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US-Africa Collaboration in a Chemical Education Project Involving Analysis of Amaranthus Grain Oil from Selected Agro-Ecological Zones in Kenya

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Abstract: Amaranthus is a cosmopolitan genus of annual and perennial plants. Worldwide, amaranths are cereals, leaf vegetables, or ornamental plants. Two African species of amaranthus grain; *Amaranthus cruentus* and *Amaranthus hypochondriacus* from selected regions in Kenya (Bondo, Bureti, Embu, Kenyatta University (KU), Kisumu, Kitale, Meru and Nyeri) were tested for oil content and fatty acid profiles. The oils from the grains were extracted using Soxhlet method for the total lipid content and Dyer and Bligh method for the fatty acid profile and oil oxidative stability. The fatty acid composition was determined using gas chromatography. The study showed that statistically there was no difference in the composition of oil among the various regions. The fatty acid profile for *Amaranthus cruentus* included: linoleic acid (35–38%), oleic acid (32–36%), palmitic acid (22–24%), stearic acid (2–4%), and linolenic acid (1–2%). There was no significant difference in the composition of fatty acids in the two species of amaranth studied except oleic and linoleic acids. The fatty acid profile for *Amaranthus hypochondriacus* was: linoleic (41–44%), oleic (26–34%), palmitic (19–24%), stearic (2–3%), and linolenic acid (1–3%) with no significant differences in the selected regions. The present study reported capric, lauric, myristic, pentadecanoic, palmitoleic and heptadecanoic acids for the first time though in small amounts. The total lipid component ranged from 7 to 10% for *Amaranthus hypochondriacus* and 7 to 9% for *Amaranthus cruentus*. The results of the study compared well with those done earlier in other countries.

Introduction

Worldwide, we rely on just 30 crops for 95% of our nutrition [1–3]. As wheat, rice, and maize (corn) displace the more and more indigenous crops and market gardeners resort to an increasingly smaller number of vegetable species, the security of the world's food supply is becoming more tenuous. Malnutrition is a threat facing the greater part of Africa, and Kenya is no exception. Beyond the overwhelming odds caused by malnutrition and protein deficiency, Amaranthus (family Amaranthaceae) trees have the under exploited potential to provide the loudest single glimmer of hope at the horizon.

Amaranth is defined as a "never fading flower" in Greek. Amaranthus species were grown by the Aztecs 5000 to 6000 years ago, prior to the disruption of the South American civilization [4, 5]. Both the grain and leaves are utilized for human as well as animal food [4, 6, 7]. Amaranth grain consists of 6–9% of oil which is higher than most other cereal [8–11]. Amaranth oil contains approximately 77% unsaturated fatty acids and is high in linoleic acid, which is necessary for human nutrition. Other fatty acids present in considerable amounts include oleic acid, palmitic acid, stearic acid and linolenic acid. In amaranth, phospholipids represent 3.6% of total lipids, mainly phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [12–14]. The lipid fraction is unique due to the high squalene content. The squalene concentration in the oil ranges from trace to 7.3%, which is much higher than in other oil seeds [15]. The iodine value is found to vary from 80–115, acid value between 1–5, peroxide value that gives degree of rancidity 2–5, and saponification value of 140–210 [15]. Amaranth is a drought tolerant crop and has the earliest maturity period in the cereal class ranging between 45–75 days for fast maturing species. It does well in poor and infertile soils and thus recommended for the vast Kenyan arid and semi arid zones to curb the problem of food insecurity.

Amaranth was found to grow in diverse geographical areas. It was equally necessary to establish if the diversity affected the oils composition which was investigated in the present study. It is recommended to replace Anti-retroviral (ARV) drugs due to its immune boosting properties [16–21]. There are

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over 60 species and 4000–6000 different varieties of amaranth [16]. However, *A.* hypocondriacus [21–25] and A. cruentus [26–28] are the most common in Kenya. *Amaranthus cruentus* was an ancient food and in the Tehuacan caves in Central Mexico, over 5500 years ago [8–11]. It is thought to be the most adaptable of all amaranths species. *Amaranthus hypochondriacus* is the most robust, highest-yielding of the grain types and is thought to have been domesticated in Central Mexico about 1500 years ago [8–11]. The species is particularly useful for tropical areas, high altitudes and dry conditions. In this paper, oil from grains of two species, *A. hypocondriacus* and *A. cruentus* from selected regions: Bureti, Embu, Kitale, Meru, Nyeri, Kisumu and Bondo in Kenya were studied to determine their oil composition and total lipids extracted.

The present research is part of an ongoing US-Africa collaborative Natural Products Program directed by professor Joseph Rugutt at Missouri State University, West Plains (USA) [29–39, 40].

Experimental

The "Experimental" Garden. In this project, a small plot of 5 m by 10 m was planted with Amaranthus to act as an experimental garden. This was done at Kenyatta University (KU). The garden was divided into two equal portions of 5 m by 5 m and *Amaranthus hypochondriacus* planted on one potion and *Amaranthus cruentus* planted on the other portion. The grains from amaranth head were harvested after 75–90 days, dried and cleaned in readiness for extraction of oil and analysis.

Sampling and Sample Pre-treatment. Samples of the two species of amaranth, *A. cruentus* and *A. hypochondriacus* were obtained from the following locations: Bondo, Bureti, Embu, Kisumu, Kitale in Transnzoia, Meru and Nyeri districts and KU. The map [41] of Kenya shows these locations. The raw seeds were cleaned to remove any foreign matter and milled. The raw seed flour was then stored in closed glass bottles at four degrees Celsius awaiting extraction and analysis [14].

Crude Oil Extraction. The oil was extracted from the amaranth flour (10 g) by Soxhlet extraction method in which oil was extracted by repeated washing (percloration) with petroleum ether under a reflux in a special glass. After 12-hr extraction, the solvent was evaporated in a rotary evaporator. The oil was kept in a desiccator for 3 hours. The weight of the oil extracted was expressed as percentage of lipids in the dry matter of seed powder [42].

Fatty Acids Composition of Grain Amaranths. Extraction of lipids was done using modified Bligh and Dyer method (1959) [43]. The sample was then injected into the GC machine for the fatty acid profile. Identification of fatty acids was done by comparing with known methyl ester standards [42, 44]. The analysis were performed using a Shimadzu GC -9A (Shimadzu Co., Tokyo, Japan) fitted with glass column, pre-packed and pre-conditioned by Shimadzu; Synchrom E-715% Shimallite (80–100 AW), 3.1 m in length by 3.2 mm internal diameter and flame ionization detector temperature of 220 ^{0}C, injector temperature of 170 ^{o}C and detector temperature of 220 ^{o}C were used. Flow rate was 8 mL minute, injection volume 1 µl. Gases used were nitrogen carrier gas at 2.5 kg/cm^3; hydrogen at 0.68 kg/cm^3 and air at 0.5 kg/cm^3. Shimadzu integrator software was used to calculate the peak areas [42].

Iodine Value Determination. Samples of oil 1.0 g each were weighed into 250 mL conical flasks and 10 mL chloroform added and the two mixed together. The blank only contained 10mL of chloroform. A 25 mL portion of wij's solution was then added into each of the flasks. The flasks were then stoppered and placed at a dark place for 1 hour. A 20 mL portion of 10% KI was added to each flask followed by 100 mL of distilled water and the mixture shaken. The solution was then titrated with 0.1 N sodium thiosulphate until the color changed to faint yellow after which 5-drops of starch solution were added. Titration was continued until the blue color disappeared and the results recorded, same treatment occurred for the blank [45].

Acid Value Determination. A 1.0 g portion of amaranth oil was weighed into 250 mL conical flask. Three drops of phenolphthalein were then added to ethanol-diethyl ether mixture (1:1 v/v) before neutralizing with 0.1 N alcoholic KOH. A 40 mL volume of the solvent mixture was then added to the oil and dissolved. Three drops of indicator were added then titrated with 0.1 N KOH solution.

Peroxide Value Determination. Amaranth oil (1 g) for analysis was weighed on an analytical balance. A 25 mL of acetic acid-chloroform mixture (3:2 v/v) was added and dissolved for each of the oil sample and blank. 1 mL of saturated KI was added to each sample and mixed. The samples were then placed in a dark place for 10 minutes. Water (30 mL) was added to each sample followed by 1 mL of starch solution. They were then titrated with 0.01 N sodium thiosulphate.

Saponification Value Determination. A portion of 1.0 g oil sample was weighed into 50 mL quick fit conical flasks. A volume of 25 mL of 0.5 N alcohol KOH was then pipetted into each of the flasks having oil. Another 25 mL of KOH was pipetted as a blank. They were then boiled for 1 hour under reflux. After cooling to room temperature, 3 drops of phenolphthalein were added followed by titration with 0.5 N HCl [15, 42].

Statistical Analysis. Statistical analysis of data derived from the various regions was performed using the SPSS statistical program [46] with significance level at $P< 0.05$.

Results and Discussion

Fatty acids composition of Amaranthus *cruentus* grain oils from Bondo, Meru, Bureti, Embu, KU, Nyeri and Bureti. Table 1 gives the fatty acid content of *Amaranthus cruentus* grains from Bondo, Meru, Bureti, Embu, Kitale, KU, Nyeri and Kisumu. From Table 1, linoleic acid was the predominant fatty acid in almost all regions except for Nyeri area where oleic acid was the major fatty acid. Linoleic acid ranged from 35.32% in Nyeri to 38.41% in Meru. These values compared well with those reported by Dhellot [15] and [47] of 37–40%. However, they were significantly lower than those reported [8–11] of 50%. There was a significant difference in the values reported across the regions. Oleic acid ranged between 32.40% in Meru sample and 35.95% in Nyeri sample.

Linoleic acid or oleic acid also slightly increase the amount of HDL, "the good" cholesterol which assists in the removal of TAGs from the blood stream [48]. This high quantity of oleic acid could be beneficial to diabetic patients, those with heart and vascular problems and patients with disorders in metabolism of fats. Palmitic acid was equally in high amounts in amaranth grain oils, ranging from 22.43% in Bondo and 24.26% in Meru. Palmitoleic acid is contained in the fatty acids of avocado (12%) and in macadamia nut oil (20%) which is the reason for the excellent skin care features of these oils [49]. Linolenic acid (ω-3) was found to vary between 0.93% in Embu oil and 1.39% in Bureti. The study reported the following fatty acids in grain amaranth for the first time; myristic 0.25–0.39%, heptadecanoic 0.44–0.69%, palmitoleic acid 0.30–0.75%, capric, lauric and pentadecanoic acid 0.06–0.15%. Very low quantities of capric (0.00–0.05%), lauric (0.01–0.14%) and arachidic (0.00–0.19%) acids were however detected. *Amaranthus cruentus* oil had higher linoleic acid content (35.32–38.41%) in comparison to that found in peanut oil (30.5%), rapeseed oil (21.2%), olive oil (12.9%) and linseed (24%) [50]. However, the same quantity was far much

Table 1. Fatty acids composition of *Amaranthus cruentus* grain oils from different ecological zones (mean±SE), P= 0.05

Fatty acid (%)	Bondo	Embu	KU	Meru	Bureti	Nyeri	p-value
Capric	0.02 ± 0.01^{ab}	0.02 ± 0.01^{ab}	0.01 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.00 ± 0.00^{a}	0.05 ± 0.02^{b}	0.03
Lauric	0.07 ± 0.02^{a}	0.09 ± 0.04^{a}	0.08 ± 0.05^{a}	0.14 ± 0.02^{a}	0.01 ± 0.01^{a}	0.09 ± 0.03^{a}	0.216
Myristic	0.28 ± 0.02^{ab}	0.30 ± 0.06^{ab}	0.25 ± 0.00^{a}	0.39 ± 0.03^{b}	0.23 ± 0.01^{a}	0.31 ± 0.02^{ab}	0.034
Pentadecanoic	0.12 ± 0.01^{ab}	0.15 ± 0.03^{bc}	0.08 ± 0.00^{ab}	0.19 ± 0.01^{c}	0.06 ± 0.00^{a}	0.11 ± 0.01^{ab}	0.002
Palmitic	22.43 ± 0.08^{a}	23.02 ± 1.80^{a}	22.84 ± 0.61^{a}	24.26 ± 1.16^{a}	23.16 ± 0.72^{a}	22.46 ± 0.19^{a}	0.778
Palmitoleic	0.75 ± 0.03^{a}	0.43 ± 0.17^{a}	0.30 ± 0.13^{a}	0.34 ± 0.11^{a}	0.34 ± 0.11^{a}	0.48 ± 0.11^{a}	0.157
Heptadecanoic	0.44 ± 0.03^{a}	0.53 ± 0.03^{ab}	0.64 ± 0.01^{ab}	0.45 ± 0.01^{ab}	0.69 ± 0.00^{c}	0.65 ± 0.14^{ab}	0.042
Stearic	2.12 ± 0.13^{a}	2.36 ± 0.10^{a}	2.94 ± 0.38^{ab}	2.62 ± 0.32^{ab}	3.49 ± 0.13^{b}	2.98 ± 0.08^{ab}	0.012
Oleic	35.80 ± 0.13^{a}	33.70 ± 1.43^{a}	35.00 ± 0.94^{a}	32.42 ± 0.53^{a}	34.28 ± 0.53^{a}	35.96 ± 0.15^{a}	0.051
Linoleic	37.07± 0.32^{ab}	38.41± 1.07^{b}	36.65± 0.18^{ab}	38.17 ± 0.39^{b}	36.21±0.37^{ab}	35.32 ± 0.19^{a}	0.009
Linolenic	0.97 ± 0.08^{a}	0.93 ± 0.05^{a}	1.30 ± 0.02^{b}	0.98 ± 0.07^{a}	1.39 ± 0.08^{b}	1.37 ± 0.15^{b}	0.004
Arachidic	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.19 ± 0.05^{a}	0.00 ± 0.00^{a}	0.12 ± 0.04^{a}	0.19 ± 0.10^{a}	0.067

NB: Same superscripts mean no significant difference and different superscripts indicate significant difference at 95% confidence level

Table 2. Fatty acids composition of *Amaranthus hypochondriacus* (mean±SE), P= 0.05

	Bondo	KU	Kitale	Kisumu	Bureti	Nyeri	p-value
Capric	0.01±0.00^{a}	0.00±0.00^{a}	0.05±0.02^{b}	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.017
Lauric	0.04±0.02^{a}	0.04±0.02^{a}	0.14±0.05^{a}	0.04±0.02^{a}	0.07±0.01^{a}	0.04±0.01^{a}	0.083
Myristic	0.31±0.02^{b}	0.34±0.03^{b}	0.33±0.03^{b}	0.20±0.03^{a}	0.27±0.01^{ab}	0.26±0.02^{ab}	0.012
Pentadecanoic	0.13±0.02^{a}	0.09±0.01^{a}	0.20±0.02^{a}	0.07±0.01^{a}	0.13±0.02^{a}	0.19±0.08^{a}	0.162
Palmitic	19.25±0.37^{a}	22.04±0.78^{a}	21.09±0.50^{a}	19.95±0.44^{a}	20.96±0.16^{a}	23.61±2.86^{a}	0.253
Palmitoleic	0.54±0.06^{a}	0.20±0.07^{a}	0.40±0.13^{a}	0.55±0.09^{a}	0.66±0.10^{a}	0.75±0.23^{a}	0.094
Heptadecanoic	0.66±0.06^{a}	0.56±0.03^{a}	0.66±0.03^{a}	0.65±0.06^{a}	0.70±0.05^{a}	0.88±0.28^{a}	0.608
Stearic	2.24±0.09^{a}	2.73±0.16^{a}	2.72±0.07^{a}	2.79±0.08^{a}	2.64±0.11^{a}	2.17±0.63^{a}	0.462
Oleic	33.21±0.19^{d}	31.32±0.30^{c}	26.92±0.47^{a}	32.59±0.20^{d}	30.75±0.08^{c}	29.26±0.51^{b}	0.000
Linoleic	42.40±0.17^{a}	41.33±0.32^{a}	43.92±0.68^{a}	41.92±0.28^{a}	42.50±0.21^{a}	41.79±1.36^{a}	0.172
Linolenic	1.15±0.04^{a}	1.27±0.05^{a}	3.04±1.84^{a}	1.49±0.41^{a}	1.17±0.06^{a}	0.99±0.10^{a}	0.470
Arachidic	0.06±0.03^{a}	0.05±0.05^{a}	0.01±0.01^{a}	0.04±0.04^{a}	0.12±0.08^{a}	0.00±0.00^{a}	0.439

NB: same superscript mean no significant difference and different superscripts indicate significant difference

Table 3. Acid, iodine, peroxide and saponification values and total lipid extraction of *Amaranthus cruentus* (mean±SE), P= 0.05

Parameter	Bondo	Embu	KU	Meru	Bureti	Nyeri	p-value
Saponification value mg KOH g^{-1}	168.30±1.36^{a}	179.02± 2.55^{ab}	177.75± 5.55^{ab}	189.90±3.20^{c}	168.24±7.83^{a}	168.05 ± 2.56^{a}	0.024
Peroxide value meq O_2 kg^{-1}	2.66± 0.52^{a}	3.23±0.01^{a}	2.63±0.57^{a}	3.17±0.06^{a}	3.30±0.01^{a}	3.07±0.13^{a}	0.552
Iodine value g I_2 100 g^{-1}	81.82±0.17^{a}	88.5±0.46^{a}	81.57±0.52^{a}	78.77±0.80^{a}	101.17±9.77^{b}	79.08 ± 0.36^{a}	0.014
Acid value mg KOH g^{-1}	3.63±0.03^{a}	3.51±0.01^{a}	3.54 ± 0.09^{a}	3.57±0.01^{a}	3.69±0.03^{a}	3.65±0.04^{a}	0.076
Total lipid extraction (%)	7.55±0.23^{a}	7.38±0.39^{a}	6.98±0.40^{a}	7.16±0.24^{a}	7.29±0.24^{a}	8.35±0.64^{a}	0.244

NB: Same superscripts mean no significant difference and different superscripts indicate significant difference at 95% confidence level

Table 4. Iodine, acid, peroxide and saponification values and total lipid extraction of *Amaranthus hypochodriacus* (mean±SE), P= 0.05

Parameter	Bondo	KU	Kitale	Kisumu	Bureti	Nyeri	p-value
Saponification value mg KOH g^{-1}	176.85±2.77^{a}	180.51±3.10^{a}	173.52±0.58^{a}	176.34±0.67^{a}	171.33±4.92^{a}	176.51±0.72^{a}	0.298
Peroxide value meq O_2 kg	4.10±0.79^{a}	3.26±0.04^{a}	2.65±0.56^{a}	3.27±0.03^{a}	3.20±0.06^{a}	3.25±0.04^{a}	0.304
Iodine value g I_2 100 g^{-1}	93.02±3.41^{c}	85.37±0.83^{ab}	80.31±0.36^{a}	80.88±0.50^{a}	87.48±0.65^{b}	81.52±0.76^{a}	0.000
Acid value mg KOH g^{-1}	3.91±0.22^{a}	3.72±0.02^{a}	3.87±0.02^{a}	3.72±0.02^{a}	4.07±0.26^{a}	4.05±0.31^{a}	0.666
Total lipid extraction (%)	7.00±0.70^{a}	7.56±0.47^{a}	7.38±0.27^{b}	9.80±0.07^{a}	8.59±0.66a^{ab}	8.31±0.29^{ab}	0.013

NB: Same superscripts mean no significant difference and different superscripts indicate significant difference at 95% confidence level

less than that recorded for corn oil (55.9%), soybean oil (52.6%), walnut oil (56.7%) and sunflower oil (52.6%) [15, 51].

Fatty acids composition of *Amaranthus hypochondriacus* grains from Bondo, Meru, Bureti, Embu, Kitale, KU, Nyeri and Kisumu. From Table 2, linoleic acid was the most abundant in quantity among the fatty acids. The quantity of linoleic acid ranged from (41.33%) in KU to (43.92%) in Kitale with no significant difference between the regions.

Peroxide, iodine, saponification and acid values and total lipid extraction of *Amaranthus hypochondriacus* from Bondo, KU, Kitale, Kisumu, Bureti and Nyeri. A summary of results of analysis of *Amaranthus hypochondriacus* oil from Bondo, KU, Kitale, Kisumu, Bureti and Nyeri are shown in Table 4. There was no significant difference in the acid, peroxide and saponification values of oils from the selected regions. Saponification value was lowest in Bureti 171.33 mg KOH g^{-1} of oil and highest in KU 180.51 mg KOH g^{-1} of oil while the peroxide value ranged from 2.65 meq O_2 kg^{-1} for

Kitale to 4.10 meq O_2 kg^{-1} for Bondo, values that were within those prescribed for good oil. These peroxide values compared well with those reported 3–5 meq O_2 kg^{-1} by Dhellot [15]. Acid values from the selected regions ranged from 3.72 mg KOH g^{-1} in KU to 4.07 mg KOH g^{-1} in Bureti with no significant difference between the regions. Iodine values [52–54] ranged from 80.31 g I_2 100 g^{-1} for Kitale to 93.02 g I_2 100 g^{-1} oil for Bondo. Total lipids quantity range of 7.00–9.80% is much lower than those of tropical plants such as Canarium schwenfurthii that had an average lipid content of 36.1% and Balanites aegyptiaca almonds, 48.3% [55]. It is also lower than that of some usual oilseeds such as cotton (16–28%) and corn ranging from 30–65% [55].

Peroxide, iodine, saponification and acid values and total lipid extraction of *Amaranthus cruentus* from Bondo, Meru, Embu, KU, Nyeri and Bureti. The results of some of the physicochemical parameters of oil in *Amaranthus cruentus* from various regions extracted using Dyer and Bligh (1959) method [43] are shown in Table 3. Saponification value is highest for oil from Meru of 189.90 mg KOH g^{-1} of oil while the lowest in oil from Nyeri of 168.05 mg KOH g^{-1} of oil. Bureti, an agriculturally productive area receiving a lot of rain (1400–2100 mm) annually [56] had a saponification value of 168.30 mg KOH g^{-1} and thus the saponification values may not be subject to agricultural productivity of an area. The saponification values in Table 3 compared well with those obtained elsewhere, 147.9–185.6 mg KOH g^{-1} oil. [15] The peroxide values were between 2.63-3.30 meq O_2 kg^{-1}.This was less than those reported elsewhere of 3–5 meq O_2 kg^{-1}. [15] There was no significant difference in the peroxide values across the regions. The iodine value was lowest in grain oil from Meru 78.77 g I_2 100 g^{-1} oil and highest in grain oil from Bureti 101.17 g I_2 100 g^{-1}. The acid value was found to be between 3.51 mg KOH g^{-1} for Embu and 3.69 mg KOH g^{-1} for Bureti. The total lipid extracted was highest for Nyeri (8.35%) and lowest for KU (6.98%), though there was no significant difference as indicated in Table 3. The second most abundant oil was from Bondo that experiences low amounts of rainfall (480–600 mm) besides other conditions not favourable for agriculture. Thus, agro-ecological region did not affect the amounts of oil in the grains. Total lipid extracted compared well with those reported of between 5.7–11% [8–11].

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