The vitamin C analogue 2-O-β-D-glucopyranosyl-L-ascorbic acid in fruits from the *Solanaceae* family.

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Abstract

Goji berries from the *Solanacea* family are used in traditional Chinese medicine for their bioactive compounds, mostly antioxidants like ascorbic acid. Recently, an ascorbic acid analogue from goji, the 2-O-β-D-glucopyranosyl-L-ascorbic acid has been reported. In rats, this analogue is absorbed intact and as free ascorbic acid and has been consequently proposed as provitamin C. Since synthesis of the analogue is not promising, reliable natural sources are searched. However, knowledge concerning the analogue's occurrence in other *Solanacea* fruits is lacking. In the present study, the presence of the analogue is evaluated in cherry tomatoes, cape gooseberries, different eggplants, and green chili peppers. Surprisingly, the analogue was found in all fruits investigated, but in considerably lower contents when compared to goji berries. For the first time, the presence of 2-O-β-D-glucopyranosyl-L-ascorbic acid is confirmed in fruits from the Solanacea family, other than goji berries.

Keywords: *Solanacea*, fruits, 2-O-β-D-glucopyranosyl-L-ascorbic acid, ascorbic acid, ascorbic acid analogue

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Introduction

For centuries, goji berries (*Lycium* spp.) from the *Solanacea* family have been used in the traditional Chinese medicine for their bioactive compounds content [1]. Most of these bioactives are antioxidants such as ascorbic acid (AA), which are known to protect lipids, proteins and nucleic acids against oxidation. Other *Solanaceae* fruits are also well known for their antioxidant activities. According to Toor et al. [2], tomatoes present an antioxidant capacity, partly due to their AA content. Similar properties have been reported for cape gooseberries [3], for chili peppers [4, 5], and for different kinds of eggplants [6].

Rather recently, the interest has been put on the 2-O-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA), a stable AA analogue, which is one of the main biologically active components of goji berries [7]. As an analogue of AA, 2-β-gAA possesses antioxidant activities and prevents oxidative stress [8, 9]. Toyoda-Ono et al. reported an increased level of AA and of intact 2-β-gAA in the portal vein blood of rats, after oral administration of the analogue [10]. According to their findings, 2-β-gAA to some extend maintains the level of AA in the rat tissues and hence acts as a provitamin C. They even proposed the 2-β-gAA as a stable AA substitute for clinical applications. Synthetic access to this promising compound by either chemical or enzymatic approach is not efficient [11, 12]. Therefore, reliable natural sources are needed. The presence of 2-β-gAA in different goji berries has been studied extensively [12–15]. However, analogue's occurrence in other fruits or berries have never been confirmed despite some unsuccessful studies on solanacea species.

The aim of this study was to evaluate the content of 2-β-gAA in other Solanaceae fruits than in goji berries. Tomatoes, such as cherry tomatoes (*Solanum lycopersicum* var. cerasi-

Fig. 1: **The 2-β-gAA contents in cape gooseberries, Thai white eggplants, pea eggplant and chili peppers from the Solanaceae family were determined.**

forme), cape gooseberries *(Physalis peruviana)*, eggplants as Thai white eggplants *(Solanum xanthocarpum)* and pea eggplant *(Solanum torvum)*, and chili peppers such as red chili and green eye's bird chili *(Capsicum annuum)* are all part of the *Solanacea* family and therefore of interest in this study (◆ Figure 1).

Materials and methods

Materials

Cape gooseberries (product of Colombia), and red cherry tomatoes (product of Italy) were acquired in a local supermarket. Red chili peppers (product of Vietnam), Bird's Eye chili peppers (product from Vietnam), Pea eggplants (product from Thailand) and Thai white eggplants (product from Thailand) were bought in a local Chinese supermarket. Fruits were stored in the fridge at 4 °C before experiments. Cape gooseberries were directly analyzed after purchase.

Reagents

AA was obtained from Fluka Analytical (Damstadt, Germany), Dithiolthreitol (DTT) from PanRec AppliChem (Damstadt, Germany), and sulfuric acid was acquired from Acros Organic (Geel, Belgium). Acetonitrile of HPLC grade was purchased from Macron Fine Chemicals Avantor (Ankara, Turkey) and formic acid from Sigma Aldrich (Buchs, Switzerland). Deionized water was used from Milli-Q purification system.
DTT solution: 200 mg DTT was solubilized in 5 mmol/L aqueous sulfuric acid. DTT protects the AA from oxidation and the same aqueous sulfuric acid was also used as HPLC eluent.

Sample homogenization

One fresh cape gooseberry without the calyx was mixed (Ultraturax T25, IKA, Staufen, Germany) for 1 min at 9,500 rounds/min with 5 mL of the DTT solution (4 g of sample in 20 mL of solution). To prepare the calyx from cape gooseberry, the Ultraturax was used for 2 min at 9,500 rounds/min with 20 mL of the DTT solution (600 mg/20 mL). Regarding the cherry tomatoes (13 g/20 mL), Ultraturax was used at 20,500 rounds/min for 2 min with the same amount of DTT. Stems were removed from chili peppers and eggplants before the further preparation. For chili peppers (red 2 g/20 mL; green 1 g/20 mL), the Ultraturax was used for 3 min at 2,000 rounds/min, for pea eggplants (1 g/20 mL), 13,500 rounds/min for 2 min and for the Thai white eggplants' extraction (20 g/20 mL), 20,500 rounds/min for 3 min each with 10 mL of DTT solution. For an optimal homogenization, the mixer speed for the sample preparation and the quantity of the DTT solution were adapted to the matrix (more DTT solution for a dry matrix).

Extraction procedure

The method described by Toyodo-Ono et al. [12] with modifications proposed by Kosińska-Cagnazzo et al. [7] was used to extract 2-β-gAA and AA. Briefly, the homogenized sample was ultrasonicated for 10 min at 35 kHz (VWR, Dietikon, Switzerland). After centrifugation for 15 min at 2,800 g (NUVE, Ankara, Turkey), the supernatant was collected. To the deposit, 2.5 mL of DTT solution were added and blended using a vortex genius 3 (IKA, Chavannes-de-Bois, Switzerland). For the Thai white eggplants and the calyx, 5 and 10 mL, respectively, were used. The deposit was extracted twice under the same conditions. The supernatants were combined and adjusted to 20 mL. For the Thai white eggplants and the calyx, the volume was adjusted to 25 and 50 mL, respectively. After filtration (0.45 μm nylon syringe filter ChromafilR, Machery-Nagel, Düren, Germany), the extracts were directly analyzed by HPLC.

HPLC analysis

An Agilent 1220 Infinity series liquid chromatograph (Agilent Technologies, CA, USA) comprised of an auto-sampler, a binary pump and a G4294B UV-DAD detector (Agilent Technologies 110 Series, Agilent Technologies, CA, USA) was employed for the chromatographic separation as described previously by Kosińska-Cagnazzo et al. [13]. Briefly, 5 µL were injected onto an amino column (Aminex HPX-87H Ion exclusion, 300 × 7.8 mm i.d., particle size 5 µm, Bio-Rad, Hercules, CA, USA) equipped with a precolumn (30 × 4.6 mm, cation H cartridge for amino column). The mobile phase was composed of 5 mmol/L sulfuric acid and was delivered in an isocratic mode at a constant flow rate of 0.5 mL/min. The column temperature was set to 40 °C. AA and 2-β-gAA were detected at 254 nm. Chromatographic peaks were identified by comparison of retention times and UV spectra with those obtained for the standard AA. The retention time was 8.6 min for AA and 11.3 min for 2-β-gAA with a total run time of 30 min. AA was quantified by external calibration. Quantification of 2-β-gAA was done with the AA calibration curve and a conversion factor reported by Tai and Godha [16].

Dry weight and presentation of results

Dry weight (dw) of the raw material was determined with a halogen moisture analyzer (Mettler Toledo, Greifensee, Switzerland). Temperature was set at 110 °C. All samples were analyzed in triplicate. Results of dry weight, AA and 2-β-gAA are indicated as mean ± standard deviation from triplicate analysis of the fruits or the calyx.

Results and discussion

In a recent study on mice, the vitamin C analogue 2-β-gAA showed palliating effects on induced inflammatory bowel disease, an increased production of short-chain fatty acids and a modulation of the gut microbiota composition [17]. Another study on 2-β-gAA isolated from *L. barbarum* confirmed the excellent free radical scavenging activity in vitro [9]. The 2-β-gAA seems to be a promising natural AA derivative for further pharmacological evaluation. These studies and the difficulties to synthesize the vitamin C analogue explain the interest to identify natural sources of this promising compound.

Samples	2-β-gAA	AA	AA-Reference value	Reference
Cape gooseberry, without calyx	9.94 ± 0.60	149 ± 3.7	90–195	[3, 21]
Calyx	8.63 ± 0.23	nd	–	
Green bird's eye chili pepper	19.5 ± 3.7	10 740 ± 3 580	413	[24]
Red chili pepper	7.45 ± 0.43	501 ± 49.0	476–933	[5, 18]
Thai white eggplant	3.27 ± 0.55	4.06 ± 0.89	56–129	[25]
Peas eggplant	16.7 ± 2.7	nd	56–129	[25]
Cherry tomato	4.82 ± 0.84	275.6 ± 54.7	24–233	[2, 18, 19]
Goji berry	35–280	na	–	[13]

Tab. 1: **Contents of ascorbic acid (AA) and (2-β-gAA) compared to published reference values.**
In mg/ 100 g dry weight, mean value and standard deviation, n = 3.
nd = not dedectable (> 0,5 mg/110 g), na = not analyzed

The contents of AA and 2-β-gAA of the different fruits and the calyx from cape gooseberry are summarized in ◆ Table 1. Surprisingly, the 2-β-gAA was found in all the five fruits analyzed and even in the cape gooseberry calyx. The analogue's content ranged from 3.27 mg/100 g dw for Thai white eggplants up to 19.5 mg/100 g dw for green bird's eye chili peppers. However, the amounts are far below when compared to goji berries, ranging from 35 to 280 mg/100 g dw [13]. Moreover, all the fruits showed a significant higher content in AA than in 2-β-gAA, except for the Thai white eggplants. The AA contents of cape gooseberries and red chili peppers were within the range reported in literature [3, 18]. Hanson et al. who analyzed 35 varieties of eggplants reported a much higher AA contents than found in the present study for Thai white eggplants [6]. A similar situation was observed for green bird's eye chili peppers and cherry tomatoes showing much higher values in the present study than in the literature [5, 18–21]. The differences might be explained by the different geographical origin, varieties, cultivation conditions, degree of ripeness or storage time [4, 22, 23]. Additionally, diverse extraction and analysis methods give varying results.

The highest relative standard deviations are observed in Bird's Eye chili peppers, Thai white eggplants and cherry tomatoes. This is a result of the low extraction reproducibility of the plant matrix, coming from the difficulty to break and shear the hard and resistant fruit peel. The difficulties of AA degradation by oxidation was mastered by the protecting and stabilizing effect of the DTT solution. Ultraturax treatment heated the plant preparation, which did most probably not affect the analogue content; its high temperature stability has been shown recently [7]. This high stability is also confirmed by the analogue's presence in naturally dried calyx of the cape gooseberries, which is completely devoid of AA. Traces of the analogue were also found in fruits from other families than the Solanacea such as grapes *(Vitis vinifera)* and blueberries *(Vaccinium myrtillus)* but not in raspberries *(Rubus idaeus)* (data not shown). Consequently, carrying out more studies on other fruits to identify new sources of the analogue and to get the whole picture would be of interest.

Conclusions

The present study confirmed for the first time that fruits from the *Solanacea* family, other than goji berries, contain 2-β-gAA. However, content in these fruits are lower when compared to goji berries [13]. AA is a very sensitive compound and can be easily oxidized during processing and storage. Therefore, the presence of its more stable analogue in fruits is pertinent for the nutrients profile of these foods and products made thereof. In addition to this study, bioavailability and bioactivity of 2-β-gAA in human body still needs to be entirely investigated for a better understanding of its physiological behavior. Studies into this direction are underway.

Conflict of Interest
The authors declar no conflict of interest.

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