A Quantitative Analytical Chemistry Research-based Experiment Involving Comparative Analysis of *Amaranth* Grain Oils Extracted from Embu and Meru Districts in Kenya

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Abstract: *Amaranthus cruentus* grains from Meru and Nyeri regions in Kenya were milled and oil extracted by the Bligh and Dyer method. Samples of oil from grains in the same regions extracted by cold pressing method were obtained from All Grain Company in Nairobi. Triacylglycerides (TAG) was the major component of neutral lipids in oil extracted by the two methods. In Dyer and Bligh method, TAG had a mean of 76.12% while cold pressed oil had 72.09%. Phosphatidylethanolamine (PE) was the major polar lipids component in both methods of extraction. In both methods of oil extraction, linoleic acid was the most abundant fatty acid. Other fatty acids that were also found to be present in high amounts in the two methods included: oleic acid (30–34%), palmitic acid (20–23%), stearic acid (2–3%) and linolenic acid (0.8–1.2%). There was no significant difference in the saponification, acid, peroxide and iodine values. The saponification values were quite high, 187.61–189.90. The peroxide values from both methods ranged from 3.10 to 3.17 while acid values from the same analysis ranged between 1.74 and 3.92. Iodine value was found to be between 77.02 in cold pressed oil to 78.77 in oil from Dyer and Bligh method.

Introduction

Amaranthus cruentus found in Mexico, about 4000 BC [1, 2] made it one of the oldest cash crops. *Amaranth* grains can be used as cereals or ground into flour for such purposes as making bread [3]. Apart from being staple foodstuff, it was used by ancient Aztecs who called it "huautli" to prepare ritual drinks and foods [4, 5]. *Amaranth* seeds, like buckwheat and quinoa, contain protein that is unusually complete for plant sources. It has high levels of protein, 13–19%, among the highest protein levels of grain in the world [6]. *Amaranth* seed contains 7–8% oil which is about 77% unsaturated, similar to wheat 77%, oat 77%, corn 83%, brown rice 75% and olive 87%. The free lipid content of *Amaranth* ranges from 5.7–7.2% while bound lipid ranged from 0.4–0.9% for eight *Amaranth* varieties. Triacylglycerols (TAGs) represent the major non-polar compounds of the free lipids while glycolipids and the phospholipids represent the main fraction of bound lipids [7, 8]. Natural membranes have a mixture of lipid types, which determine the bilayer structures; Glyceropholipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) together with the sphingolipids such as sphingomyelin, glycosphingolipids and cholesterol are essential structural elements of all the biological membranes that provide the permeability barriers between cells and between organelles within cells [9, 10]. The glycolipids represent 6.4% of the total lipids, mainly monogalactosyl and digalactosyl compounds while the phospholipids represent 3.6%, mainly phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [3, 7, 8].

The aim of this study was to determine the composition and oxidative stability of cold pressed *Amaranth* oil, and to compare them with those of oil obtained from Bligh and Dyer method [11]. This project is part of an ongoing US-Africa collaborative project involving several institutions including: University of Kabianga, Kapkatet University, Kenyatta University, Egerton University, the University of Nairobi, Catholic University of East Africa, Moi University, Kenya Medical Research Institute, Pyrethrum Board of Kenya, St. Patrick's High School Iten, Kapsogut Boys High School, Kericho Teachers College, and Baringo Teachers College.

Experimental

Sampling and Sample Pre-treatment. Samples of *Amaranthus cruentus* were obtained from Embu and Meru [12]. 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 [13].

Fatty Acids Composition of Grain *Amaranths.* Extraction of lipids was done using modified literature procedure [11]. Methyl esterification of lipids for fatty acids test by GC was done by refluxing

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2–5 mg of oil in 2 mL of 95% methanolic hydrochloric acid for 1 hour [14, 15]. Methyl esters formed were extracted thrice using n-hexane. It was then filtered through anhydrous sodium sulphate and the solvent evaporated to concentrate the extract to about 0.3 mL using a stream of nitrogen. The sample was then injected to the GC machine for the fatty acid profile.

Identification of fatty acids was done by comparing with known methyl ester standards. 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°C, injector temperature of 170°C and detector temperature of 220°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 [14].

Iodine Value Determination. Samples of oil 1 g each were weighed into 250 mL conical flasks and 10 mL chloroform added and the two mixed together. The blank only contained 10 mL 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 till the colour changed to faint yellow after which 5-drops of starch solution were added. Titration was continued till the blue colour disappeared and the results recorded, same treatment occurred for the blank.

Acid Value Determination. A 1 g portion of *Amaranth* oil was weighed into 250 mLl conical flask. Three drops of phenolphthalein were then added to ethanol-diethyl ether mixture (1:1 v/v) and the mixture neutralized with 0.1 N alcohol KOH. A 40 mL volume of the solvent mixture was then added to the oil and dissolved. Three drops of indicator were added then titration done with 0.1 N KOH solution. These were done in triplicates.

Peroxide Value Determination. Oil for analysis was weighed on the analytical balance (1 g). 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 then the samples 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 until the blue colour disappeared. The result for triplicate titrations was then recorded.

Saponification Value Determination. A portion of 1 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 a reflux condenser then cooled, 3 drops of phenolphthalein was then added and titration with 0.5 N HCl done till the pink colour disappeared. The analysis was done in triplicate [14, 16].

Polar and Neutral Lipid Classes. With the aid of TLC plates, polar and neutral lipids of *Amaranth* were separated. The polar solvent system was chloroform: methanol: water (65:25:4 v/v) while for the neutral lipids, the solvent system was Hexane: diethyl ether: acetic acid (80:20:1 v/v). After development, the plates were dried in the fume oven then sprayed with 50 % sulphuric acid in the fume chamber [9, 10, 15, 17, 18]. The plates were then heated in the oven at 180°C for 30 minutes. The spots were identified using standards and quantitative determination achieved using a densitometer (Densitograph Lumino-CCD, ATTO corp.) [14].

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

Restults and Discussions

Table 1 gives a summary of results obtained from the analysis of the various parameters of the cold pressed *Amaranthus cruentus* oil and oil obtained by extraction by Dyer and Bligh method [11]. Triacylglycerides (TAG) was the major component of neutral lipids in oil extracted by the two methods. TAG was higher in the oil extracted by Dyer and Bligh (76.12%) compared to that by the cold pressing method. Diacylglycerols ranges from (17.28%) in cold pressed oil to (19.24%) in oil from Dyer and Bligh method. Free fatty acids (FFA) was the least present neutral lipid in oil from the Dyer and Bligh method (0.12%) while fatty alcohol (0.45%) was the least present neutral lipid. Among the polar lipids, PE was found to be the major component in both methods of extraction though the amount of phosphatidylethanolamine (PE) from the cold pressing method was far much higher (68.17%) as compared to that of Dyer and Bligh method. The second most abundant component of polar lipids was phosphatidylcholine (PC) from both methods of analysis while the least present polar lipid from Dyer and Bligh method was phosphatidylinositol (PI) with that of cold pressing being digalactosyl diglyceride (DGDG).

Table 2 gives a comparison of fatty acid composition of oil from cold pressed and Dyer and Bligh methods of *Amaranthus* grains from Embu [12]. No significant difference was noted among almost all the fatty acid components of the oils detected as shown in Table 2. In both methods, linoleic acid was the most abundant fatty acid. A mean of 38.41% was recorded for Dyer and Bligh method while oil from cold pressing method had a slightly higher concentration of the same acid (42.63%). These results were in agreement with those of research done elsewhere of (32–50%) as reported [6, 8, 16]. Other fatty acids that were also found to be present in high amounts in the two methods included: oleic acid (30–34%), palmitic acid (20–23%), stearic acid (2–3%) and linolenic acid (0.8–1.2%). It is important to note that other fatty acids such as capric, lauric, myristic, pentadecanoic, heptadecanoic and arachidic that were rarely reported in research done earlier were all present in oil extracted from the two methods though their compositions were relatively low.

Table 3 gives a comparison of acid, iodine, peroxide and saponification values of *A. cruentus* oil from cold pressing and Dyer and Bligh method [11]. There was no significant difference in saponification, peroxide and acid value. Saponification values of oil from the two methods had means of 179, the peroxide values with means of 3.2 and acid values of 3.5 showing that these parameters are not affected by the method of oil extraction.

Our data also confirmed the suitability of oil from cold pressing, from the low peroxide value recorded as one would fear for rancidity due to the heat used in extraction. The difference in iodine values could be attributed to the slight differences in un-saturation levels of the oils. The iodine values were found to be much slightly than those got by Dhellot 109–113 but compared well with iodine values of other oils such as *Coula edulis* 90–95, *Dacrydodes edulis* 60–80 and *Canarium schweinfurthii* 71.7–94.9 [16].

Table 1. Comparison of Polar and Neutral Lipids of Cold Pressed Oil and Oil from Dyer and Bligh Method of Grains from Embu [12]

	Dyer and Bligh method			**Cold-pressed method**		
Parameter (%)	Mean ± SE	min	max	Mean ± SE	min	max
PI	4.05±1.35	0.81	7.33	3.03±1.11	1.62	5.22
PC	18.33±4.11	13.74	30.63	18.54±1.47	15.92	20.99
DGDG	11.86±1.83	6.37	14.05	1.88±0.54	1.33	2.96
PE	25.49±1.76	20.68	29.12	68.17±0.55	67.32	69.19
CMH	17.43±5.09	2.17	23.14	2.31±0.12	2.06	2.44
ASG	10.16±3.34	2.80	19.02	3.09±0.02	3.05	3.12
SG	12.68±2.53	7.61	17.63	2.98±0.99	1.38	4.78
Neutral lipids						
TAG	76.12±0.81	74.50	76.97	72.09±0.46	71.11	73.30
FFA	0.12±0.01	0.11	0.15	0.82±0.36	0.14	1.47
Fatty alcohol	1.05±0.15	0.90	1.20	0.45±0.15	0.18	0.71
Sterols	0.44±0.12	0.29	0.67	1.60±0.60	0.54	2.70
Diacylglycerides	19.24±1.17	17.04	21.04	17.28±1.02	15.16	19.73
Monoacylglycerides	3.37±0.49	2.68	4.31	7.78±0.70	6.46	9.60

Table 2. Comparison of Fatty Acids of Oil from Cold Pressed and Dyer and Bligh methods of *Amaranthus* Grains from Embu [12]

	Dyer and Bligh method			**Cold-pressed method**		
Fatty acid (%)	Mean ± SE	min	max	Mean ± SE	min	max
Capric	0.02±0.01	0.01	0.03	0.07± 0.03	0.04	0.14
Lauric	0.09±0.04	0.03	0.18	0.23±0.01	0.21	0.25
Myristic	0.30±0.06	0.23	0.42	0.29±0.02	0.26	0.33
Pentadecanoic	0.15±0.03	0.11	0.22	0.07±0.01	0.06	0.07
Palmitic	23.02±1.80	20.70	26.58	20.98±0.64	19.85	22.08
Palmitoleic	0.43±0.17	0.22	0.77	0.07±0.03	0.00	0.10
Heptadecanoic	0.53±0.03	0.46	0.57	0.65±0.08	0.54	0.81
Stearic	2.36±0.10	2.18	2.50	2.42±0.18	2.13	2.76
Oleic	33.70±1.43	31.37	36.30	30.34±0.98	28.39	31.56
Linoleic	38.41±1.07	37.20	40.54	42.63±1.87	40.45	46.35
Linolenic	0.93±0.05	0.84	1.02	1.02±0.08	0.88	1.16
Arachidic	0.03±0.03	0.00	0.08	0.00±0.00	0.00	0.00

Table 3. Comparison of Acid, Iodine, Peroxide and Saponification Values of Oil from Cold Pressed and Dyer and Bligh Methods of Grains from Embu [12]

	Dyer and Bligh method			**Cold-pressed method**		
Parameter	Mean ± SE	min	max	Mean ± SE	min	max
Saponifcation value	179.02±2.55	174.59	183.44	179.52±1.32	177.45	181.96
Peroxide value	3.23±0.01	3.21	3.25	3.21±0.03	3.14	3.26
Iodine value	88.55±0.46	87.63	89.14	79.47±0.42	78.70	80.12
Acid value	3.51±0.01	3.50	3.52	3.51±0.07	3.38	3.62

Table 4. Comparison of Polar and Neutral Lipids of Oil from Cold Pressed and Dyer and Bligh Methods of Grains from Meru [12]

	Dyer and Bligh Method			**Cold-pressed method**		
Parameter (%)	Mean	min	max	mean	min	max
PI	4.98±0.07	4.89	5.11	10.90±1.17	9.36	14.33
PC	15.66±0.08	15.51	15.76	18.61±4.40	13.77	31.76
PGDG	6.82±0.07	6.69	6.91	6.41±1.80	2.80	9.54
PE	40.40±0.55	39.84	41.50	48.92±6.57	29.60	59.07
CMH	15.00±0.89	13.23	15.90	4.16±0.61	2.54	5.52
ASG	15.64±0.19	15.39	16.02	7.36±2.69	3.49	14.92
SG	1.32±0.10	1.12	1.42	4.62±1.95	1.41	9.32
Neutral lipids						
TAG	75.77±2.22	72.36	82.28	84.10±0.29	83.56	84.53
FFA	0.43±0.17	0.16	0.89	0.21±0.00	0.20	0.21
Fatty alcohol	0.23±0.08	0.08	0.38	0.29±0.15	0.09	0.58
Sterols	1.88±0.60	0.68	3.16	0.48±0.21	0.11	0.82
Diacylglycerol	17.43±1.39	13.34	19.47	9.66±0.18	9.43	10.01
Monoacylglycerol	4.27±0.78	3.34	6.57	5.27±0.10	5.08	5.42

Table 5. Comparison of Fatty Acids of Oil from Cold Pressed and Dyer and Bligh Methods of Grains from Meru [12]

	Dyer and Bligh Method	**Cold-pressed Method**	
Fatty acid (%)	mean	mean	*t*
Lauric	0.14±0.02	0.13±0.04	0.36
Myristic	0.39±0.03	0.30±0.01	2.85
Pentadecanoic	0.19±0.01	0.07±0.01	9.13
Palmitic	24.26±1.16	21.53±0.13	2.34
Palmitoleic	0.34±0.11	0.06±0.01	2.46
Heptadecanoic	0.45±0.01	0.55±0.05	1.88
Stearic	2.62±0.32	2.60±0.08	0.05
Oleic	32.42±0.53	33.88±0.25	2.52
Linoleic	38.17±0.39	39.25±0.80	1.21
Linolenic	0.98±0.07	0.73±0.32	0.74

Table 6. Comparison of Acid, Iodine, Peroxide and Saponification Values of Oil from Cold Pressed and Dyer and Bligh Methods of Grains from Meru [12]

	Dyer and Bligh Method			**Cold-pressed Method**				
Parameter	mean	min	max	mean	min	max	*t*	R
Saponification value mg KOH g^{-1}	189.90±3.20	184.50	195.62	187.61±5.9	181.33	199.40	0.34	N
Peroxide value meq O_2 kg	3.17±0.06	3.05	3.23	3.10±0.06	3.00	3.21	0.85	N
Iodine value g I_2 100 g^{-1}	78.77±0.80	77.47	80.23	77.02±0.49	76.17	77.87	1.87	N
Acid value mg KOH g^{-1}	3.57±0.01	3.56	3.59	3.60±0.03	3.54	3.64	1.00	N

S- Significant, N- Non-significant, R- result

Comparison of results of analysis of *Amaranthus cruentus* oil from Meru, extracted by cold pressing and Dyer and Bligh methods. From Table 4, a summary of the results of analysis of *Amaranthus* oil extracted from Dyer and Bligh and cold pressing methods can be derived [11]. Among the polar lipids, Phosphatidylethanolamine (PE) was the major component. The PE in Dyer and Bligh method was found to be (40.4%) while in cold pressed oil it was found to be (48.9%) and though cold pressing was found to have a higher value, they were not significantly different at ($p<0.05$). However, steryl glycoside was found to be the least present in both methods of extraction. Among the neutral lipids, TAG was predominant in both methods of extraction. It varied between (75.77%) for Dyer and Bligh and (84.10%) for cold pressed oil. This still confirms that most lipids in both animals and plants are stored in form of TAGs. There was a significant difference in the means of the TAG components. Diacylglycerides was the second most abundant oil (9.66–17.43%) in both oils followed by the monoacylglycerides (4.27–5.27%).

Table 5 gives the fatty acid profile of Meru, cold pressed oil and oil extracted by Dyer and Bligh method [11]. Among the fatty acids, linoleic acid had the highest composition, (38.17%) in oil from Dyer and Bligh method and (39.25%) in cold pressed oil and just like *Amaranth* oil analysed in other countries, the sequence of abundance is linoleic (38.17–39.25%), oleic (32.42–33.88%), palmitic (21.53–24.26%), stearic (2.60–2.62%) and linolenic (0.73–0.98%). Palmitic acid from this study (21.53–24.26%) was higher than those obtained by Dhellot (14–18%) but compared well with those obtained by Saunders and Becker (20–22%) [6].

Table 6 gives a comparison of acid, iodine, peroxide and saponification values of oil from cold pressed and Dyer and Bligh methods of grains from Meru. There was no significant difference in the saponification, acid, peroxide and iodine values. The saponification values were quite high 187.61–189.90 compared to those obtained by Dhellot [16] of 160 but they compared well with those of other usual oils such as soybeans 189–195, peanut 187–196 and cotton 189–198. The peroxide values from both methods compared well with those found elsewhere of 2.3–4.8 [16] while acid values from the same analysis ranged between 1.74–3.92.

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References and Notes

1. Martirosyan, D. M.; Miroshnichenko, L. A.; Kulalkova, S. N.; Pogojeva, A. V.; Zoloedov, V. I. *Lipids in Health* **2007**, *6*, 1–10.
2. Sauer, J. D. *Ann. Missouri Bot. Gard.* **1967**, *54*, 103–137.
3. Becker, R.; Wheeler, E. L.; Lorenz, K.; Stafford, A. E.; Grosjean, O. K.; Betschart, A. A.; Saunders, R. M.; Saunders, R. M. *Journal of Food Science* **1981**, *46*, 1175–1180.
4. Teutoniko, R. A. and Knorr, D. *Journal of Food Technology* **1985**, *29*, 49–60.
5. Paulina, B. d. l. R. A.; Cecilia, S.-S. n.; Elvira, G. l. d. M. In *Hispanic Foods*, 946 ed.; American Chemical Society, 2006, pp 103–116 SE - 109.
6. Saunders, R.; Becker, R. *Advances in Cereal Science and Technology* **1984**, *6*, 357–396.
7. Lorenz, K. a. Hwang, Y. S. *Nutr Reports Intl.* **1985**, *1*, 83–89.
8. Tamer, G. H.; Mesallam, A. S.; Damir, A. A.; Shekib, L. A.; Linssen, J. P. *Journal of Food Lipids* **2996**, *14*, 323–334.
9. Lerno, L. A.; German, J. B.; Lebrilla, C. B. *Anal. Chem.*, **2010**, *82*, 4236–4245.
10. Brooks, S. P. J.; Ratnayake, W. M. N.; Lampi, B. J.; Hollywood, R. *J. Agric. Food Chem.* **1998**, *46*, 4214–4217.
11. Bligh, E. G. and Dyer, W. J. *J. Biochem.* **1959**, *37*, 911–917.

12. *Map of Kenya*. http://www.masai-mara.com/mmvmk.htm (accessed February 2013).
13. Bressani, R.; Gonzales, J. M.; Brauner, M.; Elias, L. G. *J. Sci. Food Agric*. **1987**, *38*, 347–356.
14. Karanja, P. N.; Hiroshi, K.; Michiyuki, K.; Naomichi, B.; Ishibashi, K. *Journal of Food Preservation Science* **2002**, *28*, 76–80.
15. Harvey, D. T. *J. Chem. Educ*. **2006**, *83*, 385.
16. Dhellot, R. J.; Matouba, E.; Maloumbi, M. G.; Nzikou, J. M.; Safou, D. G.; Linder, M.; Desobry, S.; Parmentier. *African Journal of Biotechnology* **2006**, *5*, 1095–1102.
17. Berger, D. *J. Chem. Educ*. **2001**, *78*, 1336.
18. Storfer, S. J.; Becker, E. I. *J. Chem. Educ*. **1959**, *36*, 614.