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Physico-Chemical Characterization and Fatty Acid Profiling of Seed Oils of Grain *Amaranthus* Cultivars of India -A Nutritional Perspective

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ABSTRACT

Seed oils of seven Amaranthus varieties, representing two species, namely, A. hypochondriacus and A. caudatus were obtained by solvent extraction method. Oil content for the varieties ranged from 4-8%. Physico-chemical characterization of seed oils showed them to be of good quality with trace moisture content (≤0.03%), with low acid values (2.45-4.08 mg KOH/g) and peroxide values (2.17-3.89 meq O_2/kg). High iodine values (104.40-113.81 g I_2/g) showed presence of high unsaturated fats. The saponification values were also high (173.15-190.50 mg KOH/g). Lipid profiles of Amaranthus seed oils were determined using GC-MS which demonstrated that linoleic acid, oleic acid and palmitic acid were the major fatty acids of oils. The unsaturated fatty acid content of Amaranthus seed oils was less than 70%, while the essential fatty acid content for Amaranthus seed oils was <40%.

Key words: *Physico-chemical properties, fatty acid composition, Amaranthus seed oils, Gas Chromatography-Mass Spectrometry (GC-MS).*

INTRODUCTION

Amaranthus is a dicotyledonous pseudocereal and one of the New World's oldest crop, having originated in Meso-America around 400 A. D.[19]. Presently, it is grown in many areas of the world, including Central and South America, Africa, India, China, and the United States[10]. Popularity in the cultivation and consumption of *Amaranthus* seed in the modern era began in the mid-1970s with the rediscovery and promotion of amaranth due to its superior nutritional attributes as compared to cereal grains [9, 15]. Recently, current interest in amaranth also resides in the fact that it has a great amount of genetic diversity, phenotypic plasticity and is extremely adaptable to adverse growing conditions, resists heat and drought, has no major disease problem, and is among the easiest of plants to grow in agriculturally marginal lands[22]. More recently, research activities have focused on examining and characterizing the lipid components of *Amaranthus* seed.

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Amaranth oil is light to medium colored, clear liquid that is pourable at room temperature, highly unsaturated with delicate, agreeable aroma and taste, allowing greater versatility[12, 16]. Although the lipid content of *Amaranthus* seed is typically 6–9%, some species such as *A. spinosus* and *A. tenuifolius* have been reported to contain as much as 19.3%.[9] Nutraceutical value of *Amaranthus* seeds has already been affirmed due to its lipid lowering, anti-diabetic, immune modulatory and cytoprotective properties and the ability to activate membrane function and to increase the heart rate variability. The diverse health effects of amaranth oil have been attributed to its specific chemical composition comprising of high level of linoleic acid (upto 50%), tocopherols/ tocotrienols and squalene (upto 8%), which take part in redox reactions[25].

The objective of this study was to evaluate the physico-chemical properties of the extracted *Amaranthus* seed oils. Determination of the fatty acid profiles of *Amaranthus* seed oils was also performed using GC-MS. The study will help exploit the nutritive and dietetic potentialities of the *Amaranthus* seed oils to synthesize blended edible oils with enhanced lipid profile for specific health benefits[23]. The characterization of *Amaranthus* seed oils will also help enhance its human consumption and its use in industrial applications.

MATERIAL and METHODS

Chemicals

Liquid chemicals like acetic acid, chloroform, cyclohexane, petroleum ether and heptane; solid chemicals like potassium iodide, sodium thiosulphate, anhydrous sodium chloride, anhydrous sodium sulfate, sodium hydroxide, potassium hydroxide and indicators like starch soluble and phenolphthalein were procured from Merck KGaA (Germany). Specialty chemicals like Boron Trifluoride (BF_3) in methanol (12% solution) and Karl Fisher Reagent were procured from Sigma-Aldrich Co. (USA).

Materials

Seeds of *Amaranthus* varieties which belonged to two grain *Amaranthus* species, namely *Amaranthus hypochondriacus* and *Amaranthus caudatus* and representing different agro-ecological regions of India were procured from various agricultural institutions as mentioned in table-1.

Table 1: List of *Amaranthus* grain varieties

Sr. No.	*Amaranthus* Species	Name of the Variety	Source and Year of Release
1.	*Amaranthus hypochondriacus*	Gujarat Amaranth-1 (GA-1)	SardarKrushinagar Dantiwada Agricultural University (SDAU), Gujarat – 1991.
2.		Gujarat Amaranth-2 (GA-2)	SardarKrushinagar Dantiwada Agricultural University (SDAU), Gujarat – 2000.
3.		Gujarat Amaranth-3 (GA-3)	SardarKrushinagar Dantiwada Agricultural University (SDAU), Gujarat – 2008.
4.		KAPILASA (BGA-2)	Orissa University of Agricultural and Technology, Bhubaneshwar -2005.
5.		VL Chua 44	ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VKPAS), Uttarakhand- 2006.
6.	*Amaranthus caudatus*	Suvarna	University of Agricultural Sciences (UAS), Bangalore- 1992.
7.		DURGA (IC35407)	National Bureau of Plant Genetic Resources (NBPGR) regional station Shimla- 2006.

Methods

Oil Extraction from Amaranthus Seeds

30±0.1 g non-heat treated seeds were weighed for each of the 7 *Amaranthus* varieties collected for the study. The seeds were then crushed to fine powder using a miller (IKA, A11 Basic). Soxhlet extraction of the powdered samples was carried out at 60 ºC using petroleum ether as the extraction solvent. The extraction was continued for 6-8 hours. The oil was separated from the solvent using a rotary evaporator (IKA, RV 10) and stored in glass vial at 2º-8ºC for further analysis.

Physico-Chemical Characterization of *Amaranthus* Seed Oils

The following parameters were studied using the standard procedures of American Oil Chemist Society (AOAC, 1995).

Lipid content - The weight of oils extracted from 30 g of seed powder was determined in a pre-tarred glass vial to calculate the lipid content. The total oil/lipid content was expressed in percentage (%).

Color - The seed oils of seven *Amaranthus* varieties were analyzed using the spectrocolorimeter, Tintometer (PFXi-995, Lovibond) for determination of their color.

Moisture content-The extracted oils were analyzed to determine their moisture content using Karl Fischer Moisture Analyzer (836 Titrando, Metrohm, USA).

Acid Value (AV) - The free fatty acid content of the *Amaranthus* seed oils was determined by AOAC Official Method 940.28 (Titrimetric Method)[4].

Iodine Value (IV) - The amount of unsaturated fatty acids present in the *Amaranthus* seed oils was determined by AOAC Official Method 993.20 (Wijs Method)[7] .

Peroxide Value (PV) - As a measure of oxidative rancidity, peroxide value for *Amaranthus* seed oils was determined by AOAC Official Method 965.33 (Titrimetric Method)[5].

Saponification Value (SV) - The average fatty acid chain length of the *Amaranthus* seed oils was determined by AOAC Official Method 920.160 (Titrimetric Method)[3].

Fatty acid profiling by Gas Chromatography Mass-Spectrometry (GC-MS)

Preparation of methyl ester - The seed oils extracted for the 7 *Amaranthus* varieties were used for preparation of methyl ester. 100 ± 0.1 mg test portion of each oil was accurately weighed in 250ml round bottom flasks and converted to methyl esters (FAMEs) using boron trifluoride (BF_3) in methanol as per AOAC Official Method 969.33 (Determination of Fatty Acids (FA) in Oils and Fats)[6]. In the final step, 1 µl of the heptane portion containing FAMEs was injected into Gas Chromatography (GC) coupled with Mass Spectrometer (MS).

GC-MS analysis conditions - A Shimadzu Gas Chromatograph, GC-2010 system comprising of an AOC-20i auto-sampler, equipped with a polar fused capillary column, DB-Wax (100% Polyethylene Glycol, 30 mt Length × 0.25 mm ID × 0.25 µm df) and interfaced to a Mass Spectrometer (QP 2010 Plus) was used for the analysis. For GC-MS detection, an Electron Ionization system was operated in Electron Impact (EI) mode with ionization energy of 70 eV. Helium gas (99.999% purity) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed with a split ratio of 50:1. The injector temperature was maintained at 250°C, and the Ion-Source temperature at 230°C. The column oven temperature was programmed from 60°C with an increase of 12 °C/min to 150°C (isothermal for 1 min), and then was increased at a rate of 5°C/min upto 240°C (isothermal for 5 min). Mass spectra were taken for fragments ranging from 50 m/z to 1000 m/z. Identification of the Fatty Acid Methyl Ester was conducted by comparing the mass spectrum with NIST library. The compound showing more that 90% Similarity Index (SI) was identified and recorded. The fatty acid composition was reported as a relative percentage (%) of the total peak area.

Statistical Analysis - The experimental design of the physicochemical properties and fatty acid profiles of the seed oils was completely randomized, with three replicates for all treatments.

RESULTS AND DISCUSSION

Table 2: Physico-chemical properties* of *Amaranthus* seed oils

Parameter	*A. hypochondriacus*					*A. caudatus*	
	GA-1	**GA-2**	**GA-3**	**BGA-2**	**VL- 44**	**Suvarna**	**DURGA**
Lipid (%)	4.83 ± 0.15	5.30 ± 0.23	4.97 ± 0.10	6.40 ± 0.28	5.93 ± 0.17	7.07 ± 0.21	5.23 ± 0.13
Color	4.3 R + 50.0 Y	2.4 R + 70.0 Y	1.1 + 32.0 Y	6.2 R + 50.0 Y	9.6 R + 70.0 Y	3.3 R + 70.0 Y	2.4 R + 2.5 Y
Moisture (%)	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
AV	2.63 ± 0.04	2.59 ± 0.03	2.45 ± 0.05	3.95 ± 0.09	4.00 ± 0.11	4.08 ± 0.07	3.76 ± 0.05
IV	108.5 ± 0.70	106.76 ± 0.44	107.44 ± 0.40	110.15 ± 0.67	111.75 ± 0.90	113.81 ± 0.82	104.40 ± 0.92
PV	2.17 ± 0.12	2.36 ± 0.23	2.20 ± 0.19	3.41 ± 0.16	3.58 ± 0.10	3.89 ± 0.15	3.72 ± 0.10
SV	178.32 ± 0.50	175.60 ± 0.77	173.15 ± 0.92	187.61 ± 0.56	187.10 ± 0.51	190.50 ± 0.61	185.43 ± 0.66

***Mean±SD, n=3**
AV: Acid Value as mg KOH/g
IV: Iodine Value as g I_2/100g
PV: Peroxide Value as meq O_2/kg
SV: Saponification Value as mg KOH/ g

Lipid Content

The lipid content for the 7 *Amaranthus* grain varieties studied, ranged from 4.0-8.0%, which co-related with the results of Saunders and Becker (1985)[24]. Seeds of the variety Suvarna had the highest content of oil-7.07%, followed by BGA-2 (6.40%) and VL Chua 44 (5.93%). The seeds of variety GA-1 contained the least amount of oil- 4.83%. However, the oil content varies in a wider range depending on the species, cultivar, agrotechnological practices, and growing location and may be present at remarkably higher concentrations [10].

Color

The color of the *Amaranthus* seed oils as determined using the AOCS Tintometer color scale, varied distinctively[20]. In general, the color of all the seed oils was towards the yellow scale. The oil of variety GA-1 was yellow in color, while that of varieties GA-2 and GA-3 were golden in color. The oils for varieties BGA-2 and VL Chua 44 had a blend of red color with that of yellow and hence appeared reddish-brown in color while that of Suvarna and DURGA were light brown in color [27].

Moisture Content

The trace amounts of water in the seed oils extracted was determined using the most sensitive technique of Karl Fischer titration. The moisture content of the seed oils was trace being $\leq 0.03\%$, which was in accordance to the requirements of the codex standard[11]. The relative low moisture content of the oils will increase its shelf-life, because oxidative rancidity, microbial growth and infestation are prevented or reduced by moisture removal [21].

Acid Value

The free fatty acid content of the oils varied between 2.45 and 4.08 mg KOH/g of oil. The acid value was higher for the reddish-brown colored oils than compared to that of the others. The low acid value was indicative of the fact that the rancidity of the oils was very low. This characteristic of the oils could be attributed due to the presence of natural anti-oxidants in the seeds like vitamin C and A as well as other phytochemicals like flavonoids[12]. There was no significant difference in the acid values of *Amaranthus* seed oils for the selected agro-ecological regions.

Iodine Value

The iodine value for the *Amaranthus* seed oils varied over a range of 104.40 and 113.81g I_2/100g of oil. Higher iodine values for the oils, pointed the oils to be rich in Unsaturated Fatty Acid (UFA). The iodine values of *Amaranthus* oils were nearly similar to other edible plant oils like cottonseed oil (100-123 g I_2/100g), sesame oil (104-120 g I_2/100g) and maize oil (103-135 g I_2/100g)[11]. A good drying oil should have an iodine value of 180 g I_2/100g, and hence the *Amaranthus* seed oils having iodine values above 100 g I_2/100g were classified as semi-drying oils. They were found to be unsuitable for use in the paint and coating industries[1].

Peroxide Value

Peroxide value is the most common indicator of lipid oxidation. The peroxide values for the *Amaranthus* seed oils ranged between 2.17 to 3.89 meq O_2/ kg of oil. The low values of peroxide value are indicative of low levels of oxidative rancidity of the oils and also suggest strong presence of high levels of antioxidants. The oils would be thus less susceptible to deterioration. The low levels of rancidity present in the oils could be further reduced by use of natural antioxidants like sesamol, shikonin etc. or synthetic antioxidants like propyl gallate and butyl hydroxyl anisole[26]. The WHO/ FAO,[11] has stipulated a permitted maximum peroxide level of not more than 10 meq O_2/ kg of oil; therefore the *Amaranthus* seed oils extracted and having low peroxide values were suitable for consumption.

Saponification Value

The saponification value for the *Amaranthus* seed oils ranged between 173.15 and 190.50 mg KOH/ g of oil, which were similar to that of typical seed oils like maize, sesame, soybean oil and safflower oils whose average saponification value range from 180-200 mg KOH/g[11]. The oils thus had high potential for use in the production of liquid soap and shampoos apart from human consumption[17].

Fatty Acid Composition of *Amaranthus* Seed Oils

The fatty acid profiling results for seed oils of varieties GA-1, GA-2 and GA-3 were similar, while only a minor difference was observed in the results of BGA-2, VL Chua 44 and Suvarna seed oil. The seed oil of variety DURGA had the most distinct fatty acid profile amongst the seven seed oils analyzed as shown in table-3. The major fatty acids were linoleic acid (30.16-38.35%), oleic acid (27.30-33.13%) and palmitic acid (19.31-21.51%). The highest content of Saturated Fatty Acid (SFA) was found in the seed oil of variety DURGA (37.20%), comprising mainly of palmitic and stearic acid. The seed oils were high in total Unsaturates (UFA) ranging from 62.80-69.71%. The Saturate/Unsaturate ratio (S/U) was $\leq$ 0.6 for oils of all the varieties. The total Mono-Unsaturated Fatty Acid (MUFA) content varied between 28.07-34.05%, while the total Poly-Unsaturated Fatty Acid (PUFA) content varied between 31.51-40.37%. The α-linolenic acid content for the *Amaranthus* seed oils was lower and ranged from 1.16-1.84%. The relationship between saturated and polyunsaturated fatty acid content is expressed as P/S index. Oils and Fats with P/S Index value greater than 1 are considered to be nutritional. It also indicates less deposition of lipid in the body. P/S Index for all the *Amaranthus* seed oils was greater than 1, except for the seed oil of variety DURGA (0.847)[18, 30]. The seed oils of all the *Amaranthus* varieties analyzed proved to be good source of omega fatty acids. The essential fatty acid content (sum of ω-3 + ω-6) of *Amaranthus* oils ranged from 31.51-40.37%. The two essential ω-3 fatty acids, namely Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were totally absent for the seed oils of the Indian varieties studied. The seed oils had good content of ω-7 and ω-9 fatty acids with their contents varying between 0.24 – 0.31% and 27.78 – 33.74% respectively.

Table 3: Percent Fatty acid composition* of seed oils of seven *Amaranthus* varieties

Fatty Acid	*A. hypochondriacus*					*A. caudatus*	
	GA-1	**GA-2**	**GA-3**	**BGA-2**	**VL-44**	**Suvarna**	**DURGA**
C 14:0	0.39±0.036	0.39±0.035	0.38±0.038	0.36±0.032	0.38±0.038	0.36±0.015	0.36±0.051
C 15:0	0.15±0.025	0.15±0.026	0.15±0.029	0.12±0.031	0.14±0.040	0.11±0.029	0.13±0.050
C 16:0	21.27±0.112	21.12±0.261	21.31±0.140	21.51±0.176	21.35±0.196	21.36±0.110	19.31±0.137
C 16:1	0.29±0.025	0.31±0.021	0.29±0.030	0.30±0.021	0.31±0.015	0.31±0.015	0.24±0.021
C 17:0	0.16±0.010	0.18±0.049	0.19±0.061	0.25±0.035	0.18±0.038	0.16±0.010	0.16±0.006
C 18:0	6.65±0.061	6.96±0.517	6.99±0.569	6.53±0.227	6.33±0.575	5.92±0.414	14.74±0.151
C 18:1	27.59±0.236	27.57±0.270	27.30±0.684	32.46±0.314	32.46±0.364	33.13±0.339	30.55±0.032
C 18:2	38.29±0.099	38.23±0.012	38.35±0.208	34.51±0.352	34.31±0.145	34.24±0.125	30.16±0.116
C 18:3 (n-3)	1.84±0.091	1.82±0.107	1.81±0.110	1.36±0.138	1.36±0.015	1.23±0.066	1.16±0.025
C 20:0	1.86±0.061	1.81±0.086	1.79±0.115	1.35±0.105	1.61±0.076	1.59±0.038	1.72±0.070
C 20:1	0.49±0.044	0.52±0.090	0.47±0.015	0.48±0.021	0.60±0.035	0.61±0.046	0.50±0.061
C 21:0	0.20±0.010	0.19±0.025	0.21±0.015	0.18±0.031	0.21±0.015	0.19±0.020	0.18±0.031
C 22:0	0.80±0.015	0.77±0.068	0.77±0.068	0.59±0.085	0.77±0.057	0.78±0.046	0.78±0.046
SFA	31.29±0.116	31.37±0.215	31.56±0.527	30.71±0.462	30.75±0.531	30.29±0.348	37.20±0.040
MUFA	28.38±0.238	28.40±0.220	28.07±0.700	33.23±0.303	33.37±0.364	34.05±0.369	31.29±0.087
PUFA	40.33±0.125	40.23±0.125	40.37±0.180	36.06±0.249	35.88±0.168	35.66±0.145	31.51±0.122
UFA	68.71±0.116	68.63±0.215	68.44±0.527	69.29±0.462	69.25±0.531	69.71±0.348	62.80±0.040
S/U	0.46±0.002	0.46±0.005	0.46±0.011	0.44±0.010	0.44±0.011	0.43±0.007	0.59±0.001
ω-3	1.84±0.091	1.82±0.107	1.81±0.110	1.36±0.138	1.36±0.015	1.23±0.066	1.16±0.025
ω -6	38.49±0.098	38.41±0.029	38.56±0.219	34.70±0.324	34.52±0.156	34.43±0.105	30.35±0.144
ω -7	0.29±0.025	0.31±0.021	0.29±0.030	0.30±0.021	0.31±0.015	0.31±0.015	0.24±0.021
ω -9	28.08±0.214	28.08±0.214	27.78±0.671	32.93±0.323	33.06±0.362	33.74±0.384	31.05±0.086
ω-3 + ω-6	40.33±0.125	40.23±0.125	40.37±0.180	36.06±0.249	35.88±0.168	35.66±0.145	31.51±0.122
P/S Index	1.288	1.282	1.279	1.174	1.166	1.177	0.847

***Mean±SD, n=3**

S/U= Saturate/ Unsaturate ratio,

n-6= Linoleic acid,

n-3= Linolenic acid,

ω-3, 6, 7 and 9 = Omega series of fatty acids,

ω-3 + ω-6 = Sum of essential fatty acids

P/S Index= Ratio of Polyunsaturated fatty acid to Saturated fatty acid

CONCLUSION

The physico-chemical characterization of *Amaranthus* seed oils concluded that the oils were of good quality[11]. Low moisture content, acid value and peroxide value of the oils confirmed that the oils extracted had low rancidity and presence of natural antioxidants in good quantity. High iodine value of the oils pointed the oils to be rich in Unsaturated Fatty Acid (UFA), which was later confirmed by fatty acid profiling results using GC-MS.

Fatty acid profiling of *Amaranthus* seed oils revealed various health beneficiary aspects of the oils. The oils containing high amounts of UFA (both MUFA and PUFA), help reduce the risk of cardiovascular diseases, diabetes and cancer. They also help lower cholesterol levels and maintain good brain health. They also possess anti-inflammatory properties[13]. *Amaranthus* seed oils studied proved to be a good source of essential fatty acid namely linoleic acid (ω-6) and α-linolenic acid (ω-3). Presence of significant amounts of α-linolenic acid in the seed oils which acts as a precursor for synthesis of EPA and DHA in the body, helps the body to compensate their absence from the *Amaranthus* seed oils. Essential fatty acids also help in the treatment and prevention of various diseases like heart disease, diabetes, cancer, obesity, multiple sclerosis, lupus, asthma and various allergies[14]. Because of its health beneficial properties, *Amaranthus* seed oils, like safflower oil, tigernut oil and garden cress oil can be used for supplementation of vegetable oils to prepare "blended edible oils", with enhanced health effects like lowering LDL cholesterol levels and enhancement of anti-inflammatory properties of the oil[29], for increasing UFA content of vegetable oils, thereby enhancing HDL level and controlling total and LDL-cholesterol levels[2] or for decreasing n6/n3 ratio of the oils in order to decrease total cholesterol and modulate the lipid profile[28]. Because no particular oil has all the nutritional requirements and ideal fatty acid profile, mixing vegetable oils is a cost effective practice to modify their fatty acid composition and physico-chemical properties[8].

Hence, the nutraceutical properties of *Amaranthus* seed oils of the varieties used in the study can be exploited to enhance the lipid profiles of vegetable oils consumed in India. The oils can be used for direct consumption or can even be used for industrial applications.

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