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Short communication

Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties

Lixia Liu[a,b], Peter Howe[a], Ye-Fang Zhou[b], Zhi-Qiang Xu[b], Charles Hocart[c], Ren Zhang[a,b,*]

[a]*Smart Foods Centre, University of Wollongong, Wollongong, NSW 2522, Australia*
[b]*Department of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia*
[c]*Research School of Biological Sciences, Australian National University, Canberra, ACT 2600, Australia*

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Abstract

The fatty acid profile and β-carotene content of a number of Australian varieties of purslane (*Portulaca oleracea*) were determined by GC and HPLC. The total fatty acid content ranged from 1.5 to 2.5 mg/g of fresh mass in leaves, 0.6 to 0.9 mg/g in stems and 80 to 170 mg/g in seeds. α-Linolenic acid ($C_{18:3\omega3}$) accounted for around 60% and 40% of the total fatty acid content in leaves and seeds, respectively. Longer-chain omega-3 fatty acids were not detected. The β-carotene content ranged from 22 to 30 mg/g fresh mass in leaves. These results indicate that Australian purslane varieties are a rich source of α-linolenic acid and β-carotene.

Keywords: *Portulaca oleracea*; Fatty acids; Carotenes; Linolenic acid

1. Introduction

Common purslane (*Portulaca oleracea* L.), is a member of the Portulacaceae, which consists of more than 120 species of often succulent herbs and shrubs [1]. *P. oleracea*, hereafter referred to as purslane, is widespread as a weed and has been ranked the eighth most common plant in the world [2]. It is fast growing, self-compatible and produces large numbers of seeds that have a long viability.

Purslane has a long history of use for human food, animal feed and medicinal purposes [3]. These days, however, most people in modern societies limit their diet to a few cultivated vegetables so that wild plants such as purslane tend to be under-utilised. Recent research indicates that purslane offers better nourishment than the major cultivated vegetables. In particular, it has a high percentage of α-linolenic acid (LNA) [4–8] and is a richer source of this fatty acid than any other green leafy vegetable investigated to date [4,5,9].

LNA, an omega-3 fatty acid, is an essential fatty acid because it cannot be synthesised by humans but has to be ingested. It plays an important role in human growth, development and disease prevention. It is the precursor of the longer-chain omega-3 fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), found predominantly in marine organisms, to

*Corresponding author. Department of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia. Fax: +61-2-4221-4135.

E-mail address: rzhang@uow.edu.au (R. Zhang).

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which a wide range of health benefits have been ascribed [10]. The ability of LNA to convey these health benefits is limited by its poorer conversion to EPA and DHA in humans. Thus recent reports claiming the existence of longer-chain omega-3 polyunsaturated fatty acids in purslane have attracted considerable interest in this plant as an alternative source of these nutrients for human consumption [11,12].

Purslane has been described as a ''power food'' of the future because of its high nutritive and antioxidant properties [5,13]. It is an excellent source of antioxidants such as vitamins A, C and E and β-carotene, which, through their ability to neutralise free radicals, have the potential to prevent cardiovascular disease, cancer and infectious diseases. Taken as supplements, they can decrease the rate of oxidation of low-density lipoprotein, a significant contributor to atherosclerosis [14].

In Australia, purslane is a native plant found in all mainland states [15]. Moreover, it has been used as a traditional food and medicine by Aborigines and its health attributes were noted by early European settlers [16]. Surprisingly, however, there has been no attempt to evaluate the nutritional attributes of Australian varieties of purslane. In this study, the fatty acid profile and β-carotene content of several Australian varieties of purslane were analysed by chromatographic methods and compared with North American varieties.

2. Experimental

2.1. Plant materials

Seeds for all the purslane varieties tested in this study were provided by the Reality Check Group (Sydney, Australia). They included three cultivars (C1, C2 – North American origin and C3 – local) and nine Australian wild varieties (W1, W2, W3 and the others). The plants were grown in a glasshouse at day/night temperatures of 28/20°C in pots filled with soil mixture (composition+fertiliser). Irrigation was on a need basis, about twice a week. Samples for fatty acid and β-carotene analyses were collected at 45, 60 (leaves and stems) and 70 days (seeds) after planting. Cultivar C3 was also grown under field conditions on a farm in Felham, New South Wales during October to December 1999.

2.2. Fatty acid extraction and methylation

The standard procedure used for analysing the fatty acid contents of plants was as following. Fatty acids were extracted by a method modified from that described by Lepage and Roy [17]. A 1-g amount of fresh plant tissue was frozen in liquid nitrogen and ground in a mortar and pestle with 6 ml of chloroform–methanol (2:1, v/v) containing internal standard (I.S.; $C_{21:0}$; Sigma, USA; 0.2 mg/ml) and 5% butylhydroxytoluene (BHT) to prevent oxidation of fatty acids. The mixture was vortex-mixed for 5 min and centrifuged at 3000 *g* for 10 min at 5°C. The lower phase (1 ml) was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 2 ml of methanol–toluene (4:1, v/v) and acetyl chloride (0.2 ml) was slowly added while vigorously stirring. The mixture was heated at 100°C for 1 h, then cooled in water and the reaction stopped by addition of 6% K_2CO_3 solution (5 ml). The samples were vortex-mixed, centrifuged, and the upper phase was collected prior to GC analysis. The efficiency of fatty acid extraction from the plants was compared with two alternative methods [18,19] normally used for plant samples. In one case [18], fatty acids were extracted with a mixture of methanol–chloroform (2:1, v/v) and shaken overnight at 2°C. In the other case [19], fatty acids were extracted with a mixture of phosphate buffer (0.05 *M*, pH 7.2; 1 ml), acetic acid (0.15 *M*; 2 ml) and methanol–chloroform (2:1, v/v; 3.5 ml). Other aspects of these two methods were similar to the method used in this experiment.

2.3. Gas chromatography

Samples (1 μl) were injected via an autosampler onto a fused-silica capillary column (J & W Scientific; DB-Wax; 30 m×0.25 mm I.D., 25 μm film thickness) in a Shimadzu Model GC-17A gas chromatography (GC) system fitted with a flame ionisation detector and eluted with H_2 at 30±1 ml/min. with a split ratio of 1:17. The injector and detector were heated to 250°C. The column was temperature programmed from 150°C (hold 1 min) to 180°C at

25°C/min, then to 220°C (hold 3 min) at 2.5°C/min and then to 250°C (hold 4 min) at 15°C/min. Fatty acid methyl esters were identified by comparing GC retention times with those of a mixture of standard fatty acids (Sigma). Fatty acids were quantified using peak areas, against internal standard.

2.4. Gas chromatography–mass spectrometry

Samples were injected (0.2–0.8 μl injection volume) via an autosampler onto the same GC column as used for GC. This was eluted with He (inlet pressure 15 p.s.i.; 1 p.s.i.=6894.76 Pa) directly into the ion source of a Fisons MD800 GC–mass spectrometry (MS) system (injection port 250°C; interface 250°C; source 200°C). The column was temperature programmed as described above. The mass spectrometer was operated in the electron impact ionisation (EI) mode with an ionisation energy of 70 eV. Mass spectra were acquired either as full scans from m/z 50 to m/z 400 in 0.45 s or by selected ion monitoring (SIM) with a dwell time of 100 ms and span of 0.1 u (m/z 326, from 13.0 to 15.5 min; m/z 318 and 320, from 16.5 to 18.5 min; m/z 350 and 354, from 19.0 to 22.3 min; m/z 342, 344, 380 and 382, from 23.5 to 25.5 min). Fatty acids were identified by comparison of retention times and mass spectra with those of authentic standards (Sigma).

2.5. β-Carotene determination

β-Carotene was analysed following the procedures of Thayer and Björkman [20]. Plant tissues (fresh mass 300 mg) were frozen in liquid N_2 and ground in a mortar and pestle with cold acetone (1.5 ml). The extract was placed on ice for 20 min prior to centrifugation (10 000 *g*, 6 min at 5°C). The pellet was re-extracted with a small volume (0.5 ml) of acetone–water (85:15, v/v) and centrifuged as before. The supernatants were combined and filtered through a 0.45-μm syringe filter. β-Carotene was analysed by high-performance liquid chromatography (HPLC) (Shimadzu SPD-M10A) on a Spherisorb ODS-1 column (150×4.6 mm) fitted with an ODS-1 guard cartridge (Alltech Associates, Deerfield, IL, USA). The column was equilibrated with solvent A (acetonitrile–methanol–0.1 *M* Tris, pH 8; 19:3:1, v/v/v) and eluted as follows: solvent A at a flow-rate 1.5 ml/min for 6 min followed by solvent B (methanol–hexane; 4:1, v/v) at 2 ml/min for 10 min and then back to solvent A at 1.5 ml/min for 8 min. Quantification was against a β-carotene external standard using peak areas.

2.6. Statistics

Data are presented as mean±standard error (S.E.) in all tables and figures and analysed by general analysis of variance (ANOVA) and *t*-test (Statistica Version 4.0; StatSoft, USA).

3. Results and discussion

3.1. Simplified extraction of fatty acids from plant tissues

To deal with the large number of plant samples to be analysed, the simplified procedure of Lepage and Roy [17] was adapted for this study. It has been used for extracting fatty acids from animal tissues like plasma, liver and bile, but not plants. This method proved to be simpler and faster with comparable extraction efficiency when compared with the other two extraction methods commonly used for plant tissues [18,19]. No significant difference in the contents of different fatty acids was detected ($P<0.05$; data not shown).

3.2. Fatty acid profile

The fatty acids in the leaves (Table 1), stems (Table 2) and seeds (Table 3) of the various purslane cultivars were identified by GC–MS and quantified by GC (Fig. 1). In general, the major fatty acids present in all tissues were LNA (18:3ω3), linoleic acid (18:2ω6) and palmitic acid (16:0). Smaller amounts of 14:0, 18:0, 18:1ω1, 20:0, 22:0, and 24:0 were also detected.

Significant differences existed in total fatty acid contents between different plant tissues. The total fatty acid contents in leaves, relative to fresh plant mass, ranged from 1.5 to 2.5 mg/g with an average of 2.0 mg/g (Table 1). These figures agree with those reported by Guil et al. [6] but were significantly less than those reported by Simopoulos and

Table 1
Comparison of fatty acid contents (mg/g fresh mass) in leaves of different purslane varieties at 60 days after planting[1]

Fatty acid	C1	C2	C3	W1	W2	W3
14:0	0.08±0.003	0.05±0.002	0.10±0.003	0.09±0.005	0.06±0.005	0.07±0.001
16:0	0.41±0.020	0.30±0.090	0.27±0.010	0.32±0.080	0.35±0.020	0.30±0.090
18:0	0.07±0.010	0.07±0.005	0.07±0.003	0.08±0.010	0.11±0.005	0.11±0.020
18:1	0.14±0.020	0.14±0.003	0.06±0.010	0.05±0.010	0.05±0.003	0.15±0.020
18:2	0.29±0.020	0.31±0.010	0.14±0.008	0.19±0.020	0.27±0.010	0.31±0.050
18:3	1.47±0.060[a]	1.60±0.060[a]	0.97±0.050[b]	1.02±0.092[b]	1.24±0.071[a]	1.46±0.120[a]
20:0	n.d.	0.03±0.001	n.d.	n.d.	n.d.	0.001±0.000
22:0	n.d.	0.04±0.010	n.d.	n.d.	n.d.	0.02±0.001
24:0	0.02±0.003	0.02±0.010	0.01±0.001	0.01±0.002	0.01±0.001	0.05±0.010
Total	2.48±0.121[a]	2.56±0.616[a]	1.62±0.058[b]	1.76±0.160[ab]	2.09±0.112[a]	2.47±0.193[a]
18:3 (%)	59.3[a]	62.5[a]	59.9[a]	58.0[a]	59.3[a]	59.1[a]

[1] Data are mean±S.E. ($n=5$). Values followed by different letters denote significant difference and those followed by same letters denote no significant difference at $P<0.05$. n.d., Not detected.

co-workers [4,5]. On a fresh mass basis, the total fatty acid contents in stems ranged from 0.5 to 0.9 mg/g (Table 2), about one-third of those in leaves. The total fatty acid contents in seeds were 8–18% mg/g (Table 3). This range is about 20% lower than the value reported by Miller et al. [21]. On a dry mass basis, the contents of total fatty acids and LNA were highest in the seeds, then in the leaves and lowest in stems (Fig. 2). The total fatty acid content in seeds was 1.2–3.8-times higher than in leaves and 22–32-times higher than in stems.

LNA accounted for 58–62.5% of the total fatty acids in leaves and 9.6–24% in stems. It was also the predominant fatty acid in purslane seeds, accounting for 31–44% of total fatty acids. Except for linseed, in which LNA is 50–60% of the total fatty acids, most other oil crop seeds contain only 3–10% LNA. Thus our results confirm that purslane is an excellent source of LNA.

3.3. Absence of EPA, DPA or DHA

We could not detect the longer-chain omega-3 fatty acids such as EPA, DPA or DHA in any of the leaf, stem or seed samples from the six purslane varieties grown in the glasshouse. The leaf and stem samples were harvested at two different growth stages, 60 days (Tables 1 and 2) and 45 days (data

Table 2
Comparison of fatty acid contents (mg/g fresh mass) in stems of different purslane varieties at 60 days after planting[1]

Fatty acid	C1	C2	C3	W1	W2	W3
14:0	0.03±0.001	0.05±0.001	0.04±0.004	0.03±0.005	0.03±0.001	0.02±0.003
16:0	0.18±0.040	0.21±0.040	0.23±0.010	0.21±0.010	0.15±0.010	0.25±0.020
18:0	0.06±0.005	0.05±0.003	0.07±0.010	0.10±0.007	0.07±0.004	0.06±0.004
18:1	0.08±0.003	0.08±0.006	0.10±0.008	0.05±0.004	0.04±0.003	0.09±0.005
18:2	0.25±0.040	0.24±0.020	0.20±0.020	0.23±0.020	0.14±0.010	0.21±0.007
18:3	0.07±0.010[a]	0.16±0.020[ab]	0.10±0.010[a]	0.20±0.010[b]	0.12±0.010[a]	0.21±0.007[b]
20:0	0.01±0.001	0.02±0.001	0.01±0.001	n.d.	0.004±0.000	0.001±0.000
22:0	0.01±0.001	0.01±0.004	n.d.	n.d.	0.004±0.002	0.002±0.002
24:0	0.04±0.002	0.05±0.008	0.01±0.003	0.03±0.010	0.03±0.006	0.03±0.011
Total	0.73±0.086[a]	0.87±0.061[a]	0.76±0.034[a]	0.85±0.055[a]	0.59±0.042[b]	0.87±0.040[a]
18:3 (%)	9.6[a]	18.4[ab]	13.2[a]	23.5[b]	20.3[b]	24.1[b]

[1] Data are mean±S.E. ($n=5$). Values followed by different letters denote significant difference and those followed by same letters denote no significant difference at $P<0.05$. n.d., Not detected.

Table 3
Comparison of fatty acid contents (mg/g fresh mass) in seeds of different purslane varieties at 70 days after planting[1]

Fatty acid	C1	C2	C3	W1	W2	W3
14:0	0.17±0.001	0.20±0.004	0.19±0.006	0.23±0.008	0.31±0.010	0.29±0.005
16:0	14.01±0.260	18.42±0.400	17.47±0.580	22.83±0.760	22.45±0.180	23.73±0.580
18:0	2.71±0.020	3.40±0.130	4.90±0.370	6.02±0.160	6.10±0.190	6.80±1.370
18:1	8.68±0.130	11.44±0.330	15.88±1.070	13.63±0.950	16.61±0.860	19.04±1.070
18:2	28.22±0.580	38.34±0.890	37.09±2.320	42.55±3.050	50.88±0.750	54.72±2.320
18:3	26.78±0.670[a]	36.42±0.800[a]	35.33±1.150[a]	68.66±2.750[b]	68.71±1.690[b]	68.82±1.150[b]
20:0	1.00±0.020	1.20±0.030	1.30±0.040	2.50±0.150	2.90±0.070	3.20±0.040
22:0	0.10±0.001	0.16±0.008	0.16±0.005	0.34±0.007	0.39±0.006	0.40±0.010
24:0	0.15±0.050	0.21±0.030	0.21±0.005	0.36±0.017	0.42±0.010	0.43±0.010
Total	81.82±1.67[a]	109.79±2.50[a]	112.53±5.16[a]	157.12±7.58[b]	168.77±3.70[b]	177.43±5.16[b]
18:3 (%)	32.7	33.2	31.4	43.7	40.7	38.8

[1] Data are mean±S.E. ($n=5$). Values followed by different letters denote significant difference and those followed by same letters denote no significant difference at $P<0.05$.

not shown). Field-grown C3 plants were also analysed but no EPA, DPA or DHA was detected. In addition, our analysis was extended to six other Australian wild purslane varieties with the same result (data not shown).

This finding is in agreement with those of Simopoulos et al. [5], Guil et al. [6] and Guil and Rodriguez [7]. The presence of low levels of longer chain omega-3 fatty acids in purslane had been reported in early studies by Simopoulos and Salem [12] and Omara-Alwala et al. [11] using GC analysis, but confirmation of chemical identity by MS was not undertaken. Peaks with retention times corresponding to EPA and DHA were also observed in our GC chromatograms but this tentative identification was not confirmed by MS analysis.

3.4. β-Carotene contents

The contents of β-carotene in the purslane leaves and stems were in the range of 21–30 and 3.6–6.5 μg/g fresh mass, respectively (Fig. 3), which are about the same as reported in literature [5,7]. The contents of β-carotene in leaves were 3.2–8.3-fold higher than that in stems. There were some significant differences between the varieties. C1 and C3

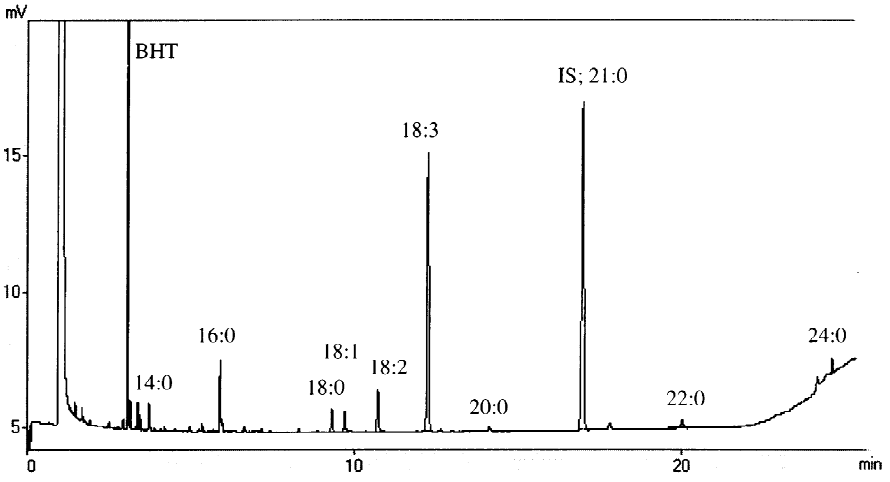

Fig. 1. Typical gas chromatogram of fatty acids extracted from leaves of an Australian variety of purslane.

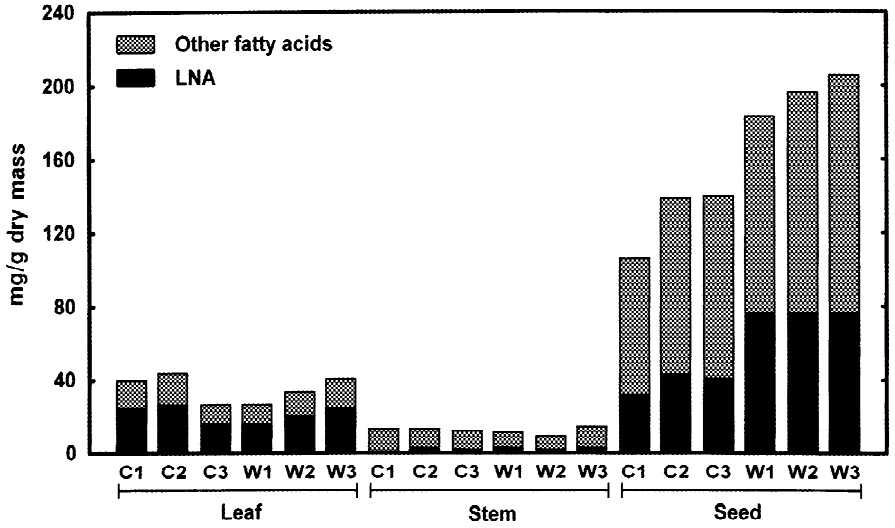

Fig. 2. Total fatty acid and LNA contents (relative to dry mass) of leaves, stems and seeds of different purslane varieties.

have relatively high contents of β-carotene in leaves than others.

4. Conclusion

This study demonstrates that Australian varieties of purslane contain substantial amounts of the antioxidant β-carotene and the omega-3 fatty acid LNA, which is essential for animal and human nutrition. The levels detected in leaves, stems and seeds were comparable to those of imported cultivars. However, the presence of EPA, DPA and DHA in purslane previously reported by other researchers could not be confirmed in either local or imported varieties. The Australian purslane varieties have great potential as a

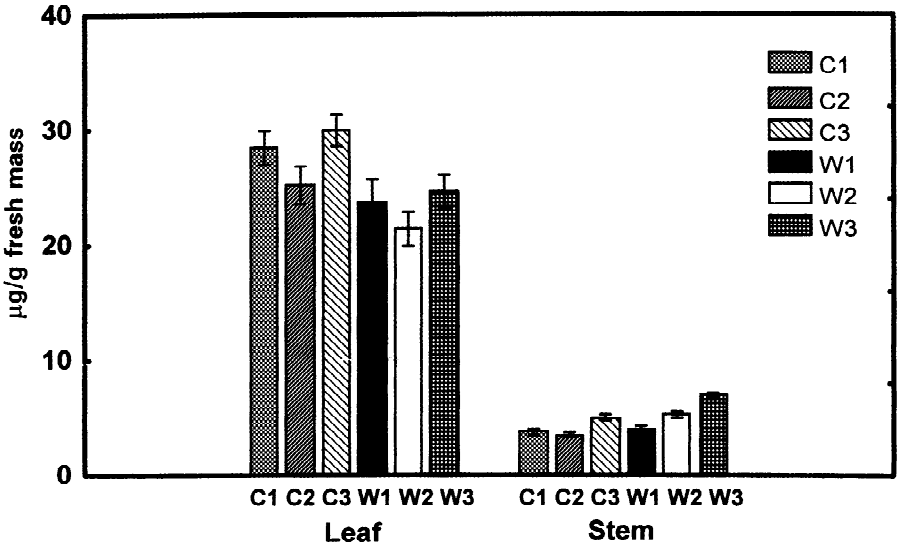

Fig. 3. β-Carotene contents of leaves and stems of different purslane varieties.

hitherto unexploited source of β-carotene and LNA for human and animal consumption.

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