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# The antioxidant and phylloquinone content of wildly grown greens in Crete

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#### Abstract

The traditional Mediterranean diet of Crete is renowned for its very high consumption of olive oil, vegetables, legumes, fruit, fish, whole-wheat cereals and a moderate consumption of dairy products and meat. Wild greens play an important role in the traditional diet and are eaten either fresh in salads, boiled or cooked in pies, thus increasing the daily vitamin and antioxidant intake of the population that adheres to the traditional diet. Six cultivated and 48 wildly grown greens were collected and analyzed for their carotenoid, L-ascorbic acid, phylloquinone,  $\gamma$ -tocopherol,  $\alpha$ -tocopherol and total polyphenol content. In most cases, the wild greens had higher micronutrient contents than those cultivated. Certain wild greens, such as Rumex obtusifolius, Prasium majus and Lathyrus ochrus had higher concentrations of vitamin  $K_1$ , vitamin C, lutein,  $\beta$ -carotene,  $\gamma$ -tocopherol and total polyphenol content than those cultivated; proving the significance of these wildly grown greens for the well being of the Cretan population.

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Keywords: Carotenoids; Tocopherols; Polyphenols; Ascorbic acid; Phylloquinone; Wild greens; Cretan diet

# 1. Introduction

The traditional diet of Crete is said to be one of the most important reasons for the health and long life expectancy of its population. It first came to scientific attention with the seven countries study 40 years ago. The results of the study showed that the population of Crete had a low prevalence of cancers and coronary heart disease in comparison to the other participating populations (Keys, 1970). Recent studies have also found similar results (Ferro-Luzzi, James, & Kafatos, 2002; Renaud et al., 1995). The traditional Mediterranean diet of Crete is renowned for its very high consumption of olive oil, vegetables, legumes, fruits, fish, whole-wheat cereals and a moderate consumption of dairy products and meat.

The large variety of wildly grown edible greens differ every season with most varieties grown in spring and usually collected and eaten fresh in salads with plenty of virgin olive oil, or mixed and cooked with tomatoes, onions, meat or fish. They are also mixed and prepared as pies, thus increasing the daily vitamin and antioxidant intake.

Edible wild plants are a common food source for the older generations of the population of Crete and continue today in families which maintain the traditional lifestyle. The Greek Orthodox religion plays an important role in the traditional diet, recommending approximately 180 or more days of fasting from meat, eggs and dairy products per year. This periodic vegetarian diet of legumes, nuts, fruits, olives, bread, snails; and seafood plays an important role in the population's health (Sarri, Linardakis, Bervanaki, Tzanakis, & Kafatos, 2004), especially by reducing serum lipid values over the fasting periods (Sarri, Tzanakis, Linardakis, Mamalakis, & Kafatos, 2003). During the main fasting period of Lent, which coincides with spring,

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wildly grown greens are particularly popular. These edible plants most likely provide health benefits, therefore the objective of the present research was to collect, chemically analyze and summarize the nutritional composition of 54 commonly eaten greens, six that are cultivated and 48 that grow wildly in Crete, for carotenoid (lutein and  $\beta$ -carotene), vitamin C (L-ascorbic acid), total polyphenols,  $\gamma$ tocopherol,  $\alpha$ -tocopherol and phylloquinone (vitamin K<sub>1</sub>) content. Furthermore, the present study aims to identify potential health benefits associated with the adherence to a diet rich in these wild greens.

## 2. Methods

## 2.1. Field collection

The wild greens were collected between mid January and early March in 2002 from three different prefectures of Crete. In Heraklion, from the village of Venerato and from the Valley of Messara; in Rethymno, from the villages of Panormo, Monastiraki and Drimiskos. Finally, collections were also made from the Lasithi plateau in the prefecture of Lassithi, at an altitude of 1200 m. Forty-eight wild plants were collected. For reason of comparison, samples of 6 commonly cultivated vegetables in Crete were also taken from plants bought from the supermarket. Table 1 shows the complete list of plant samples along with their common and scientific names where available.

## 2.2. Laboratory processing

The wild Cretan greens were sent to the Department of Nutritional Sciences in Vienna (Austria) for the analysis. Immediately after receipt the samples were coded, homogenized, freeze dried and frozen at -80 °C until analysis.

#### 2.3. Analytical methods

# 2.3.1. Polyphenols

The concentration of total phenolics was determined photometrically by the method of Folin–Ciocalteu, using gallic acid as standard (Linkens & Jackson, 1988). The absorbance of the sample was measured at 720 nm after 1 h of reaction. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of greens. The intra-assay variation was 3.9%.

## 2.4. Vitamin C (L-ascorbic acid)

The content of vitamin C (as an ascorbic acid) was determined photometrically by using the commercial enzymatic bio-analysis/food analysis test of Boeringer Menheim.

# 2.5. Vitamin $K_1$ (pylloquinone)

Sample extraction and the HPLC assay (Jakob & Elmadfa, 1996) for the determination of phylloquinone

Table 1		
Common	and	scient

Common and scientific names of the Cretan plai	its
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Common name	Latin name (where available)
Cultivated greens	
Lattuce, organic	Lactuca sativa
Lattuce, non-organic	Lactuca sativa
Spinach	Spinacea oleracea var. glabra
Broad beans	Vicia faba
Artichokes	Cynara carduncelus var. scolymus
Kokkinogoulia	Beta vulgaris
Wild greens	
Radikia	Taraxacum spp.
Stafilinakes	Daucus carota
Fasoulides	_
Lapatha	Rumex obtusifolius
Pikrorodika	Taraxacum spp.
Wild leeks	Allium scoenoprasm
Petrahatziki	Daucus carota
Papoules	Lathyrus ochrus
Agriopapoules	Silene vulgaris subsp. macrocarpa
Akoumopodl	-
Glikorodika	Taraxacum spp
Galatsides	Taraxacum spp. Baihandia pianaidas
	Reihardia picroides
Miridouses (wild)	Apium graveolens
Maratha	Foeniculum vulgare
Lapsana	Sinapis spp. gruciferae
Ahatziki	_
Parsley (wild)	Petroselinum sativum
Agrioselino	—
Kalogeros	-
Avizitis	-
Stravoksilo	Scabiosa cretica
Goules or Askolibri	Scolumus hispanicus
Agoglossi	Cynoglossum creticum
Hiromourides	Hypochoeris cretensis
Avronies	Bryonia cretica
Lagoudohorto	Prasium majus
Maroulides	_
Volvi or Askordoulaki	Muscari commosum
Tsohi	Sonchus oleraceus
Pigounites	Tragopogon sinuatus
Roka	Eruca sativa
Artichokes (wild) (stems only)	Cynara comigera
Radish bitter, (semi-cultivated)	Cichorium intibus
	Cichorium
Radish (wild)	Cicnorium
Pikroussa	—
Petsetes	-
Stamnagathi	Cichorium spinosum
Strufoulia	Solanum nigrum
Pahies or pikrorodiko	_
Glikossirides	Crepis commutata
Koutsounades	Papaver rhoeas
Skouloi	Tragopogon sinuatus
Spinach (wild)	_
Harakoulia	_
Katsoules	_
Pontikines	_
	Crepis vesicaria
Pikrosirides	Crepts vesicaria

was carried out according to the slightly modified (modification: filtration after extraction and addition of methanol; only dichloromethan/methonal was taken as an extraction mixture; for details see the text below) HPLC method. One to two g of the homogenized samples were taken for extraction with a mixture of dichloromethane/methanol (2:1 v/v, sample/solvent 1:30). The extract was filtered through dehydrated sodium sulfate and made up to 100 ml with methanol. One millilitre of this sample was taken, and 1 ng of the internal standard (2',3'-dihydrophylloquinone dissolved in ethanol) was added and then be solved evaporated. The residue was redissolved in hexane, which was followed by purification, as described below. A volume of 4 ml of the mixture of methanol/water (9:1 v/v) was added to the hexane extracts, vigorously mixed for 2 min and centrifuged for 5 min at 3000 rpm. The upper hexane layer was removed and evaporated to dryness (15 min, 40°C, vacuum). After dissolving the residue in 150  $\mu$ l of the eluent, it was injected into the HPLC.

## 2.6. HPLC system

The HPLC system consisted of a high precision pump model 300B, an analytical column Gynkotek ODS Hypersil ( $250 \times 4.6 \text{ mm}$  i.d.,  $5 \mu \text{m}$ ), a guard-column Gynkotek ODS Hypersil ( $20 \times 4.6 \text{ mm}$  i.d.,  $5 \mu \text{m}$ ) – all from Gynkotek, Germering, Germany, a reduction-column dry filled with zink powder ( $20 \times 4.0 \text{ mm}$  i.d., Bischoff, Leonberg, Germany), injection device (Rheodyne, Cotati, USA), column-heating, cosytherm (Labortechnik Barkey, Bielefeld, Germany) and U3 (Julabo, Seelbach, Germany), Fluoroscence Spectrophotometer F-1050 and Chromato-Integrator D-2500 (both from Merck, Darmstadt, Germany).

The mobile phase contained 100 ml of dichloromethane and 900 ml of methanol. This solvent was then combined with 5 ml of a methanolic solution, containing 1.37 g of zinc chloride, 0.41 g of sodium acetate and 0.30 g of acetic acid. Detection was carried out with an excitation wavelength of 243 nm and an emission wavelength of 430 nm. The concentrations were calculated by peak height ratios, using a linear regression curve from standard solutions containing 1.0 ng 2',3'-dihydrophylloquinone as an internal standard. The intra-assay variation was 5.6%.

#### 2.7. Carotenoids and tocopherols

Lipid phases from wild plants were obtained according to the method of Folch, Less, and Stoane-Stanley (1957). After the extraction of a sample with chloroform–methanol (2:1, v/v, sample/solvent: 1/30) the lipid phase was separated from the water phase by addition of a 0.05 molar calcium chloride solution. The lower chloroform phase (lipid phase) was filtered through dehydrated sodium sulfate and then 1 ml was taken, evaporated with nitrogen, reconstituted in 150  $\mu$ l of eluent and used for analysis.

# 2.8. HPLC (high performance liquid chromatography)

Carotenoids and tocopherols were determined according to the slightly modified isocratic HPLC method by Jakob and Elmadfa (1995). Carotenoids and tocopherols were separated with an analytical column VYDAC 201TP54 (RP-18, 300 Å, 5  $\mu$ , 250 × 4.6 mm). The mobile phase consisted of methanol/acetonitrile/dichloromethane 85:10:5 (v/v). Peak responses were measured at 450 nm for all carotenoids and at 295 nm for  $\alpha$ - and  $\gamma$ -tocopherol using UV/Vis detector. Carotenoids and tocopherols were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of external standards. The intra-assay variation was about 2% for both tocopherols, 4% for lutein, 5.8% for  $\beta$ -carotene.

## 3. Results

#### 3.1. Vitamin $K_1$ (pylloquinone)

Table 2 shows the concentration of vitamin  $K_1$ . The highest amount of vitamin K was found in *Lagoudohorto* with 373 µg/100 g of fresh matter. Very high amounts were also found in *Rumex obtusifolius* (*Lapatha*) with 329 µg/100 g of fresh matter closely followed by *Daucus carota* (*Stafilina-kas*) at 328 µg/100 g of fresh matter. As for the cultivated greens, spinach had the highest vitamin K content (194 µg/100 g). The lowest amount of vitamin  $K_1$  was detected in Artichokes with a mere 13 µg/100 g for the stems and 18 µg/100 g for the rest of the plant. Low concentrations were also observed in *Muscari commosum* (*Volvi*) with 22 µg/100 g. The remaining plants contained different amounts ranging between 31 and 298 µg/100 g of fresh mass.

## 3.2. Vitamin C (L-ascorbic acid)

Based on the results of Table 2 the highest concentrations of ascorbic acid were found in *Lathyrus ochrus (Papoules)* with 140 mg/100 g of fresh matter, closely followed by *Petrahatziki* and *Eruca sativa (Roka)* with 127 mg and 125 mg/100 g of fresh matter, respectively. As for the cultivated greens, again spinach had the highest level of ascorbic acid, 50 mg/100 g of fresh leaves. The lowest levels of ascorbic acid were found in *Hypochoeris cretensis* (*Hiromourides*) and wild Radish with a mere 9 mg and 13 mg/100 g of fresh matter, respectively.

#### 3.3. Lutein

According to Table 3 the highest lutein content was found in *Lagoudohorto* (4128  $\mu$ g/100 g) followed by *Ahournopodi* with 3843  $\mu$ g/100 g and *Foeniculum vulgare* (*Marathd*) with 3669  $\mu$ g/100 g. High amounts of lutein were also detected in *D. carota* (*Petrahatziki*) with 3507  $\mu$ g/100 g and *R. obtusifolius* (*Lapatha*) (3438  $\mu$ g/100 g). As for the cultivated plants, the highest level of lutein was observed in spinach containing 3052  $\mu$ g/100 g of fresh mass.

#### 3.4. $\beta$ -Carotene

The highest  $\beta$ -carotene content was found in *Lagoudo*horto with 2168 µg/100 g of fresh mass followed by *L. ochrus* 

Table 2

Vitamin K1 and vitamin C (ascorbic acid) content of wild edible Cretan greens

Plant names	Vitamin $K_1 \mu g/100 g$ of fresh matter	Vitamin C mg/100 g of fresh matter	
Cultivated greens			
Lactuca sativa (Lettuce, organic)	78	39	
Lactuca sativa (Lettuce, non-organic)	81	35	
Spinacea oleracea var. glabra (Spinach)	194	50	
Vicia faba (Broad beans)	48	23	
Cynara carduncelus var. scolymus (Artichokes)	18	41	
Beta vulgaris (Kokkinogoulia)	95	39	
Wild greens			
Taraxacum spp. (Radikia)	198	51	
Daucus carota (Stafilinakas)	328	29	
Fasoulides	163	25	
Rumex obtusifolius (Lapatha)	329	32	
<i>Taraxacum</i> spp. (Pikrorodlka)	157	34	
Allium scoenoprasm (Wild leeks)	188	55	
Daucus carota (Petrahatziki)	250	127	
Lathyrus ochrus (Papoules)	298	140	
Silene vulgaris subsp. macrocarpa (Agriopapoules)	172	140	
Akournopodi	277	28	
<i>Taraxacum</i> spp. (Glikorodika)	155	18	
<i>Reihardia picroides</i> (Galatsides)	108	33	
Apium graveolens (Mirldouses)	159	18	
	239	101	
Foeniculum vulgare (Maratha)			
Slnapls spp. gruciferae (Lapsana)	204	52	
Ahatziki	132	39	
Petroselinum sativum (Agriomaindanos)	270	60	
Agrioselino	172	51	
Kalogeros	101	32	
Avizitis	130	19	
Scabiosa cretica (Stravoksilo)	165	33	
Scolymus hispanicus (Goules or Askolibri)	38	22	
Cynoglossum creticum (Agoglossi)	59	24	
Hypochoeris cretensis (Hiromourides)	87	9	
Bryonia cretica (Avronies)	95	20	
Prasium majus (Lagoudohorto)	373	70	
Maroulides	201	16	
Muscari commosum (Volvi or Askordoulaki)	22	52	
Sonchus oleraceus (Tsohi)	175	20	
Tragopogon sinuatus (Pigounites)	135	23	
Eruca sativa (Roka)	31	125	
Cynara cornigera (Artichokes wildly grown-stems)	13	21	
Cichorium intibus (Radish bitter, semi-cultivated)	173	23	
Beta vulgaris (Radish Wildly grown)	129	13	
Pikroussa	135	24	
Petsetes	65	19	
Cichorium spinosum (Starmnagathi)	240	27	
Solanum nigrum (Strufoulia)	136	21	
Pahies or Pikrorodiko	107	34	
Crepis commutata (Glikossirides)	148	16	
Papaver rhoeas (Koutsounades)	145	17	
Tragopogon Sinuatus (Skouloi)	137	20	
Spinach wildly grown	158	29	
Harakoulia	150	51	
Katsoules	217	16	
Pontikines	177	41	
Crepis vesicaria (Pikrosirides)	143	24	
Kofta	161	33	

(*Papoules*) with 2057µg/100 g. High amounts of  $\beta$ -carotene were also observed in *D. carota* (*Petrahatziki*) with 1770 µg/ 100 g of fresh matter and both in cultivated and wild spinach (1678 and 1663 µg/100 g). The lowest levels of  $\beta$ -carotene were found in cultivated Broad Beans with 78 µg/100 g

followed by *Scolymus hispanicus* (*Goules*) and *C. creticum* (*Agoglossi*) at 97 and 195  $\mu$ g/100 g, respectively. According to Table 3, all other plants have  $\beta$ -carotene levels between those found in *Lagoudohorto* and those found in cultivated Broad Beans.

# 3.5. Total polyphenols

Cultivated Broad Beans showed the highest total polyphenol content, with 550 mg/l00 g of fresh matter. *Cyno*-

#### Table 3

Lutein and  $\beta$ -carotene content of cretan edible wild plants

glossum creticum (Agoglossi) had 373 mg and Kalogeros had 339 mg of total polyphenols per 100 g of fresh matter. Cultivated Artichokes followed with 325 mg total polyphenols/100 g and Ahatziki contained 298 mg/100 g (Table 4).

Plant names	Lutein $\mu$ g/100 g of fresh matter	$\beta$ -Carotene $\mu$ g/100 g of fresh matte
Cultivated greens		
Lactuca sativa (lettuce, organic)	941	511
Lactuca sativa (lettuce, non-organic)	1379	801
Spinacea oleracea var. glabra (spinach)	3052	1678
Vicia faba (Broad beans)	305	78
Cynara carduncelus var. scolymus (Artichokes)	64	n/a
Beta vulgaris (Kokkinogoulia)	1669	803
Wild greens		
Taraxacum spp. (Radikia)	2459	1209
Daucus carota (Stafilinakas)	3217	1173
Fasoulides	2511	1047
Rumex obtusifolius (Lapatha)	3438	1439
<i>Taraxacum</i> spp. (Pikrorodika)	1451	515
Allium scoenoprasm (wild leeks)	3013	1342
Daucus carota (Petrahatziki)	3507	1770
Lathyrus ochrus (Papoules)	3015	2057
Silene vulgaris subsp. macrocarpa (Agriopapoules)	2012	1029
Akournpodi	3843	1525
<i>Taraxacum</i> spp. (Glikorodika)	2855	989
	1499	586
Reihardia picroides (Galatsides)		
Apium graveolens (Miridouses)	1938	831
Foeniculum vulgare (Maratha)	3669	1196
Sinapis spp. gruciferae (Lapsana)	1799	546
Ahatziki	1880	816
Petroselinum sativum (Agriomaindanos)	2600	988
Agrioselino	1666	908
Kalogeros	571	732
Avizitis	2138	718
Scabiosa cretica (Stravoksilo)	1472	821
Scolymus hispanicus (Goules or Askolibri)	330	97
Cynoglossum creticum (Agoglossi)	463	195
Hypochoeris cretensis (Hiromourides)	1262	576
Bryonia cretica (Avronies)	1063	331
Prasium majus (Lagoudohorto)	4128	2168
Maroulides	2336	1183
Muscari commosum (Volvi or Askordoulaki)	n/a	n/a
Sonchus oleraceus (Tsohi)	1826	1051
Tragopogon sinuatus (pigounites)	2085	997
Eruca sativa (Roka)	2483	1155
Cynara cornigera (Artichokes wildly grown -stems)	91	n/a
Cichorium intibus (Radish bitter, semi-cultivated)	3036	1443
Beta vulgaris (Radish wildly grown)	1471	684
Pikroussa	1054	491
Petsetes	625	326
Cichorium spinosum (Stamnagathi)	1160	595
Solanum nigrum (Strufoulia)	1450	572
Pahies or pikrorodiko	1544	732
Crepis commutata (Glikossirides)	1902	1002
Papaver rhoeas (Koutsounades)	1154	750
Tragopogon sinuatus (Skouloi)	2521	1313
Spinach wildly grown	2894	1663
Harakoulia	2084	812
Katsoules	1938	991
Pontikines	1852	1007
	2009	1071
Crepis vesicaria (Pikrosirides)		
Kofta	969	507

n/a, not applicable.

The lowest polyphenol content was found in the stems of wild artichokes and in *Beta vulgaris* (Kokkinogoulia) with 41 mg and 55 mg/100 g, respectively.

#### 3.6. To copherols ( $\alpha$ - and $\gamma$ -to copherol)

As seen in Table 4, *E. sativa* (*Roka*) had the highest  $\alpha$ -tocopherol level of all the wild greens with 3.07 mg/100 g of fresh mass followed by *Allium scoenoprasm* (*Agrioprassa*) with 2.1 mg/100 g. The lowest levels of  $\alpha$ -tocopherol were found in wild artichokes, which contained 0.04 mg/ 100 g and *S. hspanicusi* (*Goules* or *Askolibri*) with a mere 0.06 mg/100 g. As for the cultivated plants, results varied from a very high 3.86 mg in 100 g of fresh Broad Beans to a very low 0.05 mg in 100 g of Artichoke. On the average, most of the wild greens had higher concentrations of  $\alpha$ -tocopherol than the cultivated of our study.

As for  $\gamma$ -tocopherol, the highest concentration was found in wild radish with 1.25 mg/100 g of fresh matter, but most of the selected greens had much lower concentrations of  $\gamma$ -tocopherol (23 of the investigated 54 plants had  $\gamma$ -tocopherol concentrations lower than 0.1 mg/100 g of fresh matter). Non-organic Lettuce had the highest  $\gamma$ tocopherol level of the cultivated plants with a substantial 0.62 mg/100 g of fresh lettuce leaves.

## 4. Discussion

This study has focused on the vitamin and antioxidant contents of commonly eaten wild Cretan greens which play a vital role in supplementing the population's daily diet with essential micronutrients and antioxidants. By managing to cover the recommended daily allowances in vitamins and by enriching their diet with valuable antioxidants, the population of Crete has been able to enjoy a healthier and longer life as seen in the studies of Keys (1970) and Renaud et al. (1995).

Other studies also have demonstrated that the wildly grown Cretan greens are potentially a rich source of antioxidants in the Greek diet. Trichopoulou et al. (2000) presented a similar study analyzing seven Cretan edible wild plants for their flavonoid and flavone content, their mineral content, dietary fiber and mineral concentration. Their results also show that wild Cretan greens are rich in antioxidant nutrients. Zeghini, Kallithraka, Simopoulos, and Kypryotakis (2003) analyzed 25 wild plants for their  $\alpha$ tocopherol, total phenol, antioxidant activity, antiradical power as well as their mineral and nitrate content and also concluded that wild Cretan greens are rich in antioxidants micronutrients. Our results differ than the results presented by Zeghini et al. (2003) in that we determined wild greens that have not been analyzed before and we found the  $\alpha$ tocopherol and total polyphenol levels to be substantially higher for the same plants, but also in greens that have not been analyzed before. Schaffer, Schmitt-Schillig, Muller, and Eckert (2005) collected and analyzed three plants that are included in our study (Chicorium intybus, Sonchus

oleraceus and Papaver rhoeas) from Italy, Spain and Crete and examined them for their antioxidant and polyphenol content. We found slightly higher results regarding the polvphenol content of the Cretan greens than the greens collected from Crete in their study, but similar polyphenol content to the plants collected in Spain and Italy. When comparing the results in both studies, it becomes obvious that the same wild greens eaten in Crete also exist in other parts of the Mediterranean, although we are not sure at what extent they are consumed in other population. Kapiszewska, Soltys, Visioli, Cierniak, and Zajac (2005) recently studied the protective ability of six Mediterranean plant extracts against oxidative damage, caused by H<sub>2</sub>O<sub>2</sub> in lymphocytes, in relation to their polyphenolic content. One of the plants in their study, S. hispanicus is included in our study and was shown in certain concentrations to protect DNA destruction. The wild greens analyzed in our study were found mostly to have similar or higher  $\alpha$ tocopherol,  $\gamma$ -tocopherol and total polyphenol contents than those cultivated.  $\alpha$ -Tocopherol and  $\gamma$ -tocopherol are two of the major chemical forms of vitamin E. Like other antioxidants, vitamin E can scavenge free radicals and may, as a result, prevent oxidative tissue damage by trapping organic free radicals. By including large quantities of fresh greens into their diet the population of Crete was able to guarantee a substantial daily intake of these valuable antioxidants.

Vitamin K plays a major role in many body functions, especially in normal blood clotting, due to its role in producing prothrombin and factors VII,IX and X (Fairfield & Fletcher, 2002). According to Dietary Reference Intakes and recommended Dietary Allowances (2001), the current RDI for vitamin K is 90–120  $\mu$ g depending on the gender. Taking into account the quantities of vitamin K found in 100 g of fresh matter in most of the investigated greens, it becomes obvious that by following a traditional Cretan diet, one can adequately cover the recommended amount.

Vitamin C also plays an important role in body functions. In mice it has been shown to stimulate the immune system by enhancing T-cell proliferation in response to infection (Campbell, Cole, Bunditrutavorn, & Vell, 1999). It is also known to prevent the oxidation of LDL, therefore, preventing the development of atherosclerosis (Akhilender, 2003). Epidemilogic evidence of a protective effect of vitamin C for non-hormone-dependent cancers is also strong (Block, 1991).

In comparison with cultivated green vegetables, one can deduce that most of the wild greens have similar and even higher vitamin C levels then those recorded by Favel (1998) and When taking into account the recorded levels of vitamin C, it becomes obvious that a diet high in wild greens, such as the traditional Cretan diet, can easily contribute towards exceeding the recommended daily intake of vitamin C (75–90 mg/day) suggested by Dietary Reference Intakes and recommended Dietary Allowances (2001).

Lutein, unlike other carotenoids cannot be transformed into vitamin A in the human body and it is believed to

Table 4

 $\alpha$ -Tocopherol,  $\gamma$ -tocopherol and total polyphenol content of cretan edible wild plants

Plant names	α-Tocopherol mg/100 g of fresh matter	γ-tocopherol mg/100 g of fresh matter	Total polyphenol mg/100 g of fresh matter
Cultivated greens			
Lactuca sativa (Lettuce, organic)	0.46	0.49	79.9
Lactuca sativa (Lettuce, non-organic)	0.25	0.62	69.6
Splnacea oleracea var. glabra (Spinach)	2.06	0.19	148
Vicia faba (Broad beans)	3.86	0.20	550
<i>Cynara carduncelus</i> var. <i>scolymus</i> (Artichokes)	0.05	n/a	325
Beta vulgaris (Kokkinogoulia)	0.55	0.01	55
	0.00	0.01	55
Wild greens	0.81	0.50	136.6
<i>Taraxacum</i> spp. (Radikia)			
Daucus carota (Stafilinakas)	0.35	0.01	111.8
Fasoulides	0.79	0.10	154.1
Rumex obtusifblius (Lapatha)	0.85	0.04	153.3
Taraxacum spp. (Pikrorodika)	0.86	0.26	88.8
Allium scoenoprasm (Wild leeks)	2.10	0.48	133.2
Daucus carota (Petrahatziki)	0.53	0.15	274.8
Lathyrus ochrus (Papoules)	0.49	0.02	198.1
Silene vulgaris subsp. macrocarpa (Agriopapoules)	0.92	0.09	117.4
Akournopodi	0.22	0.04	89.3
Taraxacum spp (Glikorodika)	0.45	0.04	126.7
Reihardia picroides (Galatsides)	0.18	0.04	67.7
Apium graveolens (Miridouses)	0.51	0.09	267.9
Foeniculum vulgare (Maratha)	0.63	0.14	155.2
Sinapis spp. gruciferae (Lapsana)	1.02	0.03	205.2
Ahatziki	0.43	0.09	298.1
Petroselinum sativum (Agriomaindanos)	0.75	0.05	249.1
Agrioselino		0.03	122
6	1.45		
Kalogeros	0.61	0.04	339
Avizitis	0.53	0.06	88
Scabiosa cretica (Stravoksilo)	0.73	0.01	118
Scolymus hispanicus (Goules or Askolibri)	0.06	0.02	56
Cynoglossum creticum (Agoglossi)	0.77	0.29	373
Hypochoeris cretensis (Hiromourides)	0.40	0.02	105
Bryonia cretica (Avronies)	1.11	0.41	276
Prasium majus (Lagoudohorto)	1.62	0.11	126
Maroulides	0.52	0.03	82
Muscari commosum (Volvi or Askordoulaki)	0.73	n/a	88
Sonchus oleraceus (Tsohi)	0.97	0.14	71
Tragopogon Sinuatus (Pigounites)	1.53	0.27	126
Eruca sativa (Roka)	3.07	0.09	211
<i>Cynara comigera</i> (Artichokes wildly grown-stems)	0.04	n/a	41
<i>Cichorium intibus</i> (Radish bitter, semi-cultivated)	0.98	0.58	119
Beta vulgaris (Radish wildly grown)	1.17	1.25	274
	0.89		
Pikroussa		0.23	143
Petsetes	1.10	0.03	161
Cichorium spinosum (Stamnagathi)	1.23	0.83	132
Solanum nigrum	0.91	0.29	126
Pahies or pikrorodiko	0.54	0.15	91
Crepis commutata (Glikossirides)	0.82	0.15	163
Papaver rhoeas (Koutsounades)	1.37	0.03	269
Tragopogon sinuatus (Skouloi)	0.73	0.25	81
Spinach wildly grown	1.49	0.04	82
Harakoulia	0.72	0.15	113
Katsoules	0.77	0.06	155
Pontikines	1.58	0.58	176
Crepis vesicaria (Pikrosirides)	1.53	0.32	190
Kofta	1.15	0.52	229

n/a, not applicable.

function in two ways; first as a filter of high energy blue light and second as an antioxidant according to Alves-Rodrigues and Shao (2004). Khachik, Beecher, Goli, Lusby, and Datich (1992) showed that it is the most prevalent carotenoid in human serum and is highly concentrated in the macula. Due to its high concentration in the macula, it is known mainly for its importance in eye health protection. Lyle, Mares-Perlman, Klein, Klein, and Greger (1999) Gale, Hall, Phillips, and Martyn (2001) studied the effects of consumption and serum levels of lutein and suggested that they are inversely related to age related macular degeneration and cataract, both in men (Brown et al., 1999) and in women (Hankinson et al., 1992). Lutein also plays an important role by reducing the risk of atherosclerosis and coronary heart disease as suggested by Dwyer et al. (2001). Lutein also has a protective effect against skin cancer as shown by Lee et al. (2004) and Stahl and Sies (2002). It can be found in abundance in vegetables, especially in green leaf vegetables like spinach, E. sativa (Roka), Akournopodi and in the leaves (not the stem) of F. vulgare (Maratha). Such green leaf vegetables play a major role in traditional Cretan diet, enriching it with high levels of lutein in a natural way.

Out of all the carotenoids (600 of them),  $\beta$ -carotene has received the most attention from scientific studies due to its provitamin A activity, unlike lutein, and to its prevalence in many foods. β-Carotene acts as a free radical scavenger and evidene, although not completely consistent, has suggested that high plasma levels might lower the risk of coronary heart disease according to Jha, Flather, Lonn, Farkouth, and Yusuf (1995). Human health benefits from high levels of antioxidant vitamins that inhibit the oxidative process of LDL cholesterol into atherogenic forms and, therefore, preserve endothelial function as stated by Tavani and La Vecchia (1999). Van Poppel and Goldbohm (1995) stated that high plasma levels of  $\beta$ -carotene have an inverse relationship with lung cancer risk. The wild greens that we analyzed had substantial β-carotene contents and when eaten fresh with olive oil important role in protecting the population that adheres to the traditional diet from the negative effects of high plasma LDL levels.

We find it necessary to note that there are a few wildly grown greens with different names between the prefecture of Heraklion and Rethymnon. Therefore, this is an excellent opportunity to check the validity of laboratory methods. Specifically, one plant with two different common name is *Tragopogon sinatus*, which is known in Heraklion as Skouloi and in Rethymnon as Pigounites. Although they give similar results for vitamin K<sub>1</sub>, vitamin C and  $\gamma$ tocopherol, substantial differences were found between the two for lutein,  $\beta$ -carotene,  $\alpha$ -tocopherol and total polyphenol results. It is possible that the noted differences are due to environmental factors that differ between the two different areas of collection.

Our study also focused on comparing 6 popularly cultivated greens with the selected 48 wild greens. On most occasions, the wild greens had higher micronutrient contents than those cultivated. Specifically, certain wild greens had higher concentration of vitamin K<sub>1</sub>, vitamin C (as an ascorbic acid), lutein,  $\beta$ -carotene,  $\gamma$ -tocopherol and total polyphenol content than those cultivated, proving the significance of these wildly grown greens to the well being of the Cretan population.

In the present study, it was not possible to measure the folate, metal, trace elements and fiber content of the greens; therefore, there is no complete nutrient and non-nutrient profile. Also, there is insufficient information to ascertain the exact variety and the quantities of the wild greens consumed by the children, the adults and the elderly. The pesticide and nitrate content of the wild greens is also unknown, but it is expected that the amount of toxic substances are limited, since in most cases the wildly grown greens are collected from non-cultivated fields and mountainous areas.

## 5. Conclusion

The traditional diet of Crete, which is high in local greens, whether eaten with olive oil in salads, in pies or other recipes, plays an important role in the health of the elderly and rural population of Crete. According to our study of 48 wild and 6 cultivated greens of Crete, the wild Cretan greens are rich sources of vitamin C, K, E and carotenoids, and capable of significantly contributing to the RDA needs of the population. In most cases, it was found that the wild greens had higher micronutrient content than those cultivated. We must emphasize though, that further studies are required to evaluate the feasibility of commercially growing some of the wild greens so that non-rural population can also reap the benefits of enriching their diet with traditional greens.

#### References

- Akhilender Naidu, K. (2003). Vitamin C in human health and disease is still a mystery. An overview. *Nutrition Journal*, 2, 7.
- Alves-Rodrigues, A., & Shao, A. (2004). The science behind lutein. Toxicology Letters, 150, 57–83.
- Block, G. (1991). Vitamin C and cancer prevention: the epidemiological evidence. American Journal of Clinical Nutrition, 53, 270S–282S.
- Brown, L., Rimm, E. B., Seddon, J. M., Giovannucci, E. L., Chasan-Taber, L., Spiegelman, D., et al. (1999). A prospective study of carotenoid intake and risk of cataract extraction in US men. *American Journal of Clinical Nutrition*, 70, 517–524.
- Campbell, J. D., Cole, M., Bunditrutavorn, B., & Vell, A. T. (1999). Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. *Cell Immunology*, 194, 1–5.
- Dietary Reference Intakes and recommended Dietary Allowances (2001). Food and nutrition information centre. National Academies Press. Available from http://www.nal.usda.gov/fnic/etext/000105.html.5-10-2004.
- Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P., Shircore, A., et al. (2001). Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles Atherosclerosis Study. *Circulation*, 103, 2922–2927.
- Fairfield, K., & Fletcher, R. (2002). Vitamins for chronic disease prevention in adults. *Journal of the American Medical Association.*, 287(23), 3116–3126.
- Favel, D. J. (1998). A comparison of the vitamin C content of fresh and frozen vegetables. *Food Chemistry*, 62(1), 59–64.
- Ferro-Luzzi, A., James, W., & Kafatos, A. (2002). The high fat greek diet: a receipe for all? *European Journal of Clinical Nutrition*, 56, 796–809.
- Folch, J., Less, M., & Stoane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.

- Gale, C. R., Hall, N. F., Phillips, D. I., & Martyn, C. N. (2001). Plasma antioxidant vitamins and carotenoids and age-related cataract. *Oph-thalmology*, 108, 1992–1998.
- Hankinson, S. E., Stampfer, M. J., Saddon, J. M., Colditz, G. A., Rosner, B., Speizer, F. E., et al. (1992). Nutrient intake and cataract extraction in women: a prospective study. *British Medical Journal*, 305, 335–339.
- Jakob, E., & Elmadfa, I. (1996). Aplication of simplified HPLC assay for the determination of phylloquinone (vitamin Kl) in animal and plant food items. *Food Chemistry*, 56, 87–91.
- Jakob, E., & Elmadfa, I. (1995). Rapid HPLC assay for the assessment of vitamin Kl, A, E and β-carotene status in children (7-19 years). International Journal of Vitamin and Nutrition Research, 65, 31–35.
- Jha, P., Flather, M., Lonn, E., Farkouth, M., & Yusuf, S. (1995). The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Annals of Internal Medicine*, 123, 860.
- Kapiszewska, M., Soltys, E., Visioli, F., Cierniak, A., & Zajac, G. (2005). The protective ability of the Mediterranean plant extracts against the oxidative DNA damage. The role of the radical oxygen species and the polyphenol content. *Journal of Physiology and Pharmacology*, 56(Suppl. 1), 183–197.
- Keys, A. (1970). Coronary heart disease in Seven countries. American Heart Association Monograph Number 29. New York: American Heart Association.
- Khachik, F., Beecher, G. R., Goli, M. B., Lusby, W. R., & Datich, C. E. (1992). Separation and qualification of caretenoids in human plasma. *Methods in Enzymology*, 213, 205–219.
- Lee, E. H., Faulhaber, D., Hanson, K. M., Ding, W., Peters, S., Kodali, et al. (2004). Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosuppression. *Journal of Investigative Dermatology*, 122(2), 510–517.
- Linkens, H. F., & Jackson, J. F. (1988). Wine analysis. Modern methods of plant analysis. Chapter: Wine phenols (Vol. 6). Springer-Verlag.
- Lyle, B. J., Mares-Perlman, J. A., Klein, B. E., Klein, I. L., & Greger, J. L. (1999). Antioxidant intake and risk of incident age related nuclear

cataracts in the Beaver Dam Eye Study. American Journal of Epidemiology, 149, 801-809.

- Renaud, S., de Lorgeril, M., Delaye, J., Guidollet, J., Jacquard, F., Mamelle, N., et al. (1995). Cretan Mediterranean diet for prevention of coronary heart disease. *American Journal of Clinical Nutrition*, 61(Suppl.), 1360S–1367S.
- Sarri, K., Linardakis, M., Bervanaki, F., Tzanakis, N., & Kafatos, A. (2004). Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. *British Journal of Nutrition*, 92(2), 277–284.
- Sarri, K., Tzanakis, N., Linardakis, M., Mamalakis, G., & Kafatos, A. (2003). Effects of Greek orthodox Christian church fasting on serum lipids and obesity. *BMC Public Health*, 3(May), 16.
- Schaffer, S., Schmitt-Schillig, S., Muller, W. E., & Eckert, G. P. (2005). Antioxidant properties of Mediterranean food plant extracts: geographical differences. *Journal of Physiology and Pharmacology*, 56(Suppl. 1), 115–124.
- Stahl, W., & Sies, H. (2002). Canotenoids and protection against solar UV radiation. Skin Pharmacology and Applied Skin Physiology, 15, 291–296.
- Tavani, A., & La Vecchia, C. (1999). β-carotene and risk of coronary heart disease. A review of observational and intervention studies. *Biomedicine and Pharmacotherapy*, 53, 409–416.
- Trichopoulou, A., Vasilopulou, E., Hollman, P., Chamalides, C., Foufa, E., Kaloudis, T., et al. (2000). Nutritional composition and flavonoid content of edible wild greens and green pies: A potential rich source of antioxidant nutrients in the Mediterranian diet. *Food Chemistry*, 70, 319–323.
- Van Poppel, G., & Goldbohm, R. A. (1995). Epidemiologic evidence for β-carotine and cancer prevention. *American Journal of Clinical Nutrition*, 62, 1393s–1402s.
- Zeghini, S., Kallithraka, S., Simopoulos, A., & Kypryotakis, Z. (2003). Nutritional composition of selected wild plants in the diet of Crete. World Review of Nutrition and Dietetics, 91, 22–40.