Proximate Composition, Proteins and Lectins

Amaranthus caudatus is a gluten-free pseudocereal widely found in all temperate-tropical areas of the world. Its seeds have long been used as a source of food in South America. Like other *Amaranthus* species, *Amaranthus caudatus* has several features that make it attractive as a potential nutraceutical crop, both in the Western world and in developing countries. From nutraceutical point of view, products derived from plants belonging to the *Amaranthus* genus are known to be antihpercholsterolemic (Bruni *et al.*, 2001). Both the seed and leaf of *Amaranthus caudatus* were eaten in several countries (e.g. Watt and Breyer-Branwijk, 1962; Parades-Lopez, 1994; Tyokumbur and Okorie, 2011).

The proteins, carbohydrates, fats and ash constituents of various samples of *Amaranthus caudatus* seeds have been early reported (Murri and Ermakov, 1936). The mineral elements, crude fiber, total N, crude protein, available lysine, total phosphorus, calcium, nitrate and oxalic acid levels of 3 species of *Amaranthus* (*Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus mantegazzianus*) were: 20.53-22.15%, 12.31-13.01%, 3.68-4.23%, 23.00-26.44%, 4.0-4.29 g/kg N, 0.43-0.54%, 1.55-2.10%, 1.15-2.05% and 2.24-3.92% respectively (Gomez *et al.*, 1986). According to Ezeala (1985), the leaves of *Amaranthus caudatus* contained 27.2% protein; 11.1% fiber, 5.4% fat, 20.1% ash and a gross energy of 4.66 kcal/g (all on dry matter basis). The crude protein and starch contents of *Amaranthus caudatus* seeds were 15.4 and 54.3% respectively (Gamel *et al.*, 2005) Protein content of *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* was 14.5-16.1% (Bressani and Garcia-Vela, 1990). Total ash and water soluble ash of *Amaranthus caudatus* leaves are 5.02 and 3.92% respectively (Urmila, 2012). The proximate composition of *Amaranthus caudatus* grain are: crude protein, 13.1-21.0; crude fat, 5.8-10.9; crude fiber, 2.7-4.9; carbohydrates, 63.7-76.5; and ash, 2.5-4.4% (Mlakar *et al*., 2009). The protein and fat content of 41 lines of amaranth (*Amaranthus* species, including *Amaranthus caudatus* and

Table 23. Average composition of fatty acids in *Amaranthus* species grains[a]

Species	N[b]	FAs in oil (%)	Palmitic 16.0	Stearic 18.0	Oleic 18.1	Linoleic 18.2	S/U ratio[c]
1. *Amaranthus blitoides*	2	84.8±19.3	17.2±8.5	1.5±0.3	22.2±3.5	56.3±11.8	0.26±0.13
2. *Amaranthus cruentus*	7	53.5±13.2	27.0±3.1	1.4±0.8	27.9±2.4	38.1±3.1	0.44±0.07
3. *Amaranthus hybridus*	6	48.2±12.9	22.0±5.0	1.3±1.0	26.3±2.8	47.4±7.2	0.34±0.08
4. *Amaranthus hypochondriacus*	5	63.8±6.4	24.0±1.7	0.9±0.4	33.7±6.5	38.9±5.1	0.37±0.02
5. *Amaranthus palmeri*	4	64.9±9.3	22.3±0.9	3.9±6.8	21.0±1.5	52.4±1.7	0.37±0.10
6. *Amaranthus retroflexus*	8	69.5±5.3	14.3±1.8	0.7±0.2	27.1±2.5	55.2±4.3	0.20±0.03
7. *Amaranthus spinosus*	4	67.0±6.0	24.9±0.4	0.9±0.1	27.4±0.5	44.6±0.3	0.37±0.01
8. *Amaranthus tricolor*	8	56.8±16.2	24.3±1.6	1.1±0.4	25.9±2.9	46.4±2.7	0.36±0.03
9. *Amaranthus viridis*	9	60.1±12.6	23.0±1.3	1.3±0.1	34.4±2.8	38.7±2.9	0.35±0.03
Overall mean and standard deviation		61.3±10.1	21.3±5.1	1.1±1.4	28.2±4.5	46.5±7.9	0.32±0.09

[a]Means of duplicate determinations. [b]Number of genotypes in relevant species. [c]S/U ratio = saturated/unsaturated = (14:0 + 16:0 + 18:0 + 20:0)/(18:1 + 18:2).
He and Corke (2003)

Table 24. Oil and squalene in mature *Amaranthus* species leaves*

Species	Genotype	Oil % (DB)	Squalene in oil (%)
1. *Amaranthus blitoides*	Pl 608663	1.54	0.33
2. *Amaranthus cruentus*	Ames 5604	1.90	0.24
3. *Amaranthus hybridus*	Ames 2028	1.68	0.26
	Ames 5684	1.66	0.17
	Pl 604574	1.63	0.28
4. *Amaranthus hypochondriacus*	Ames 5158	1.75	0.28
5. *Amaranthus retroflexus*	Ames 5328	1.38	0.14
	Ames 21767	1.50	0.16
	Ames 23890	1.75	0.26
	Pl 607465	1.77	0.14
6. *Amaranthus spinosus*	Ames 2043	1.64	0.18
	Pl 482057	1.08	0.4
	Pl 500294	1.13	0.21
7. *Amaranthus tricolor*	Ames 1980	1.36	0.20
	Ames 15330	1.28	0.37
	Ames 18049	1.81	0.44
	Pl 607446	1.49	0.31
8. *Amaranthus viridis*	Ames 23388	2.18	0.104
	Ames 25413	1.91	0.16
Overall mean		1.63	0.26
Standard deviation		0.30	0.13

* He and Corke (2003)

Amaranthus graecizans) including both the grain and vegetable types, varied from 103 to 183 g kg^{-1} and 8 to 68 g kg^{-1} respectively (Prakash and Pal, 1992). The leaves of *Amaranthus caudatus* contain high amount of β-carotene (Booth *et* al., 1992).

The bioconcentration of trace metals in *Amaranthus caudatus* (widely consumed in Nigeria), from a cultivated flood plain receiving effluent from diverse factories in Ibadan, has been reported. The leaves had the highest bioconcentration in the following order Ba > Mn > Zn > Cu > Pb > Cr > Co > Ni > Cd > U > Sb; stems: Ba > Zn > Mn > Cu > Cr > Pb > Ni > Co > Cd > U > Sb; and roots: Mn > Ba > Zn > Cr > Pb > Ni > Co > U > Cd > Sb (Tyokumbur and Okorie, 2011). The mineral content of *Amaranthus caudatus* (spinach) has been reported by several others (e.g. Faboya, 1983; Bawa and Yadav, 1986; Booth *et al*., 1992; Aboho *et al*., 2010; Abdu *et al*., 2011; Uwah *et al*., 2011). Red amaranth (*Amaranthus caudatus*), green amaranth (*Amaranthus viridis*) and edible amaranth (*Amaranthus mangostans*) have been found to accumulate Ni and Zn and hence have the potential to be hyperaccumulator plants (Lin *et al*., 2005a).

The study of the yield, grain size, chemical composition and protein quality of 25 varieties of amaranth (*Amaranthus caudatus*) showed a large variability. The average moisture, protein, and fat content were 11.81, 12.66 and 8.44% respectively (Imeri *et al*., 1987). Seeds of *Amaranthus caudatus* contain an average 16% protein, with an ideal amino acid balance. Flour made from such seeds can complement the protein intake from other cereals (Bressani, 1989). Moreover, its high lysine content can make up for the lack of this

Table 25. Quantification and distribution of simple (nonacylated) and acylated betacyanins in *Amaranthus* species*

Species	Genotype name	Origin	Plant part	Individual betacyanin composition (%)						Total pigment content (mg/g)
				Amaran-thine	Isoama-ranthine	Betanin	Isobet-anin	Celosi-anin I	Celosi-anin II	
1- *Amaranthus aspera*	Am 10	China	S	94.7	2.6	1.7				
2- *Amaranthus albus*	A1002	Spain	S	94.1	5.4					0.57
3- *Amaranthus blitoides*	B1001	Hungary	St	95.7	tr	3.1				0.12
4- *Amaranthus caudatus*	Ca 1657	U.K.	S	95.3	2.8	0.7				0.73
	San 119	China	If	97.7	2.0					1.22
5- *Amaranthus cruentus*	Cr 071	U.S.	S	97.0	1.6	1.0	0.2			1.36
	Cr 072	India	L	96.1	1.7	1.8				1.27
6- *Amaranthus graecizans*	Gr 001	U.S.	S	93.3	tr	tr				0.08
7- *Amaranthus hybridus*	Hr 008	Zimbabwe	S	96.1	1.2	2.5				0.76
8- *Amaranthus hypochondriacus*	Hy 041	U.S.	If	95.5	2.0	0.8	tr			1.08
9- *Amaranthus lividus*	Lv 003	India	S	97.0	1.9	0.5				0.45
10- *Amaranthus mangostanus*	Xian C.	China	S	95.6	2.2	1.3				0.36
11- *Amaranthus palmeri*	Pa 002	U.S.	St	96.4	tr				1.9	0.24
12- *Amaranthus paniculatus*	Tibet Y.	China	S	97.3	1.1	0.8				0.87
13- *Amaranthus retroflexus*	Re 003	Turkey	S	96.6	tr	2.6				0.50
14- *Amaranthus spinosus*	Sn 002	Taiwan	S	94.9	2.2	1.0				0.26
15- *Amaranthus tricolor*	Tr 011	China	S	95.6	4.1					1.05
	Beijing R.	China	L	93.5	4.6	1.2	tr			1.28
16- *Amaranthus viridis*	Vd 002	Maldives	St	90.7	2.7	tr		tr	1.8	0.18

If: inflorescences, L: leaves, S: seeds, St: stems, tr: trace amount.

* Cai *et al.* (2001)

amino acid from the more common cereals. All these characteristics make the plant an attractive, complete dietary crop (Bruni *et al*., 2001). Evalution of the nutritional value of three pale-seeded variety of *Amaranthus caudatus*, showed that the pale seeds contained 14% protein, 10% fat, 2.5% ash, 64% starch and 8% of dietary fiber. The black seeds has a much higher content of fiber (16%) (Pedersen *et al*., 1987a). Addition of *Amaranthus caudatus* to cereal flours improved protein quality without affecting energy utilization. It is an effective source of protein to combine with cereal protein (Pederesen *et al*., 1987b). The dried leaves have been reported to contain 21.51% protein (Oshodi, 1993). The proximate composition, amino acid pattern and mineral content of the leaves of *Amaranthus caudatus*, growing in Mozambique are shown in Tables 26 - 28 (Oliveira and De Carvalho, 1975). According to Imeri *et al*. (1987), the average values for methionine, threonine, cystine, leucine, and lysine were 168, 276, 74, 381, and 370 mg/g N respectively in 25 varieties of *Amaranthus caudatus*. Lysine ranged from 5.2-6.0 g/16 g N in the seeds and the limiting amino acids were leucine, followed by valine or threonine (Pedersen *et al*., 1987a). Stanimirović *et al*. (1983) found methionine and isoleucine to be limiting amino acids in the green mass protein of *Amaranthus hypochondriacus*. Amino acid score was reported by Becker *et al*. (1981) 81 and by Dodok *et al*. (1997) 75.

Computational analysis of amino acid residue sequences of *Amaranthus caudatus* and some other proteins, showed that the amino acid sequences of the amaranth (100% identity) had degree similarity in the range of 71.4 to 52.2% with rice, garden pea, job's teats, maize and yam (Gorinstein *et al*., 1998). Digestibility of dietary fiber (DF) and energy of 3-pale-seeded and 1 dark-seeded variety of *Amaranthus caudatus* were studied in balance experiments with growing rats (Pedersen *et al*., 1990). The pale seeds contained ~ 8% of DF and the black seeds about twice as much. The soluble DF fraction made up 33-44% of the total DF (TDF) fraction in the pale-seeded varieties, but only 18% in the black seeds. The monomer sugar composition of the DF was very similar in all products. However, the black seeds were very high in lignin, and DF of the black-seeded products were more resistant to digestion than that of the pale-coloured products. In the pale amaranth products, digestibility energy (DE) varied between 86 and 91%. In the dark-seeded products, DE was lower, and there was a significant correlation between DE and TDF. In general, the pale seeds had a lower content of DF than most cereal grains, and the DF was more easily digested (Pedersen *et al*., 1990). Amino acid content before and after heat treatment was assessed in grain of six selected amaranth varieties and four species: *Amaranthus caudatus, Amaranthus cruentus,*

Table 26. Proximate composition of the leaves of some *Amaranthus* species

Species	*Amaranthus caudatus*	*Amaranthus gracilis*	*Amaranthus graecizans*	*Amaranthus spinosus*
Moisture %	80	82	84	79
Food energy*	265	256	269	267
Nitrogen**	4.28	5.10	3.70	4.54
Total protein**	26.74	31.90	23.16	28.38
Fat**	2.81	2.96	3.20	4.49
Cellulose**	8.66	9.18	13.46	10.41
Nitrogen free extract**	40.77	33.75	38.53	34.59
Ash** %	21.02	22.21	21.65	22.13

* Calories 100 grams of dry matter. ** % of Dry matter (Oliveira and De Carvalho, 1975)

Table 27. Amino acids pattern (mg/g dry matter) of some *Amaranthus* species*

Amino acids	*Amaranthus caudatus*		*Amaranthus gracilis*		*Amaranthus graecizans*		*Amaranthus spinosus*	
	a	b	a	b	a	b	a	b
1. Arginine	12.86	4.81	14.57	4.57	11.52	4.98	16.66	5.87
2. Histidine	6.77	2.53	6.23	1.95	5.57	2.41	7.86	2.81
3. Lysine	12.72	4.76	11.58	3.63	11.26	4.84	14.77	5.20
4. Methionine	2.96	1.11	3.07	0.96	2.80	1.21	3.28	1.16
5. Cystine	2.57	0.96	1.31	0.41	2.14	0.93	3.91	1.38
6. Phenylalanine	13.58	5.08	11.99	3.76	9.41	4.06	16.09	5.67
7. Tyrosine	8.24	3.08	7.87	2.47	7.34	3.17	9.81	3.46
8. Leucine	25.56	9.56	23.80	7.46	19.01	8.21	28.22	9.94
9. Isoleucine	12.44	4.65	12.63	3.96	12.21	5.27	16.87	5.94
10. Valine	14.05	5.75	13.82	4.33	12.71	5.49	15.03	5.30
11. Threonine	9.28	3.47	9.57	3.00	7.96	3.44	13.00	4.58
12. Tryptophan	2.32	0.87	2.97	0.93	2.92	1.26	2.88	1.01

a) mg/g dry matter; b) g/16g nitrogen.
* Oliveira and De Carvalho (1975)

Table 28. Mineral content of some *Amaranthus* species (mg/100 g dry matter)*

Species	Ca	P	Mg	Na	K
1. *Amaranthus caudatus*	2341	304	1341	11	263
2. *Amaranthus gracilis*	2877	559	1352	14	403
3. *Amaranthus graecizans*	1827	455	1448	11	226
4. *Amaranthus spinosus*	1795	430	2195	13	337

* Oliveira and De Carvalho (1975)

Amaranthus hypochondriacus and *Amaranthus hybridus*, cultivated in Czech Republic. High content of lysine and arginine was detected in both heat treated and untreated grains, as well as satisfactory contents of cystine and lower levels of methionine, valine, isoleucine and leucine. Chemical scores of essential amino acids and essential amino acid index (EAAI) showed EAAI value of 90.4% of amaranth protein, which is almost comparable with egg protein (Pisarikova *et al*., 2005). On the basis of protein characterization of 18 amaranth (including *Amaranthus caudatus*) genotypes, it has been concluded that seed protein can be useful for varietal identification (Kadam *et al*., 2010). The study of glutelin protein fractions of 59 amaranth accessions (*Amaranthus australis, Amaranthus caudatus, Amaranthus cruentus, Amaranthus deflexus, Amaranthus hypochondriacus, Amaranthus retroflexus, Amaranthus tuberculatus* and *Amaranthus wrightii*) showed their use as a tool for clear identification of these accessions (Dzunkova *et al*., 2011). The study of protein fractions in grains of *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus hypochondriacus*

revealed that albumins averaged 20.7, globulins 19.2, prolamins 2.2, glutelins 44.4 with 13.4% in the total protein residue. Albumins were high in tryptophan, threonine, and lysine; globulins in S-containing amino acids and lysine; prolamins in threonine and leucine; and glutelins in tryptophan and leucine (Bressani and Garcia-Vela, 1990).

Gross *et al*. (1989) reported that the seeds of *Amaranthus caudatus* (among other studied cereal-like grains) are characterized by a protein content higher than that found in cereals and an excellent protein quality. The effects of processing on nutritive value and amaranth protein complementary effect to cereal grains and other protein sources were reviewed by Bressani (1994). The review also discussed the results of evaluation of the protein quality of amaranth in human subjects and its uses in various food products. The *in vitro* digestibility of protein and starch of *Amaranthus caudatus* grain demonstrated its potential for nutritional applications (Repo-Carrasco-Valencia *et al*., 2009). Partial substitution of wheat flour (*Triticum aestivum* L.) by amaranth flour (*Amaranthus caudatus*) constitutes a viable option to improve the nutrtional value of the breads (Rosell *et al*., 2009). The obtained bread had a protein efficiency ratio value of 0.76 (Chagman and Huaman, 2010).

Two small (29 and 30 residues) basic proteins named Ac-AMP1 andAc-AMP2, with strong antifungal and antimicrobial properties were isolated from the seeds of love-lies-bleeding, *Amaranthus caudatus* (Broekaert *et al*., 1992). They are probably involved in the protection of seeds or seedlings against fungi and microorganisms. ^{1}HNMR study of the interaction of *N*,*N*',*N*''-triacetyl chitotriose with Ac-AMP$_{2,}$ a sugar binding antimicrobial protein isolated from *Amaranthus caudatus*, was reported (Verheyden *et al*., 1995). The conformation in water of the antimicrobial protein Ac-AMP2 was determined (Martins *et al*., 1996). El Bouyoussfi *et al*. (1997) determined the disulphide bridge pairing of Ac-AMP2, using a fast method involved enzymic fragmentation followed by identification of the fragments with FAB mass spectrometry. The results obtained confirmed the location of three disulphide bridges as previously established by Martins *et al*. (1996). The antimicrobial peptide Ac-AMP2 is processed from a precursor preprotein and preproprotein (De Bolle *et al*, 1996).

The relationship of leaf nitrate reductase and proteinase activities to the grain protein level and grain yield was investigated in 4 species of grain amaranth (including *Amaranthus caudatus*). The study revealed positive correlation between the leaf proteinase activity and the grain protein content (Naidu *et al*., 1982).

The lectin, purified by Singh *et al*. (1993) is a dimeric protein composed of subunits having a molecular weight of 35,800 Da, which are not held together by disulphide linkages. The lectin was found to be non-specific and reacted with human and various animal erythrocytes. It is a glycoprotein having no metal ion requirement for its activity. A lectin for lactose specifity was also isolated from the seeds of *Amaranthus caudatus* (Antonyuk *et al*., 1983). Amaranthin is the lectin present in the seeds, (Rinderle *et al*., 1989, 1990), which specifically binds the T-disaccharide (Galβ1,3GalNAcα-*O*-). This lectin is composed of a single type of subunit with M_r = 33,000-36,000. The studies showed that this homodimer lectin is highly compact relative to typical globular proteins. Amaranthin does not appear to be present in stems or leaves of the plant (Rinderle *et al*., 1990). Amaranthin contains high amounts of acidic and hydroxyamino acids and relatively large amounts of lysine, methionine, and tryptophan for a plant protein (Rinderle *et al*., 1989). Later, Transue *et al*. (1997) reported that *Amaranthus caudatus* agglutinin (amaranthin) is a lectin tightly associated homodimer of 66,000 molecular weight and contains two identical carbohydrate-binding sites. Unlike most lectins, it is not glycosylated and does not require metal cations for sugar binding. The agglutinin contains a novel arrangement of four β-trefoil domains. The sugar-binding site provides a specifity for the carcinoma-associated T-antigen disaccharide

even when 'masked' by other sugars (Transue *et al*., 1997).

There are several other reports on the *Amaranthus caudatus* agglutinin (amaranthin) (Goldstein *et al*., 1990; Atillasoy *et al*., 1998; Akiyoshi *et al*., 2011) and its reactivity as biomarkers in colorectal cancer (Boland *et al*., 1991, 1992).

Carbohydrates

Alcohol-soluble carbohydrates (ASCHs), a water-soluble polysaccharide (WSPs) and pectin substances (PcSs) were isolated from the seeds. The ASCHs (yield 1.5%) contained glucose, galactose and two oligosaccharides. The WSPs was obtained in the highest yield (15.2%), and PC revealed glucose in the products of its complete acid hydrolysis. The yield of PcSs was 2.7%. The monosaccharide composition of the PcSs was represented by galacturonic acid, glucose, galactose, arabinose, rhamnose, and xylose (Chernenko *et al*., 1997).

The starch content of the seeds is 543g kg^{-1} (Gamel *et al*., 2005). *Amaranthus caudatus* starches (normal and waxy types) consisted of mainly typical amylopectin and 5.7% amylose (Tomita *et al*., 1981). The starch type was completely nonglutinous (Okuno and Sakaguchi, 1981). The partial characterization of the starch (60-63%) of two types of grains of *Amaranthus caudatus* L. var. Oscar Blanco, cultivated in Peru has been studied. The starch of opaque grains was identified as waxy starch with 6.8 % amylose. However, the starch of translucent grains showed characteristic of nonwaxy starch (Cotos *et al*., 2004). The gelatinization heat of the nonwaxy starch (with 11.3% amylose) was three times more elevated than the waxy starch (Konishi *et al*., 2006). Ferulic acid was found predominantly bound to pectic substances and galactans in *Amaranthus caudatus* dietary fiber (Bunzel *et al*., 2005).

Lipids

The lipids (4.8%) of the seed of *Amaranthus caudatus* (love-lies-bleeding) consisted of 72% neutral lipids (NLs), 16.3% phospholipids (PLs) and 11.2% glycolipids. The NLs contain the following main classes of lipids (% by weight): hydrocarbons, 0.8; sterol esters, 0.5; squalene, 2.8; triacylglycerols (TAGs), 82.4; epoxyacylglycerols (EP-TAGs) with free fatty acids (FFAs), 6.9; sterols + diacylglycerols, 3.6; and unidentified components, 3.0. Predominating in the NLs were TAGs and the unsaturated aliphatic hydrocarbon squalene. According to mass spectra, the hydrocarbons consisted of a mixture of the homologs $C_{20:2}$ (M^+ 278), $C_{16:3}$-$C_{17:3}$ (M^+ 220-234), $C_{22:4,}$ $C_{29:4}$, $C_{31:4}$ (M^+302, 400, 414, 428), $C_{30:5}$ (M^+412), and $C_{19:6}$ (M^+256). The sterol fraction was composed of β-sitosterol stigmasterol, campesterol, and cholesterol. The predominant fraction was β-sitosterol. Six phospholipids, of which four were identified: phosphatidylcholine (PhC), phosphatidylethanolamine (PhE), phosphatidylinositol (PhI) and phosphatidic acid (PhA). Four types of compounds: sulfoquinovosyldiglycerides, digalactosyldiglycerides, ceramidooligosides, and monogalactosyldiglycerides were identified. Table 29 gives the fatty acid compositions of the lipid classes. All of them contained six fatty acids, with the 16:0, 18:1, and 18:2 species predominating. The NLs were distinguished by a greater degree of unsaturation, and the total amounts of saturated and unsaturated acids in the PLs and GLs were the same, although they differed in the amounts of the 18:1 and 18:2 acids. In the FFAs, the proportion of saturated fatty acids was higher than in the other classes because of the greater proportion of the 16:0 species (Chernenko *et al*., 1997).

Later, Chernenko and Glushenkova (1998) reported the composition and levels of the fatty acids of *Amaranthus caudatus* in the triacylglycerols (TAGs) and the monoacylglycerols

Table 29. Composition of the fatty acids of *Amaranthus caudatus* seeds *

Acid	NLs	PLs	GLs	FFAs
14:0	1.4	2.0	2.8	2.3
15:0	0.3	0.5	0.4	0.7
16:0	21.2	26.9	27.2	32.2
16:1	0.5	0.8	0.2	3.1
18:1	32.4	33.9	35.7	37.2
18:2	44.2	35.9	33.7	24.5
$\sum_{sat}$	22.9	29.4	30.4	35.2
$\sum_{unsat}$	77.1	70.6	69.6	64.8

FFAs: free fatty acids; Gls: glycolipids; NLs: neutral lipids; PLs: phospholipids
* Chernenko *et al.* (1997)

(Table 30). As seen from the Table 30, with the presence in the TAGs of 71.8% of unsaturated and 28.2% of saturated acyls, more than 92% of the *sn*-2 positions of the TAGs were acylated with unsaturated acids and only 7.8% with saturated acids. Of the unsaturated acids, the middle position was occupied predominantly by linoleic acid (Table 31). The latter table includes 34 types of TAGs present in amounts greater than 0.1%, their total amounting to 97.9%; only 2.1% remained for other types of TAGs. In the majority of TAG molecules (90.5%), the *sn*-2 position was occupied by unsaturated acyls. Gamel *et al*. (2007) reported that the oil contents of *Amaranthus caudatus* and *Amaranthus cruentus* were 7.1 and 8.5%, and consisted of 80.3-82.3% of triacylglycerols.

Table 30**.** Composition of the fatty acids of *Amaranthus caudatus* seeds

Fraction	14:0	16:0	18:0	18:1	18:2	Σ unsat.	Σ sat.
TAGs	0.6	24.0	3.6	33.6	38.2	71.8	28.2
MAGs	1.0	6.8		31.8	60.4	92.2	7.8

MAGs: Monoacylglycerols; TAGs: Triacylglycerols
* Chernenko and Glushenkova (1998)

Table 31. Position-species composition* of the triacylglycerols of *Amranthus caudatus*

TAG	%	TAG	%	TAG	%	TAG	%	TAG	%
PPP	0.7	LPS	0.2	POLP	3.4	PLOL	13.7	LLL	3.8
PPSt	0.3	OLML	0.2	MLP	0.2	PLL	11.7	LOLOL	6.0
PMOL	0.2	OLPOL	0.8	PLP	6.5	LLM	0.2	LOLL	2.3
PML	0.2	OLPL	1.3	POLSt	1.1	OLOLSt	1.2	OLLOL	7.2
PPOL	1.5	LPL	0.5	StLSt	0.2	LOLSt	1.8	OLLL	11.3
PPL	1.2	PLSt	2.2	POLOL	3.6	LLSt	1.7	LLL	4.4
OLPSt	0.3	MOLP	0.2	POLL	5.6	StLOL	2.2		
SSS	1.0	SSU	3.6	USU	2.8	SUS	13.8	SUU	41.7
VVV	35.0								

*M : 14:0, P: 16:0, St: 18:0, OL: 18:1, L: 18:2, S: saturated, U: unsaturated
Chernenko and Glushenkova (1998)

Dixit and Varma (1971) identified palmitic, stearic, oleic and linolenic acids in the fixed oil from the seeds of *Amaranthus caudatus*. The prevalent 6 fatty acids of *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus mantegazzianus* were $C_{18:1}$ and $C_{18:2}$ (Bertoni *et al*., 1984b). Hexadecanoic, octadecenoic and octadecadienoic acids were reported as the major fatty acids of the oil (Prakash and Pal, 1992). The study of the seeds of *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus mantegazzianus* indicated the presence of β-eleostearic acid [(9*E*, 11*E*,13*E*)-octadecatrienoic acid] (Bertoni *et al*.,, 1984a). The triacylglycerols of the seeds comprised 71.8 and 28.2 % unsaturated and saturated fatty acids respectively, with > 92 % of *sn*-2 positions acylated with unsaturated acids.

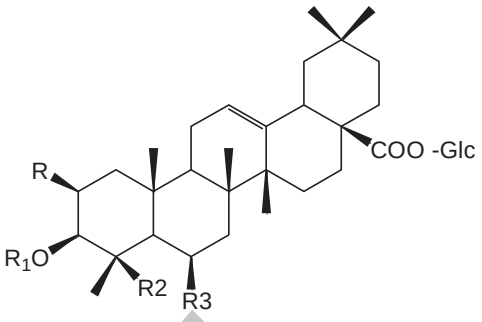
Of the unsaturated acids, the middle position was occupied predominantly by linoleic acid (Chernenko and Glushenkova *et al*, 1998). The fatty acid compositions of the neutral lipids and of the phospho- and glycolipids of the seeds have been determined. The hydrocarbon components have been identified (Chernenko *et al*., 1997). Also, the compositions of the lipids of the leaves, stems, roots and inflorescences of *Amaranthus caudatus* have been investigated. The fatty acid compositions of the total lipids and of the free fatty acids were determined. Nine types of phthalic acid esters have been found in the leaf and stem lipids (Chernenko *et al*., 1998a). Significant levels of squalene (2-5%) and a combined linoleic acid and oleic acid occurrence of 70-80% were found in 18 varieties of amaranth, including *Amaranthus caudatus* (Tables 32 and 33) (Ayorinde *et al*., 1989). The lipid content of the grains has been reviewed by Becker (1994).

β-Sitosterol, campesterol, stigmasterol, Δ^5-avenasterol and Δ^7-avenasterol were identified from the seed oil (Dixit and Varma, 1971; Ologunde *et al*., 1992). The study of the sterol mixtures from *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus mantegazzianus* seed oils showed that the three species contain C_{29} and C_{29}-Δ^7-sterols with saturated and unsaturated side chains as the main components and traces of cholest-5-en-3β-ol. The main sterols were identified as: 24ζ-methyl-5α-cholesta-7,22(*E*)-dien-3β-ol; 24ζ-methyl-5α-cholest-7-en-3β-ol; 24ζ-ethyl-5α-cholesta-7,22(*E*)-dien-3β-ol(main component in the three classes); 24ζ-ethyl-5α-cholest-7-en-3β-ol; and 24-ethyl-5α-cholesta-7,24(28)-dien-3β-ol (Seldes *et al*., 1987). The sterol fraction was composed of β-sitosterol, stigmasterol, campesterol, and cholesterol. The predominant fraction was β-sitosterol (Chernenko *et al*., 1997). The unsaponifiable substances of the leaves, roots, stems and inflorescences of *Amaranthus caudatus* have been investigated. Squalene, ergost-7-en-3β-ol, chondrillasterol, chondrillastanol and 24-ethylidenecholest-7-en-3β-ol were identified from the unsaponifiable fraction of the seeds (Bruni *et al*., 2001). The compositions of four classes of compounds were determined: hydrocarbons, polyprenols, triterpenols and sterols (Chernenko *et al*., 1998b, 1999). Other sterols, identified from *Amaranthus caudatus* are shown in Table 34.

Saponins

The following seven triterpenoid saponins were isolated from the seeds of *Amaranthus caudatus*: 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-2β,3β-dihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester; 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-methyl-β-D-glucopyranosyl-2β,3β-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester; 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-2β,3β-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester; 3-β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-2β,3β,6α-trihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester; 2β,3β-dihydroxy-30-norolean-12,20(29)-dien-23,28-doic acid 28-*O*-β-D-glucopyranosyl ester; 2β,3β-dihydroxy-30-norolean-12,20(29)-dien-23,28-dioic acid 23,28-di-O-β-D-glucopyranosyl ester; and 3-*O*-β-D-glucopyranosyl-2β,3β-dihydroxy-30-norolean-12,20(29)-dien-23,28-dioic acid 23,28-di-*O*-β-D-gluco-pyranosyl

ester (Rastrelli *et al*., 1995). Seven triterpenoid saponins (**73-79**) and three ionol-derived glycosides were isolated from the leaves (Rastrelli *et al*., 1998).



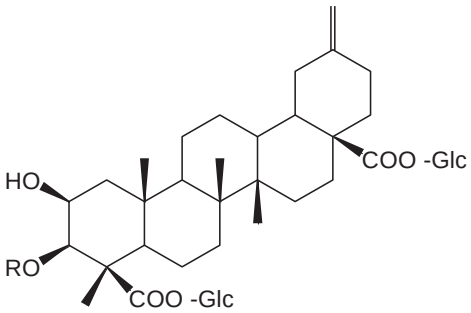
73 R =OH, $R_{1\,=Glc}$ (Ara, Glc) R_2= CH_2OH, R_3 =OH

74 R =OH, $R_{1\,=Glc \rightarrow Glc}$, R_2= CH_2OH, R_3 =OH

77 R =H, R_1 =Glc , R_2= CH_3, R_3 =H

78 R=OH, R_1=Glc, R_2=CH_3, R_3=H

79 R =H, R_1 =Glc, R_2= COOH, R_3 =H



75 R=Glc

76 R = H

Table 32. Weight percent oils in *Amaranthus* grains and percent squalene in the seed oils

Species	Breeding line[α]	Trial planting dates	% Oil	% Squalene in oil
1. *Amaranthus caudatus*	713	6/4/87	3.49	3.78
	988	6/4/87	1.70	NA
2. *Amaranthus cruentus*	Local[b]	6/4/87	3.49	3.78
	Local[b]	6/4/87	2.42	NA
	1011	6/4/87	2.92	4.05
	1034	6/4/87	2.12	2.24
	434	6/4/87	3.85	NA
	1011	9/16/87	3.16	ND
	434	9/16/87	2.77	NA
3. *Amaranthus hybridus*	1047	9/16/87	3.06	4.66
	1004[b]	6/4/87	2.25	2.40
4. *Amaranthus hypochondriacus*	674[b]	6/4/87	1.94	1.88
	718	6/4/87	3.29	3.35
	646	6/4/87	4.08	2.68
	1046	6/4/87	3.18	NA
	1046	9/16/87	1.86	2.29
	674[b]	9/16/87	1.71	2.95
	1023	9/16/87	3.74	2.20
	1024	9/16/87	3.13	2.94

[a]From Rodale Research Center, Kutztown, Pennsylvania, ND: not detected [b]Dark-seeked variety, NA = not analyzed; ND = none detected (Ayorinde *et al.* 1989).

Table 33. Relative weight percent of fatty acids in *Amaranthus* grains[a]

Species	Breeding line[b]	Trial planting dates	C16:0	C17:0	C18:2	C18:1	C18:0	C20:0
1. *Amaranthus caudatus*	713	6/4/87	18.04(0.52)	ND	42.03(0.52)	36.62(2.32)	2.79	0.53
	988	6/4/87	17.65(0.22)	ND	55.97(5.15)	25.87(4.31)	0.57	ND
2. *Amaranthus cruentus*	Local[c]	6/4/87	19.96(0.70)	0.02	52.83(3.17)	24.52(4.07)	2.68	0.01
	Local[c]	6/4/87	19.31(1.00)	ND	52.18(4.01)	25.11(2.21)	3.40	ND
	1011	6/4/87	21.14(1.51)	ND	43.94(3.15)	33.23(2.40)	1.61	ND
	1034	6/4/87	19.39(2.13)	0.11	47.71(1.00)	29.46(0.99)	2.67	0.65
	434	6/4/87	19.07(0.40)	0.08	42.30(1.18)	34.68(0.15)	3.15	0.71
	1011[d]	9/16/87	23.17(2.11)	0.40	37.61(0.86)	34.07(1.86)	4.93	0.10
	434	9/16/87	19.25(1.68)	ND	40.02(2.54)	36.90(2.61)	3.83	ND
3. *Amaranthus hybridus*	1004[c]	6/4/87	19.69(2.14)	ND	54.15(1.65)	23.98(1.11)	2.19	ND
	1047	9/16/87	14.32(0.21)	ND	50.72(3.13)	31.02(2.96)	3.91	ND
4. *Amaranthus hypochondriacus*	674[c]	6/4/87	15.06(4.32)	ND	44.67(4.71)	38.72(1.36)	1.36	ND
	718	6/4/87	17.36(1.59)	ND	58.74(1.69)	21.30(0.32)	2.54	0.06
	646	6/4/87	16.96(0.62)	ND	44.68(1.18)	37.20(0.92)	1.07	0.09
	1046	6/4/87	19.52(2.38)	ND	50.44(1.42)	27.43(2.24)	2.62	ND
	1046[d]	9/16/87	20.34(0.37)	ND	47.97(1.36)	27.01(1.72)	4.68	ND
	674[c]	9/16/87	18.00(2.50)	ND	56.68(2.15)	24.46(1.83)	0.86	ND
	1023	9/16/87	19.55(1.26)	0.14	47.09(0.90)	29.85(1.01)	2.94	0.44
	1024	9/16/87	18.96(1.03)	ND	48.03(1.18)	30.32(1.45)	2.70	ND

[a]Minimum of triplicate determination for each sample. Standard deviations given in parentheses (+). ND: Not detected. [b]From Rodale Research Center, Kutztown, Pennsylvania. [c]Dark-seeded variety. [d]Contain traces of linolenic acid (Ayorinde *et al.*, 1989).

Table 34. Sterol composition of some species and varieties of *Amaranthus*

Species	Individual sterol* (as % of total sterol)						
	1	2	3	4	5	6	7
1- *Amaranthus caudatus* (Kiss me)	0.6	1.1	3.4	7.1	55.1	22.1	4.7
2- *Amaranthus caudatus*	8.7	3.2	5.6	4.9	55.7	16.0	3.6
3- *Amaranthus cruentus* (79-R123)		7.6	6.4	6.1	55.4	15.6	9.0
4- *Amaranthus gangeticus*	1.5	1.9	2.2	6.3	68.7	17.1	1.7
5- *Amaranthus hypochondriacus* (79-R101)		1.5	1.6	6.6	69.3	15.7	5.9
6- *Amaranthus hypochondriacus* (seed)	0.5	0.2	1.5	22.5	46.7	24.3	3.6
7- *Amaranthus hypochondriacus* (callus)		tr	8.8	2.3	70.5	11.3	4.2
8- *Amaranthus tricolor* Hong Kong	0.9	0.8	4.3	5.4	66.7	31.1	8.7
9- *Amaranthus tricolor* 9 Ind.		tr	3.7	5.3	73.8	12.1	5.0
10- *Amaranthus tricolor* 'chin'		tr	1.8	4.7	76.1	15.0	2.5
11- *Amaranthus tricolor* P.R.C.	tr	4.0	5.1	6.1	68.2	15.0	2.0
12- *Amaranthus tricolor* 'coz'	tr	0.5	0.2	4.4	66.1	15.7	8.0
13- *Amaranthus tricolor* (Splendor)	2.0	tr	4.0	7.1	67.2	16.2	0.4

1, Cholesterol; 2, campesterol; 3, stigmasterol; 4, 7-ergostenol; 5, spinasterol; 6, 7-stigmastenol; 7, 24-methylenecycloartanol (Xu *et al*., 1986).

Other Constituents

Flavour compounds of raw and popped seeds of *Amaranthus caudatus* were identified. The main volatile compounds of raw seeds were 2,4-dimethyl-1-heptene, 4-methylheptane, branched $C_{11}H_{24}$ alkane and dodecane $C_{12}H_{24}$ isomer (representing about 70 % of the total volatile compounds). Most of the volatiles identified in popped seeds were aldehydes formed by Strecker degradation including 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and phenylacetaldehyde. Also, alkylpyrazines such as methylpyrazine, vinylpyrazine, 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were found. These compounds are cornlike, nutty, hazelnutty and having roasty odours, and they were not present in the raw seeds (Gamel and Linssen, 2008).

Glyceinebetaine and trigonelline were identified in *Amaranthus caudatus* (Table 35) (Blunden *et al*., 1999). The betacyanins, amaranthin and isoamaranthin were isolated from the leaves and petioles of *Amaranthus caudatus* (Zakharova *et al*., 1995). Two betacyanins, amaranthin and betanin and two betaxanthins (present in small quantities) tentatively identified as miraxanthin and vulgaxanthin, were isolated from *Amaranthus caudatus* var. *pendula* seedlings (Colomas, 1977). Amaranthine was shown to be resistant to pH and high temperature: the colour of the pigment did not change within a wide range of pH (3.5-9.3), and only a 15-20% decrease in the colour intensity was induced by amaranthine incubation for 20 minutes at 80^{o}C. Similar physicochemical properties of amaranthine and betanine and comparable contents of these pigments in plants suggest that amaranthine is a promising food colour and amaranth is a promising source of amaranthine (Gins *et al*., 1998). The pigment distribution of fresh plant material (seedlings, leaves, inflorescences or branches and stem skin) of *Amaranthus caudatus* is shown in Table 36 (Cai *et al*., 1998a).

Table 35. Betaines in some *Amaranthus* species*

Species	Origin of plant material	Betaine yields (% dry weight)	
		Glycinebetaine	Trigonelline
1- *Amaranthus caudatus*	Havant, Hampshire, UK	1.74	0.040
2- *Amaranthus graecizans*	Vácrátót, Hungary	1.33	0.009
3- *Amaranthus hypochondriacus*	Vácrátót, Hungary	1.01	0.008
4- *Amaranthus mangostanus*	Wuchang, China	1.24	0.009
5- *Amaranthus retroflexus*	Vácrátót, Hungary	1.67	0.10
6- *Amaranthus spinosus*	Vácrátót, Hungary	1.21	0.010
7- *Amaranthus viridis*	Wuchang, China	1.54	—

* Blunden *et al*. (1999)

The synthesis of pigments in *Amaranthus caudatus* cultured in agar, under different periods of illumination, and the effect on their concentrations have been studied (Koehler, 1965). The distribution of amarantin in the seedlings after illumination or treatment with kinetin was also examined. The pigment was mainly located in the cotyledons and in the upper 3rd of the hypocotyls, whereas no pigment was found in the rootlets. The pigment formation in cotyledons but not in the hypocotyls was disturbed by illumination; the disturbance was diminished by kinetin, but not by tyrosine (Koehler, 1970). Isolated hypocotyls synthesized betacyanin after light exposure. Pigment synthesizing capacity was reduced in the hypocotyls with increased incubation of seedlings in dark after 24 hours. Both cotyledons and hypocotyls were the sites of betacyanin synthesis. Betacyanin synthesizing capacity was progressively lost from the base of hypocotyls and precursors (L-tyrosine and dopa) could not induce pigment synthesis in these regions (Guprasad and Laloraya, 1976).

Light affected the biosynthesis of both betalain and cinnamic acid from L-phenylalanine in dark-grown *Amaranthus caudatus* in the same way. On the other hand, it stimulated an increase in amaranthin, phenylalanine ammonia lyase activity and caffeic acid and, later ferulic acid. Gentisic and *p*-hydroxybenzoic acids were hardly affected by light, whereas vanillic acid showed an immediate increase (Woodhead and Swain, 1974). The total amount of phenolic acids in Andean indigenous amaranth grains (including *Amaranthus caudatus*) varied from 16.8 to 59.7 mg/100 g (Repo-Carrosco-Valencia *et al*., 2010). Several phenolic acids have been detected in both *Amaranthus caudatus* var. *albiflorus* and *Amaranthus caudatus* var. *atropurpurea* (Table 21).

Amarantin (red pigment) and vulgaxanthin (an orange pigment) were extracted from the inflorescences of *Amaranthus caudatus* var. *atropurpureus*. The dye content in the flower depends on the growth age of the plant (Ubillas Sanchez and Lock de Ugaz, 1989).

Four flavonoids were isolated from the flowers of the plant *viz.* 3,5,7-trihydroxy-6-methyl-4'-methoxydihydroflavanol, 5,7-dihydroxy-8-methyl-4'-methoxyflavanone, 5,7-dihydroxy-8-methyl-4'-methoxyisoflavone and kaempferide (Srivastava and Reddy, 1994). Flavonoids of quercetin type were detected in the leaves (Tekelova and Marlianova, 2002). The total quercetin content of *Amaranthus caudatus* is shown in Table 37 (Kalinova and Dadakova, 2009).

Tocopherols (α, β and δ-tocopherols) and tocotrienols (vitamin E isomers), well known natural antioxidants are detected in the seeds of *Amaranthus caudatus* (Bruni *et al*., 2001, 2002).

Table 36. Pigment distribution and harvest time for 21 genotypes of some *Amaranthus* species*

Species	Genotypes	Red-colored parts[a]	Suitable harvest period for betacyanins[b] (days)			Fresh wt[c] (kg/ha)
			seedlings	leaves	inflorescence	
1. *Amaranthus caudatus*	2	Sl, Lv, In*, Sd			74±5	42520 (In)
2. *Amaranthus cruentus*	12	Sl*, Lv*, In*, St, Sd, or Tp	32±6	42±8	81±10	56390±7225 (Lv.In)
3. *Amaranthus hybridus*	1	Sl, In*, Sd			80±4	39285 (In)
4. *Amaranthus hypochondriacus*	1	Sl*, In*, Sd	30±3		76±4	29160 (In)
5. *Amaranthus lividus*	1	Sl*, Lv*, Sd	28±3	32±4		17370 (Sl)
6. *Amaranthus paniculatus*	1	Sl*, In*, St, Sd	30±3		71±5	44550 (In)
7. *Amaranthus tricolor*	3	Sl*, Lv*, Sd, or Tp	27±3	33±4		20520 (Lv)

Lv, leaves; In, inflorescences; St, stem skin; Sd, seeds; Tp, total plant; *, particularly high pigments in the parts. [a] Sl, seedlings; [b] Days after sowing; [c] Estimated as Sl, Lv, or in weight per plant x planting density (Cai *et al.*, 1998a)

Table 37. The total quercetin content (mean±SD) in some *Amaranthus* species and plant parts (mg/kg DW)*

	Total quercetin	Quercetin released from rutin
Amaranthus hypochondriacus (Koniz):		
Seeds	68±3	65±5.77
Flowers	5,155±205	5,765±70
Stems	3,083±152	3,411±76
leaves at the beginning of the growth	6,765±191	6,531±433
leaves at harvest time	8,750±566	7,322±541
Leaves at stage of full flowering:		
Amaranthus hypochondriacus (Koniz)	7,375±262	7,704±289
Amaranthus hybridus (K-526)	15,600±424	16,913±505
Amaranthus caudatus (Oscar blanco)	6,695±219	7,755±685
Amaranthus tricolor (Ames 1983)	1,395±78	1,217±105

* Kalinova and Dodakova (2009)