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Linolenic acid and folate in wild-growing African dark leafy vegetables (morogo)

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Abstract

Background: Transition from a low-fat vegetable-rich rural diet to a high-fat Westernised diet is considered a factor in the escalating occurrence of vascular-related diseases and type 2 diabetes in urban black South Africans. Consumption of morogo is a distinguishing feature of rural African diets.

Objective: To determine fatty acid profiles and folate contents of three widely consumed, wild-growing, African dark green leafy vegetables (morogo).

Design: GC–MS was applied for analysis of fatty acid composition and a validated microbiological assay conducted to determine folic acid contents of wild-growing morogo sampled from deep rural villages in three different geographical regions of South Africa.

Results: Measured fatty acids ranged from 1610·2 to 2941·6 mg/100 g dry mass, with PUFA concentrations 1·4 to 2·8 times those of SFA. Calculated from the relative percentages of linoleic acid (18 : 2*n*-6) and linolenic acid (18 : 3*n*-3), the ratio of 18 : 2*n*-6 to 18 : 3*n*-3 PUFA was 1·0:3·4 to 1·0:8·9. The only MUFA was palmitoleic acid (16 : 1), measured at 34·7 (SD 0·3) to 79·0 (SD 9·3) mg/100 g dry mass, and the predominant SFA was palmitic acid (16 : 0), measured at 420·6 (SD 83·3) to 662·0 (SD 21·2) mg/100 g dry mass. Folic acid concentration varied from 72 to 217 μg/100 g fresh sample.

Conclusion: Morogo is low-fat food item high in folate and with 18 : 3*n*-3 in excess of 18 : 2*n*-6, the proposed anti-inflammatory effects of which may lower risks of vascular-related chronic diseases and type 2 diabetes.

Keywords
African diet
Traditional vegetables
Morogo
Polyunsaturated fatty acids
Folate

Chronic lifestyle-related cardio- and cerebrovascular diseases (CVD) and type 2 diabetes are becoming more pronounced in urbanised black South Africans[1]. Levitt and Mollentze[2] predicted that type 2 diabetes could affect over 3 million South Africans by 2010. The occurrence of these lifestyle-related chronic diseases apparently does not discriminate on a socio-economic basis[1,3,4]. Currently, dietary transition from a traditional low-fat, plant protein-rich rural diet towards a high-fat, animal protein-rich Westernised diet receives much attention as a factor contributing to the increased occurrence of chronic diseases of lifestyle in urbanised black South Africans[5,6]. In rural diets, a variety of cultivated and/or wild-growing African dark green leafy vegetables (morogo) supplement traditional maize-based staples[7]. Concerning micronutrient levels, morogo vegetables compare well with spinach, Swiss chard and cabbage[8–10]. Dark green leafy vegetables (DGLV) are also indicated as rich sources of folate[11,12] and linolenic acid (18 : 3*n*-3)[13–15]. Nutritional data on cultivated varieties of traditional African vegetables are fragmentary and almost non-existent for wild-growing morogo species. The present study reports on the fatty acid profiles and folate contents of three wild-growing morogo species: cowpea (munawa), vegetable amaranth (thepe) and spider flower (lerotho; also known as African cabbage or cat's whiskers).

Materials and methods

Sample collection and preparation

Sampling sites were situated in three geographically separated and climatically distinct areas of South Africa. One fresh field sample of each morogo type, i.e. amaranth, spider flower and leafy cowpea, were collected from rural villages respectively in the Rustenburg District (North-West Province), as well as Vhembe and Capricorn Districts (Limpopo Province). One traditionally sun-dried, household sample of spider flower was obtained from

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a rural family in Capricorn District. Samples were transported to the laboratory in Ziploc® plastic bags on ice. Upon arrival at the laboratory, the fresh samples were freeze-dried and stored at −20°C until analysis. Finely ground, freeze-dried sample material and the traditionally sun-dried material were subsequently used for fatty acid analysis. Samples for folic acid determinations were weighed accurately before oven-drying at 105°C for approximately 1 h until oven-dried weight remained constant. Moisture loss was recorded for use as a dry to wet mass conversion factor in calculations of folate concentrations in fresh leaves.

Chemicals

All organic solvents used were of GC grade and, together with fatty acid standards, were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Folic acid analysis

Freeze-dried morogo samples were sent to the South African Bureau of Standards (Pretoria, South Africa) for folic acid analysis. A standard method for the microbiological assay of folic acid in foods and pharmaceutical products was employed[16,17]. *Streptococcus faecalis* (ATCC 8043; American Type Culture Collection, Manassas, VA, USA) was the test organism. Materials included USP folic acid standard (Merck, Darmstadt, Germany), Difco Bacto-folic AOAC medium (code 0967; Becton Dickinson Microbiology Systems, Sparks, NV, USA) and Oxoid MRS agar (code CM 361; Oxoid Ltd, Basingstoke, UK) as culture media; and Oxoid MRS agar (code CM 359; Oxoid Ltd) to prepare the inoculum. Folic acid detection limit was 0·0005 μg/ml. Dry mass concentrations were converted for expression as μg/100 g wet mass.

Fatty acid determinations

Heptadecanoic acid (72 mM), as an internal standard, was added to 25 mg of lyophilised sample followed by 100 μl of a 45 mM solution of butylated hydroxytoluene and 2 ml of methanolic HCl (3 M). The mixture was subsequently vortexed before incubation at 90°C for 4 h. Following cooling to room temperature, the sample was extracted twice with 2 ml of hexane, dried under an N_2 stream and finally re-suspended in 100 μl of hexane, 1 μl of which was injected into the GC–MS system via split-less injection. An Agilent 6890 gas chromatograph ported to a 5973 mass selective detector (CA, USA) was used for identification and quantification of individual fatty acids. For the acquisition of an electron ionisation mass spectrum, an ion source temperature of 200°C and electron energy of 70 eV were used. The gas chromatograph was equipped with a SE-30 capillary column (Agilent, Palo Alto, CA, USA), a split/split-less injection piece (250°C) and a direct GC–MS coupling (260°C). Helium (1 ml/min) was used as the carrier gas. An initial oven temperature of 50°C was maintained for 1·5 min and then allowed to increase to 190°C at a rate of 30°C/min. The oven temperature was maintained at 190°C for 5 min and then allowed to increase at a rate of 8°C/min to 220°C, this temperature being maintained for 2 min. Finally, ramped at a rate of 3°C/min, the oven temperature was maintained at 230°C for 24 min. All samples were analysed in triplicate and reported as the mean and standard deviation of concentration in mg/100 g.

Statistical analyses

Data of fatty acid analyses were processed using the STATISTICA Data Analysis Software System version 7·1 (StatSoft, Inc., Tulsa, OK, USA). Statistical evaluation of measured folic acid concentrations employed statistical techniques included in a computerised data processing program of the South African Bureau of Standards.

Results

Botanical species identification

Amaranth (thepe) samples were identified as *Amaranthus hybridus* L. subsp. *cruentus* (L.) Thell. (Rustenburg), *Amaranthus hybridus* L. subsp. *hybridus* var *hybridus* (Vhembe) and *Amaranthus thunbergii* Moq. (Capricorn). Spider flower (lerotho) from Rustenburg and Capricorn were in both instances identified as *Cleome gynandra* L. and cowpea (munawa) as *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* (Table 1).

Folate contents

Results of the folic acid determinations (Table 1) indicated considerable variation in folic acid concentrations in

Table 1 Folic acid contents and botanical species identification of wild-growing amaranth, spider flower and cowpea: sampling sites were situated in three geographically separated and climatically distinct areas of South Africa (Rustenburg District, North-West Province; Vhembe and Capricorn Districts, Limpopo Province)

Plant species	Study area	Folic acid content (μg/100 g fresh sample)
Amaranthus hybridus L. subsp. *cruentus* (L.) Thell. (amaranth/thepe)	Rustenburg	72
Amaranthus hybridus L. subsp. hybridus var *hybridus* (amaranth/thepe)	Vhembe	122
Amaranthus thunbergii Moq. (amaranth/thepe)	Capricorn	130
Cleome gynandra L. (spider flower/lerotho)	Capricorn	217
Vigna unguiculata (L.) Walp. subsp. *unguiculata* (cowpea/munawa/dinawa)	Vhembe	154

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morogo: 217 μg/100 g fresh weight (spider flower, Capricorn), 154 μg/100 g fresh weight (leafy cowpea, Vhembe), 122 μg/100 g fresh weight (amaranth, Vhembe), 130 μg/100 g fresh weight (amaranth, Capricorn) and 72 μg/100 g fresh weight (amaranth, Rustenburg).

Fatty acid profiles

Total measured fatty acid concentrations (Table 2) ranged from 1610·2 mg/100 g dry mass in Capricorn amaranth to as high as 2941·6 mg/100 g dry mass in spider flower of Rustenburg. The major PUFA detected in all samples included linolenic acid (18 : 3*n*-3), ranging from 753·8 (SD 48·6) to 1629·7 (SD 77·1) mg/100 g dry mass, and linoleic acid (18 : 2*n*-6), ranging from 110·8 (SD 7·1) to 506·3 (SD 47·2) mg/100 g dry mass. Palmitic acid (16 : 0), the predominant SFA, varied between 420·6 (SD 83·2) and 662·0 (SD 21·2) mg/100 g dry mass and the only MUFA, palmitoleic acid (16 : 1), ranged between 34·7 (SD 0·3) and 79·0 (SD 9·3) mg/100 g dry mass. Table 3 indicates the relative percentages of SFA and PUFA in terms of the total measured fatty acid concentrations. The ratio of SFA to PUFA ranged from 1·0:1·4 to as high as 1·0:2·8, and PUFA contents from 51·5% (Vhembe amaranth) to 73·5% (Vhembe cowpea leaves). Notable variations were observed in measured concentrations of both 18 : 2*n*-6 and 18 : 3*n*-3 in samples of the same plant genus but from different species or different localities. The highest dry mass concentrations of PUFA (Table 2) were found for Rustenburg samples of amaranth and spider flower, with values for 18 : 2*n*-6 of 506·3 (SD 47·2) and 385·7 (SD 9·7) mg/100 g dry mass, respectively and for 18 : 3*n*-3 of 1183·9 (SD 114·9) and 1554·3 (SD 59·4) mg/100 g dry mass, respectively. Expressed in percentages of total measured fatty acids (Table 3), ratios of 18 : 2*n*-6 to 18 : 3*n*-3 PUFA in Vhembe amaranth (1:9), spider flower (1:4·5) and Rustenburg amaranth and Vhembe cowpea leaves (1:3·4) were in favour of 18 : 3*n*-3 PUFA.

Discussion

Steyn[6] describes the low-fat, plant protein-rich diet of rural black South Africans as most prudent. Wild-growing DGLV, such as amaranth, spider flower and cowpea, feature prominently in this type of diet[7,18]. DGLV are important sources of food folate[11,12]. Table 1 shows that folic acid concentrations of amaranth varied between 72 and 130 μg/100 g fresh weight, while its concentration was 217 μg/100 g fresh weight in spider flower and 154 μg/100 g fresh weight in cowpea. South African food composition data (SAFCOD) tables[10] report the folic acid concentrations in raw samples of these vegetables respectively as 64, 346 and 141 μg/100 g fresh weight. The folic acid concentrations in raw leaves of other morogo vegetables such as amadumbe, black jack and nightshade[7] are respectively 126, 351 and 404 μg/100 g fresh

Table 2 Fatty acid profiles of some traditional leafy vegetables consumed in South Africa

	Measured fatty acid concentrations (mg/100 g dry mass; *n* 3)																		
	Myristic acid (14 : 0)		Palmitic acid (16 : 0)		Stearic acid (18 : 0)		Arachidic acid (20 : 0)		Behenic acid (22 : 0)		Lignoceric acid (24 : 0)		Palmitoleic acid (16 : 1)		Linoleic acid (18 : 2*n*-6)		Linolenic acid (18 : 2*n*-3)		Total fatty acids
Vegetable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Amaranth*	5·8	0·5	552·0	40·7	73·8	5·9	13·6	3·3	16·4	2·4	60·2	6·5	79·0	9·3	506·3	47·2	1183·9	114·9	2491·2
Amaranth**	18·0	1·2	570·6	40·2	192·5	15·4	83·5	5·4	12·7	2·4	29·1	2·8	40·2	1·7	110·8	7·1	964·7	60·5	2081·5
Amaranth***	9·5	0·2	422·1	16·5	50·6	21·5	14·1	1·3	13·0	11·0	37·6	3·6	58·1	14·3	217·4	74·7	753·8	48·6	1610·2
Spider flower*	8·6	0·3	662·0	21·2	127·7	2·5	38·3	3·6	35·6	3·0	38·0	1·9	91·4	0·5	385·7	9·7	1554·3	59·4	2941·6
Spider flower***	16·6	5·2	420·6	83·2	70·0	13·0	23·6	4·7	0·7	0·3	24·2	4·4	45·3	9·7	171·0	33·7	899·1	159·0	1696·8
Spider flower***,†	11·2	7·4	593·8	26·3	107·1	1·0	24·3	0·8	0·4	0·01	25·2	0·04	65·1	5·9	379·4	2·1	1629·7	77·1	2864·7
Cowpea**,‡	5·2	0·1	576·4	3·5	75·9	1·6	7·0	0·01	0·4	0·01	24·6	0·5	34·7	0·3	474·1	2·8	1599·8	26·3	2817·5
Cowpea**,‡	10·6	0·4	450·6	13·5	45·4	21·0	10·1	2·3	5·3	5·7	38·2	4·0	68·8	16·6	243·0	74·6	822·3	85·3	1728·5

Sample from Rustenburg*, Vhembe** and Capricorn***.
†Traditionally sun-dried household sample.
‡Collected from two different villages in the Nzhelele Valley.

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Table 3 Relative amounts of the various fatty acids measured in South African samples of wild-growing amaranth, spider flower and cowpea

	Relative amount of fatty acid (%)												
	Myristic acid (14:0)	Palmitic acid (16:0)	Stearic acid (18:0)	Arachidic acid (20:0)	Behenic acid (22:0)	Lignoceric acid (24:0)	Palmitoleic acid (16:1)	Linoleic acid (18:2*n*-6)	Linolenic acid (18:2*n*-3)	Ratio of 18:2*n*-6 to 18:2*n*-3	SFA	MUFA	PUFA	Ratio of SFA to PUFA
Amaranth*	0·23	22·1	3·0	0·55	0·65	2·40	3·1	20·3	47·5	1·0:2·3	28·9	3·1	67·8	1·0:2·3
Amaranth**	0·86	27·4	2·9	4·00	0·61	1·40	1·9	5·2	46·3	1·0:8·9	37·2	1·9	51·5	1·0:1·4
Amaranth***	0·59	26·2	3·1	0·87	0·80	2·33	3·6	13·5	46·8	1·0:3·5	33·9	3·6	60·3	1·0:1·8
Spider flower*	0·29	22·5	4·3	1·30	1·20	1·30	3·1	13·1	52·8	1·0:4·0	31·9	3·1	65·9	1·0:2·1
Spider flower***	0·98	24·8	4·1	1·40	0·04	1·40	2·7	10·1	52·9	1·0:5·2	32·7	2·7	63·0	1·0:1·9
Spider flower***,†	0·39	20·7	3·7	0·85	0·01	0·88	2·3	13·2	56·9	1·0:4·3	26·5	2·3	70·1	1·0:2·6
Cowpea**,‡	0·18	22·0	2·7	0·25	0·01	0·87	1·2	16·8	56·7	1·0:3·4	26·0	1·2	73·5	1·0:2·8
Cowpea**,‡	0·61	26·1	2·6	0·58	0·30	2·20	4·0	14·0	47·5	1·0:3·4	32·4	4·0	61·5	1·0:1·9

Sample from Rustenburg*, Vhembe** and Capricorn***.
†Traditionally sun-dried household sample.
‡Collected from two different villages in the Nzhelele Valley.

weight and that of raw commercial Swiss chard, 52 μg/100 g fresh weight[10]. Boiled amaranth contains 5 μg folic acid/100 g fresh weight, cowpea leaves 60 μg/100 g, sweet potato leaves (also consumed as morogo) 124 μg/100 g and commercial spinach 146 μg/100 g[10]. Values reported from the National Food Consumption Survey for the percentage of energy derived from plant and animal sources[19] indicate that rural populations in South Africa consume a larger amount of plant foods than their urban contemporaries. Families in the Mopani District of Limpopo Province indicated that, when available, morogo is eaten on a daily basis[20]. Results from the present study and information provided by the SAFCOD tables[10] suggest that, if consumed on a regular basis in normal amounts, morogo vegetables could be an important source of dietary folate.

In addition, DGLV generally contain small amounts of fat predominantly in the form of PUFA[10]. In the present study, six SFA, one MUFA and two PUFA (18:2*n*-6 and 18:3*n*-3) featured in differing ratios in the fatty acid profiles of the three types of morogo (Table 2). In all samples, 16:0 was the predominant SFA measured in concentrations higher than 18:2*n*-6 but lower than 27 % of 18:3*n*-3. The ratio of SFA to PUFA in wild-growing morogo vegetables ranged from 1·0:1·4 to 1·0:2·8 (Table 3). Calculated from the SAFCOD tables[10] the ratios of SFA to PUFA in other morogo and commercial vegetables are of the same order (between 1·0:1·1 and 1·0:2·5).

The following percentages of energy from total fat are indicated for various groups of adult black South African males[6,21]: rural areas 22·9 %; informal settlements 24·3 %; urban middle class 26·0 %; urban upper class 30·6 %. Steyn *et al.*[22] found that a rural black population derived 3·7–4·4 % of energy from SFA compared with 8·5–9·2 % for an urban black population. The following consumption pattern is indicated for black South Africans in terms of the percentage of the population consuming a food item respectively in rural and urban settings[6]: meat 46 and 74 %; full cream milk and products 10 and 60 %; vegetable fats and oils 26 and 62 %. The adoption of a Westernised diet due to urbanisation[5,21] appears to be linked with a propensity among black South Africans to consume greater amounts of fat. Moreover, the fat content of their diet seems to increase with the degree of affluence. Simopoulos[23] describes the Western diet as 'deficient' in 18:3*n*-3 mainly because of the high intakes of cereals and vegetable oils rich in 18:2*n*-6 and the low intakes of fruits, nuts and DGLV containing large amounts of 18:3*n*-3. Sanders[24] maintains that, consumed in normal amounts, DGLV would contribute little to the dietary intake of 18:3*n*-3 because of the overall low fat content. In rural settings, morogo vegetables are prepared mixed with other plant foods, including groundnuts and traditional legumes such as cowpeas, which are expected to contribute to the overall 18:3*n*-3 intake[7,10,20,23,25].

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Adequacy in dietary 18 : 3*n*-3 is essential for the biosynthesis of DHA, the long-chain PUFA derivative with vital membrane, brain and cardiac functions[14,24]. In all the morogo vegetables 18 : 3*n*-3 was the predominant PUFA with ratios of 18 : 2*n*-6 to 18 : 3*n*-3 ranging between 1·0:2·3 to 1·0:8·9 (Table 3). EPA, an intermediate in the synthesis of DHA, and DHA are both absent from plant foods and very low in ruminant fats such as milk and dairy products[14]. Since the synthesis of DHA from 18 : 3*n*-3 via EPA occurs at a slow rate in man, EPA and DHA sufficiency is dependent on dietary intake from sources such as oily fish and seafood[14]. With limited access to oily fish and seafood, poultry and eggs would serve as an alternative, though less efficient, source of EPA and DHA, provided animals are not fed on grain concentrates high in 18 : 2*n*-6 which competitively inhibits the conversion of EPA to DHA[14]. Dietary studies in South Africa indicate the following relevant consumption pattern in rural and urban black South Africans, respectively[6]: grain-based food (maize porridge, sorghum and bread) 98·6 and 98·5 %; fish (canned) 3·2 and 10·6 %; eggs and egg products 7·4 and 16·2 %; vegetables 48·1 and 42·8 %. Rural and urban black South Africans thus have equally high 18 : 2*n*-6 intakes from grain staples, and seem to derive EPA and DHA primarily from poultry, eggs and canned fish. However, raised on commercial corn mixtures, poultry and eggs purchased from urban retail outlets are expected to have a higher 18 : 2*n*-6 content[23,24] than similar items consumed by rural farm-based families. Furthermore, the percentage of urban black South Africans consuming vegetable fats and oils is 2·4 times the rural subjects[6], implying a higher overall 18 : 2*n*-6 intake by urban populations. Rural populations furthermore consume a wider range of vegetables, including DGLV (morogo) which is absent from the vegetable list of their urban contemporaries[6].

By comparison, urban black South Africans seem more likely to consume a diet high in SFA and 18 : 2*n*-6 and lower in folate than rural families in subsistence settings. This dietary trend could relate to findings of epidemiological studies suggesting that the high fat content of their Westernised diet might be a prominent factor in the rise of Western lifestyle diseases in urbanised black South Africans[1,2,26,27]. Notwithstanding underlying genetic factors, obesity in black populations is attributed to overnutrition and linked with the consumption of a Westernised diet[26,28]. In conjunction with other negative factors (e.g. excessive alcohol use, cigarette smoking and a sedentary lifestyle) obesity enhances risks of chronic diseases among adults[6,21,27,29]. Biological functions of long-chain fatty acids respectively derived from 18 : 2*n*-6 and 18 : 3*n*-3 differentially modulate risks of CVD[12,15,23], degenerative and inflammatory diseases[14,30] as well as type 2 diabetes[31]. Proposed antioxidant and anti-inflammatory actions of folate[12] may further enhance the health protective value of morogo consumption. In combination with tetrahydrobiopterin and insulin, folate suppresses superoxide anion generation and increases endothelial nitric oxide and prostacyclin production, both of which are potent platelet anti-aggregators and vasodilators[30]. The inhibiting effect exerted by dietary 18 : 3*n*-3 intake on the clotting activity of platelets[32] appears to act complementary to mechanisms by which folate lowers risks of CVD. Mandatory folic acid fortification of various food items, and national strategies aimed at increasing fruit and vegetable intake and reducing saturated fat consumption in Western countries, emphasise the importance of folate and 18 : 3*n*-3 in disease prevention[12,33].

The present study underscores the value of morogo vegetables as low-fat food items that could contribute notable amounts of folate and provide 18 : 3*n*-3 in excess of 18 : 2*n*-6. Lowering fat intakes and including morogo vegetables could adjust the diet of urbanised black South Africans to a more prudent one. Reporting the health beneficial qualities of morogo could improve the image of these valuable vegetables and enhance their marketability in wider society and urban centres. Future research should focus on expanding the nutritional database, including possible anti-nutritional properties. Epidemiological studies could accurately assess the role of morogo vegetables in health and disease.

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supervised the PhD project and contributed to interpretation of the results. D.T.L. supervised the analytical procedures and data processing.

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