

Content of Higher Fatty Acids in Green Vegetables

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Abstract: Green vegetables are considered an important source of some nutritionally important constituents that have health benefits (e.g. vitamins, minerals, antioxidants, fibre). Epidemiological data suggest that consuming a diet rich in fruit and vegetables can lower the risks for chronic diseases, such as cardiovascular diseases and cancer. Over the past 100–150 years, there have been enormous increases in the consumption of omega-6 fatty acids due to the increased intake of vegetable oils from various seeds. Studies have indicated that a high intake of omega-6 fatty acids shifts the physiological state to one that is prothrombotic and pro-aggregatory, whereas omega-3 fatty acids have anti-inflammatory, antithrombotic, anti-arrhythmic, hypolipidemic and vasodilatory properties. Literature data regarding the contents of higher fatty acids (e.g. omega-6 fatty acids) in vegetables are scarce, although vegetables are known to contain a high proportion of n-3 fatty acids. Here, the fatty acid content and composition was determined for 26 green vegetables that are commonly available in Slovenia, by gas-liquid chromatography and *in situ* transesterification. The fatty acid analysis revealed C16:0, C16:1, C18:0, C18:1, C18:2n-6 and C18:3n-3. The total fatty acid content in the vegetables ranged from 500 mg/100 g fresh weight (f.w.) in red cabbage, to 4.000 mg/100 g f.w. in tarragon. The proportion of saturated fatty acids (as g/100 g total fatty acids) ranged from 12% to 35%. All of the vegetables contained a high proportion of poly-unsaturated fatty acids (PUFAs), ranging from 45% to 81% of total fatty acids. The omega-3 PUFA proportion ranged from 5% in carrot to 60% in tarragon. The content of mono-unsaturated fatty acids ranged from 1% to 25%. French beans, tarragon and radish sprouts contained the highest concentrations of C16:1, at 5 mg/100 g f.w. Consumption of 100 g of tarragon meets 13.2% of daily requirements for α -linolenic acid; similarly, for radish sprouts 9.4%, for mangold 6.9%, for rucicola 5.4%, for green salad 5.0%, and for kale 4.7%. Green vegetables are an important source of 18:3n-3 PUFAs, especially for vegetarian populations.

Keywords: higher fatty acids; green vegetables; gas-liquid chromatography

INTRODUCTION

The omega-3 (n-3) fatty acids are essential fatty acids, and they are necessary from conception throughout life (INNIS 1993; SIMOPOULOS 1997; SIMOPOULOS *et al.* 1999; CONNOR 2000; BAHRI *et al.* 2002; DE LORGERIL & SALEN 2004). Indeed, a dietary recommendation for prevention of cardiovascular diseases is the regular consumption of n-3 fatty acids. Fish and fish oils contain long-chain polyunsaturated fatty acids (PUFAs; e.g. eicosapentaenoic and docosahexaenoic acid) that have preventive roles in coronary heart diseases (BROPHY 2009). In the past, the human diet contained approximately equal amounts of omega-6

(n-6) and n-3 essential fatty acids (SIMOPOULOS 2002); a reduced consumption of foods rich in n-3 fatty acids and an increased consumption of n-6 fatty acids results in an imbalance in the ratio of n-6/n-3 fatty acids. An n-6/n-3 ratio from 4 to 10 has been recommended in various countries.

While the n-3 series of PUFAs are present mainly in fish oils (e.g. from cod, salmon, sardine, sole), the n-6 series are more common in vegetable oils (e.g. from seeds of grape, corn, sunflower). GUIL *et al.* (1996) have shown high n-3/n-6 ratios in some wild plants, which would thus indicate good nutritional qualities with respect to these fatty acids. α -linolenic acid (C18:3) is part of the n-3 series, and it is known to be present in many plants

(GHAFOORUNISSA & PANGREKAR 1993; PEREIRA *et al.* 2001; LIU *et al.* 2002), along with C16:3n-3 in some plant species (MONGRAND *et al.* 1998). α -linolenic acid can also act as a precursor of long-chain PUFAs. However, although α -linolenic acid has been shown to increase the synthesis of long-chain PUFAs (LIOU *et al.* 2007), according to INDU and GHAFOORUNISSA (1992), high long-term intake of α -linolenic acid provides only modest benefits compared to fish oils. It has also been shown that supplementation of high doses of α -linolenic acid, in the form of linseed oil, produce antiaggregatory effects (BUDOWSKI 1988). The aim of the present study was to determine the importance of green vegetables as a source of fatty acids, and especially of α -linolenic acid.

MATERIAL AND METHODS

Samples. All of the green vegetable, mushroom and legume samples were purchased fresh in a market in Ljubljana. The edible portions were separated from those non-edible, and washed under running tap water. Excess water was removed in a kitchen centrifuge; the edible portion was then dehydrated immediately by lyophilisation.

Preparation of methyl esters of fatty acids. Fatty acid methyl esters (FAMES) were prepared from the lyophilised samples of green vegetables, mushrooms and some legumes according to the method described by GARCES and MANCHA (1993).

Analytical conditions. The FAMES were separated using an HP 6890 N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector and a Supelco SPB-PUFA capillary column (No: 24314; 30 m \times 0.25 mm \times 0.5 μ m); the column temperature was kept at 210°C. The injector temperature was 220°C and the detector temperature 280°C. The flow

rate of the carrier gas (He, Istrabenz 99.9999%) was 1.0 ml/min.

The following fatty acids were detected in green vegetables: C16:0, C16:1, C18:0, C18:1, C18:2n-6, C18:3n-3. Reliability and accuracy of the analytical methods for the detection of fatty acids were ensured by the use of the certified reference matrix that consisted of a mixture of 37 FAME standards (Supelco 37 Component FAME mix, Sigma-Aldrich, St. Louis, MO, USA). The amounts of each of the fatty acids were calculated from the areas of the internal standards (heptadecanoic acid). The contents of the particular fatty acids are expressed as percentages of the sum of all of the fatty acids analysed.

Statistical methods. The data were evaluated using the method of variance analysis with the Duncan test ($P < 0.05$) of the SAS/SAT Software programme.

RESULTS AND DISCUSSION

Following the methods described by GARCES and MANCHA (1993), the average contents of the fatty acids in samples from green vegetables, mushrooms and some legumes are presented in Table 1. The most abundant was linoleic acid (C18:2), followed by α -linolenic acid (C18:3). The average ratio of n-6n/n-3 is 1.45, and it is thus very suitable according to nutritional recommendations.

The total fatty acid concentrations of the vegetables under study ranged from 500 mg/100 g f.w. in red cabbage, to 4.000 mg/100 g f.w. in taragon. The differences among the different fatty acid contents (as g/100 g total fatty acids) were statistically significant ($P < 0.05$), with the exception of C16:1.

According to the fatty acid compositions listed in Table 2, these vegetables can be divided in the following groups:

Table 1. Average fatty acid composition of the green vegetables, mushrooms and legumes analysed, and average relative levels of these fatty acids

Average content of fatty acids in all samples (mg/100 g f.w.)					
C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
402.58	22.96	61.70	258.07	833.42	623.44
Average relative levels of fatty acids (% ^a)					
22.15	1.20	3.66	7.67	38.70	26.62

^a expressed as g/100 g total fatty acids

Table 2. Fatty acid composition of vegetables

	Fatty acid (%)*					
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Green leafy vegetables						
Artichoke	29.3 ± 3.3 ^{cde}	2.3 ± 3.8 ^{abc}	4.1 ± 2.3 ^d	3.1 ± 2.7 ^{ghij}	46.8 ± 10.7 ^e	14.4 ± 2.3 ^l
Asparagus	25.6 ± 0.6 ^{def}	0.7 ± 0.2 ^{bc}	1.1 ± 0.1 ⁱ	4.2 ± 0.2 ^{gh}	54.3 ± 1.0 ^d	14.0 ± 2.0 ^l
Mangold	16.9 ± 2.3 ^{hij}	1.7 ± 23.6 ^{abc}	1.2 ± 2.5 ^{hi}	2.3 ± 0.0 ^{hij}	21.2 ± 1.6 ^{hijk}	56.6 ± 0.8 ^b
Broccoli	25.0 ± 1.1 ^{ef}	1.6 ± 1.7 ^{abc}	3.8 ± 1.8 ^d	2.3 ± 1.6 ^{hij}	16.9 ± 5.9 ^{kl}	50.2 ± 1.8 ^d
Lettuce	16.3 ± 0.5 ^{hijk}	0.9 ± 0.5 ^{abc}	1.3 ± 0.1 ^{ghi}	1.3 ± 0.1 ^j	20.2 ± 0.4 ^{ijk}	59.9 ± 0.6 ^a
French beans	31.5 ± 10.3 ^{bc}	3.4 ± 5.2 ^a	3.8 ± 3.3 ^d	3.2 ± 2.8 ^{ghij}	28.2 ± 5.1 ^{fg}	29.8 ± 5.8 ^g
Parsley	32.1 ± 8.7 ^{bc}	1.0 ± 1.3 ^{abc}	1.7 ± 1.7 ^{fghi}	2.5 ± 2.6 ^{ghij}	44.8 ± 9.3 ^e	17.9 ± 3.1 ^k
Kale	23.3 ± 0.8 ^{fg}	3.2 ± 0.3 ^{ab}	2.9 ± 0.2 ^{defgh}	1.6 ± 0.2 ^{ij}	24.0 ± 0.9 ^{fghi}	44.8 ± 0.9 ^e
Red cabbage	43.8 ± 1.5 ^a	0.9 ± 0.2 ^{abc}	4.1 ± 0.1 ^d	2.7 ± 0.2 ^{ghij}	19.2 ± 1.1 ^{jk}	29.2 ± 2.4 ^{gh}
Tarragon	12.5 ± 0.3 ^{ijkl}	1.2 ± 0.1 ^{abc}	1.0 ± 0.1 ⁱ	1.0 ± 0.1 ^j	23.3 ± 0.5 ^{ghij}	60.9 ± 1.1 ^a
Sprouts						
Alfalfa sprouts	14.7 ± 0.9 ^{ijkl}	0.6 ± 0.1 ^{bc}	3.4 ± 0.1 ^{def}	7.4 ± 0.6 ^f	44.1 ± 0.5 ^{fghi}	29.6 ± 0.7 ^{ij}
Radish sprouts	10.2 ± 1.2 ^l	0.6 ± 0.1 ^c	3.1 ± 0.5 ^{defg}	37.7 ± 3.5 ^f	25.0 ± 0.5 ^{fghi}	23.4 ± 2.3 ^{jk}
Soya sprouts	35.9 ± 0.7 ^b	0.6 ± 0.1 ^{bc}	9.7 ± 1.1 ^b	8.4 ± 0.5 ^a	24.5 ± 0.6 ^e	20.8 ± 0.7 ^g
Legumes						
Chick-pea	11.7 ± 25.1 ^{kl}	0.5 ± 9.8 ^c	1.6 ± 2.3 ^{fghi}	31.5 ± 0.7 ^b	52.1 ± 87.4 ^d	2.4 ± 1.9 ^o
Red lentils	17.4 ± 0.3 ^{hij}	1.2 ± 0.3 ^{abc}	2.0 ± 0.1 ^{defghi}	26.6 ± 0.6 ^d	41.5 ± 0.2 ^e	11.3 ± 0.1 ^{lm}
Brown lentils	16.8 ± 0.2 ^{hij}	1.2 ± 0.5 ^{abc}	2.3 ± 0.1 ^{efghi}	24.0 ± 0.1 ^d	43.9 ± 0.8 ^e	11.6 ± 0.1 ^m
Green lentils	17.6 ± 0.1 ^{hij}	1.6 ± 0.4 ^{abc}	2.3 ± 0.1 ^{defghi}	23.6 ± 0.3 ^c	45.3 ± 0.6 ^e	9.6 ± 0.1 ^{lm}
Other						
Carrot	22.8 ± 0.2 ^{fg}	0.2 ± 0.1 ^c	1.6 ± 0.2 ^{fghi}	3.0 ± 0.1 ^{ghij}	66.5 ± 0.14 ^{bc}	5.9 ± 0.1 ⁿ
Fennel	23.1 ± 0.5 ^{fg}	1.2 ± 0.2 ^{abc}	2.5 ± 0.3 ^{defghi}	2.9 ± 0.3 ^{ghij}	63.7 ± 0.6 ^c	6.5 ± 0.2 ⁿ
Cucumber	29.9 ± 1.8 ^{cd}	1.4 ± 0.8 ^{abc}	3.3 ± 0.1 ^{def}	3.1 ± 0.3 ^{ghij}	26.2 ± 0.4 ^{fgh}	36.1 ± 2.4 ^f
Zucchini	26.0 ± 0.2 ^{def}	0.6 ± 0.3 ^{bc}	1.8 ± 0.2 ^{fghi}	1.4 ± 0.1 ^{ij}	16.8 ± 0.1 ^{kl}	53.3 ± 0.6 ^c
Egg plant	27.7 ± 0.9 ^{cdef}	1.0 ± 0.5 ^{abc}	21.4 ± 0.5 ^a	8.7 ± 0.7 ^f	29.6 ± 0.8 ^f	11.5 ± 0.9 ^{lm}
Kohlrabi	30.9 ± 1.4 ^c	1.9 ± 0.3 ^{abc}	2.7 ± 0.7 ^{defghi}	3.7 ± 0.2 ^{ghi}	26.1 ± 1.9 ^{fgh}	34.6 ± 4.1 ^f
Radish	23.5 ± 0.2 ^{fg}	1.1 ± 0.2 ^{abc}	3.3 ± 0.1 ^{def}	16.3 ± 0.6 ^e	12.1 ± 0.5 ^l	43.7 ± 0.8 ^e
Red bell pepper	19.8 ± 0.13 ^{gh}	1.3 ± 0.1 ^{abc}	6.3 ± 0.1 ^c	4.7 ± 0.2 ^g	41.4 ± 0.3 ^d	26.4 ± 0.6 ^{hi}
Green pepper	15.5 ± 0.8 ^{hijk}	1.1 ± 0.3 ^{abc}	7.2 ± 0.3 ^c	2.5 ± 0.6 ^{ghij}	53.2 ± 1.3 ^e	20.4 ± 0.9 ^{jk}
Mushrooms						
Oyster mushroom	11.4 ± 0.4 ^{kl}	0.6 ± 0.1 ^c	2.2 ± 0.4 ^{defghi}	14.8 ± 0.3 ^e	70.8 ± 0.3 ^b	0.05 ± 0.1 ^o
Champignons	13.5 ± 0.1 ^{ijkl}	0.2 ± 0.2 ^c	3.7 ± 0.1 ^{de}	1.2 ± 0.1 ^j	81.2 ± 0.3 ^a	0.2 ± 0.1 ^o
Shiitake	14.5 ± 0.1 ^{ijkl}	0.7 ± 0.1 ^{bc}	1.2 ± 0.4 ^{hi}	2.7 ± 0.1 ^{ghij}	80.7 ± 0.7 ^a	0.2 ± 0.12 ^o
Significance (P-value)	0.0001	0.4439	0.0001	0.0001	0.0001	0.0001

*expressed as g/100 g total fatty acids

1. Vegetables with high levels of C16:0 (> 30%): red cabbage (43.9%), soya sprouts (35.9%), parsley (32.1%), French beans (31.5%), and kohlrabi (31.0%);
2. Vegetables with high levels of C16:1 (> 2%): French beans (3.4%), kale (3.2%), artichoke (2.3%);
3. Vegetables with high levels of C18:0 (> 7%): egg plant (21.4%), soya sprouts (9.7%), and green pepper (7.3%);
4. Vegetables with high levels of C18:1 (> 30%): radish sprouts (37.7%), chick-pea (30.5%);
5. Vegetables with high levels of C18:2 (> 60%): shiitake (81.2%), champignons (80.7%), oyster mushroom (70.8%), carrot (66.5%), fennel (63.7%);
6. Vegetables with high levels of C18:3 (> 50%): tarragon (61.0%), green lettuce (59.9%), mangold (56.7%), zucchini (53.3%), and broccoli (50.2%).

Our data of the fatty acid contents generally correspond well to those of previous studies (SOUCI *et al.* 2000; SALVINI *et al.* 2002 and USDA National Nutrient Database for Standard Reference 2005), although a slightly higher variation was seen for C18:2 and C18:3 in most of the vegetables. In mushrooms, in particular, our data for C18:2 show substantially higher values compared to those of SOUCI *et al.* (2000) and SALVINI *et al.* (2002).

Tarragon, lettuce, rucola, mangold and radish sprouts have the highest amounts of α -linolenic acid. According to current dietary recommendations, 100 g of tarragon would cover 13% of the daily needs for α -linolenic acid. In countries like China, where daily vegetable consumption can amount to 1 kg (personal communication), vegetable intake may indeed satisfy the needs for α -linolenic acid.

Rucola, radish, courgette, green lettuce, broccoli, mangold, tarragon, kale, red cabbage, cucumber, kohlrabi and French beans all have an n-6n/n-3 ratio below 1. In developed countries, the actual n-6n/n-3 ratio in food is between 10 and 20 to 1, with the current recommendations for the n-6n/n-3 ratio as less than 10 to 1.

Only carrot, chick-pea, champignons, shiitake mushrooms and oyster mushrooms have an n-6/n-3 ratio above 10. Green vegetables in general have a suitable n-6n/n-3 ratio, which corresponds to the current nutritional recommendations (SIMOPOULOS 1999; SIMOPOULOS *et al.* 1999; SOUCI *et al.* 2000; BAHRI *et al.* 2002; SALVINI *et al.* 2002;

USDA National Nutrient Database for Standard Reference, 2005).

CONCLUSIONS

The method developed in the present study has proven to be reliable for the analysis of fatty acids in these vegetables. Our data correspond well with those of other studies, with the exception of mushrooms, where our data show higher values for C18:2. In general, higher variations were seen in our data for C18:2 and C18:3n-3.

From the nutritional point of view, the most important components in vegetable are antioxidants (vitamins) and dietary fibre. The green vegetables in our study appear to be a good source of α -linolenic acid, and they also have a suitable n-6n/n-3 ratio. Although the content of fatty acids in vegetables is rather low, they still represent an important nutritional factor, which will be especially important in vegetarian populations.

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