The α-linolenic Acid Content of Green Vegetables Commonly Available in Australia

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Received for publication: February 21, 2001

Abstract: Green vegetable consumption has long been considered to have health benefits mainly due to the vitamins, minerals and phytonutrients (such as vitamin C, folate, antioxidants etc) contained in a vegetable-rich diet. Additionally, green vegetables are known to contain a relatively high proportion of omega-3 polyunsaturated fatty acids (PUFAs), primarily in the form of α-linolenic acid (18:3n-3). However, there are no data available on the fatty acid composition and concentration of green vegetables commonly consumed in Australia. The present study determined the fatty acid content of 11 green vegetables that are commonly available in Australia. The total fatty acid concentrations of the vegetables under study ranged from 44 mg/100 g wet weight in Chinese cabbage to 372 mg/100 g in watercress. There were three PUFAs in all vegetables analyzed; these were 16:3n-3, 18:2n-6, and 18:3n-3 fatty acids. Sample vegetables contained significant quantities of 16:3n-3 and 18:3n-3, ranging from 23 to 225 mg/100g. Watercress and mint contained the highest amounts of 16:3n-3 and 18:3n-3, and parsley had the highest amount of 18:2n-6 in both percentage composition and concentration. Mint had the highest concentration of 18:3n-3 with a value of 195 mg/100 g, while watercress contained the highest concentration of 16:3n-3 at 45 mg/100 g. All 11 green vegetables contained a high proportion of PUFAs, ranging from 59 to 72% of total fatty acids. The omega-3 PUFA composition ranged from 40 to 62% of total fatty acids. Monounsaturated fatty acid composition was less than 6% of total fatty acids. The proportion of saturated fatty acids ranged from 21% in watercress and mint to 32% of total fatty acids in Brussels sprouts. No eicosapentaenoic and docosahexaenoic acids were detected in any of the samples. Consumption of green vegetables could contribute to 18:3n-3 PUFA intake, especially for vegetarian populations.

Key words:

Introduction

Diet has long been considered to play a critical role in human health. Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are becoming of increasing nutritional interest, as evidenced in the medical research literature of the last two decades. Increased n-3 PUFA intake from marine sources has been shown to reduce the risk for coronary heart disease [1–3], improve inflammatory conditions such as rheumatoid arthritis [4], reduce plasma triacylglycerol levels [5], and lower blood pressure [6, 7]. Symptoms of depression may also be improved by an increased n-3 PUFA intake [8, 9].

Marine foods such as fish and shellfish are the main dietary sources of long chain n-3 PUFA, such as eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic

Abbreviations: 18:3n-3, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

acid (DHA or 22:6n-3). The precursor of these long chain n-3 PUFA is a-linolenic acid (18:3n-3), which is mainly found in flaxseed oil, perulla oil, canola oil, walnut oil, soy oil, baked beans and most green leafy vegetables [10]. Most animals can convert 18:3n-3 to 20:5n-3 and 22:6n-3, however this conversion does not occur in plants, therefore there are no C20 and C22 n-3 LC PUFAs in vegetable-based vegan diets. Vegans must obtain EPA and DHA by endogenous synthesis from 18:3n-3, by desaturation and elongation [11]. A recent study conducted in Spain [12] on some common native coastal edible vegetable species reported that these species contained a higher lipid content than more common vegetables. These vegetables also contained small amounts (≈ 0.1–3.8% of total fatty acids) of the long chain n-3 fatty acids EPA and DHA, although the identification was based on GC retention time and not on mass spectrometry.

Australian food composition databases lack information on n-3 PUFAs in edible green vegetables [13]. The aim of this study was to determine the content of 18:3n-3 and other fatty acids, and also any long chain n-3 PUFAs such as EPA and DHA in edible green vegetables commonly available in Australia. In the present study, we have analyzed the fatty acid content in 11 commonly consumed green vegetables (Fig. 1): spinach (Spinacea oleracea), watercress (Nasturtium officinale), parsley (Petrolelinum crispum), Chinese cabbage (Brassica chinensis), Brussels sprouts (Brassica oleracea var. gemmifera), bok choy (Brassica chinensis), cos lettuce (Lactuca sativa), broccoli (Brassica oleracea), Chinese broccoli (Brassica alboglabra), baby bok choy (Brassica chinensis) and mint (Mentha viridis, M. spicata, M. Crispa).

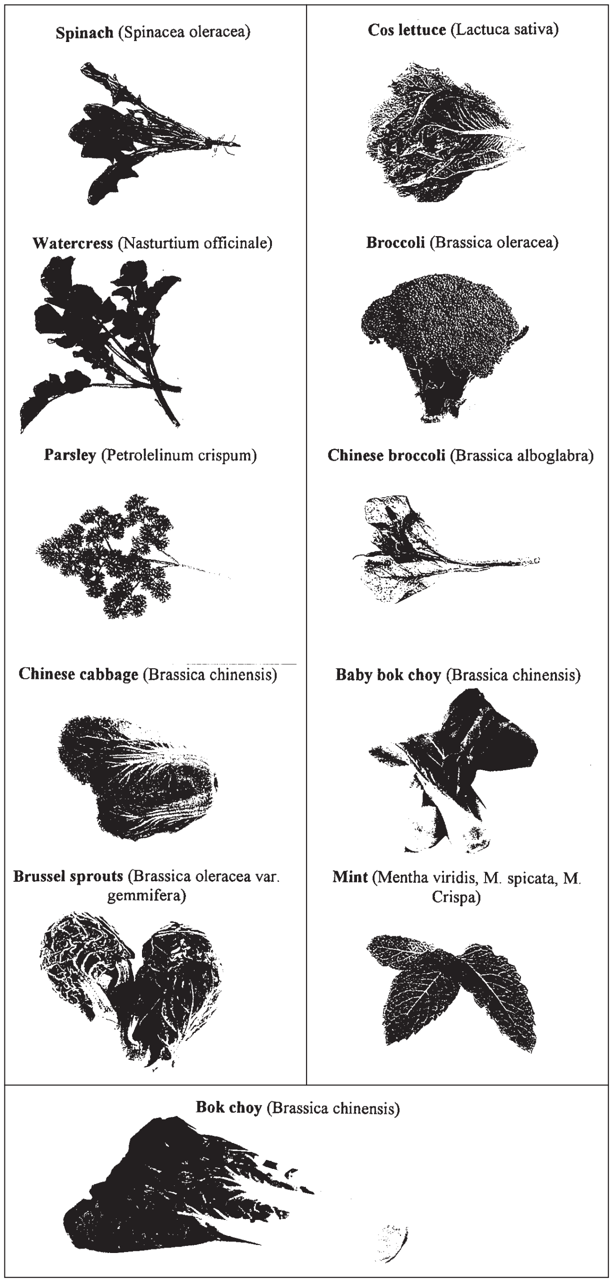

Figure 1: Eleven commonly available green vegetables.

Methods and Materials

Samples: All vegetables were purchased from the Queen Victoria Market, Melbourne. The green vegetables chosen for analysis were based on the predominance in the stalls at the market. The samples chosen for analysis were: bok choy, spinach, watercress, parsley, cos lettuce, Chinese broccoli, baby bok choy, mint, broccoli, Brussels sprouts, Chinese cabbage. All samples were analyzed on the morning of purchase. Prior to analysis, the samples were blotted to remove adhering moisture and then the leaves or heads were chopped and blended. To determine any variation in lipid content, six sub-samples, each weighing approximately 10 g, were analyzed for each of the 11 vegetables. If results were inconsistent in terms of the total fatty acid content, a further sample was purchased and the analysis was repeated.

Lipid analysis: Approximately 10 g of sample was extracted with 50.0 mL of methanol-chloroform (2:1 v/v) containing 10 mg/L of butylated hydroxytoluene and 0.2 mg/mL of tricosanoic acid (C23:0, Nu-Chek-Prep, Elysian, MN, USA) as an internal standard. Each sample was filtered; the residue from the filter paper was then scraped off and blended with about 30 mL of methanol-chloroform (2:1 v/v) and 10 mL of distilled water. This was filtered again using the same filter paper and buchner funnel, washed with about 15 mL of methanol-chloroform

(2:1 v/v). The combined filtrates were then transferred into a 500 mL separating funnel. The buchner funnel was washed with about 25 mL of chloroform and 30 mL of distilled water, and the wash added into the separating funnel. The samples were shaken well and then stored in a fume hood overnight to allow the phases to separate. The lower phase was evaporated under a vacuum and the dried extract was then washed with chloroform and transferred into a 10 mL methylation tube; the chloroform was then evaporated under nitrogen. The lipid extraction method was based on the method described by Kates [14].

The fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by saponification by using KOH (0.68 mol/L in methanol) followed by transesterification in BF_3 in methanol [15]. The FAMEs were separated by capillary gas liquid chromatography using a 50×0.22 mm (I.D.) fused silica column bonded phase column (BPX70, SGE, Melbourne, Australia). Fatty acids were identified by comparison with standard mixtures of FAMEs and results were calculated using response factors derived from chromatograph standards of known composition (Nu-Chek-Prep, Elysian, MN, USA). Silver ion TLC was used to identify any peaks on the GC traces that could not be identified using the standards [16]. Four areas, each containing a different group of FAMEs, were scraped off the plates for each sample: the first area from the top contained saturated FAMEs, the second area contained monounsaturated FAMEs, the third area contained two double-bond FAMEs, and the fourth area contained three double-bond FAMEs. The FAMEs were extracted with diethyl ether, dried under N_2, then reconstituted with petroleum ether and analyzed by GC.

Results and Discussion

The objectives of this study were to quantify 18:3n-3 and other fatty acids in commonly available green vegetables. The data obtained could contribute to the Australian food composition database to provide information for further research and to the general public. For all samples in this study, only the chloroplast-rich leaves or heads were analyzed , and stems were omitted from analysis. The 18:3n-3 fatty acid was the most predominant fatty acid in all analyzed samples.

There were 3 PUFAs in all vegetables analyzed: 16:3n-3, 18:2n-6, and 18:3n-3. The 16:3n-3 was identified based on its retention time and occurrence in the same Ag fraction as 18:3n-3. For all vegetables, 18:3n-3 had the highest concentration and 18:2n-6 had the second highest concentration, except for bok choy and watercress, in which the 16:3n-3 concentration was higher (Table I). The 18:3n-3 concentration of ranged from 22 mg/100 g fresh weight in Chinese cabbage to 195 mg/100 g in mint. The concentration of 18:2n-6 for the samples ranged from 3 mg/100 g in baby bok choy to 97 mg/100 g in parsley. The concentration of 16:3n-3 ranged from 0.6 mg/100 g in Chinese cabbage to 45 mg/100 g in watercress. Cos lettuce and mint were only the vegetables in which 16:3n-3 was not detected.

The percentage composition of 16:3n-3 and 18:3n-3 ranged from 40% in Brussels sprouts to 59% in watercress (Table II). Mint had the highest proportion of 18:3n-3 at 58% of total fatty acids. Parsley had the lowest proportion of 18:3n-3 at 30% of total fatty acids. Although baby bok choy and Chinese cabbage had low concentrations of 18:3n-3, the percentage composition of this fatty acid was high, at 51% for both. The percentage composition of 18:2n-6 was higher in parsley than in any other vegetable at 24%. The vegetable with the lowest proportion of 18:2n-6, at 6% of total fatty acids, was baby bok choy. The percentage composition of 16:3n-3 of analyzed vegetables ranged from 1% in Chinese cabbage and Brussels sprouts to 12% in watercress. Mint and cos lettuce were only the analyzed vegetables with no 16:3n-3 detected. No EPA or DHA was detected in any of the green vegetable samples in the present study, based on the limits of detection in this analysis of 0.05 mg fatty acid per 100 g sample.

The total saturated fatty acid concentration ranged from 12 mg/100 g in Chinese cabbage to 102 mg/100 g in parsley, and the percentage composition ranged from 21% of total fatty acids in spinach, watercress and mint to 28% in Chinese cabbage and Chinese broccoli. The most predominant saturated fatty acid was 16:0, which ranged from 9 mg/100 g in Chinese cabbage to 72 mg/100 g in Brussels sprouts. The percentage composition of 16:0 of analyzed vegetables ranged from 14% in mint to 29% in Brussels sprouts. Brussels sprouts had the highest level of 16:0 in both concentration and composition.

Spinach had the highest concentration (41 mg/100 g) and percentage composition (16% of total fatty acids) of total monounsaturated fatty acids. The main monounsaturated fatty acid in analyzed vegetables was 18:1 for spinach, Chinese cabbage, Brussels sprouts, broccoli and baby bok choy, while in watercress, parsley, bok choy, cos lettuce, Chinese broccoli and mint, the predominant monounsaturated fatty acid was 15:1. There was no 15:1 detected in Chinese cabbage or Brussels sprouts.

The differences in the total fatty acid content between the samples may be due to the greener samples such as watercress and mint having more chloroplasts, in which fatty acids are synthesized [17]. The Chinese cabbage and baby bok choy were lighter green in color and had a lower concentration of fatty acids.

The concentrations of fatty acids varied quite significantly for the vegetables analyzed, between and within

Table I: Fatty acid concentration (mg/100 g wet weight) of common consumed fresh green vegetables[a]

	Saturates							Monounsaturates					Polyunsaturates n-6 series		Polyunsaturates n-3 series				
	15:0	16:0	18:0	20:0	22:0	24:0	Total	15:1	16:1	18:1	24:1	Total	18:2	Total	16:3	18:3	Total	Total PUFA	Total fatty acid
Spinach (n=12)	5.9 (±1.0)	45.3 (±4.5)	2.2 (±0.5)	0.8 (±0.0)	1.5 (±0.1)	3.7 (±0.0)	59.5[b] (±6.0)	16.8 (±4.4)	9.2 (±0.9)	17.7 (±2.1)	nd	40.7[d] (±6.9)	32.8 (±4.0)	32.8 (±4.0)	20.5 (±2.4)	129.2 (±16.4)	149.6 (±18.5)	182 (±22)	287 (±34)
Watercress (n=18)	10.6 (±1.5)	62.2 (±12.8)	2.8 (±0.5)	0.4 (±0.1)	0.7 (±0.0)	1.6 (±0.3)	77.5[b] (±13.7)	25.5 (±4.6)	7.3 (±2.2)	4.2 (±1.2)	1.0 (±0.2)	36.6[d] (±7.4)	34.9 (±8.6)	34.9 (8.6)	45.4 (±13.7)	179.6 (±70.5)	225.0 (±83.1)	260 (±91)	374 (±107)
Parsley (n=12)	8.6 (±1.9)	67.3 (±23.8)	2.5 (±0.7)	1.4 (±0.3)	6.1 (±0.6)	7.8 (±0.5)	101.7[c] (±28.1)	20.0 (±5.0)	8.2 (±3.1)	3.3 (±1.1)	nd	31.6[d] (±6.9)	97.2 (±30.2)	97.2 (±30.2)	44.0 (±12.1)	124.8 (±33.9)	168.8 (±45.9)	266 (±76)	399 (±106)
Chinese cabbage (n=6)	nd	9.0 (±0.8)	1.3 (±0.2)	0.3 (±0.1)	0.7 (±0.3)	0.8 (±0.4)	12.1 (±0.8)	nd	0.2 (±0.0)	4.3 (±0.3)	0.6 (±0.2)	5.0 (±0.3)	3.5 (±0.3)	3.5 (±0.3)	0.6 (±0.3)	22.4 (±3.4)	22.9 (±3.8)	26 (±4)	44 (±4)
1.4 Brussels sprouts (n=6)	nd	71.7 (±15.8)	5.8 (±1.4)	1.4 (±0.1)	nd	1.4 (±0.0)	78.2 (±0.3)	nd	7.1 (±1.1)	13.0 (±2.5)	2.5 (±0.3)	22.2 (±2.9)	47.2 (±11.5)	47.2 (±11.5)	2.8 (±1.5)	97.8 (±24.5)	100.5 (±24.0)	148 (±35)	248 (±54)
Bok choy (n=8)	4.1 (±0.3)	34.1 (±2.4)	4.2 (±0.3)	0.6 (±0.0)	0.8 (±0.1)	1.3 (±0.1)	43.5 (±2.0)	11.3 (±1.8)	2.3 (±0.2)	4.7 (±0.9)	1.1 (±0.1)	17.5 (±3.2)	15.7 (±2.0)	15.7 (±2.0)	22.4 (±3.9)	103.0 (±16.1)	125.4 (±19.9)	141 (±22)	202 (±22)
Cos lettuce (n=5)	2.4 (±0.1)	32.2 (±2.7)	2.0 (±0.2)	0.5 (±0.3)	1.7 (±0.1)	3.2 (±0.2)	42.6 (±3.4)	5.2 (±0.3)	2.3 (±0.3)	2.0 (±0.4)	0.9 (±0.1)	10.2 (±01.2)	42.6 (±5.4)	42.6 (±5.4)	nd	94.0 (±19.0)	94.0 (±19.0)	137 (±24)	189 (±27)
Broccoli (n=6)	1.4 (±0.2)	61.3 (±3.5)	5.3 (±0.2)	1.3 (±0.1)	0.8 (±0.1)	2.7 (±2.4)	73.9 (±3.7)	3.1 (±0.7)	0.8 (±0.3)	16.4 (±0.6)	1.3 (±0.1)	21.6 (±0.9)	45.4 (±3.8)	45.4 (±3.8)	3.6 (±0.6)	110.3 (±16.4)	114.0 (±17)	159 (±21)	255 (±20)
Chinese broccoli (n=12)	3.3 (±0.5)	29.2 (±3.4)	3.5 (±0.3)	0.5 (±0.0)	0.7 (±0.0)	16.9 (±14.2)	52.7 (±16.6)	6.6 (±1.4)	2.5 (±0.4)	3.8 (±1.1)	nd	11.3 (±4.3)	33.9 (±4.8)	33.9 (±4.8)	15.1 (±2.4)	67.5 (±10.0)	82.6 (±12.4)	117 (±17)	180 (±32)
Baby bok choy (n=6)	0.4 (±0.0)	10.8 (±1.4)	1.2 (±0.2)	0.3 (±0.0)	0.4 (±0.0)	0.7 (±0.0)	13.5 (±1.6)	0.8 (±0.1)	0.5 (±0.1)	2.5 (±1.0)	0.4 (±0.1)	4.3 (±0.9)	3.1 (±0.6)	3.1 (±0.6)	3.2 (±0.7)	27.7 (±5.6)	30.8 (±6.3)	34 (±7)	52 (±7)
Mint (n=6)	7.5 (±0.3)	46.3 (±6.0)	7.7 (±0.9)	3.3 (±0.3)	2.7 (±0.2)	0.8 (±0.1)	68.9[c] (±7.2)	17.3 (±2.2)	8.2 (±1.3)	3.2 (±0.5)	nd	29.1[d] (±3.4)	31.2 (±5.1)	31.2 (±5.1)	nd	194.9 (±38.9)	194.9 (±38.9)	226 (±44)	325 (±52)

[a] Mean ± SD , [b] includes 14:0, [c] includes 14:0 and 17:0, [d] includes 20:1, nd = not detected.

Table II: Fatty acid composition (% of total fatty acid) of common consumed fresh green vegetables[a]

	Saturates							Monounsaturates					Polyunsaturates n-6 series		Polyunsaturates n-3 series			
	15:0	16:0	18:0	20:0	22:0	24:0	Total	15:1	16:1	18:1	24:1	Total	18:2	Total	16:3	18:3	Total	Total PUFA
Spinach	2.0	15.8	0.8	0.3	0.6	1.3	21.0[b]	5.9	3.2	6.3	nd	15.9[d]	11.7	11.7	7.2	43.5	50.7	62.4
(n=12)	(±0.6)	(±1.8)	(±0.1	(±0.0)	(±0.1)	(±0.2)	(±2.7)	(±1.5)	(±0.3)	(±0.6)		(±2.4)	(±0.4)	(±0.4)	(±0.5)	(±2.7)	(±3.2)	(±3.0)
Watercress	2.9	16.8	0.7	0.1	0.2	0.5	20.9[b]	7.0	1.9	1.0	0.2	9.8[d]	9.2	9.2	11.8	47.6	59.3	68.6
(n=18)	(±0.6)	(±3.5)	(±0.2)	(±0.0)	(±0.0)	(±0.1)	(±3.5)	(±1.9)	(±0.3)	(±0.3)	(±0.0)	(±1.9)	(±0.6)	(±1.5)	(±3.3)	(±4.5)	(±4.5)	(±4.4)
Parsley	2.2	16.1	0.6	0.3	1.6	2.0	24.2[c]	5.1	2.0	0.8	nd	7.9[d]	23.8	23.8	10.8	30.3	41.0	64.8
(n=12)	(±0.8)	(±1.8)	(±0.0)	(±0.0)	(±0.3)	(±0.5)	(±1.9)	(±1.7)	(±0.3)	(±0.1)		(±1.7)	(±1.3)	(±1.3)	(±0.6)	(±2.0)	(±2.5)	(±3.6)
Chinese cabbage	nd	20.7	3.1	0.7	1.6	1.9	28.0	nd	0.6	10.1	1.4	11.7	8.0	8.0	1.2	50.9	51.9	59.5
(n=6)		(±3.1)	(±0.6)	(±0.0)	(±0.5)	(±0.6)	(±2.6)		(±0.1)	(±1.5)	(±0.3)	(±1.4)	(±0.6)	(±1.4)	(±0.4)	(±2.9)	(±(3.4)	(±3.3)
Brussels sprouts	nd	28.8	2.4	0.5	nd	0.9	31.6	nd	2.8	5.3	1.1	8.8	19.1	19.1	1.2	38.9	40.0	59.1
(n=6)		(±2.5)	(±0.3)	(±0.0)		(±0.4)	(±2.6)		(±0.3)	(±0.5)	(±0.2)	(±1.1)	(±0.5)	(±0.5)	(±0.6)	(±2.2)	(±2.2)	(±2.3)
Bok choy	2.0	16.9	2.2	0.3	0.4	0.7	21.6	5.7	1.1	2.3	0.6	8.8	7.8	7.8	11.0	50.5	61.7	69.4
(n=8)	(±0.2)	(±1.7)	(±0.2)	(±0.0)	(±0.0)	(±0.0)	(±0.3)	(±1.4)	(±0.0)	(±0.4)	(±0.0)	(±2.0)	(±0.3)	(±2.0)	(±1.1)	(±2.8)	(±3.7)	(±3.7)
Cos lettuce	1.3	17.0	1.1	0.3	0.9	1.7	22.6[b]	2.8	1.2	1.1	0.5	5.5	22.7	22.7	nd	48.9	48.9	71.7
(n=5)	(±0.2)	(±1.6)	(±0.1)	(±0.0)	(±0.1)	(±0.3)	(±2.4)	(±0.4)	(±0.1)	(±0.2)	(±0.0)	(±0.8)	(±0.6)	(±0.6)		(±3.6)	(±3.6)	(±3.1)
Broccoli	0.5	22.6	1.8	0.5	0.3	1.0	27.1[c]	1.1	0.3	5.6	0.5	8.0	17.0	17.0	1.3	40.3	41.6	58.6
(n=6)	(±0.1)	(±2.6)	(±0.4)	(±0.1)	(±0.0)	(±0.8)	(±2.4)	(±0.3)	(±0.1)	(±0.3)	(±0.0)	(±0.7)	(±0.2)	(±0.2)	(±0.1)	(±3.1)	(±3.2)	(±3.4)
Chinese broccoli	1.6	15.4	1.9	0.3	0.3	8.7	27.6	3.4	1.3	1.9	nd	5.8	18.2	18.2	7.9	35.6	43.5	61.7
(n=12)	(±0.1)	(±1.0)	(±0.2)	(±0.0)	(±0.0)	(±9.1)	(±8.0)	(±1.4	(±0.1)	(±0.3)		(±2.7)	(±1.1)	(±1.1)	(±0.3)	(±2.6)	(±2.9)	(±3.9)
Baby bok	0.8	20.2	2.4	0.6	0.8	1.3	25.9	1.5	0.9	5.6	0.8	8.9	5.9	5.9	5.9	51.1	57.0	62.9
choy (n=6)	(±0.2)	(±3.2)	(±0.4)	(±0.1)	(±0.1)	(±0.2)	(±3.8)	(±0.3)	(±0.1)	(±0.6)	(±0.1)	(±0.8)	(±0.3)	(±0.3)	(±0.6)	(±3.7)	(±4.2)	(±4.3)
Mint	2.2	13.9	2.4	1.0	0.7	0.3	20.9	5.3	2.4	1.0	nd	8.9[d]	9.5	9.5	nd	57.9	57.9	67.3
(n=6)	(±0.4)	(±1.3)	(±0.2)	(±0.1)	(±0.3)	(±0.0)	(±2.1)	(±1.1)	(±0.3)	(±0.1)		(±1.1)	(±0.2)	(±0.2)		(±3.2)	(±3.2)	(±3.2)

[a] Mean ± SD , [b] includes 14:0, [c] includes 14:0 and 17:0, [d] includes 20:1, nd = not detected.

each experiment. For some of the vegetables such as Chinese cabbage and Brussels sprouts, the outer leaves were of a darker green color and the inner leaves were a lighter green, a difference which may have contributed to variability. The moisture content between different samples of the same vegetables may have also varied. Bok choy was one of the vegetables that had some dry patches on the leaves; this attribute may have contributed to variable moisture content.

Although concentration in fatty acids varied between experiments for the same vegetable, the percentage composition of total fatty acids was very similar between subsamples foreach vegetable. For example, the spinach had a CV of 13% for 18:3n-3 concentration, while it was only 6% when the 18:3n-3 was expressed as percentage composition of total fatty acids. This suggests that concentration can vary between different samples of vegetables but the percentage composition can remain quite constant. For future studies, the moisture and chlorophyll content of vegetables should be measured in order to express the fatty acid concentration data on either a dry weight or chlorophyll basis.

In conclusion, green vegetables contained a relatively high level of polyunsaturated fat, and low saturated fat content. The most predominant PUFA in all vegetables was 18:3n-3. Other important PUFAs found in the vegetables were 18:2n-6 and 16:3n-3. This latter fatty acid may be a precursor of 18:3n-3 in humans, based on data showing that 16:3n-3 may be chain- elongated to 18:3n-3 in animals, thus contributing to the total body pool of 18:3n-3 [18]. The long chain n-3 fatty acids EPA and DHA were not detected in the present study. Consumption of several servings per day of these green vegetables could contribute to n-3 PUFA intake, especially important for vegetarian populations.

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