Indian Journal of Animal Sciences **81** (8): 864–869, August 2011

Fatty acid profile of chaya (*Cnidoscolus aconitifolius*) leaves and fodder

ANIL KUMAR[1], MAMUN M OR-RASHID[2], OUSAMA ALZAHAL[3] and BRIAN W MCBRIDE[4]

University of Guelph, Guelph, Ontario, N1G 2W Canada

Received: 15 September 2011; Accepted: 15 March 2011

ABSTRACT

Chaya (*Cnidoscolus aconitifolius*) is a perennial shrub that is used as vegetable in Central America by the Maya people. The traditional knowledge also points to several medicinal properties of chaya. Being perennial in nature, yielding copious amount of foliage it can also be used as forage for livestock. The present study was carried out to determine the fatty acid profile of chaya leaves and fodder. Ether extract in chaya leaves and fodder were 9.27% and 3.61% of total dry matter, respectively. Chaya leaves had 58.9 g/100 g of total fatty acid, polyunsaturated fatty acids (PUFA). The omega-3 (n-3) FA in chaya leaves was 50.0%. Chaya fodder had 42.6% n-3 FA content. The n-3/n-6 ratio in chaya leaves was 5.6. A rare omega-3 PUFA in higher plants, eicosaterienoic acid (20:3n-3) was detected in chaya leaves (0.26% of total FA) and fodder (0.38% of total FA). Fresh chaya leaves (100 g) can provide about 50% of the acceptable intake of omega-3 fatty acids in human beings. Chaya fodder can provide an n-3 rich ration for cows and provide precursors for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) rich milk. The medicinal properties attributed to chaya may be due to its fatty acid content. It was concluded that chaya has an optimal fatty acid profile for human consumption as well for healthy livestock production.

Key words: Chaya, *Cnidoscolus aconitifolius*, Fatty acids

Chaya (*Cnidoscolus aconitifolius*), a perennial shrub of Mesoamerica (Central America), is used as vegetable by the indigenous Maya people in the region of Guatemala, Belize, South-east Mexico, the Yucatan peninsula and parts of Honduras (Martin and Ruberte 1978). It belongs to the family Euphorbeaceae and is also known as 'Tree spinach'. Chaya leaves are rich in nutrient having 28–30% crude protein (Kuti and Kuti 1999, Sarmiento-Franco *et al.* 2003). Use of chaya as fodder for livestock has not been reported, although occasionally it is fed to the sheep, pig and poultry.

Polyunsaturated fatty acids (PUFAs) are essential lipids, that cannot be synthesized by human beings organisms and hence these fatty acids must come from the diet. PUFA and especially very long chain polyunsaturated fatty acids (i.e, VLCPUFAs <18C) such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are the subject of much interest. They play important roles in human health and in neonatal retinal and brain development, as well as being important factors in cardiovascular health and disease prevention (Crawford 2000). The diet of most modern societies is nowadays relatively low in n-3 PUFAs with a concomitant increased level of n-6 PUFAs intake, largely resulting from a preference for plant-seed oils. The oils derived from fish are sometimes contaminated with a range of pollutants, heavy metals and toxins (Qi *et al.* 2004, Coreen *et al.* 2005). Omega-3 fatty acids are also found in legumes such as soy and pinto beans, cereals, some nuts, flaxseed, walnut, and canola oils (Meyer 2003). Efforts are also being made to improve the VLCPUFA through transgenic plants (Qi *et al*. 2004), by feeding custom-designed protein supplement (Wright 2003) to cows and diets rich in omega-3 fatty acid such as linseed oil (Loor 2005).

For Maya people, chaya was also offered protection against diabetes (Kuti and Torres 1996, Noel *et al.* 1997, Ross-Ibarra and Molina-Cruz 2002), arteriosclerosis, gall bladder stones, high cholesterol, high blood pressure, and treatment of migraine (Gonzalez-Laredo *et al.* 2003, Arnaud-Vinas and Lorenz 1999). The supposed medicinal benefits may have been derived from the fatty acids and other metabolites contained in the plant. This paper aims to evaluate the fatty acid content of chaya in the light of the traditional knowledge of Maya people and explores its use as livestock fodder to enhance the nutritional quality of milk.

MATERIALS AND METHODS

Plant sample: Forage samples of chaya containing leaves and succulent stems (40–50 days old growth) were harvested

Present address: [1]Senior Scientist (e mail: anil.igfri @gmail.com), Department of Livestock Production Management, Indian Grassland and Fodder Research Institute, Jhansi, India 284 003.

[2-4]University of Guelph.

from 2 year old plants growing at the central research farm of Indian Grassland and Fodder Research Institute, Jhansi, India. Chaya was recently introduced in India in 2006 and over 2000 plants are now growing. The climate of Jhansi is semi arid with temperature going up to 42°C in summer and down to 2°C in winter. The average annual rainfall in Jhansi is 900 mm. The harvested forage was chopped and dried in an oven. The dried samples were grounded, brought to University of Guelph, Canada and analyzed in triplicate for ether extract (AOAC 1996) and fatty acid contents.

Analysis of fatty acid: Lipids for fatty acid analysis were extracted according to Blig and Dyer (1959) with minor modifications as suggested by Or-Rashid *et al.* (2009). About 30 mg of sample plus 1 ml of water was taken in a 15 ml culture tube with a screw-cap Teflon lining and vortexed several times. 2.5 ml of methanol (CH_3OH) and 1.25 ml of choloform (CH_3Cl) were added and the contents were vortexed and kept for 30 min at room temperature. Additionally 66.66 µl of HCl (3N) was added and vortexed. Subsequently, 1.25 ml of choloroform and 1.25 ml of water were added, vortexed, and centrifuged. The acid was added to ensure the pH of the extract was acidic. The choloform layer (bottom phase) containing fat was removed using two Pasteur pipettes, one inserted into another. Another 1.25 ml of choloroform was added to the lipid-containing solution and centrifuged. The contents were then transferred into a 4 ml vial passing through a Pasteur-pipet-column containing a glass wool plug [washed with choloroform and methanol (1:1, v/v) and dried] and a 2 cm bed of anhydrous Na_2SO_4 to absorb any moisture within the sample. The contents were dried under an N_2 steam at room temperature, and then 3 to 4 drops of benzene were added and vortexed. The fat content in the vial was methylated as follows: 200 µl of sodium methoxide ($NaOCH_3$) (0.5 M solution in methanol) was added for methylation. The vials were kept at room temperature for 25 min. Then, 1 ml of 1 N methanolic sulphuric acid (2.8 ml of 96% sulphuric acid in 100 ml methanol) was added. After vortexing the vials were heated at 50°C for 15 min. After cooling at –20 °C for 10 min (Cruz-Hernandez *et al.* 2004, Muller *et al.* 2005) 1.0 ml hexane were added, vortexed, and centrifuged. The upper portion containing fatty acid methyl ester (FAME) i.e. hexane layer was transferred into another vial for GLC analysis.

Analysis of FA by GLC: The hexane containing FAME was analysed by GLC and subsequently identified as described by Odongo et al. (2007) with a different temperature program. Briefly, FAME analysis was performed with a split-splitless injector at 259°C, a flame-ionization detector at 250°C, and a CP Sil 88 column (100 m × 0.25 mm, 0.2 µm of film thickness). Hydrogen was used as a carrier gas at a constant flow rate of 1 ml/min. The temperature of the GLC oven was set to 45°C for 4 min, increased at 13°C/min to 170°C and held for 30 min, and increased@ 2°C/min to a final temperature of 218°C and held for 25 min. Agilent Technologies Chemstation software was used for the data analysis. A 1-µl sample was injected at splitless mode. Peaks of fatty acids were routinely identified by comparision of retention times with fatty acid methyl ester standards. Fatty acid composition was expressed as g/100g FA.

RESULTS AND DISCUSSION

The chaya plant was investigated to analyse (i) the leaves which is used as human food and (ii) chaya fodder (containing leaves and soft stem) for livestock feeding. The leaves and fodder of chaya had 9.27 and 3.61% of ether extract (EE) on a dry matter (DM) basis, respectively (Table 1). The EE content in leaves of chaya is similar to commonly used vegetable such as spinach and amaranthus (USDA 2008). Similarly, fodder of chaya also had higher content of EE than fodders like alfafa (3.0%), barley hay (2.5%) and oat hay (2.23%). Chaya leaves and fodder have (Table 1) saturated fatty acid (30.32%) in leaves and 35.46% in fodder. Palmitic acid (16:0, 22-24%) and stearic acid (C18:0, 4-5%) were the main saturated fatty acids in the leaves and fodder.

The polyunsaturated fatty acid content in leaves was 58.89% and in the fodder 56.55%, which is similar to safflower and soybean oil (60%) but is higher than sunflower oil (36.3%) and lower than linseed oil (70%) (Table 2). However, the omega-3 fatty acid content of chaya leaves (50.0%) is similar to linseed oil (55.9%). Chaya fodder (42.6%) contained less omega-3 fatty acids compared to linseed oil. Linseed is the best known source of omega-3 fatty acid in plants for use by human beings and chaya appears to be next best source of omega-3 fatty acid surpassing walnut and canola oils. However, oat hay has a similar omega-3 fatty acid percentage (49.9%) but the EE content is only 2.2% and it is primarily used as livestock fodder. The content of n-6 fatty acid (linoleic acid, LA) in chaya leaves and fodder were 8.89% and 13.96%, respectively. Chaya leaves and fodder contained 49.7% and 42.2% α-linolenic acid (ALA), respectively. Linoloeic acid in chaya is lower than linseed oil (14.15%). Thus, chaya leaves have favourable ratio of n-6: n-3 (1: 0.18) than linseed oil (1: 0.25).

Several studies (Okuyama 2001, Griffin 2008) indicated that the ingested ratio of n-6 to n-3 (especially linoleic vs alpha linolenic) fatty acids is important to maintain cardiovascular health. The human body cannot synthesize n-3 fatty acids de novo, but it forms long chain omega-3 fatty acids like 20-carbon eicosapentaenoic acid (20:5n-3, EPA) and 22-carbon docosahexaenoic acid (22:6n-3, DHA) from the 18 carbon n-3 fatty acid α-linolenic acid (Braud 1997). n-3 and n-6 fattty acids compete for the same metabolic enzymes, thus the n-6:n-3 ratio will significantly influence the ratio of the ensuing eicosanoids (hormones), (e.g. prostaglandins, leukotrienes, thromboxanes etc.), and will alter the body's metabolic functions (Lee 2006). Synthesis of the longer n-3 fatty acids from linolenic acid within the body is competitively slowed by the n-6 analogue.

Table 1. Fatty acid content of chaya leaves and fodder (g/100 g of total fatty acid)

Fatty acids	Leaf		Fodder	
	Mean	± SD	Mean	± SD
8:0	0.206	±0.010	0.315	±0.035
10:0	0.186	±0.018	0.273	±0.044
12:0	0.185	±0.013	0.098	±0.002
14:0	0.848	±0.008	0.509	±0.016
9c-14:1	0.108	±0.005	0.172	±0.039
16:0	22.725	±0.711	24.369	±0.155
9c-16:1	0.366	±0.025	0.517	±0.021
17:0	0.847	±0.011	0.548	±0.002
18:0	4.839	±0.028	5.439	±0.081
9c-18:1	8.307	±0.563	5.920	±0.084
11c-18:1	0.951	±0.038	0.616	±0.000
18:2n-6 (LA)	8.891	±0.277	13.955	±0.490
20:0	0.459	±0.023	1.276	±0.002
11c-20:1	0.130	±0.007	0.124	±0.011
18:3n-3 (ALN)	49.744	±1.255	42.210	±0.539
20:2n-6	0.075	±0.006	0.092	±0.007
22:0	0.268	±0.022	1.329	±0.008
20:3n-3	0.258	±0.009	0.383	±0.015
24:0	0.396	±0.028	1.087	±0.020
26:0	0.210	±0.010	0.768	±0.041
Omega-3 fatty[a] acid	50.003	±1.264	42.593	±0.553
PUFA[b]	58.893	±0.987	56.548	±0.197

[a],Omega-3 fatty (18:3n-3 and 20:3n-3). [b], PUFA (18:2n-6; 18:3n-3 and 20:3n-3).

Thus accumulation of long-chain n-3 fatty acids in tissues is more effective when they are obtained directly from food or when competing amounts of n-6 analogue do not greatly exceed the amounts of n-3. Metabolites of n-6 are significantly more inflammatory (esp. arachidonic acid) than those of n-3. This necessitates that n-3 and n-6 be consumed in a balanced proportion and healthy ratios of n-6:n-3 range from 1:1 to 4:1 (Department of Health 1994). The fatty acid content of chaya leaves have even better ratio (n-6: n-3, 1: 0.18) favouring α-linolenic acid than linoleic acid (n-6). Less than 8% of ALA absorbed is transformed to EPA and 0.2 – 4.0% of ALA is converted to DHA (Burdge 2003). Chaya leaves with lower (0.18) n-6/n-3 ratio than linseed oil (0.25) may have better conversion efficiency of ALA to EPA and DHA.

Another polyunsaturated fatty acid of the omega-3 series, eicosatrienoic acid (20:3n-3; EtrA) which is rare in higher plants was detected in chaya. The contents of EtrA in chaya leaves was 0.26% of total FA and in the fodder 0.38% of total FA. Microalga *Odontella aurita* (Eupodiscaceae) upon which sea fish feed contains EtrA 1.72% of total FA (Braud 1997). EtrA is an intermediate product in the biosynthesis of ALA to EPA brought about by elongation and desaturation (n-8 and n-5) (Qi *et al.* 2004, Braud 1997). Eicosanoids as described earlier are signaling molecules made by oxygenation of 20-carbon essential fatty acids, (EFAs). They exert complex control over many bodily systems, mainly in inflammation or immunity, and as messengers in the central nervous system. The networks of controls that depend upon eicosanoids are among the most complex in the human body. Eicosanoids are derived from either n-3 or n-6 EFAs. The n-6 eicosanoids are generally pro-inflammatory but the effect of n-3 is much less. The amounts and balance of these fats in a person's diet affects the body's eicosanoid controlled functions, with effects on cardiovascular disease, triglycerides, blood pressure, and arthritis. The presence of an C20 n-3 eicosatrienoic acid in chaya may be helpful in carrying out the functions of the signaling molecules in a less inflammatory way.

Humans can synthesize omega-6 and omega-3

Table 2. Ether extract (% of dry matter) and fatty acid (% of total fatty acid) contents of chaya leaves, chaya fodder and other feedstuffs

Feed name		Chaya leaf	Chaya fodder	Linseed oil	Canola oil	Soybean meal	Alfafa	Oat hay	Peanut meal
Ether extract		9.27	3.61	100	100	2.8	3	2.23	1.6
Fatty acids									
Lauric	12:0	0.19	0.10	0	0.1	0	1.36	1.19	0
Myristic acid	14:0	0.85	0.51	0.16	0.1	0.83	0.85	0.43	0
Palmitic acid	16:0	22.73	24.37	5.74	4.36	17.28	25.01	16.44	9.24
Palmitoleic acid	9c-16:1	0.37	0.52	0.18	0.28	0	2.23	0.48	0.08
stearic acid	18:0	4.84	5.44	4.3	2.05	4.45	4.01	1.33	2.32
Oleic acid	9c-18:1	8.31	5.92	18.88	57.28	13.22	2.43	2.53	66.61
Vaccenic	11c-18:1	0.95	0.62	0	3.53	0.43	0.35	0.06	0
Linoleic acid	18:2n6 (LA)	8.89	13.96	14.15	18.99	54.16	18.49	23.38	19.3
Linolenic acid	18:3n3 (ALN)	49.74	42.21	55.95	7.64	8.43	36.79	49.9	2.45
Others		3.14	6.37	0.64	5.67	1.2	8.47	4.26	0
PUFA*		58.64	56.17	70.10	26.63	62.59	55.28	73.28	21.75

*PUFA, polyunsaturated fatty acid.

VLCPUFAs from linoleic acid and α-linolenic acid by a n-6-desaturation pathway in which the characteristic first step is the n-6-desaturation of LA and ALA to yield α-linolenic acid (C18:3n-6) and stearidonic acid (C18:4n-3), respectively. Further fatty acid elongation and desaturation steps give rise to arachidonic acid (AA, C20:4n-6) and EPA. DHA is the product of further elongation and desaturation of EPA (Le-Gal 1997).

However, these pathways are very inefficient as previously mentioned and hence to obtain these VLCPUFAs directly from the diet is considered necessary. An alternative pathway for the biosynthesis of AA and EPA operates in some organisms. Here, LA and ALA are first elongated specifically to eicosadienoic acid (EDA, C20: 2n-6) and eicosatrienoic acid (EtrA, C20:3n-3), respectively. Subsequently, n-8 and n-5 desaturation of these products yield AA and EPA (Qi *et al.* 2004). It appears that the enzyme responsible for the n-9-specific elongating enzyme as found in *Isochrysis galbana* (Qi 2002) is present in chaya plant. The presence EtrA together with higher content of ALA and the favourable ratio of n-6/n-3 may have a better efficiency of bioconversion to EPA and DHA in animal systems.

The acceptable intake (AI) for n-3 is 1.6 g/day for men and 1.1 g/day for women while the acceptable macronutrient distribution range (AMDR) is 0.6% to 1.2% of total energy (FNDB 2005). Approximately, 10% of the AMDR can be consumed as EPA and/or DHA (FNDB 2005). 100 g of chaya leaves will provide 16 g DM; 1.4 g EE; 0.7 g n-3 fatty acid, which is about 50% of the acceptable intake. Western diets provide n-6/n-3 ratios of between 10:1 and 30:1, that is highly skewed toward n-6 (Hibbeln 2006). Incorporating chaya leaves in the diet will result in higher intake of n-3 as compared to n-6, thus providing a more beneficial effect of omega-3 fatty acid.

It is interesting to examine the traditional knowledge of Maya people about the benefits of eating chaya in the light of the fatty acid profile of chaya and the role of omega-3 fatty acids in human health. An early report (Roys 1931) recommends the use of chaya in case of haemorrhoids and other inflamed protuberances. Use of chaya has been also advocated in a number of ailments such as diabetes (Kuti and Torres 1996, Noel *et al.* 1997, Ross-Ibarra and Molina-Cruz 2002), arteriosclerosis, gall bladder stones and high cholesterol, high blood pressure and treatment of migraine (Gonzalez-Laredo *et al.* 2003, Arnaud-Vinas and Lorenz 1999). Some of the medicinal properties claimed in traditional beliefs may be due to various other chemical constituents such as recently identified dihydromyricetin flavanol in chaya (Gonzalez-Laredo *et al.* 2003) which is known have antimutagenic, anti-inflammatory, melanin inhibitor and anti-oxidative activities. But the omega-3 fatty acid present in higher quantities and favourable n-6/n-3 ratio may also be contributing to some of the claims. There is strong scientific evidence that *n*-3 fatty acids reduce blood triglyceride levels (Harris 1997, Sanders *et al.* 1997) and the risk of secondary and primary heart attacks. It has been recently reported that the tea of chaya (2% chaya leaves in water) caused a reduction of 30% in triglycerides in rats (Palos-Suárez *et al.* 2006). In addition some benefits have also been reported in conditions such as rheumatoid arthritis, cardiac arrhythmias (Morris *et al.* 1993, Mori *et al.* 1993) and blood pressure (Oladeinde 2007).

The traditional knowledge regarding beneficial effect of chaya in diabetes was put to test and it was observed that chaya leaf extract does lower the blood glucose in diabetic rats (Kuti and Torres 1996) and rabbits (Pace-Asciak 2003), but no reason was offered for this decline. It was reported that hepoxilins, a C20 lipid (hydroxyl epoxide-containing derivatives of arachidonic acid) caused the release of insulin from isolated pancreatic islets of Langerhans (Cattaneo *et al.* 2006). As reported above, chaya contains a rare C20 fatty acid, eicosatrienoic acid which may have a role in lowering the glucose in diabetic animals.

Importance of chaya fodder as livestock feed: Use of chaya as a fodder offers a good opportunity to increase the content of omega-3 fatty acids especially the VLCPUFAs such as EPA and DHA. Milk and meat obtained from animals fed on grain based diets are deficient in n-3 fatty acids. There have been attempts to increase the VLCPUFAs content in cow's milk by supplementing linseed oil (Loor 2005) and formulating special rations incorporating fishmeal (Wright *et al.* 2003). Cows fed on typical rations have about 0.05% DHA of total fatty acid which was increased to 0.59% by supplementing linseed oil (Loor 2005) and to 0.72% by supplementing fishmeal (Wright *et al.* 2003). Such naturally fortified milk has the potential to improve the health of consumers and they command premium price in the market (Cruz-Hernandez et al. 2004). Fish oils have also been used to increase DHA from 0.07% (control) to 0.51% of total FA in goat's milk (Cattaneo *et al.* 2006). Feeding chaya fodder to cows can provide a ration rich in omega-3 fatty acids and having a favourable n-3/n-6 ratio together with the presence of eicosatrienoic acid (EtrA, 20:3n-3) may result in higher DHA content in the milk.

Omega-3 fatty acid rich egg and poultry products can also be obtained by feeding α-linolenic acid rich diets such as flax and canola seeds (Cherian and Sim 1991) to chickens. Chaya leaf meal provides another alternative feed to increase the omega-3 fatty acid in eggs and poultry products.

Chaya is rich in unsaturated omega-3 (α-linolenic acid) fatty acid that is similar to linseed. It has better n-6/n-3 ratio than linseed which is important in the conversion of LA and ALA to EPA and DHA. A rare eicosatrienoic acid (C20:n-3 fatty acid) was detected in chaya0. Traditional knowledge of the Maya people about the medicinal benefits of chaya appears to be because of its fatty acid content. Feeding chaya fodder to livestock may result in better nutritional profile in terms of fatty acid content of milk and meat.

ACKNOWLEDGEMENT

The first author is thankful to the Director, Indian Grassland and Fodder Research Institute, Jhansi and National Bureau of Plant Genetic Resources, New Delhi for facilitating the import of chaya and its introduction in India. To undertake this study, the award of Boycast Fellowship by the Department of Science and Technology, Government of India is also duly acknowledged (AK). The support of National Science and Engineering Research Council of Canada is also gratefully acknowledged (BWM).

REFERENCES

AOAC. 1996. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists, Arlington, VA.

Arnaud-Vinas M D R and Lorenz K. 1999. Pasta products containing taro (*Colocasia esculenta* L. Schott) and chaya (*Cnidoscolus chayamansa* L. McVaugh). *Journal of Food Processing and Preservation* **23**: 1–20.

Braud I P. 1997. Simultaneous culture in pilot tanks of the macroalga *Chondrus cripus* (Gigrtinaceae) and the microalga *Odontella aurita* (Eupodiscaceae) producing EPA. *Marine Microorganisms for Industry*. Pp.47. (Eds) Le-Gal Y and Feuga A M. French Research Institute for Exploration of Sea, Rousseau, France.

Burdge G C, Finnegan Y E, Minihane A M, Williams C M and Wooton S A. 2003. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [^{13}C]α–linolenic acid to longer-chain fatty acids anf partitioning towards ß-oxidation in older men. *British Journal of Nutrition* **90**: 311–21.

Cattaneo D, Dell'Orto V, Varisco G, Agazzi A and Savoini G. 2006. Enrichment in n-3 fatty acids of goat's colostrum and milk by maternal fish oil supplementation. *Small Ruminant Res*earch **64**: 22–29.

Cherian G and Sim J S. 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acids composition of eggs, embryos, and newly hatched chicks. *Poultry Sci*ence **70**: 917–22.

Coreen M H, Ronald A H, Steven J S, Jeffery A F, Barbara A K and David O C. 2005. Lipid composition and contaminants in farmed and wild salmon. *Environmental Science and Technology* **39***:* 8622–29.

Crawford M. 2000. Placental delivery of arachidonic acid and docosahexaenoic acids: implication for the lipid nutrition of preterm infants. *American Journal of Clinical Nutrition* **71**: 275S–284S.

Cruz-Hernandez C, Deng Z, Zhou J, Hill A R, Yurawecz M P, Delmonte P, Mossoba M M, Dugan M E R and Kramer J K G. 2004. Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. *Journal of AOAC International* **87**: 545–62.

Deapartment of Health. 1994. *Nutritional Aspects of Cardio-vascular Diseases: Reports on Health and Social Subjects 46*, H. M. Stationery Office: London, U K.

FNDB. 2005. *Dietary Reference Intakes For Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Report no. 423, Pp.770, The National Academies Press, Washington D C.

Gonzalez-Laredo R F, Flores-De-La-Hoya M M, Quintero-Ramos M J and Karchesy J. 2003. Flavonoid and cyanogenic contents of chaya (spinach tree). *Plant Foods for Human Nutr*ition **58**: 1–8.

Griffin B A. 2008. How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. *Current Opinion in Lipidology* **19**: 57–62.

Harris W S. 1997. n-3 fatty acids and serum lipoproteins: human studies. *American Journal of Clinical Nutrition* **65**: 1645S–1654S.

Hibbeln J R, Ferguson T A and Blasbalg T L. 2006. Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. *International Review of Psychiatry* **18**: 107–18.

Kuti J O and Kuti H O. 1999. Proximate composition and mineral content of two edible species of *Cnidoscolus* (tree spinach). *Plant Foods for Human Nutrition* **53**: 275–83.

Kuti J O and orres E S. 1996. Potential nutritional and health benefits of tree spinach. *Progress in New Crops.* (Ed.) Janick J. ASHS Press, Arlington VA.

Kuti J O and Torres E S. 1996. Potential nutritional and health benefits of tree spinach. *Progress in New Crops* (Ed.) Janick J. ASHS Press, Arlington VA.

Lee H, Thompson R L, Harrison R A, Summerbell C D, Ness A R, Moore H J, Worthington H V, Durrington P N, Higgins J P T, Capps N E, Riemersma R A, Ebrahim S B J and Smith G D. 2006. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *British Medical Journal* **332**: 752–60.

Loor J J, Ferlay A, Ollier A, Doreau M and Chilliard Y. 2005. Relationship among trans and conjugated fatty acids and bovine milk fat due to dietary concentrate and linseed. *Journal of Dairy Science* **88**: 726–40.

Martin F W and Ruberte R. 1978. Vegetables for the hot, humid tropics. *Chaya, Cnidoscolus chayamansa*. Pp. 11 Part 3. Science and Education Administration, USDA, New Orleans, Louisiana.

Meyer B J, Mann N J, Lewis J L, Milligan G C, Sinclair A J and Howe P R C. 2003. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids* **38**: 291–98.

Mori T A, Bao D Q, Burke V, Puddey I B and Beilin L J. 1993. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* **4**: 253–60.

Morris M C, Sacks F and Rosner B. 1993. Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circultion* **88**: 523–33.

Muller A, Ringseis R, Dusterloh K, Gahler S, Eder K and Steinhart H. 2005. Detection of conjugated dienoic fatty acids in human vascular smooth muscle cells treated with conjugated linoleic acid. *Biochimica et Biophysica Acta* **1737**: 145–51.

Noel P H, Pugh J A, Larme A C and Marsh G. 1997. The use of traditional plant medicines for non-insulin dependent diabetes mellitus in South Texas. *Phytotherapy Research* **11**: 512–17.

Odongo N E, Or-Rashid M M, Kebreab E and France J, McBride B W. 2007. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *Journal of Dairy Science* **90**: 1851–58.

Okuyama H. 2001. High n-6 to n-3 ratio of dietary fatty acids rather than serum cholesterol as a major risk factor for coronary heart disease. *European Journal of Lipid Science and Technology* **103**: 418–22.

Oladeinde F O, Laditan A A, Michelin R, Bryant J L, Denaro F, Makinde J M, Williams A L, Kennedy A P and Bronner Y. 2007. Effect of *Cnidoscolus aconitifolius* leaf extract on the blood glucose and insulin levels of inbred type 2 diabetic mice. *Cellular and Molecular Biology* **53**: 34–41.

Or-Rashid M M, Odongo N E, Wright T and McBride B W. 2009. Fatty acid profile of bovine milk naturally enhanced with docosahexaeonic acid. *Journal of Agriculture and Food Chemistry* **57**: 1366–71.

Pace-Asciak C R. 2003. Development of novel anti-inflammatory and antidiabetic drugs. *Essential Fatty Acids and Eicosanoids* Pp. 247 (Eds.) Huang Y S, Lin S J, Huang P C. Champaign, AOCS Press, Illinois.

Palos-Suárez G M R, Ramos-Gomez M, Reynoso-Camacho R and González-Jasso E. 2006. Study of the administration of subcronica of the tea of chaya (Cnidoscolus chayamansa) in the diabetes induced chemically in rats. *2nd Congreso Nacional de Quimicia Medica*, Queretaro, Mexico.

Qi B, Fraser T, Mugford S, Dobson G, Syanova O, Butler J, Napier J A, Stobart A K and Lazarus C M. 2004. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. *Nature Biotechnology* **22**: 739–45.

Qi B. 2002. Identification of a cDNA encoding a novel C18-D9 polyunsaturated fatty acid-specific elongating activity from the docosahexaenoic acid (DHA)-producing microalga, Isochrysis galbana. *FEBS Letters* **510**: 159–65.

Ross-Ibarra J and Molina-Cruz A. 2002. The ethnobotany of chaya (*Cnidoscolus aconitifolius* ssp. Aconitifolius Breckon): A nutritious Maya vegetable. *Economic Botany* **56**: 350–65.

Roys R L. 1931. *The Ethno-Botany of the Maya*. Pp. 118, 137, 154, 190. The Tulane University of Louisiana, New Orleans, LA.

Sanders T A B, Oakley F R, Miller G J, Mitropoulos K A, Crook D and Oliver M F. 1997. Influence of n-6 versus n-3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. *Arteriosclerosis, Thrombosis and Vascular Biology* **17**: 3449–60.

Sarmiento-Franco L, McNab J M, Pearson R A and Belmar-Casso R. 2003. The effect of chaya (*Cnidoscolus aconitifolius*) leaf meal and of exogenous enzymes on amino acid digestibility in broliers. *British Poultry Science* **44**: 458–63.

USDA. 2008. *National Nutrient Database for Standard Reference* Release 21. Washington D C.

Wright T C, Holub B J, Hill A R and McBride B W. 2003. Effect of combinations of fish meal and feather meal on milk fatty acid content and nitrogen utilization in dairy cows. *Journal of Dairy Science* **86**: 861–69.