endosperm of some higher plants, prickly pear proved to be a rich source of dietary taurine. Using taurine as the amino compound a new betaxanthin was synthesized by partial synthesis. On the basis of chemical and spectral evidence its structure was determined to be the taurine-immonium-conjugate of betalamic acid. Also betalamic acid could be detected in yellow and orange coloured cultivars of *Opuntia ficus-indica* for the first time. In spite of the high levels of taurine accompanied by the occurrence of betalamic acid, the corresponding betaxanthin could not be detected in the fruit tissue.

Key words: *Opuntia ficus-indica* (L.) Mill., Cactaceae, fruits, taurine, carnosine, betaxanthin, betalamic acid, indicaxanthin

Introduction

Opuntia ficus-indica (L.) Mill. (Cactaceae) is a perennial shrub or tree originating from Mexico. Because of its high water-use efficiency it is widespread in the Mediterranean region. Although taurine is of growing interest as a supplement to medical foods (1) and functional drinks (2), systematic screening of potential plant sources is still lacking. To date, hardly any importance has been given to high amounts of amino acids in *Opuntia* (3). Since references on the amino acid content of prickly pear fruits (3) do not include data on taurine, the present study was undertaken to examine the taurine content of the cultivars ‘Morado‘, ‘Gymno Carpo‘ and ‘Apastillada‘.

As part of our investigations on fruits of *Opuntia ficus-indica*, we studied their spectrum of free amino acids to find out a correlation between amino compounds and corresponding profile of betaxanthins. The latter result from conjugation of betalamic acid with amino acids or biogenous amines, respectively. Hitherto, non-enzymatic condensation in betaxanthin biosynthesis is assumed (4). Therefore, quantitative information about amino compound composition in plant tissues should be indicative of betaxanthin pattern in the plant material.

To identify the betaxanthins present in fruit juices of greenish-white and yellow-orange cultivars standards were provided semi-synthetically. Condensation of proline with betalamic acid yielded two different structures. The taurine-immonium conjugate of betalamic acid and the occurrence of free betalamic acid in *Opuntia ficus-indica* are reported for the first time.

Materials and Methods

Plant material

Opuntia ficus-indica (L.) Mill. fruits were purchased from Mexico (cv. ‘Apastillada‘) and from South Africa (cv. ‘Morado‘ and cv. ‘Gymno Carpo‘). Fruits of *Opuntia ficus-indica* were manually squeezed and the filtered juice was frozen (- 80 °C) and stored for analysis.

Preparation of betaxanthin standards

Betanin was extracted for the synthesis of betaxanthin standards from the roots of red beet (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef.) purchased from the local market. Beet roots were washed and peeled in order to exclude enzymatic activity from microorganisms. The roots were sliced and homogenized in a mixture of MeOH and water (80/20, v/v). Ascorbic acid was added to reduce browning caused by polyphenoloxidases. The homogenate was centrifuged for 15 min at 4000 r. p. m. . The supernatant was collected and MeOH was removed under reduced pressure at 30 °C using a rotary evaporator. The methanol-free residue was 10-fold concentrated before isolation of betanin by reversed phase chromatography. Magenta-coloured betanin was collected, concentrated under reduced pressure at 30 °C, redissolved in water and again concentrated until the solution turned to dark. The resulting betanin was frozen (- 80 °C) before use.

Hydrolysis was modified according to (5). 20 µl of the betanin solution were hydrolyzed in 2 ml 3N NH_4OH for 30 min. Hydrolysis was controlled spectrophotometrically at 424 nm. The hydrolysate was transferred to a 25 ml flask with the amino compound in 10-fold molar excess of betanin. The alkaline hydrolysis was stopped by removal of NH_3 under reduced pressure at 30 °C to yield the betaxanthin. The resulting solution was repeatedly dissolved in water and concentrated until a pH between 6 and 7 was reached. The yellow semi-synthetic standards were kept frozen (- 80 °C) for analysis.

HPLC analysis

The HPLC system (Shimadzu) was as follows: a system controller SCL- 10A*vp*, an auto injector SIL-10 AD*vp*, a solvent delivery module LC-10 AT*vp*, an auto injector SIL- 10 AD*vp* fitted with an injection loop (100µl), a pump FCV- 10AL*vp* and a column oven CTO-10A*vp* keeping a constant temperature (30 °C). A steel column (250 x 4 mm i.d.) packed with LiChrospher® 100 RP-18 (5 µm) equipped with a guard column was used for chromatography. Eluent analysis was performed using a diode-array-detector SPD-M10A*vp* (Shimadzu). Detection was carried out at 405, 475 and 540 nm. Mobile phases were prepared using HPLC grade water, phosphoric acid and MeCN.

HPLC procedure was modified according to (6). Separation was achieved with stepwise gradient elution. All gradient steps were performed linearly. Development was achieved in 60 min using the following gradient program: 100 % solvent A (1.5 % phosphoric acid) and 0 % solvent B (100 % MeCN) at time zero and ramped to 80 % A : 20 % B within 40 min; 0 % A : 100 % B in a further 5 min; 100 % A : 0 % B in another 5 min followed by isocratic elution of 100 % A. The system was operated at a flow rate of 1.0 ml / min.

LC-MS

The HPLC system (Hewlett-Packard Series 1100) was as follows: an autosampler ALS G1313A, a quat pump G1311A, a degasser G 1322 A and a column oven ColComp G1316A keeping a constant temperature (30 °C). A steel column (250 x 4 mm i.d.) packed with LiChrospher® 100 RP-18 (5 µm) equipped with a guard column were used for chromatography. Eluent analysis was performed using a diode-array-detector G1315A (Hewlett Packard). Mobile phases were prepared using HPLC-grade water, formic acid (adjusted to pH 1,93) and MeCN.

Separation was performed with stepwise gradient elution. All gradient steps were performed linearly. Development was achieved in 60 min using the following gradient program: 100 % solvent A (formic acid adjusted to pH 1.93) and 0 % solvent B (100 % MeCN) at time zero

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The MS-system was a Micromass Platform II equipped with a crossflow interface. The tuning parameters for positive ion spray (ES+) were 3.50 kV for capillary and 35 V for cone at a source temperature of 120 °C.

Amino acid analysis

Amino acid analysis was carried-out according to the procedure published by FÜRST et al. (7). For detection by RP-HPLC, proline was pre-column derivatized with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl-Cl) while all other amino acids were detected as their *o*-phthaldialdehyde (OPA) adducts.

Results and Discussion

Whereas Pasantes-Morales et al. (8) did not detect taurine in fruits and stems of prickly pear, our investigation showed *Opuntia ficus-indica* to be a rich source of amino acids, with high yields of proline (883.4 – 1143.5 mg / l) and taurine (323.6 – 572.1 mg / l). Beside the predominant free amino acids in pressed juices of *Opuntia ficus-indica*, carnosine could be found in smaller amounts (4.0 – 7.3 mg / l) in juices of all cultivars examined (Table 1). Taurine, as well as carnosine are unusual components in most plants, especially in fruits. To our knowledge, this is the first report about the occurrence of taurine in unprocessed fruits (9), especially in cacti (3, 8). Some authors even noted the virtual absence of taurine in plants (10, 11). Taurine has been scarcely detected in higher plants and reported to be present only in processed prunes (9), nuts, seed grains and leguminous seeds (8, 12, 13). As shown in table 1 the average taurine levels in the juices by far exceed the most important concentrations found in prune juices made from concentrates (155 mg / l; (9)), hazelnut (46,8 nmol / g; (8)), and leguminous seed (up to 100 nmol / g; (13)). Kataoka and Ohnishi (14) reported significant amounts of taurine only in lower plants, with maximum content of 998.7 nmol / g for Rhodophyta and up to 132,6 nmol / g for Phaeophyta.

New and easily accessible sources containing taurine could be of growing interest in the near future. Taurine is recently discussed as a conditional essential amino acid. Laidlaw et al. (15) maintain that dietary intake may play a decisive role in upholding body taurine pools since humans possess a limited ability to synthesize it. Consequently, some medical foods and drinks are already supplemented with taurine (2). Especially cats show profound alterations in structure and function of photoreceptors if their diet is deficient in taurine (16). As a consequence, taurine supplements to pet food for preventing retinal degradation is usual (17). We recognized *Opuntia ficus-indica* as an excellent source of taurine. Different cultivars of this species, but also representatives of other genera of the Cactaceae deserve further studies with regard to their taurine content.

Also the water-soluble pigments of *Opuntia ficus-indica*, the betalains, are of great importance, giving interesting colour hues to the fruit pulps and thus contributing to the overall appearance of prickly pear. For the identification of the yellow betaxanthins semi-synthetic standards had to be provided and structures had to be confirmed by mass spectrometry. The conditions for optimal positive ionization (ES+) were tested and found to be 3.50 kV for capillary and 35 V for cone at a source temperature of 120 °C. However, for the detection of betacyanins a cone voltage of 50 V was required. Hitherto, mainly betacyanins have been elucidated by mass spectrometry (18, 19) using fast atom bombardment (FAB) for ionization (20). The product from condensation of L-proline with betalamic acid yielded two distinct HPLC-peaks with identical absorption maxima at 476 nm and identical masses of $[M + H]^+ = 309$ which could not be referred to as the C_{11}-epimers. The taurine-immonium conjugate of betalamic acid ($C_{11}H_{14}N_2O_7S$) was synthesized for the first time. Its absorption maximum was 460 nm in our chromatographic system. The calculated molecular weight (MW) of 318 could be confirmed by mass spectrometry with positive ionization modus. However, the new betaxanthin (**1**) could so far not be found in the juices of *Opuntia ficus-indica*.

Up till now, it is still unknown whether the *in vivo* condensation is stereospecifically catalyzed by enzymes or whether it occurs spontaneously (4). The occurrence of two structures *in vitro* gives rise to the assumption that betalain biosynthesis is a stereospecific condensation involving enzymatic activity. If betaxanthins were generated spontaneously in the plant, the free amino acid concentration would be indicative of the formation of betaxanthins. According to the amino acid analysis, indicaxanthin and the taurine conjugate of betalamic acid should then be the predominating betaxanthins in the juice examined. In the yellow to orange-coloured cultivars ‘Gymno Carpo‘ and ‘Apastillada‘ indicaxanthin was the major yellow pigment expected by amino acid analysis. However, for the taurine conjugate, a correlation with betaxanthin formation could not be confirmed. In spite of the high taurine levels the corresponding betaxanthin was not detected. In the ripe greenish-white cultivar ‘Morado‘ no

betalain was found, although high concentrations of proline and taurine again should lead to indicaxanthin formation. From these observations the involvement of enzymatic activity in the last step of betaxanthin synthesis can be assumed.

It is well known that Centrospermae may accumulate betalamic acid for betaxanthin synthesis in contrast to exclusively betacyan generating tissues (21). Consistent with that tenet we found betalamic acid in squeezed juices of the two cultivars ‘Gymno Carpo’ und ‘Apastillada’ mainly containing yellow betalains. In the greenish-white fruits the metabolic pathway of betalamic acid synthesis is assumed to be blocked, since no betalamic acid could be detected at the absorption maximum of 405 nm. To our knowledge, this is the first time betalamic acid has been detected in *Opuntia ficus-indica* (22).

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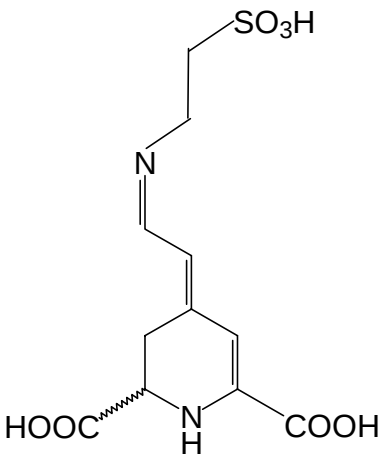
Table 1 Amino acid contents in juices of three cultivars of *Opuntia ficus-indica* (L.) Mill.

Amino acids	**cv. 'Morado'**		**cv. 'Gymno Carpo'**		**cv. 'Apastillada'**	
	µmol / l	**mg / l**	**µmol / l**	**mg / l**	**µmol / l**	**mg / l**
α-Amino-butyric acid	n.v.	n.v.	n.v.	n.v.	10.7	1.1
Alanine	937.2	83.4	917.2	81.7	1084.4	96.6
Arginine	121.5	21.2	212.9	37.1	190.5	33.2
Asparagine	265.6	35.1	344.5	45.5	334.7	44.2
Aspartic acid	n.v.	n.v.	n.v.	n.v.	n.v.	n.v.
Carnosine	28.3	6.4	32.4	7.3	17.8	4.0
Citrulline	33.9	5.9	81.1	14.2	163.7	28.7
Glutamic acid	289.2	42.5	564.5	83.0	494.8	72.8
Glutamine	1975.9	288.8	3931.9	574.6	1198.1	175.1
Glycine	123.1	9.2	115.2	8.6	216.2	16.2
Histidine	208.2	32.3	322.1	50.0	343.2	53.3
Isoleucine	165.9	21.8	250.7	32.9	296.1	38.8
Leucine	n.v.	n.v.	162.2	21.3	151.8	19.9
Lysine	113.0	16.5	125.4	18.3	n.v.	n.v.
Methionine	377.6	56.3	515.6	76.9	217.6	32.5
Ornithine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenylalanine	145.8	24.1	144.5	23.9	133.1	22.0
Proline	**7673.4**	**883.4**	**9932.4**	**1143.5**	**15362.9**	**1768.7**
Serine	1669.2	175.4	2069.9	217.5	1242.3	130.6
Taurine	**2585.4**	**323.6**	**3254.3**	**407.3**	**4571.5**	**572.1**
Threonine	105.5	12.6	126.6	15.1	97.0	11.6
Tryptophane	45.2	9.2	54.8	11.2	85.9	17.5
Tyrosine	78.9	14.3	71.5	13.0	53.1	9.6
Valine	280.6	32.9	426.5	50.0	300.8	35.2

n.v. = not valid
n.d. = not detectable



1 Taurine conjugate of betalamic aci