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Vegetables as sources of α-linolenic acid in Indian diets

Ghafoorunissa* & Jyotsna Pangrekar

National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Hyderabad–500 007, India

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Total lipids were extracted from fresh vegetables, legumes (dry beans) and fenugreek seeds and the fatty acid compositions were determined. The dry beans and fenugreek seeds contain high amounts of both linoleic and α-linolenic acids. Rajmah and cowpea provide more α-linolenic acid as compared to bengal gram and peas (linoleic/α-linolenic ratio $\leq 1{\cdot}7$ and 5·0 respectively). Fenugreek seeds contain ~2% α-linolenic acid. On an average the green leafy vegetables provide about seven times more α-linolenic acid than fresh beans and other vegetables. In cereal pulse-based lacto-vegetarian diets, inclusion of plant foods rich in α-linolenic acid on a regular basis can make important contributions to the intake of n-3 fatty acids and may ensure a better nutritional status of these fatty acids.

INTRODUCTION

The long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) namely eicosapentaenoic and docosahexaenoic acid present in fish and fish oils have hypolipidemic, antithrombotic and anti-inflammatory effects. Therefore, one of the current dietary recommendations for prevention of coronary heart diseases is to increase the intakes of n-3 fatty acids (Simopoulos, 1988; Kinsella *et al.*, 1990; Nettleton, 1991). Plant foods contain α-linolenic acid (ALNA) the precursor of LC n-3 PUFA (Tinoco, 1982) and, in a vegetarian diet, ALNA is the only source of n-3 polyunsaturated fatty acids. Recent studies in humans show that ALNA is converted to LC n-3 PUFA. The increase in LC n-3 PUFA following supplementation of high doses of ALNA in the form of linseed oil (Budowski, 1988) or a change in dietary fat source to rapeseed oil (Renaud & Nordoy, 1983; Renaud *et al.*, 1986) has been shown to produce anti-aggregatory effects. The hepatic conversion of ALNA to LC n-3 PUFA is suppressed by high levels of linoleic acid, LA (Holman, 1964). In Indian subjects maintained on cereal-based vegetarian diets, at an intake of 16 g (6 en%) LA, about 4 g ALNA (LA/ALNA = 4) increased the LC n-3 PUFA in platelets and also reduced their aggregability (Indu & Ghafoorunissa, 1992). Although the magnitude of these effects of ALNA are modest as compared to fish oils (Sanders & Roshanai, 1983; Budowski, 1988; Indu & Ghafoorunissa, 1992) long term high intakes of ALNA may provide health benefits (Nettleton, 1991). The ALNA contents of vegetables and legumes were analysed in an attempt to determine the contribution of these foods to the intakes of ALNA in Indian diets.

* To whom correspondence should be addressed.

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MATERIALS AND METHODS

The vegetables (500 g) were purchased from three different markets in the twin cities of Hyderabad and Secunderabad. The edible portion was separated from the non-edible portion, washed under running tap water, excess water removed on folds of blotting paper and weighed. The washed edible portion (50–100 g) was lyophilysed until three consecutive weights of the dry material were similar and the moisture content was calculated. The dehydrated material was ground to a fine powder and stored in stoppered screw-capped polyethylene bottles until further analysis. The dry beans were also ground to a fine powder.

A day prior to processing, the dried powders were kept overnight in an oven maintained at 40°C. The dried food sample (200 mg) and 0·5 mg heptadecanoic acid (internal standard) were digested with 4 N HCl for 30 min at 60°C and then for 30 min at 90°C. The lipids were extracted, saponified and the fatty acid methyl esters were prepared as described earlier (Ghafoorunissa, 1989). The methyl esters were analysed on a Perkin-Elmer Gas Chromatograph model 8500 equipped with a flame ionization detector. A 12 ft × ⅛ inch stainless steel column was packed with 10% silar 10C (Applied Science Division, PO Box 440, State College, PA 16901) coated on chromosorb W 80-100 A.W. (Varian Aerograph Walnut Creek, California). Ultrapure nitrogen was used as a carrier gas. The flow rate of the carrier gas was 25 ml/min. The temperatures of the injection and detector ports were kept at 230°C. The column temperature was initially 160°C and was increased at a rate of 3°C/min to 225°C and maintained at 225°C for 15 min. The individual fatty acids were identified by frequent comparisons with authentic standards. Under these conditions ALNA (18:3 n-3) and eicosenoic acid (20:1) eluted together. In order to resolve these two peaks, analysis was also done on a 12 ft × ⅛ inch column packed with 10% silar 5C coated on chromosorb W 80-100 A.W. The column temperature was initially kept at 180°C and increased at 3°C/min to 225°C and maintained at 225°C for 20 min. The amount of each fatty acid was calculated from the area of the internal standard. The standard fatty acid methyl esters and heptadecanoic acid were purchased from NuChek Laboratories (Elysian Mn). Solvents used were of analytical grade and contained 25 mg butylated hydroxytoluene per litre.

RESULTS

The fresh vegetables and legumes were grouped as: (1) green leafy vegetables, (2) fresh beans with pods, (3) other vegetables and (4) legumes (dry beans). The data on fatty acid composition are presented in Table 1. The total fat content was calculated by dividing the sum of the total fatty acids by 0·8 as used for peas in the food composition tables (Paul *et al.*, 1979). For comparison the reported values on fat, estimated by weighing the ether extractives are also given (Gopalan *et al.*, 1984). On an average, green leafy vegetables provide 0·4 g fat and fresh beans and other vegetables 0·2 g fat per 100 g food. The legumes and fenugreek seeds provide approximately 3% and 10% fat respectively.

In the green leafy vegetables, ALNA is the major fatty acid. On an average the green leafy vegetables provide 0·16 g ALNA per 100 g and, in these foods, the LA/ALNA ratio is less than 0·8 except in cabbage in which it is 1·8. In fresh beans and other vegetables, linoleic acid is the major fatty acid and the levels of ALNA are low (average 0·022 g/100 g food). The ratio of LA/ALNA ranges between 1·0 and 3·0 except in tomatoes in which the ratio is 16. Legumes and fenugreek seeds contain high amounts of both LA and ALNA. Both varieties of rajmah (black and brown) and cowpea provide more ALNA (LA/ALNA $\leq$1·7) as compared to bengal gram and peas (LA/ALNA $\geq$5). Fenugreek seeds contain ~2% ALNA.

DISCUSSION

Plant foods are the fundamental sources of essential fatty acids. It has been known for sometime now that seeds and fruits are rich sources of LA whereas in the green leaves ALNA is the predominant fatty acid (Tinoco, 1982). Much of the early information on the composition of plant lipids was on the lipid classes and the data on percentage of the total fatty acids gives ALNA along with eicosanoic (20:0) or eicosenoic (20:1) acids (Hilditch & Williams, 1964). Moreover, the methods of extraction of fat have been variable (Kinsella *et al.*, 1975; Hubbard *et al.*, 1977; Achaya, 1987). The ether extractives estimate the triglycerides and cholesterol (free fat) whereas acid hydrolysis followed by extraction with polar solvents, extracts the bound (phospholipids and glycolipids) and the structural fats (cell wall) in addition to the free fat (Kinsella *et al.*, 1975; Hubbard *et al.*, 1977; Achaya, 1987). Therefore, the latter method gives an accurate estimate of the total fat present in plant foods. These variations in the extraction of lipids make it difficult to calculate the absolute ALNA content in the edible portion of different foods. The more recent food composition tables include ALNA content of foods that are commonly consumed in western countries (Paul *et al.*, 1979; Hepburn *et al.*, 1986).

In the present study the commonly consumed fresh vegetables and legumes were hydrolysed with acid so as to enable the extraction of the total lipids. In most of the green leafy vegetables and fresh beans, the total fat content calculated from fatty acid analysis was found to be low as compared to the ether extractives (Table 1), probably because of the pigments and other unsaponifiable substances present in the ether extractives. In legumes and fenugreek seeds, total fat was more than the reported figures for ether extractives and may, therefore, be the bound and structural fats. Since the total fat in vegetables is small, these differences are, however, not important with respect to the contribution of vegetables to the total fat intake.

ALNA is synthesized in the chloroplast membranes from LA (Tinoco, 1982). Extraction of the total lipids is, therefore, important for an accurate estimate of ALNA content of plant foods. The total lipids in cabbage have been reported to vary from 0·1 to 0·7% (Peng, 1974) and the values reported here are low. The percentage composition of LA and ALNA in cabbage, spinach, amaranth and cauliflower are similar to the data in other reports (Hilditch & Williams, 1964; Allen *et al.*, 1964; Laxminarayana *et al.*, 1984). On average, the green leafy vegetables provide about seven times more ALNA than fresh beans and the other vegetables. Therefore, a daily intake of 100 g of green leafy vegetables, in addition to providing nutrients like iron and vitamins (Gopalan *et al.*, 1984), would also be an important source of ALNA.

Of the dried beans that were analysed, bengal gram contains high amounts of fat and LA and the percentages of LA and ALNA are similar to the reported values (Hilditch & Williams, 1964; Kinsella *et al.*, 1975; Tinoco, 1982). The LA/ALNA ratio in rajmah is about the same as in black gram (Ghafoorunissa, 1989). The data on fat and ALNA content in cowpea are also similar to those reported earlier (Ologhobo & Fetuga, 1983). On average, black gram, rajmah and cowpea provide about four times more ALNA than other pulses and legumes (Table 2).

Table 1. Fatty acid composition of vegetables and legumes (g/100 g food as consumed)

S. No.		Moisture	Total saturates	Total monounsaturates	Linoleic (LA)	α-Linolenic (ALNA)	LA/ ALNA	Total fat	Ether[a] extractives
1	2	3	4	5	6	7	8	9	10
Green leafy vegetables									
1.	Agathi (2) (*Sesbania grandiflora*)	73	0·24	0·05	0·09	0·48	0·19	1·08	1·4
2.	Colocasia leaves (*Colocasia antiquorum*)	81	0·16 ±0·017	0·03 ±0·002	0·15 ±0·02	0·36 ±0·05	0·42	0·88	1·5
3.	Drumstick leaves (2) (*Moringa Oleifera*)	76	0·27	0·06	0·08	0·36	0·22	0·96	1·7
4.	Fenugreek leaves (*Trigonella foenumgraecum*)	87	0·07 ±0·006	0·02 ±0·006	0·045 ±0·009	0·20 ±0·014	0·22	0·42	0·9
5.	Amaranth (*Amaranthus gangeticus*)	86	0·07 ±0·020	0·03 ±0·012	0·07 ±0·014	0·15 ±0·018	0·47	0·40	0·5
6.	Onion leaves (2) (*Allium cepa*)	92	0·04 ±0·006	0·02 ±0·009	0·06 ±0·012	0·13 ±0·029	0·48	0·31	0·2
7.	Gogu (*Hibiscus cannabinus*)	84	0·07 ±0·009	0·02 ±0·006	0·06 ±0·007	0·11 ±0·014	0·54	0·33	1·1
8.	Ambat chuka (*Rumex vesicarius*)	93	0·04 ±0·009	0·01 ±0·001	0·03 ±0·002	0·11 ±0·017	0·27	0·24	0·3
9.	Paruppu Keerai (2) (*Portulaca oleracea*)	93	0·06	0·02	0·01	0·09	0·12	0·23	0·6
10.	Cauliflower (*Brassica oleracea varbotrytis*)	90	0·05 ±0·002	0·02 ±0·002	0·06 ±0·009	0·08 ±0·009	0·75	0·26	1·3
11.	Mayalu (2) (*Basella rubra*)	91	0·07	0·03	0·05	0·08	0·67	0·29	0·4
12.	Ceylon pasali (2) (*Talinum triangulare*)	91	0·04	0·02	0·01	0·06	0·17	0·17	0·7
13.	Spinach (*Spinacia oleracea*)	92	0·05 ±0·008	0·02 ±0·005	0·02 ±0·009	0·06 ±0·014	0·37	0·19	0·7
14.	Cabbage (*Brassica oleracea varcapitata*)	92	0·02 ±0·003	0·01 ±0·004	0·01 ±0·002	0·006 ±0·003	1·83	0·06	0·1
Fresh beans									
15.	Beans, scarlet runner (*Phaseolus coccineus*)	88	0·05 ±0·004	0·01 ±0·007	0·08 ±0·023	0·03 ±0·005	2·66	0·23	1·0
16.	Cluster beans (*Cyamopsis tetragonoloba*)	86	0·06 ±0·004	0·02 ±0·003	0·05 ±0·004	0·03 ±0·003	1·5	0·20	0·4
17.	French beans (*Phaseolus vulgaris*)	90	0·04 ±0·001	0·008 ±0·001	0·05 ±0·010	0·03 ±0·002	1·5	0·15	0·1
Other vegetables									
18.	Lady's fingers (*Abelmoschus esculentus*)	89	0·06 ±0·008	0·01 ±0·004	0·07 ±0·012	0·03 ±0·001	2·0	0·22	0·2
19.	Ridge gourd (*Luffa acutangula*)	95	0·03 ±0·002	0·004 ±0·001	0·02 ±0·004	0·02 ±0·002	1·0	0·10	0·1
20.	Bottle gourd (*Lagenaria vulgaris*)	94	0·03 ±0·004	0·01 ±0·004	0·03 ±0·003	0·02 ±0·003	1·5	0·10	0·1
21.	Tomatoes (*Lycopersicon esculentum*)	94	0·06 ±0·007	0·06 ±0·013	0·16 ±0·017	0·01 ±0·002	16·0	0·35	0·2
22.	Brinjal (2) (*Solanum melongena*)	93	0·06	0·01	0·03	0·01	3·0	0·14	0·3
Dry beans									
23.	Rajmah (*Phaseolus vulgaris Brown*)	12	0·41 ±0·007	0·21 ±0·01	0·46 ±0·048	0·70 ±0·032	0·67	2·24	1·3
24.	Rajmah (Black)	12	0·43 ±0·038	0·19 ±0·00	0·44 ±0·022	0·73 ±0·032	0·60	2·24	1·3
25.	Cowpea (*Vigna Catjang*)	10	0·81 ±0·13	0·18 ±0·027	0·81 ±0·008	0·48 ±0·067	1·69	2·85	1·0
26.	Bengal gram (whole) (*Cicer Arietinum*)	10	0·7 ±0·02	1·2 ±0·12	2·73 ±0·125	0·18 ±0·02	15·2	6·01	5·3
27.	Peas (*Pisum sativum*)	15	0·32 ±0·031	0·42 ±0·046	0·76 ±0·138	0·15 ±0·013	5·07	2·06	1·1
Spices									
28.	Fenugreek seeds (*Trigonella foenumgraecum*)	13	1·22 ±0·078	1·47 ±0·267	3·44 ±0·043	1·87 ±0·009	1·84	10·00	5·8

Values are mean ± SE of three samples except where indicated.
[a] Ref. Gopalan *et al.* (1984).

Table 2. Quantities of selected plant foods which provide 100 mg ALNA

Foods	g	Foods	g
Wheat	60[a]	Green leafy vegetables	60
Bajra	80[a]	Other vegetables	400
Blackgram[b]	15[a]	Fenugreek seeds	5
Rajmah	15	Mustard/rapeseed	1·0
Cowpea	20	Soyabean oil	2·0
Redgram, greengram,[b] lentil and bengalgram	60[a]	Rice bran oil	6·0

[a] Ref. Ghafoorunissa (1989).
[b] Pulse without seed coat.

Indian diets are cereal-pulse and vegetable-based. In cereals, millets and pulses, LA is the major fatty acid. On average pulses contain more ALNA than cereals and millets (Ghafoorunissa, 1989). From the data on food intakes in the Indian population of different income groups (National Nutrition Monitoring Bureau, 1979, 1982), it is estimated that the invisible fat present in different foods provides 0·1–0·6 g ALNA per day. A more recent study on a select group of men from the urban upper middle income group showed that the daily dietary ALNA ranges from 0·3 to 0·8 g/person (Ghafoorunissa, unpublished). The results of the present study show that green leafy vegetables, legumes and fenugreek seeds can be important sources of ALNA. The current dietary guidelines for prevention of coronary heart disease in the western population emphasize the need to increase the n-3 PUFA intakes. In order to ensure adequate n-3 PUFA intakes in cereal-pulse based lacto-vegetarian diets, it is necessary to include foods which contain ALNA on a regular basis. Foods which have little fat but a high percentage of ALNA (Tables 1 and 2) can increase the intakes of n-3 PUFA.

The health benefits of increasing dietary ALNA depend on the total dietary LA. It is recommended that, in a nutritionally balanced diet, the LA/ALNA ratio should be less than 10 (Nestel, 1987). Apart from increasing dietary ALNA, it is therefore necessary to maintain a moderate intake of LA in the total diet. In the existing dietary life style of Indians, this is possible by either using a vegetable oil with moderate levels of LA (20–40%) or by including two vegetable oils (one with high and one with low LA) as sources of visible fat in the daily diet. Further, the ALNA intake can also be increased through the use of ALNA rich vegetable oils like mustard/rapeseed and soyabean (Table 2). It is possible that a regular dietary intake of ALNA may ensure a better n-3 fatty acid status and provide the health benefits associated with n-3 fatty acids.

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