Association for Aquatic Sciences of Nigeria (ISSN: 0189-8779)
http//www.journalofaquaticsciences

Journal of Aquatic Sciences, 34 (2): 117 –124 (2019) DOI: https://dx.doi.org/10.4314.jas.v34i2.14

EFFECTS OF PROCESSING METHODS ON FATTY ACID COMPOSITION, PHYSICAL AND CHEMICAL PROPERTIES OF *Moringa oleifera* SEED OIL

[*1]HARUNA, M. A., [2]IPINJOLU, J. K. AND [3]ORIRE, A. M.

[1]Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa. Nigeria.
[2]Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, Minna.
*Correspondence email: a4globalfisheries@gmail.com

ABSTRACT

The study was carried out to establish the effect of processing methods on the quality of oil extracted from *Moringa* seeds. The oil was extracted from the raw and processed (Boiling for 90 minutes and soaking for 72 hours) (BS90min/72hours) seeds using a mini oil screw press extraction machine. The Gas Chromatography Mass Spectrometer GC-MS analysis of raw and treated *Moringa* seed oil indicated presence of four saturated (lauric, myristic, palmitic and arachidonic) and five unsaturated (palmitoleic, stearic, oleic, linoleic and linolenic) fatty acids. There was a consistent trend of reduction in the concentrations of both the unsaturated and saturated fatty acids in the treated samples compared to the raw samples. Oleic acid was most abundant of the unsaturated fatty acids in both the raw (88.792%) and treated (79.946%) *Moringa* seed oil. Myristic acid recorded the lowest concentration of the saturated fatty acids in raw (0.939%) and treated (0.7640%) seed oils. The peroxide value also increased from 0.03±0.00mg/mol/kg in the raw to 73.00±0.06 mg/mol/kg in the treated oils. The quality character of the *Moringa* oil with respect to high peroxide value recorded in the oil of the processed seed indicated reduced shelf life. This calls for further studies to explore and improve on its nutritional values for possible utilization as an alternative to fish oil in fish feed production.

Keywords: Dietary oil, saturated, unsaturated, extraction, fatty acid.

INTRODUCTION

One of the major components of fish feed is fish oil, mainly because of its high content of n-3 highly unsaturated fatty acids (HUFA) which are considered essential fatty acids for fish (Sergent and Tacon, 1999). However, it has been projected that in few years, global fish oil production may not be enough to cover the increasing demand for animal feed. On the contrary, global vegetable oil production has increased in recent years, reaching volumes 100 times that of fish oil (Bimbo, 1990). As a result, prices for vegetable oils have been more stable and have even decreased in some markets, with some vegetable oils becoming less expensive than fish oil. Such oils as soybean, palm, canola and linseed are considered good alternative lipid sources for fish oil in animal feed (Ng *et al.*, 2004). Total and partial fish oil replacement had been

successfully achieved in various fish species (Babalola, 2010). Therefore, this research was conducted to establish the effect of the processing method of boiling for 90 minutes and soaking for 72 hours (BS90min/72hours) on the quality of fatty acids from *Moringa* seed oil.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto, on latitude 13^0 07' 47.6''N and longitude 05^0 12'11.3''E at 275m above sea level (Google MAP, 2015).

Seed Collection and Preparation

Dried mature fruits of *M. oleifera* were obtained from Maiyafe farm at *Kududdufawa* in Ungoggo Local Government, Kano State on Latitude 12^o 05' 26^o N and Longitude 8^o 29'48^o E. The seeds were cleaned thoroughly to remove dirt. The cleaned seeds were shelled manually to remove the kernels, dried under shade and stored until required.

Seed Preparation and Oil Extraction

The oil was extracted from the raw seed using a mini oil screw press extraction machine (rendering method). The processed method involved the following procedure: 1kg raw *M. oleifera* seeds were boiled for 90 minutes at 100^oC and then soaked for another 72 hrs (BS90min/72hours). Before commencing the extraction process, the machines in-built heating element was allowed to heat for 30 minutes to enable it generates enough heat that would eventually facilitate the separation of oil from the seeds. Thereafter, the seeds were gradually introduced into the collection tray and then into the extraction chamber. The treated seeds were then crushed and oil was separated from the cake. The exuded oil was collected and preserved for further analysis.

Determination of Fatty Acid Composition of Extracted Oil

Fatty acid compositions of oil samples were determined using gas-liquid chromatography (with omega-wax capillary column Supelco, Sigma-Aldrich, Bellefonte, PA). The lipid classes were separated by thin layer chromatography on silica gel G60 (Merck, Darmstadt, Germany), using *n*-hexane/ethyl ether/acetic acid (73/25/2/v/v/v) as developing solvent. The fatty acids of phospholipids and triglycerides were transformed with sodium methylate into methyl esters (Farooq *et al.*, 2006).

Determination of Oil Characteristics

The following oil characteristics were examined according to standard AOAC (2012) method thus:

$$Percentage\ oil\ yield = \frac{wt\ before\ extraction - wt\ after\ extraction}{wt\ before\ extraction} * 100$$

Specific gravity: A 100ml specific gravity bottle was cleaned and dried, cooled and weighed W1. This was then filled with clean water and weighed W2. The water was poured out and the bottle was allowed to dry. The specific gravity bottle was again filled with oil and the weight taken W3. The specific gravity and relative density of the oil was calculated using the formula below**:**

$$Specific\ gravity = \frac{weight\ of\ oil}{weight\ of\ equal\ volume\ of\ water}$$

Saponification Value: A 2g oil sample was weighed into a 250ml conical flask and 25ml normal alcoholic potash was added. The mixture was boiled gently under reflux on a water bath for 1hr. After boiling, the mixture was titrated with 0.5M HCl (Obikili, 2010). Saponification value was then calculated thus:

$$Saponification\ value = \frac{(B-A)\ x\ 28.05}{weight\ of\ oil\ sample}\ Mg.\ KOH/gm$$

Where; B=Blank titre, A= Sample titre

Peroxide Value: A 1g of oil sample was added with 1g of potassium iodide and 20ml glacial acetic acid chloroform in 2:1 and boiled for 1 min. The hot solution was transferred into a flask containing 20ml of 5% potassium iodide solution. Few drop of starch solution was added and titrated with 0.025 N $Na_2S_2O_3$ to a faint yellow colour. A 1ml starch indicator was added and the titration continued until blue colour disappeared (Eromosele *et al.,* 1994). The peroxide value was then calculated thus:

$$Peroxide\ value = \frac{molar\ equivalent}{weight\ of\ oil\ sample} = \frac{S\ x\ N\ x\ 100}{weight\ of\ oil\ sample}$$

Where, S = Wt of $N_2S_2O_3$ used, N = Normality of $N_2S_2O_3$

Iodine value: A 1g of oil sample was added with 5ml chloroform solution and 5ml Dan's reagent. The solution was kept in fume cupboard for 10min. A 5ml of 10% potassium iodide added with 20ml of distilled H_2O, stirred and solution titrated to a colourless end point with 0.025N $N_2S_2O_3$ (Eromosele *et al.,* 1994). Iodine number was then calculated thus:

$$\%\ iodine\ number = \frac{(B-A)\ 0.00317\ x\ 0.001269\ x100}{weight\ of\ sample}$$

Where; B = Blank Titre, A= Sample Titre, 1ml 0.025 $N_2S_2O_3$ = 0.00317g

Free Fatty acid: A 5g oil sample was weighed, poured into a conical flask and re-weighed, 50ml of hot neutral alcohol was added with a few drops of phenolphthalein and shaken vigorously. The solution was titrated with 0.5MNaOH solution with constant shaking until the pink colour remained constant. From the quantity of 0.5M alkali used, the percentage of acid present was calculated and expressed as oleic acid (Farooq *et al.,* 2006).

STATISTICAL ANALYSIS

Data obtained on fatty acids, physical and chemical properties of oils, were subjected to analysis of variance (ANOVA). Means were separated using Duncan multiple range test (Duncan, 1955) using the SPSS computer software, version 20.0. Significant difference between mean values was accepted at the 0.05 level of probability.

RESULTS

The fatty acid compositions of raw and processed *Moringa* seed and their concentrations are presented in Table 1. A total of nine fatty acids were detected at varying concentrations comprising five unsaturated and four saturated fatty acids. Palmitoleic, stearic, oleic, linoleic and linolenic acids were the unsaturated fatty acids while the saturated fatty acids consisted of lauric, myristic, palmitic and arachidonic acids. There was a consistent trend of reduction in the concentrations of both the unsaturated and saturated fatty acids in the treated samples when compared with the raw samples. Oleic acid was most abundant of the unsaturated fatty acids in both the raw and treated *Moringa* seed oil. Stearic acid recorded the lowest value of the unsaturated fatty acids in the raw and treated oil samples. Palmitic acid had the highest concentration of the saturated fatty acids in raw (14.4249 %) and treated (13.4568 %) seed oil while; myristic acid recorded the lowest concentration of the saturated fatty acids in raw 0.939 and treated 0.7640 % seed oils.

Table 1: Fatty acid compositions of raw and processed *Moringa oleifera* seed oils

Fatty acids	Peak	Time	Area	Height	Conc. (%)
Raw Seed Oil					
Palmitoleic	18	13.56	3.67	425	4.5780
Stearic	1	5.66	2.92	64	3.15
Oleic	14	11.88	3.92	827	88.792
Linoleic	2	5.80	1.54	57	4.0981
Linolenic	8	9.07	7.07	239	3.4368
Lauric	5	6.79	1.80	37	2.8375
Myristic	9	9.75	3.38	85	0.939
Palmitic	16	13.16	1.78	28	14.4249
Arachidonic	19	14.26	1.62	94	2.9772
Processed Seed Oil					
Palmitoleic	7	15.68	25.72	58	4.0184
Stearic	1	12.39	7.45	32	2.95
Oleic	10	16.90	25.27	549	79.9462
Linoleic	3	13.75	18.02	47	3.9257
Linolenic	4	14.54	4.01	110	3.0974
Lauric	2	6.79	2.20	43	1.7461
Myristic	6	9.75	2.07	43	0.7640
Palmitic	9	13.16	3.75	45	13.4568
Arachidonic	12	14.26	1.92	67	2.4786

The physical and chemical characteristics of raw and processed *Moringa* seed oils are presented in Table 2. The results indicated significant differences ($p<0.05$) between the raw and treated oils. There was a significant ($p<0.05$) increase in viscosity from $61.33\pm1.16 kg/m^3$ in the raw sample to $85.67\pm0.58 kg/m^3$ in treated oil sample, while free fatty acid increased from

0.11±0.02Mg/100g in the raw to 56.00±0.10 Mg/100g in treated sample. The peroxide value also increased from 0.03±0.00mg/mol/kg in the raw seed oil to 73.00±0.06 mg/mol/kg in the treated oil. The saponification value in the raw seed oil was significantly ($p<0.05$) enhanced from 61.79±0.70mg/KOH/g to more than double (131±0.58 mg/KOH/g) in the treated oil sample. On the contrary, specific gravity and refractive index were significantly ($p<0.05$) reduced from 0.95±0.00kg/m^3 and 1.52±0.01 kg/m^3 in the raw sample to 0.91± kg/m^3 and 1.31± kg/m^3 in the treated sample respectively.

Table 2: Physical and chemical characteristics of raw and processed *Moringa seed* oils

Parameters	Raw seed oil	Processed oil	*Standard for edible oil
Viscosity (kg/m^3)	61.33±1.16^b	85.67±0.58^a	60
Specific gravity (kg/m^3)	0.95±0.00^a	0.91±0.00^b	0.9-1.16
Refractive Index	1.52±0.01^a	1.32±0.02^b	1.46-1.47
Free fatty acid (mg/mol)	0.11±0.02^b	56.00±0.10^a	5.78-70.28
Iodine value (mg/100g)	46.19±0.16^b	97.08±0.23^a	80-106
Peroxide value (mg/mol/kg)	0.03±0.00^b	73.00±0.06^a	10-20
Saponification value (mg/g)	61.79±0.70^b	131.33±0.58^a	181.4

Mean with same letter in row are not significantly different ($p>0.05$). *FAO/WHO (2009)

DISCUSSION

The fatty acid analysis of raw and processed *Moringa* seed oil indicated the presence of both saturated and unsaturated fatty acids. The level of oleic acid recorded in this study for both raw and treated oil samples were higher than the value of 74.991% reported for *Moringa oleifera* seed oil by Ashraf and Gilani (2007), and 63.72% reported for *Luffa aegyptiaca* oil by Karaye *et al*. (2012). The major fatty acids identified in this study (oleic, linoleic and palmitic acids and their derivatives) have been described as multifunctional molecules that have a wide range of applications (Bodalo *et al*., 2005). The high levels of oleic and linoleic acids observed were in agreement with the findings of El-aziz and El-kalek (2011) who reported high contents of linoleic and oleic acids in seed oil of *Cucurbita moschata*. In earlier study, Lazos (1986) and El-Adawy and Taha (2001) observed high levels of these fatty acids in pumpkin seed oil. Linoleic acid has been reported to be one of the most important essential fatty acids, and thus must be present in animal diets (FAO, 1994). The presence of high levels of these amino acids suggested that *Moringa* seed oils were highly nutritious. The oil volume obtained for the processed oil sample was within the range of between 41.0 and 56.6% reported in other study (Madaan and Lai, 1984). The oil yield after treatment indicated that more oil was expressed from the treated seed and this could be because the treatment method involving prolonged boiling and soaking might have enhanced the separation of the oil from the seed.

Interest in the oil of *M. oleifera* seeds had grown significantly over the years. This oil, known commercially as "Ben" or "Behen" oil, is suitable for edible purposes as it contains (80%) high levels of oleic acid (Anwar and Bhanger, 2003). The viscosity value of raw *Moringa* seed oil observed in this study showed little variation from the standard value (FAO/WHO, 2009). Similar observation was made on the levels of specific gravity for the raw and processed seed oils. Refractive indices also showed little variation from the international

standard which affirmed the purity of both the raw and treated *Moringa* seed oils. The iodine value obtained for the raw seed was lower than the recommended standard range while that of the treated was within the recommended value (FAO/WHO, 2009). The higher level recorded for the treated oil indicated greater degree of unsaturation while the lower content in the raw showed lower level of unsaturation. Mabalaha *et al.* (2007) reported iodine values of 95.8 g/100g in tsama melon, and 124 g/100g in desert melon. The iodine value is a measure of the unsaturated acid present in the oil; it also indicates the non-drying qualities of the oil. The result of this study was in agreement with the findings of Olaniyan (2010) who opined that increase in heating temperature and heating time increased the free fatty acid, peroxide and iodine values of the expressed oil. Although the saponification values of raw seed oil were significantly lower ($p<0.05$) than the treated oils. Both values were lower than the recommended international standard for edible oil (FAO/WHO, 2009). The saponification value gives information concerning the character of the fatty acid present in the oil and the solubility of the soap derived from it in water. A high saponification value indicates that the oil contains low portion of fatty acids. The lower values recorded in this research for both raw and treated is an indication that *M. oleifera* seed oil may not be suitable for soap making. A significantly ($p<0.05$) higher peroxide value was recorded in the treated seed oil when compared with the level recorded in the raw sample. Peroxide value is a measure of the oxygen content used to monitor the development of rancidity. The lower value recorded in the raw *Moringa* seed oil indicated that the oil would not easily go rancid. Thus, indicative of having a long shelf life with good stability properties. This was in line with the reports of Anwar and Bhanger (2003), who noted that *Moringa* seed oil could have a shelf life of up to 5 years.

CONCLUSIONS

The results obtained from this research indicated that processing method involving boiling for 90 minutes and then soaking for another 72 hours (B90min/S72hrs) before oil extraction had significant effects on the oil quality. However, further research should be done on other processing methods that may produce better oil quality for complete replacement of fish oil in fish feed production.

REFERENCES

Abiodun, O. A. and Adepeju, A. B. (2011). Effect of processing on the chemical, pasting and anti-nutritional composition of Bambara nut (*Vigna subterranean* L. Verdc). *Advance Journal of Food Science and Technology*, 3(4): 224-227.

Anwar, F. and Bhanger, M. I. (2003). Analytical characterization of *Moringa oleífera* seed oil grown in temperate regions of Pakistan. *Journal of Agric. Food Chem.*, 51: 6558–6563.

Anwar, F., Zafar, S. N. and Rashid, U. (2006). Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab. *Grasasy aceites*, 57(2): 160-168.

AOAC (2012). Association of Analytical Chemists. Official Methods of Analysis of the AOAC International No. 994.12. 19th Chapter 4, 18-19. Official Journal of the European Communities, 19.9.98, Gaithersburg, Maryland, USA.

Ashraf, F. and Gilani, S. R. (2007). Fatty acids in *Moringa oleifera* oil. *Journal of Chemical Society of Pakistan*, 29 (4): 343-345.

Babalola, T. O. O. (2010). Utilization of dietary animal fats and vegetable oils by African catfish (*Heterobranchus longifilis*) fingerlings. Ph.D. thesis University of Ilorin, Nigeria.

Bimbo, A. P. (1990). Production of fish oil. In: M. E. Stansby (Ed.), Fish oils in nutrition, pp 141–180.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics,* 11:1-42.

Ebuchi, O. A. T., Ameh. R. A. and Oletu, F. U. (2006). Physico-chemical and fatty acid composition of two common edible vegetable oils in Nigeria. *Nigerian Food Journal,* 24 (1): 17-24.

El-Adaway, T. A. and Taha, K. M. (2001). Characteristics and compositions of different seed oils and flours. *Food Chemistry,* 74: 47-54.

El-aziz, A.B. and El-kalek, H.H. (2011). Antimicrobial protein and oil seeds from pumpkin (*Curcubita moschata*). Nature and Science, 9(3): 105-119.

Eromosele, I. C., Eromosele, C. O., Akintoye, A. O. and Komolafe, T. O. (1994): Characterization of Oils and Chemical Analysis of the Seeds of Wild Plants. *Plant Food for Human Nutrition Journal*, 5(2):30-50

FAO (1994). Assurance of sea food quality. H.H.Huss Technological Laboratory Ministry of Fisheries, Denmark FAO Fisheries Technical Paper 334. United Nations Rome, Page 4-5.

FAO/WHO (1993). Fats, oils and related product. Food standard program. Codex Alimentarius Commission. Food and Agriculture Organization of the United Nations. World Health Organization, Rome. 8: 33-35.

FAO/WHO (2009). Report of the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia, 16 – 20 February, 2009.

Farooq, A., Syeda. N. Z., and Umar, R. (2006): Characterization of *Moringa oleifera* Seed Oil from Drought and Irrigated Regions of Punjab, Pakistan. Department of Chemistry, University of Agriculture, Faisalabad-38040, Pakistan. 57(20):160-168.Google MAP (2015). http://maps.google.com/maps: 15/07/17: 20:36

Ibrahim, S.S., Ismail, M., Samuel, G., Kamel, E. and Azhari, T. (1974). Benseed, a Potential Oil Source, *Agric. Res.Rev.* 52: 47-50.

Ibrahim, A. and Onwualu, A.P. (2005): Technologies for extraction of oil from oil-bearing agricultural products. *A Journal of Agricultural Engineering and Technology,* 13(1): 58 70.

Karaye, I. U., Aliero, A. A., Muhammad, S. and Bilbis, L. S. (2012). Comparative evaluation of amino acid composition and volatile organic compounds of selected Nigerian Cucurbit seeds. *Pakistan Journal of Nutrition*, 11 (12): 1161-1165.

Lazoz, E. (1986). ''Nutritional fatty acid and oil characteristics of pumpkin and melon seeds'' *J. Food Sci*., 51: 1382-1383.

Mabalaha, M. B., Mitei, Y. C. and Yoboah, S.O. (2007). A comparative study of the properties of selected melon seeds oils as potential candidates for development into commercial edible vegetable oil. *J. Ameri. Oil Chem. Soc.*, 84: 31-34.

Madaan, T.R. and Lai, B.M. (1984). Some studies on the chemical composition of Cucurbit kernels and their seed coats. *Plant foods for Human Nutrition*, 34 (2): 81-86.

Ng, W. K., Sigholt, T. and Bell, J. G. (2004). The Influence of Environmental Temperature on the Apparent Nutrient and Fatty Acid

Digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rape seed oil and palm oil. *Aquaculture Research*, 35, 1228-1237.

Olaniyan, M. A. (2010). Effect of Extraction Conditions on the Yield and Quality of Oil from Castor Bean. *Journal of Cereals and Oilseeds*, 1(2): 24-33.

Obikili, C. M. (2010). Evaluation of The Effect of Methods of Oil Extraction On Physico-Chemical Properties of Oil from African Breadfruit Seeds, (*Treculia africana*) and Cashew Nuts (*Anarcardium occidentale*) BSc Dissertation, Federal University of Technology, Minna, Niger State. pp. 12-18.

Sargent, J. R. and Tacon, A. (1999). Development of farmed fish: a nutritionally necessary alternative to meat. Proceedings of the Nutrition Society, 58(2): 377–383.

Bodalo, A., Bastida, J., Maximo, M.F., Hidalgo, A.M. and Murcia, M.D. (2005). Production of (E) 10-hydroxy-β-octadecenoic acid with lyophilized microbial cells. *American Journal of Biochemistry. Biotechnology*, 1: 1-4.

Received: 8th August, 2019; **Accepted**: 2nd December, 2019.