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Research Article

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Vitamin E and carotenoid profiles in leaves, stems, petioles and flowers of stinging nettle (*Urtica leptophylla* Kunth) from Costa Rica

Alexander Montoya-Arroyo,[a] Camilo Toro-González,[b] Nadine Sus,[a] Jorge Warner,[c] Patricia Esquivel,[b] Víctor M Jiménez[d] and Jan Frank[a*]

Abstract

BACKGROUND: Local leafy vegetables are gaining attention as affordable sources of micronutrients, including vitamins, pro-vitamin carotenoids and other bioactive compounds. Stinging nettles (*Urtica* spp.) are used as source of fibers, herbal medicine and food. However, despite the relatively wide geographical spread of *Urtica leptophylla* on the American continent, little is known about its content of vitamin E congeners and carotenoids. We therefore investigated the particular nutritional potential of different plant structures of wild Costa Rican *U. leptophylla* by focusing on their vitamin E and carotenoid profiles.

RESULTS: Young, mature and herbivore-damaged leaves, flowers, stems and petioles were collected and freeze-dried. Vitamin E and carotenoids were determined by high-performance liquid chromatography after liquid/liquid extraction with hexane. α-Tocopherol was the major vitamin E congener in all structures. Flowers had a high content of γ-tocopherol. Herbivore-damaged leaves had higher contents of vitamin E than undamaged leaves. Lutein was the major and β-carotene the second most abundant carotenoid in *U. leptophylla*. No differences in carotenoid profiles were observed between damaged and undamaged leaves.

CONCLUSION: The leaves of *U. leptophylla* had the highest nutritional value of all analyzed structures; therefore, they might represent a potential source of α-tocopherol, lutein and β-carotene.
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Supporting information may be found in the online version of this article.

Keywords: stinging nettle; carotenoids; pro-vitamin A; tocopherols; tocotrienols; herbivory

INTRODUCTION

Urtica leptophylla Kunth is a herbaceous plant, a member of the group of stinging nettles and native to the American continent.[1] Stinging nettles (*Urtica* spp.) are perennial herbaceous plants of the Urticaceae family, characterized by the presence of trichomes ('stinging hairs') on stems and leaves that produce a stinging sensation upon contact.[2,3] Stinging nettles grow worldwide under variable climatic conditions,[2,3] but mostly in humid environments in northern temperate zones.[4] The *Urtica* genus includes more than 40 species, with *U. dioica* L. and *U. urens* L. being the most predominant ones.[5] More than 20 species have been found on the American continent, with a large distribution from the northern USA to Chile and Argentina.[6] *Urtica leptophylla* Kunth, in particular, has been reported in Latin-American countries, including Peru,[7,8] Nicaragua[8] and Costa Rica.[8-10]

Stinging nettle has been used as herbal medicine, food, feed[11] and source of fibers for textile production.[5,12] Stinging nettles become unharmful after drying or boiling[5] and have been proposed as a food additive[13,14] and as a source of bioactive compounds, such as vitamin E,[15-17] carotenoids[15,18,19] and (poly) phenols,[7,18,20] among others. Stinging nettle has low nitrogen requirements and can be grown in marginal areas,[12] and efforts to increase cultivation and commercialization of this plant have been undertaken[3]; however, up to now the use of stinging nettle in Costa Rica has been scarce and limited to some ethno-medical applications.[9,10]

* *Correspondence to: J Frank, Department of Food Biofunctionality, Institute of Nutritional Sciences, University of Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany. E-mail: jan.frank@nutres.de*

a *Department of Food Biofunctionality, Institute of Nutritional Sciences, University of Hohenheim, Stuttgart, Germany*

b *School of Food Technology, Universidad de Costa Rica, San Pedro, Costa Rica*

c *Jardín Botánico Lankester, Universidad de Costa Rica, Cartago, Costa Rica*

d *CIGRAS/IIA, Universidad de Costa Rica, San Pedro, Costa Rica*

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Vitamins E and A are both essential liposoluble micronutrients in the human diet. Vitamin E comprises eight structurally related tocochromanols (α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol) and protects membrane lipids from oxidation. Among all vitamin E congeners, α-tocopherol has the highest biological activity.[21,22] Vitamin E is mostly produced in photosynthetic tissues, and most congeners accumulate directly in the tissues where they are synthesized, while some, like γ-tocopherol, accumulate in the oil fractions of seeds.[23] Vitamin A is essential for reproduction, growth and development, immune function, vision and more.[24] Among carotenoids, all-*trans*-β-carotene is the most relevant precursor of vitamin A.[25]

Vitamin E and carotenoids play relevant roles in chloroplast integrity and function and are thus generally present in the green structures of plants, such as the leaves.[26,27] Interest in the vitamin E and pro-vitamin A contents of indigenous leafy vegetables has increased,[28] since they have been proposed as affordable sources of micronutrients to improve dietary diversity and food security in low- and middle-income populations.[29] Contents of vitamin E[15-17] and pro-vitamin A carotenoids have been reported for *U. dioica*[19,30,31] but not for *U. leptophylla*. A better knowledge of the profiles of vitamin E congeners and pro-vitamin A carotenoids in *U. leptophylla* might therefore be useful for assessing the nutritional potential of this underutilized food source, which might result in its valorization as an affordable leafy vegetable. We therefore investigated the vitamin E and carotenoid profiles in the main structures of the plant as well as the impact of herbivore-induced damage to leaves.

MATERIALS AND METHODS

Chemicals

RRR-α-Tocopherol, *RRR*-β-tocopherol, *RRR*-δ-tocopherol, *RRR*-γ-tocopherol (≥95%; Calbiochem-Novabiochem Corp. (Merck Group), Darmstadt, Germany), α-tocotrienol, β-tocotrienol, δ-tocotrienol, γ-tocotrienol (≥97%, Sigma-Aldrich (Merck Group), Darmstadt, Germany) stock solutions were prepared by dilution in ethanol and concentrations were confirmed photometrically.

β-Carotene (≥97%, Sigma-Aldrich), α-carotene (≥98%, Sigma-Aldrich), β-cryptoxanthin (ROTICHROM TLC, Carl Roth GmbH & Co. KG, Karlsruhe, Germany), lutein, zeaxanthin and lycopene (F. Hoffmann-La Roche AG, Basel, Switzerland) stock solutions were prepared by dilution in chloroform and concentrations were confirmed photometrically.

Plant material

Stinging nettle (*U. leptophylla*) samples were collected in September 2018, close to a water stream, on the side of the road to the Irazú Volcano, Santa Rosa district, Oreamuno County, Cartago Province, Costa Rica (9° 57′ 32.9″ N, 83° 50′ 33.8″ W and 2770 m above sea level (a.s.l.)). Collection was conducted with permits from the Costa Rican National System of Conservation Areas (SINAC-SE-CUSBSE-057-218 and SINAC-SE-CUSBSE-PI-R-018-2018) and University of Costa Rica (Universidad de Costa Rica, VI-6412-2018 and Resolución #159). Samples were botanically identified by Christian Trejos-Hernández and subsequently deposited in the Herbarium of the University of Costa Rica (USJ) under the reference tag *Trejos-Hernández 141* (USJ).

Sampling and sample preparation

Plant structures (stems, petioles, flowers, and young, mature and herbivore-damaged leaves) were collected from several adult plants < 1 m height growing together. Naturally herbivore-damaged leaves were characterized by visual inspection and separated from other mature leaves during collection. A mixed sample of the different plants was prepared for every structure before pretreatment. All structures were manually separated, frozen in liquid nitrogen, vacuum-sealed in laminated foil and stored at −60 °C. Subsequently, samples were lyophilized for 72 h under light protection in a laboratory freeze-dryer (Alpha 1–2 LD, Christ, Osterode, Germany). Moisture content was determined gravimetrically, and freeze-dried and vacuum-sealed samples were shipped by air to the University of Hohenheim (Germany) protected from light exposure. Samples were then ground to powder. Leaves were ground using a commercial blade coffee-grinder and petioles, stems and flowers with pistil and mortar after addition of liquid nitrogen. Ground samples were stored at −80 °C protected from light and moisture until extraction.

Extraction of vitamin E and carotenoids

Liposoluble compounds were extracted as described previously.[32] Briefly, six replicates of 100 mg (50 mg for petioles and flowers) of each freeze-dried sample were mixed with 2 mL of 1% ascorbic acid in ethanol (w/v), 900 µL deionized water and 600 µL saturated potassium hydroxide. Samples were saponified for 30 min at 70 °C under continuous agitation. After cooling on ice, 25 µL butylated hydroxytoluene in ethanol (1 mg mL^{-1}) was added. Samples were neutralized by adding 600 µL glacial acetic acid. Lipophilic compounds were extracted with 2 mL high-performance liquid chromatography (HPLC)-grade hexane by manual inversion for 1 min. After centrifugation (188 × *g* at 4 °C for 3 min), 1.5 mL of the supernatant was transferred to a glass tube. Hexane extraction was repeated three more times with 2 mL fresh hexane each and recovery of 2 mL supernatant. All supernatants (7.5 mL) were mixed together and solvent was eliminated in a centrifugal evaporator (RVC 2–25 CD Plus, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) under light protection. The dried residue was suspended in 200 µL HPLC-grade ethanol, transferred to micro-centrifuge tubes, and cooled on ice for 10 min. After centrifugation at 17 000 × *g* and 4 °C for 1 min (Heraeus Fresco 17 microcentrifuge, Thermo Fisher Scientific, Waltham, MA, USA), the clear liquid phase was further diluted with HPLC-grade ethanol and transferred to two different amber HPLC vials for vitamin E and carotenoid determination.

HPLC analysis of vitamin E

For vitamin E analysis,[32] 20 µL of the ethanolic suspension was injected into a Jasco HPLC system (LC-Net II/ADC controller, P-U2080 Plus pumps, AS-2059-SF Plus auto injector, co-2060 Plus column oven, LG-2080-02S mixer, DG-2080-53 degasser and FP-2020 Plus fluorescence detector) and separated on a Phenomenex Kinetex PFP column (2.6 µm particle size, 100 × 4.6 mm) maintained at 40 °C and a mobile phase of methanol and water (80:20, v/v), delivered at a flow rate of 1.2 mL min^{-1} for 30 min. Autosampler temperature was set to 5 °C. The fluorescence detector was set to excitation/emission wavelengths of 296/325 nm. Identification of vitamin E congeners was performed by comparing the retention times with those of the authentic standards of α-, β-, δ-, γ-tocopherol and α-, β-, δ-, γ-tocotrienol. Quantification was done by comparing areas of sample peaks to those of the corresponding authentic standards. Tocochromanol determination was performed in six biological replicates.

HPLC analysis of carotenoids

For carotenoid analysis,[33] ethanolic suspensions were diluted in acetonitrile–methanol–water (85:10:5, v/v) and 20 µL of the resulting mixture or standard was injected into a Shimadzu HPLC system (SCL-10A LP controller, SIL-20 AC HT autosampler, CTO-10AS VP

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column oven, DGV-14A degasser, SPD 20A UV–visible detector, FCV-10 AL mixer, LC-20AT pumps). For separation, a ReproSil 80 ODS-2 column (3 µm, 250 × 4.6 mm^2) maintained at 40 °C was used (Dr Maisch GmbH, Ammerbuch Entringen, Germany). Autosampler temperature was set to 5 °C. The mobile phase was a mixture of acetonitrile, 1,4-dioxane and methanol (82:15:3, v/v), 100 mmol L^{-1} ammonium acetate and 0.1% trimethylamine delivered at a flowrate of 1.5 mL min^{-1} for 20 min. Analytes were quantified with a UV–visible detector set to 450 nm. Identification of carotenoids was performed by comparing the retention times with those of the authentic standards of β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene. Quantification was done by comparing areas of sample peaks to those of the corresponding authentic standards. Carotenoid determination was performed in four biological replicates.

Statistical analyses

Values are reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine significant differences, with a significance level of 0.05 ($\alpha = 0.05$).

RESULTS AND DISCUSSION

In the present study, vitamin E and carotenoid values are expressed as mg kg^{-1} dry weight (DW). Moisture contents were calculated from water loss after freeze-drying of fresh samples and were 90.0 ± 0.2% for petioles, 89.3 ± 0.4% for stems, 77.8% for flowers, 78.4% for young leaves, 77.2 ± 1.9% for mature leaves and 76.8 ± 0.7% for herbivore-damaged leaves. Flowers and young leaves were freeze-dried in a single batch, due to limited sample availability, and therefore no standard deviation can be reported.

Vitamin E profiles in the different structures of *Urtica leptophylla*

Total tocochromanol and total tocopherol concentrations were significantly higher ($P < 0.05$) in mature leaves and flowers than in stems and petioles of Costa Rican *U. leptophylla* (Table 1), which was also observed for β- and γ-tocopherol. Stems and petioles did not differ in the contents of any of the congeners analyzed. Flowers had a higher content of γ-tocopherol ($P < 0.05$), but lower content of α-tocopherol ($P < 0.05$) than leaves (Table 1). However, the total tocopherol contents of flowers and leaves were similar. The tocotrienol contents were overall lower than those of tocopherol. No significant differences between structures were observed for total or individual tocotrienol congener concentrations (Table 1). Total tocochromanol content was 2.3, 2.0 and 1.2 times higher in mature leaves compared to stems, petioles and flowers, respectively. A similar trend was observed for α-tocopherol, with a content in mature leaves exceeding by 2.3, 2.1 and 1.7 times that observed in stems, petioles and flowers, respectively.

Tocopherols represented at least 85% of the total tocopherol content in all structures without any difference between them (Fig. 1A). α-Tocopherol was, by large, the most prevalent congener quantified. The relative content of α-tocopherol (percentage of total tocopherols) was lower in flowers (Fig. 1B), while that of γ-tocopherol was more than two times higher in flowers compared to the other analyzed tissues (Fig. 1C).

Considering a fresh/dry weight relationship of 5/1 in the tissues analyzed in this work, tocochromanol concentrations in *U. leptophylla* were close to those found in fresh *U. dioica* leaves by HPLC in Slovenia (α-tocopherol 36.7, γ-tocopherol 1.8 and δ-tocopherol 0.6 mg kg^{-1} fresh weight (β-tocopherol was not analyzed)).[15] On the other hand, higher α-tocopherol concentrations in the range of 180–740 mg kg^{-1} DW have been reported for freshly collected wild *Urtica* leaves by means of paper chromatography.[16] However, care should be taken when comparing vitamin E results obtained with different methods, especially when less specific classical approaches are involved.[34] Moreover, α-tocopherol concentrations from 50 to 300 mg kg^{-1} DW, which are within the values observed by us (Table 1), were reported for leaves from *U. dioica* cultivated in a greenhouse.[17]

Vitamin E values in the mature leaves of *U. leptophylla*, especially those of α-tocopherol – the most commonly analyzed congener – surpass those reported for dry oregano and rosemary leaves,[35,36] pointing to their potential as a source of this relevant congener. However, in the case of the other tocopherols analyzed, oregano had higher values, especially of γ-tocopherol. In addition, α-tocopherol values in the mature leaves in our work closely match those reported for dry leaves of *Piper sarmentosum*.[37]

The total tocopherol content in *U. leptophylla* leaves was higher than that reported for spinach (*Spinacia oleracea* L.) leaves (75.4–87.7 mg kg^{-1} DW), with a similar trend observed for α-tocopherol (65.5–80.1 mg kg^{-1} DW) and γ-tocopherol (7.6–9.9 mg kg^{-1} DW).[38] The total tocopherol contents of wild leafy vegetables in Spain were 30.5 mg kg^{-1} DW for *Apium nodiflorum*, 43.6 mg kg^{-1} DW for *Foeniculum vulgare*, 80.3 mg kg^{-1} DW for *Montia fontana* and 116.5 mg kg^{-1} DW for *Silene vulgaris*,[39] and thus lower than the values for *U. leptophylla* leaves (Table 1). These results show that *U. leptophylla* might have the potential to supply nutritional-relevant vitamin E into the diet.

Urtica flowers possess not just higher absolute, but also relative contents of γ-tocopherol compared to the other structures. This may suggest a specific biological role for γ-tocopherol in flowers, since higher proportions of this congener have been reported for flowers and reproductive structures in some other herbaceous plants,[40] and tobacco.[41]

Tocotrienols were detected in minor amounts in leaves, petioles, stems and flowers. Although production of tocotrienols in photosynthetically active tissues is considered uncommon,[42] analysis by liquid chromatography–mass spectrometry (LC-MS) in full-scan mode indicated the presence of ions consistent with the expected ionization of tocotrienols in both leaves and flowers. Moreover, LC-MS signals consistent with α- and γ-tocomonoenol were observed, suggesting their presence as minor tocochromanol congeners in both leaves and flowers (see Supporting Information Tables S1 and S2; see Montoya-Arroyo *et al.*[43] for a detailed description of the LC-MS^n method used for tocochromanol analyses).

Carotenoid profiles in the different structures of *U. leptophylla*

Lutein was the major carotenoid in all structures, followed by β-carotene, with 2.6–7.8 times lower concentrations depending on structure. Even lower concentrations were measured for zeaxanthin, while β-cryptoxanthin and α-carotene were detected in minor quantities only (0.3–22 mg kg^{-1}). Mature leaves had higher concentrations of all detected carotenoids than flowers, petioles and stems (Table 2). Total carotenoid contents in leaves were 4.8, 4.2 and 5.8 times higher in mature leaves than in stems, petioles and flowers, respectively. The lutein content of mature leaves was 6.1, 5.4 and 6.5 times higher than in flowers, petioles and stems, respectively. The β-carotene content of leaves was 2.1, 2.3 and 4.2 higher than in flowers, petioles and stems, respectively.

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Table 1. Tocopherol and tocotrienol contents (mg kg^{-1} DW) in different structures of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 6$)

	Stems	Petioles	Flowers	Mature leaves
α-Tocopherol	51.1 ± 14.6b	55.7 ± 18.4b	69.6 ± 6.4b	119.3 ± 21.9a
β-Tocopherol	3.2 ± 0.8b	3.0 ± 0.3b	4.7 ± 6.3a	4.6 ± 0.3a
γ-Tocopherol	2.1 ± 1.1c	2.5 ± 0.8c	29.4 ± 1.6a	11.5 ± 0.8b
δ-Tocopherol	0.4 ± 0.2b	0.3 ± 0.1b	7.7 ± 3.6a	2.3 ± 0.3b
α-Tocotrienol	1.5 ± 1.0a	2.8 ± 3.9a	4.9 ± 4.7a	1.2 ± 0.9a
β-Tocotrienol	4.1 ± 3.6a	6.3 ± 6.8a	2.2 ± 3.1a	3.4 ± 3.3a
γ-Tocotrienol	1.0 ± 0.9a	1.4 ± 1.3a	2.3 ± 2.1a	0.8 ± 0.6a
δ-Tocotrienol	0.9 ± 0.7a	1.1 ± 1.0a	2.3 ± 2.9a	0.6 ± 0.6a
Total tocopherols	56.8 ± 16.6b	61.5 ± 19.2b	111.3 ± 9.2a	137.6 ± 22.7a
Total tocotrienols	7.5 ± 5.6a	11.7 ± 11.8a	11.6 ± 9.1a	6.1 ± 5.9a
Total	**64.2 ± 13.1b**	**73.2 ± 29.1b**	**122.9 ± 16.4a**	**143.7 ± 25.3a**

Values not sharing a letter are significantly different between plant structures; ANOVA, $P < 0.05$.

The total carotenoid contents in mature leaves (714 mg kg^{-1} DW) were significantly higher than those in all other botanical structures in our samples (Table 2) and of mature *U. dioica* leaves grown in a greenhouse (74.8 mg kg^{-1} DW).[19] When considering main detected carotenoids individually (lutein and β-carotene), values in the present work also exceed those reported elsewhere for vegetative structures (<184 and < 6.7 mg kg^{-1} DW, respectively.[14] However, reported total carotenoid contents of dried and ground *U. dioica* leaves from Nepal amounted to 3497 mg kg^{-1} DW,[31] while β-carotene is reported to reach ~400 mg kg^{-1} DW in *U. dioica* leaves from the greenhouse.[17] Both reports substantially exceed all above-mentioned data. Lower total carotenoid contents found in our *U. leptophylla* samples (stems, petioles and flowers) are within the range reported for kale,[44] while the highest (detected in *U. leptophylla* leaves) are just below those reported for *Moringa oleifera*.[45]

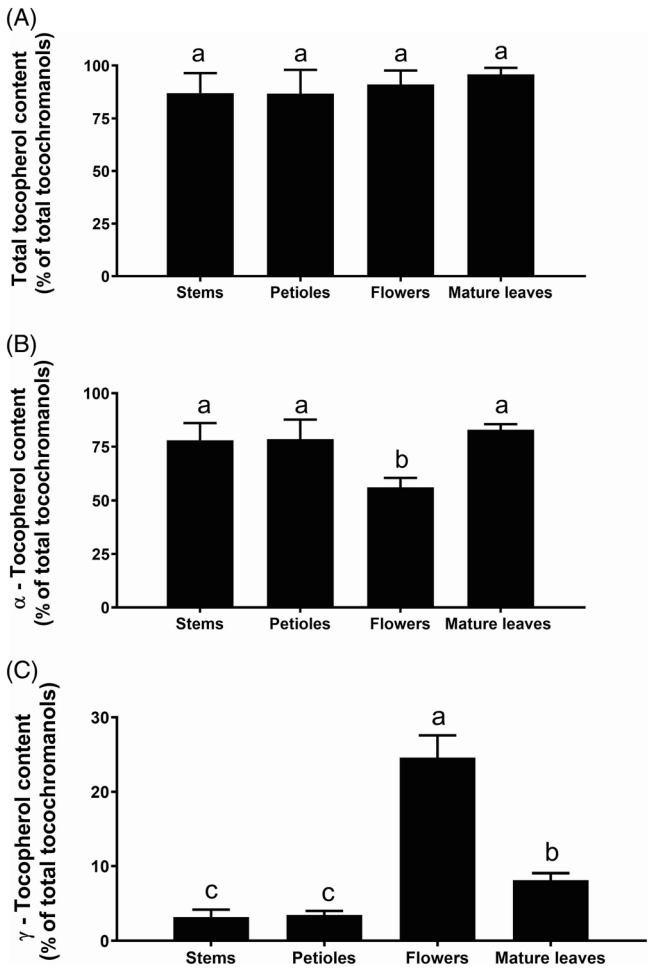

Figure 1. Relative contents of total tocopherols (A), α-tocopherol (B) and γ-tocopherol (C) calculated as percentage of total tocochromanol content of different plant structures of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 6$). Bars not sharing a letter are significantly different; ANOVA, $P < 0.05$.

The carotenoid profile in our *U. leptophylla* samples was dominated by lutein in all structures, with the highest contents in mature leaves (603 mg kg^{-1} DW; Table 2). Lutein content was much higher than the range of 23–184 mg kg^{-1} reported earlier for *U. dioica* leaves.[14,19] In agreement with our data, lutein has also been reported to be the major carotenoid in *U. dioca*.[15,19] We did not find lycopene in *U. leptophylla*, which is in contrast with previous findings reporting that lycopene amounted to ~2% of total carotenoids in mature leaves of *U. dioica*.[19] Zeaxanthin, on the other hand, was present in Costa Rican *U. leptophylla* at nearly 3% of total carotenoids, but only in minor concentrations in *U. dioica*.[15,46]

The observed differences between the vitamin E and carotenoid profiles of *U. leptophylla* and *U. dioica* may be the result of interspecific variations,[7,47] or geographical and climatic factors, such as rainfall patterns and irrigation,[12] season of collection,[16] and altitude.[48] Sufficient rainfall/irrigation is a relevant factor for proper plant development in *Urtica*,[12] and increased vitamin E content has been reported in grass plants growing in highlands compared to lowlands.[48] For *U. dioica*, seasonal variations in α-tocopherol contents have been reported, with up to four times higher α-tocopherol contents in samples collected in October (autumn) compared to April (spring).[16]

The *U. leptophylla* for the present study was sampled from a rainy area in the proximity of a water stream and over 2500 m a.s.l. Reports for *U. dioica* are widely distributed, including low-altitude zones like Ljubljana, Slovenia[15] (~307 m a.s.l.)[49] and regions like Kirtipur, Nepal[31] (1300 m a.s.l.),[50] among other collection sites. Effects of growing conditions on micronutrient profiles must be further addressed for *U. leptophylla* domestication.

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Table 2. Carotenoid contents (mg kg^{-1} DW) in different structures of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 4$)

	Stems	Petioles	Flowers	Mature leaves
Lutein	97.9 ± 4.8b	112.1 ± 12.8b	92.8 ± 7.2b	602.6 ± 122.9a
Zeaxanthin	10.4 ± 2.8b	12.2 ± 3.6b	10.4 ± 1.1b	21.5 ± 6.7a
β-Cryptoxanthin	0.3 ± 0.0b	0.3 ± 0.1b	0.3 ± 0.0b	3.3 ± 0.9a
α-Carotene	3.3 ± 0.3b	2.7 ± 0.9b	2.0 ± 0.5b	9.8 ± 7.5a
β-Carotene	37.2 ± 3.9b	33.7 ± 10.5b	18.2 ± 5.9b	76.7 ± 64.6a
Total	**148.9 ± 8.1b**	**160.9 ± 24.5b**	**123.7 ± 13.9b**	**713.9 ± 187.7a**

Values not sharing a letter are significantly different between plant structures; ANOVA, $P < 0.05$.

Lutein has been reported to be the predominant carotenoid in green vegetables of the Brassicaceae family, considered to be a relevant source of these compounds;[51] however, lutein contents in the leafy vegetables cabbage (9 mg kg^{-1} DW), Chinese cabbage (24 mg kg^{-1} DW) and pak choi (248 mg kg^{-1} DW) were lower than those of *U. leptophylla*. Moreover, *β*-carotene concentrations in cabbage (6 mg kg^{-1} DW), Chinese cabbage (8 mg kg^{-1} DW) and pak choi (97 mg kg^{-1} DW) were lower than in *U. leptophylla*.[52]

Conversely, higher lutein and *β*-carotene contents have been reported for freeze-dried spinach (1250 and 670 mg kg^{-1} DW) and kale (840 and 370 mg kg^{-1} DW)[53] than in *U. leptophylla*. Nonetheless, these values were observed under optimized growing conditions, while *U. leptophylla* plants were wild plants. Under non-optimal conditions, lutein and *β*-carotene contents in spinach (770 and 330 mg kg^{-1} DW) and kale (430 and 160 mg kg^{-1} DW)[53] were lower and closer to those observed in the present study.

In summary, *α*-tocopherol and total tocochromanol contents (2.3 and 2.0 times), total carotenoids (4.8 and 4.2 times), lutein (6.1 and 5.4 times) and *β*-carotene (2.1 and 2.3 times) in *U. leptophylla* were markedly higher in mature leaves compared to stems and petioles. Hence mature leaves of *U. leptophylla* appear to be the best source of these micronutrients among all analyzed structures.

Hence separation of the leaves from the stems, which could be used for non-nutritional applications, such as fiber production,[5,12] would enable a multipurpose production system, as suggested previously for this crop.[11] Production systems using arable land

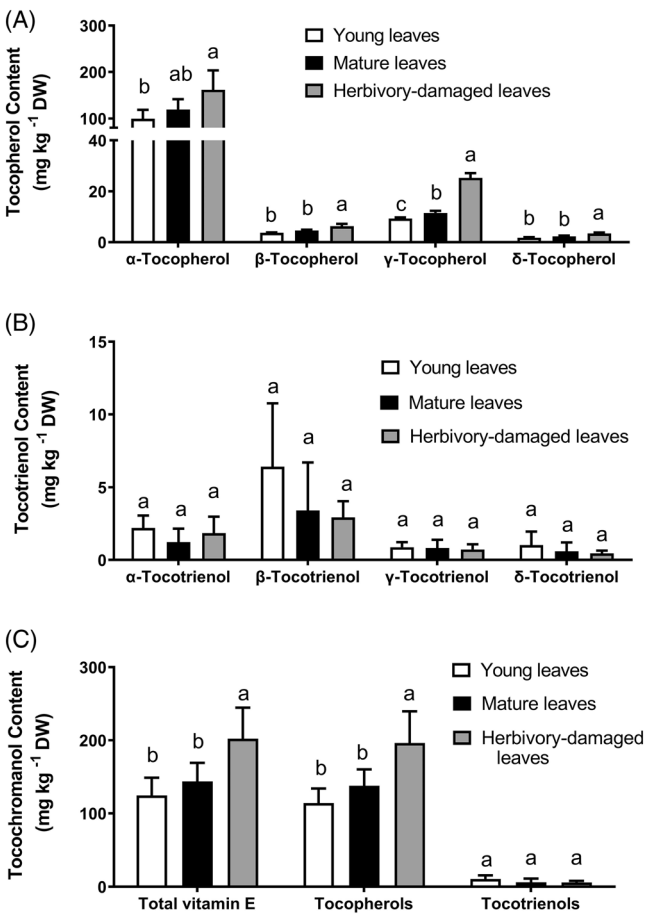

Figure 2. Tocopherol (A) and tocotrienol profiles (B) and total tocochromanol content (C) of young, mature and herbivore-damaged leaves of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 6$). Bars not sharing a letter are significantly different; ANOVA, $P < 0.05$.

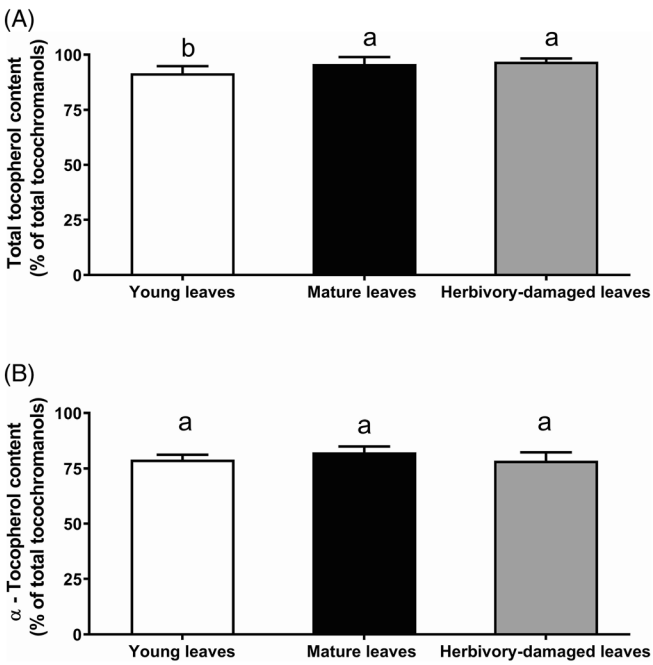

Figure 3. Relative content of total tocopherols (A) and *α*-tocopherol (B) calculated as percentage of total tocochromanol content of young, mature and herbivore-damaged leaves of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 6$). Bars not sharing a letter are significantly different; ANOVA, $P < 0.05$.

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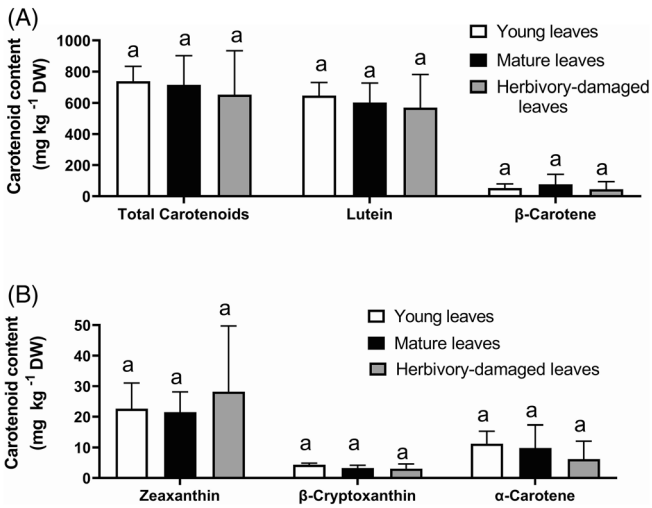

Figure 4. Major (A) and minor (B) components in carotenoid profiles of young, mature and herbivore-damaged leaves of *U. leptophylla* from Costa Rica as determined by HPLC ($n = 4$). Bars not sharing a letter are significantly different; ANOVA, $P < 0.05$.

to obtain industrial materials, such as fiber and lignocellulose, have been criticized as competitors for land use for food production.[54] The commercial cultivation of stinging nettle would open up the possibility to create cropping systems that balance both productive activities and may be a way to reduce pressure on land use. The possibility to obtain both leaves and stems of stinging nettle during different harvesting seasons during a year[11] may potentiate its use as a multipurpose crop.

In Europe, the recommended daily intake of vitamin E for adults is in the range of 11–13 mg (expressed as α-tocopherol equivalents)[22] and 580 µg of retinol equivalents for vitamin A.[55] A ratio of 12:1 is currently considered for the conversion of β-carotene to retinol equivalents.[55] Intake recommendations for lutein have not been set, despite its reported biological functions.[56] Considering the water content of mature *U. leptophylla* leaves of 77.2 ± 1.9%, a 100 g serving (fresh weight) of mature leaves from wild Costa Rican *U. leptophylla* would provide ~25% and 27% of the recommended daily intake of vitamin E and vitamin A (as provitamin A carotenoids), respectively.

Vitamin E and carotenoid profiles as a function of maturity of and herbivore-mediated damage to *U. leptophylla* leaves

Only the γ-tocopherol content was significantly higher in mature than in young leaves, while no other significant differences in the concentrations of total tocopherols, total tocotrienols, total tocochromanols or any of the other individual vitamin E congeners were observed when comparing young and mature leaves (Fig. 2). Nevertheless, tocopherols tended to be lower, whereas δ-tocotrienol and β-tocotrienol tended to be higher in young leaves compared to mature leaves. This led to a slight but significant reduction in the percentage of tocopherols of the total vitamin E in young leaves (Fig. 3A), but not of α-tocopherol, by far the most prevailing congener (Fig. 3B). Previously, lower concentrations of α-tocopherol in juvenile *U. dioica* leaves than in mature leaves were reported.[17] However, γ-tocopherol was not analyzed in this previous study and therefore further comparisons with our results are not possible. This is an example of the relevance of not assuming that related species or even genotypes within the same species share the same pattern of phytochemical accumulation, but that detailed analysis should be conducted case by case instead.

No significant differences in the concentrations of total or individual, major or minor, carotenoids as a function of maturity stage of leaves were observed (Fig. 4). Our observations that the carotenoid concentrations in leaves were not affected by maturation (Fig. 4) contrast with previous reports, where both lower[19] and

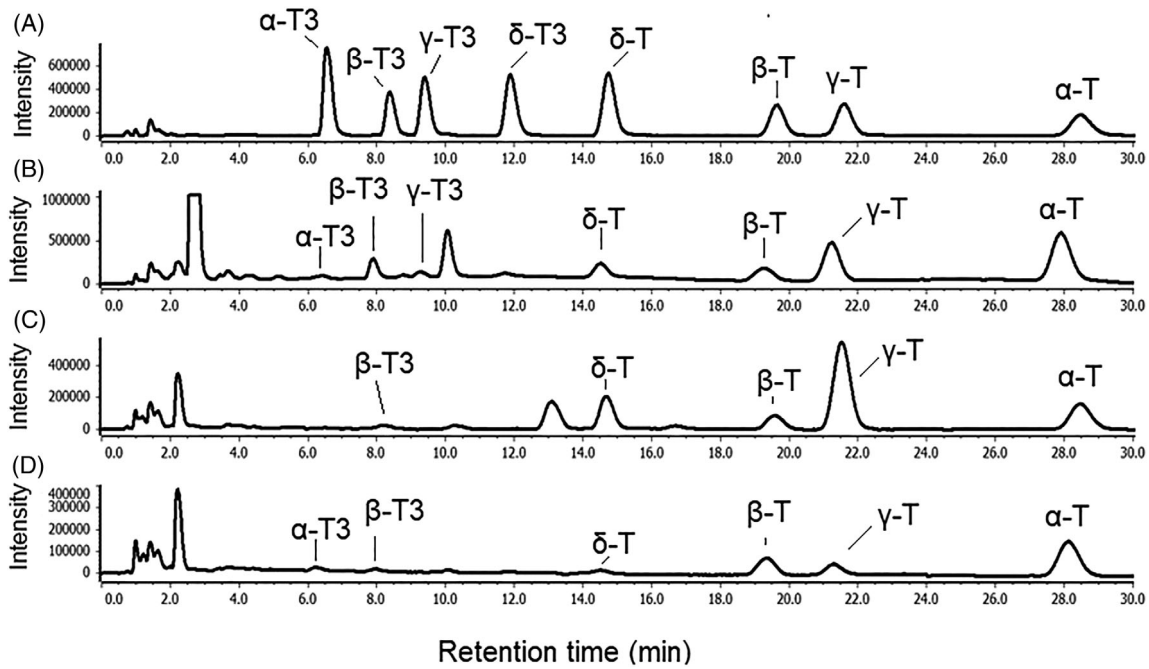

Figure 5. Representative HPLC chromatograms for the identification of tocotrienols (T3) and tocopherols (T) in standard (A), leaves (B), flowers (C) and petioles (D). Representative chromatograms do not have the same dilution factor due to sample preparation. Minor congeners are not visible under displayed conditions. The fluorescence detector was set to excitation/emission wavelengths of 296/325 nm.

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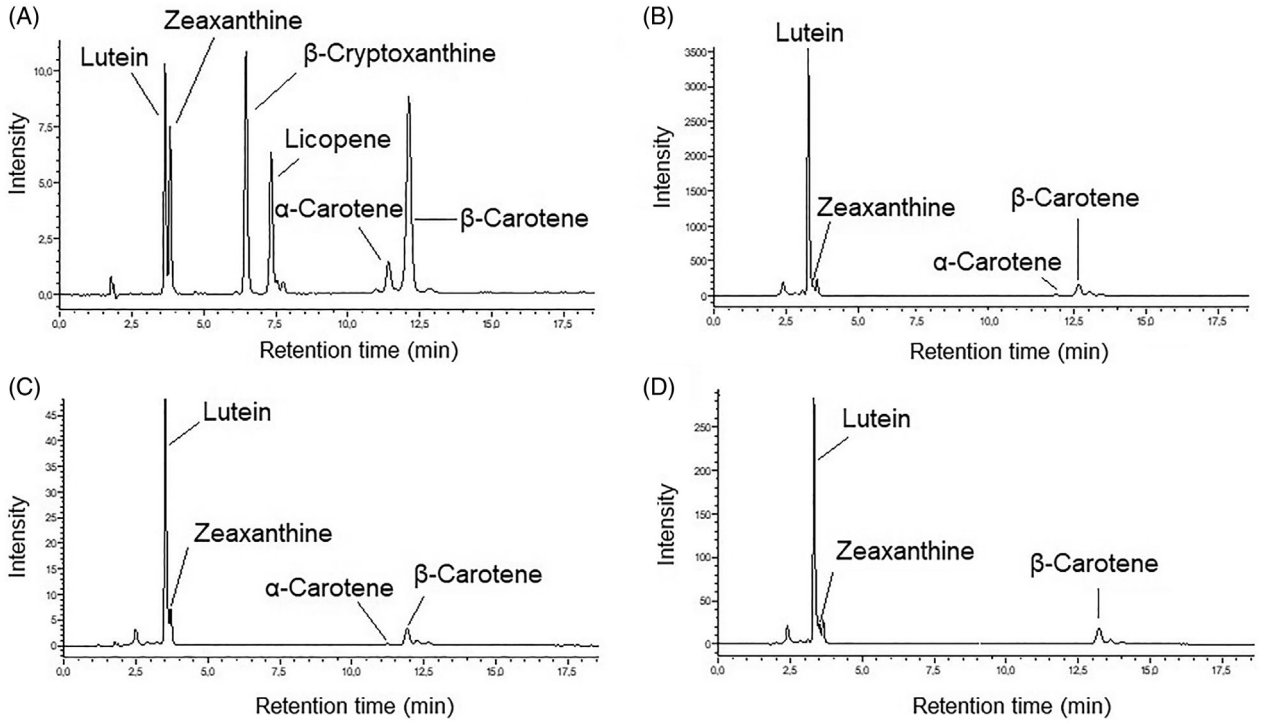

Figure 6. Representative HPLC chromatograms for the identification of carotenoids in standard mix (A), leaves (B), flowers (C) and stems (D). Representative chromatograms do not have the same dilution factor due to sample preparation. *β*-Cryptoxanthin is not visible under displayed conditions. The UV–visible detector was set to 450 nm.

higher[17] contents of total and individual carotenoids have been reported in young leaves of *U. dioica* compared to mature ones.

Herbivore-damaged leaves had significantly higher contents of total vitamin E, total tocopherol and *δ*-tocopherol, *β*-tocopherol and *γ*-tocopherol than undamaged leaves and showed a tendency to contain lower amounts of total and individual tocotrienols (Fig. 2). However, no significant differences in the proportion of total tocopherols or of *α*-tocopherol as percentage of the total tocochromanol content was observed between undamaged and damaged leaves (Fig. 3). No significant differences between herbivore-mediated damaged and undamaged leaves were observed when total or individual, major or minor, carotenoids were compared (Fig. 4).

Vitamin E concentrations have been reported to increase under stress conditions[36,57] and chlorophyll degradation and phytol recycling induce vitamin E biosynthesis.[58] Particularly, herbivore-mediated damage results in jasmonate accumulation[59] and activation of jasmonate-mediated signaling pathways[60] – cellular mechanisms that have been reported to increase tocopherol concentrations in plant cells[61,62] – and might explain the observed higher concentrations of vitamin E in damaged leaves (Fig. 2).

Representative vitamin E and carotenoid chromatograms used for isomer identification in *U. leptophylla* structures are presented in Figs 5 and 6.

CONCLUSIONS

The wild *U. leptophylla* investigated in the present study contained significant amounts of vitamin E and carotenoids, with higher vitamin E and carotenoid concentrations measured in the leaves than in the other parts of the plant. A portion size of 100 g fresh leaves would provide ~25% and 27% of the recommended daily intakes for vitamins E and A, respectively. *Urtica leptophylla* is thus a promising underutilized crop, of which the leaves could be separated from the stems to be used for their nutritional value, while the fiber-rich stems could be used for non-food applications.

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CONFLICT OF INTERESTS

None of the authors has a known conflict of interest regarding this research project.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Grosse-Veldmann B, Nürk NM, Smissen R, Breitwieser I, Quandt D and Weigend M, Pulling the sting out of nettle systematics – a

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comprehensive phylogeny of the genus *Urtica* L. (Urticaceae). *Mol Phylogenet Evol* **102**:9–19 (2016).
2 Weigend M and Luebert F, Weeding the nettles I: clarifying species limits in perennial, rhizomatous *Urtica* (Urticaceae) from southern and Central Chile and Argentina. *Phytotaxa* **2**:1–12 (2009).
3 Di Virgilio N, Papazoglou EG, Jankauskiene Z, Di LS, Praczyk M and Wielgusz K, The potential of stinging nettle (*Urtica dioica* L.) as a crop with multiple uses. *Ind Crops Prod* **68**:42–49 (2015).
4 Kregiel D, Pawlikowska E and Antolak H, *Urtica* spp.: ordinary plants with extraordinary properties. *Molecules* **23**:1664 (2018).
5 Pinelli P, Ieri F, Vignolini P, Bacci L, Baronti S and Romani A, Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of *Urtica dioica* L. *J Agric Food Chem* **56**:9127–9132 (2008).
6 Mutke J, Jacobs R, Meyers K, Henning T and Weigend M, Diversity patterns of selected Andean plant groups correspond to topography and habitat dynamics, not orogeny. *Front Genet* **5**:351 (2014).
7 Farag MA, Weigend M, Luebert F, Brokamp G and Wessjohann LA, Phytochemical, phylogenetic, and anti-inflammatory evaluation of 43 *Urtica* accessions (stinging nettle) based on UPLC–Q-TOF-MS metabolomic profiles. *Phytochemistry* **96**:170–183 (2013).
8 Weigend M, Cano A and Rodríguez EF, New species and new records of the flora in Amotape-Huancabamba zone: endemics and biogeographic limits. *Rev Peru Biol* **12**:249–274 (2005).
9 Badilla B, Mora G and Poveda LJ, Anti-inflammatory activity of aqueous extracts of five Costa Rican medicinal plants in Sprague-Dawley rats. *Rev Biol Trop* **47**:723–727 (1999).
10 Badilla B, Chaves F, Mora G and Poveda LJ, Edema induced by *Bothrops asper* (Squamata: Viperidae) snake venom and its inhibition by Costa Rican plant extracts. *Rev Biol Trop* **54**:245–252 (2014).
11 Vogl CR and Hartl A, Production and processing of organically grown fiber nettle (*Urtica dioica* L.) and its potential use in the natural textile industry: a review. *Am J Altern Agric* **18**:119–128 (2003).
12 Bacci L, Baronti S, Predieri S and Di Virgilio N, Fiber yield and quality of fiber nettle (*Urtica dioica* L.) cultivated in Italy. *Ind Crops Prod* **29**:480–484 (2009).
13 Fiol C, Prado D, Mora M and Alava JI, Nettle cheese: using nettle leaves (*Urtica dioica*) to coagulate milk in the fresh cheese making process. *Int J Gastron Food Sci* **4**:19–24 (2016).
14 Marchetti N, Bonetti G, Brandolini V, Cavazzini A, Maietti A, Meca G *et al*., Stinging nettle (*Urtica dioica* L.) as a functional food additive in egg pasta: enrichment and bioaccessibility of lutein and β-carotene. *J Funct Foods* **47**:547–553 (2018).
15 Šircelj H, Mikulic-Petkovsek M, Veberič R, Hudina M and Slatnar A, Lipophilic antioxidants in edible weeds from agricultural areas. *Turk J Agric For* **42**:1–10 (2018).
16 Booth VH and Hobson-Frohock A, The α-tocopherol content of leaves as affected by growth rate. *J Sci Food Agric* **12**:251–256 (1961).
17 Oñate M and Munné-Bosch S, Influence of plant maturity, shoot reproduction and sex on vegetative growth in the dioecious plant *Urtica dioica*. *Ann Bot* **104**:945–956 (2009).
18 Grauso L, de Falco B, Lanzotti V and Motti R, Stinging nettle, *Urtica dioica* L.: botanical, phytochemical and pharmacological overview. *Phytochem Rev* **19**:1341–1377 (2020).
19 Guil-Guerrero JL, Rebolloso-Fuentes MM and Isasa MET, Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). *J Food Compos Anal* **16**:111–119 (2003).
20 Exarchou V, Fiamegos YC, van Beek TA, Nanos C and Vervoort J, Hyphenated chromatographic techniques for the rapid screening and identification of antioxidants in methanolic extracts of pharmaceutically used plants. *J Chromatogr A* **1112**(1–2):293–302 (2006).
21 Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J *et al*., Vitamin E: emerging aspects and new directions. *Free Radical Biol Med* **102**:16–36 (2017).
22 EFSA, Scientific opinion on dietary reference values for vitamin E as α-tocopherol. *EFSA J* **13**:1–72 (2015).
23 Mène-Saffrané L and Vitamin E, Biosynthesis and its regulation in plants. *Antioxidants* **7**(1):2 (2017).
24 Meléndez-Martínez AJ, An overview of carotenoids, apocarotenoids, and vitamin A in agro-food, nutrition, health, and disease. *Mol Nutr Food Res* **63**:1801045 (2019).
25 Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G *et al*., β-Carotene is an important vitamin A source for humans. *J Nutr* **140**:2268S–2285S (2010).
26 Miret JA and Munné-Bosch S, Redox signaling and stress tolerance in plants: a focus on vitamin E. *Ann N Y Acad Sci* **1340**:29–38 (2015).
27 Domonkos I, Kis M, Gombos Z and Ughy B, Carotenoids, versatile components of oxygenic photosynthesis. *Prog Lipid Res* **52**:539–561 (2013).
28 Gowele VF, Kinabo J, Jumbe T, Kirschmann C, Frank J and Stuetz W, Provitamin A carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. *Foods* **8**:7–10 (2019).
29 Aworh OC, From lesser-known to super vegetables: the growing profile of African traditional leafy vegetables in promoting food security and wellness. *J Sci Food Agric* **98**:3609–3613 (2018).
30 Rutto LK, Xu Y, Ramirez E and Brandt M, Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.). *Int J Food Sci* **2013**:857120 (2013).
31 Adhikari BM, Bajracharya A and Shrestha AK, Comparison of nutritional properties of stinging nettle (*Urtica dioica*) flour with wheat and barley flours. *Food Sci Nutr* **4**:119–124 (2016).
32 Grebenstein N and Frank J, Rapid baseline-separation of all eight tocopherols and tocotrienols by reversed-phase liquid-chromatography with a solid-core pentafluorophenyl column and their sensitive quantification in plasma and liver. *J Chromatogr A* **1243**:39–46 (2012).
33 Stuetz W, McGready R, Cho T, Prapamontol T, Biesalski HK, Stepniewska K *et al*., Relation of DDT residues to plasma retinol, α-tocopherol, and β-carotene during pregnancy and malaria infection: a case–control study in Karen women in northern Thailand. *Sci Total Environ* **363**(1–3):78–86 (2006).
34 Pan Q, Shen M, Yu T, Yang X, Li Q, Zhao B *et al*., Liquid chromatography as candidate reference method for the determination of vitamins A and E in human serum. *J Clin Lab Anal* **34**:1–7 (2020).
35 Lagouri V and Boskou D, Nutrient antioxidants in oregano. *Int J Food Sci Nutr* **47**:493–497 (1996).
36 Munné-Bosch S, Schwarz K and Alegre L, Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. *Plant Physiol* **121**:1047–1052 (1999).
37 Ching LS and Mohamed S, Alpha-tocopherol content in 62 edible tropical plants. *J Agric Food Chem* **49**:3101–3105 (2001).
38 Pereira C, Dias MI, Petropoulos SA, Plexida S, Chrysargyris A, Tzortzakis N *et al*., The effects of biostimulants, biofertilizers and water-stress on nutritional value and chemical composition of two spinach genotypes (*Spinacia oleracea* L.). *Molecules* **24**:4494 (2019).
39 Morales P, Carvalho AM, Sánchez-Mata MC, Cámara M, Molina M and Ferreira ICFR, Tocopherol composition and antioxidant activity of Spanish wild vegetables. *Genet Resour Crop Evol* **59**:851–863 (2012).
40 Sickel H, Bilger W and Ohlson M, High levels of α-tocopherol in Norwegian alpine grazing plants. *J Agric Food Chem* **60**:7573–7580 (2012).
41 Abbasi A-R, Hajirezaei M, Hofius D, Sonnewald U and Voll LM, Specific roles of α - and γ -tocopherol in abiotic stress responses of transgenic tobacco. *Plant Physiol* **143**:1720–1738 (2007).
42 Munné-Bosch S and Alegre L, The function of tocopherols and tocotrienols in plants. *CRC Crit Rev Plant Sci* **21**:31–57 (2002).
43 Montoya-Arroyo A, Lehnert K, Lux PE, Jiménez VM, Esquivel P, Silva-Benavides AM *et al*., 11′-α-Tocomonoenol is the major α-tocomonoenol isomer in cyanobacteria and microalgae from Costa Rica. *J Food Compos Anal* **107**:104325 (2021).
44 Kaulmann A, Jonville M-C, Schneider Y-J, Hoffmann L and Bohn T, Carotenoids, polyphenols and micronutrient profiles of *Brassica oleraceae* and plum varieties and their contribution to measures of total antioxidant capacity. *Food Chem* **155**:240–250 (2014).
45 Saini RK, Shetty NP, Prakash M and Giridhar P, Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in *Moringa oleifera* leaves and preparation of a RTE product. *J Food Sci Technol* **51**:2176–2182 (2014).
46 Kudritsata SE, Filman GM, Zagorodskaya LM and Chikovanii DM, Carotenoids of *Urtica dioica*. *Chem Nat Compd* **22**:604–605 (1986).
47 Carvalho AR, Costa G, Figueirinha A, Liberal J, Prior JAV, Lopes MC *et al*., *Urtica* spp.: phenolic composition, safety, antioxidant and anti-inflammatory activities. *Food Res Int* **99**:485–494 (2017).
48 Wildi B and Lütz C, Antioxidant composition of selected high alpine plant species from different altitudes. *Plant Cell Environ* **19**:138–146 (1996).
49 Mezga K, Urbanc J and Cerar S, The isotope altitude effect reflected in groundwater: a case study from Slovenia. *Isotopes Environ Health Stud* **50**:33–51 (2014).
50 Bajrachharya N and Dyola U, Arthropod pests of pear (*Pyrus pyrifolia*, Nakai) in Central Horticulture Centre, Kirtipur, Kathmandu, Nepal. *Nepal J Zool* **3**:37–48 (2015).
51 Avato P and Argentieri MP, Brassicaceae: a rich source of health improving phytochemicals. *Phytochem Rev* **14**:1019–1033 (2015).

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52 Lee HW, Zhang H, Liang X and Ong CN, Simultaneous determination of carotenoids, tocopherols and phylloquinone in 12 Brassicaceae vegetables. *LWT – Food Sci Technol* **130**:109649 (2020).
53 Lefsrud M, Kopsell D, Sams C, Wills J and Both AJ, Dry matter content and stability of carotenoids in kale and spinach during drying. *HortScience* **43**:1731–1736 (2008).
54 Smith P, Gregory PJ, van Vuuren D, Obersteiner M, Havlík P, Rounsevell M *et al.*, Competition for land. *Philos Trans R Soc B* **365**:2941–2957 (2010).
55 EFSA, Scientific opinion on dietary reference values for vitamin a. *EFSA J* **13**:4028 (2015).
56 Ranard KM, Jeon S, Mohn ES, Griffiths JC, Johnson EJ and Erdman JW, Dietary guidance for lutein: consideration for intake recommendations is scientifically supported. *Eur J Nutr* **56**:37–42 (2017).
57 Munné-Bosch S and Alegre L, Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta* **210**:925–931 (2000).
58 Vom Dorp K, Hölzl G, Plohmann C, Eisenhut M, Abraham M, Weber APM *et al.*, Remobilization of phytol from chlorophyll degradation is essential for tocopherol synthesis and growth of *Arabidopsis*. *Plant Cell* **27**:2846–2589 (2015).
59 Reymond P, Bodenhausen N, Van PRMP, Krishnamurthy V, Dicke M and Farmer EE, A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell* **16**:3132–3147 (2004).
60 Verhage A, Rewiring of the jasmonate signaling pathway in *Arabidopsis* during insect herbivory. *Front Plant Sci* **2**:1–12 (2011).
61 Sandorf I and Holländer-Czytko H, Jasmonate is involved in the induction of tyrosine aminotransferase and tocopherol biosynthesis in *Arabidopsis thaliana*. *Planta* **216**:173–179 (2002).
62 Antognoni F, Faudale M, Poli F and Biondi S, Methyl jasmonate differentially affects tocopherol content and tyrosine amino transferase activity in cultured cells of *Amaranthus caudatus* and *Chenopodium quinoa*. *Plant Biol* **11**:161–169 (2009).

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