

ENZYME-ASSISTED AQUEOUS EXTRACTION OF NJANGSA
(*RICINIDENDRON HEUDELOTTI*) SEED OIL

By

IMMACULATE TABE ARREY

A THESIS

Submitted to the Faculty of Delaware State University
in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Food Science
in the Department of Human Ecology

DOVER, DELAWARE
May 2017

This thesis is approved by the following members of the Final Oral Committee:

Dr. Gulnihal Ozbay, Committee Chairperson, Department of Human Ecology, Delaware State University

Dr. Alberta N.A. Aryee, Committee Member, Department of Human Ecology, Delaware State University

Dr. Bettina C. Taylor, Committee Member, Department of Human Ecology, Delaware State University

Dr. Jungmi Oh, External Committee Member, Department of Human Ecology, Delaware State University

© IMMACULATE TABE ARREY
All Rights Reserved

ACKNOWLEDGMENTS

I am very grateful to GOD ALMIGHTY for without His grace, strength and blessings this study would not have been possible.

Foremost, I would like to thank Dr. Stephen Lumor for his motivation, enthusiasm, and immense knowledge. I would like to express my deepest gratitude to my advisor Dr. Gulnihal Ozbay for her excellent guidance, caring, patience, all the hours invested in making corrections and providing me with an excellent atmosphere for doing research. Many thanks to Dr. Alberta N.A. Aryee who read my numerous revisions and helped make some sense of the confusion and for her best suggestions. Thanks to my other committee members Dr. Bettina C. Taylor, Dr. Jungmi Oh and Dr. Guo for being ever generous with their time, contributions towards my thesis and for granting full access to their laboratories and instruments. Special thanks to Dr. PapaNii Asare-Okai of the university of Delaware, for his support and access to their Gas chromatography-mass spectrometry (GC-MS).

Most of all, I am fully indebted to Dr. Samuel Besong who had to bear a heavy load of responsibility and concern in bringing me to a successful end, for guiding and helping me to make the study a well-done achievement.

Anh Nguyen, your help in every aspect of my research was imperative to my completion of this degree. Thank you so much for always being there when I need you. You'll make a great professor someday. I thank my lab mates Michael, Marieme, Prince, Duchard, and Bhagya Sri, for the stimulating discussions, for the time we were working together, and for all the fun we have had.

I am also grateful to the deans of the college, Dr. Marsh and Dr. Alvarez for their support. Special thanks to Mrs. Garrison of the College of Agriculture and Related Sciences for all her assistance and for was always willing to help.

I would also like to thank my family, for their unconditional love, they were always supporting and encouraging me with their best wishes. Finally, I would like to thank my husband, Raphael Eyum, who is my champion, for his unfailing love, care and for always being there.

**Enzyme-Assisted Aqueous extraction of Njangsa
(*Ricinodendron heudelotti*) seed oil**

Immaculate T. Arrey

Faculty Advisor: Dr. Gulnihal Ozbay

ABSTRACT

Solvent extraction methods are widely used industrially to obtain oil from plant seeds, mostly due to their high efficiencies (90-98% w/w yield). However, consideration is being given to solvent-free extraction methods due to the growing concern that residual solvent in oil could pose significant health and environmental risks. As such, enzymatic extraction methods are receiving considerable interest in the oil industry due to their high specificity. Certain enzymes such as: hemicellulase, protease, pectinase and amylase are known to hydrolyze and degrade the cell wall of oilseeds, which significantly increases oil yield and quality upon extraction. Therefore, the aim of this study was to explore the effects of these four different enzymes on *Njangsa* (*Ricinodendron heudelotti*) seed. A control without any enzyme was run in tandem. These treatments were compared to solvent-extraction using hexane. The percent yield of the enzyme-assisted extraction (28.37 - 36.0%) were significantly ($p < 0.05$) higher than that of the control (19.09%) but, lower than the hexane extraction (46.40%). Quality indices of Njangsa seed oil (NSO) such as: acid, peroxide, saponification, thiobarbituric acid, para-anisidine and free fatty acid values of the enzyme-assisted extraction samples were found to be significantly ($p < 0.05$) lower than the solvent extraction samples. Predominant fatty acids of NSO were alpha-eleostearic acid

(α - ESA), linoleic, stearic, and oleic. NSO had a high polyunsaturated fatty acid content. The use of enzymes in Njangsa seed extraction were able to improve the quality parameters of Njangsa oil.

TABLE OF CONTENTS

Table of Contents	vi
List of Tables	viii
List of Figures.....	ix
CHAPTER 1: INTRODUCTION	1
1.1. Background and Significance.....	1
1.2. Statement of Problem and Hypothesis	3
1.3. Specific Objectives	4
CHAPTER 2: LITERATURE REVIEW	5
2.1. <i>Ricinodendron heudelotti</i> (Njangsa).....	5
2.2. Cell Wall Constituents of Oil Seed.....	6
2.2.1. Hemicellulose.....	7
2.2.2. Cellulose.....	7
2.3. Properties and Sources of Enzymes Used in the Study	8
2.3.1. Hemicellulase.....	8
2.3.2. Protease.....	9
2.3.3. Amylase.....	10
2.3.4. Pectinases.....	11
2.4. Physical and Chemical Properties of Seed Oil.....	11
2.4.1. Lipid Oxidation.....	13
2.4.1.1. Mechanism of Lipid Oxidation.....	14
2.5. Oxidative Stability Study of Njangsa Seed Oil.....	15
2.5.1. Peroxide Value (PV).....	16
2.5.2. Acid Value (AV).....	17
2.5.3. Saponification Value (SV).....	18
2.5.4. Thiobarbituric Acid (TBA) Value.....	19
2.5.5. Para-Anisidine Value (<i>p</i> -AV)	20
2.6. Gas Chromatography/Fatty Acid Composition and Analysis.....	21
2.7. Differential Scanning Calorimetry (DSC) Melting and Crystallization Behavior.....	22
2.8. Factors Affecting Oil Yield.....	23

2.8.1. Size Reduction	23
2.8.2. Solid: Water Ratio.....	24
CHAPTER 3: MATERIALS AND METHODS.....	25
3.1. Materials.....	25
3.2. Enzymes.....	25
3.3. Sample Preparation.....	25
3.3.1. Enzymatic Extraction.....	26
3.3.2. Solvent Extraction.....	26
3.4. Physical and Chemical Parameters of Oil.....	27
3.4.1. Peroxide Value (PV).....	27
3.4.2. Acid Value(AV).....	28
3.4.3. Saponification Value (SV).....	28
3.5. Lipid Hydroperoxide Determination.....	28
3.5.1. Para-Anisidine value(<i>p</i> -AV)	28
3.5.2. Thiobarbituric Acid Value.....	29
3.6. Determination of Fatty Acid (FA) Composition.....	30
3.7. Determination of Melting Profile.....	30
3.6.1. Preparation of FAME by Base Catalysis.....	30
3.6.2. Fatty Acid Profile Analysis.....	30
3.8. Statistical Analysis	31
CHAPTER 4: RESULT AND DISCUSSION.....	32
4.1. Oil Extraction.....	32
4.2. Quality Parameters of Njangsa Seed Oil.....	33
4.3. Oxidative Stability study of Njangsa Seed Oil.....	37
4.7. Thermal Behavior of Njangsa Seed Oil.....	41
CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS	43
REFERENCES	45

LIST OF TABLES

Table 3-1. Manufacturer Specifications for Enzymes Used in the Extraction.....	25
Table 4-1. Effect of Enzyme Type on Oil Recovery from Njangsa Seed.....	35
Table 4-2. Physicochemical Properties of Njangsa Seed Oil	35
Table 4-3. Oxidative State of Njangsa Seed Oil.....	38
Table 4-4. Fatty Acid Composition of Njangsa Seed Oil.....	38
Table 4-5. Fatty Acid Group of Njangsa Seed Oil.....	40
Table 4-6. Thermal Behavior of Njangsa Seed Oil.....	42

LIST OF FIGURES

Figure 2-1. Effect of pH And Temperature on Hemicellase Activity.....	9
Figure 2-2. Effect of pH and Temperature on Protease Activity.....	10
Figure 2-3. Effect of pH and Temperature on Amylase Activity.....	11
Figure 2-4. Free Radical Oxidation Sequence in Lipids.....	14
Figure 2-5. Schematic Diagram of a Thermogram, Plotting Heat Flow as a Function of Temperature, Showing the Parameters Typically Measured for Fats.....	23
Figure 3-1. Seeds (A), Homogenized Seeds (B) and Oil (C) from Njangsa.....	27
Figure 4-1. GC-MS Chromatogram of the Fatty Acid Profile of Njangsa Seed Oil.....	40
Figure 4-2. Melting Profiles of Njangsa oil.....	41

CHAPTER 1: INTRODUCTION

1.1. Background and Significance

Oils have always been an integral part of human foods, being essential for human health. Industrially, they play an important role in the development of different chemical products, pharmaceutical, cosmetics, paints and most importantly, food for human consumption (Atef, 2010). Oils are naturally occurring esters of long chain carboxylic acids. They belonged to the saponifiable group of lipids. Edible oils are constituted of triacylglycerol molecules, mainly formed by unsaturated (oleic, linoleic, linolenic acids etc.) and saturated fatty acids (myristic, palmitic, stearic acids etc.) esterified to glycerol units (Andersson et al., 2010). Njangsa seed has been regarded as nutritious food in most African countries and used in many sauces, soups, and condiments due to its characteristic spicy flavor (Esekwe et al., 2014; Mapongmetsen et al., 1999). Njangsa is mainly cultivated in the developing countries of West Africa for its content of good quality oil. According to Kapseu and Tchiegang (1995) and Manga et al. (2000), the oil content of Njangsa seed ranges from 45 - 67% out of which polyunsaturated fatty acids (PUFAs) constitute almost 75% of the total fatty acids, and alpha eleostearic acid (α -ESA) is the most abundant. Other fatty acids such as linoleic, oleic, and stearic acids contribute 27.9, 6.4 and 6.5%, to the total fatty acid profile, respectively (Assanvo et al., 2015; Manga et al., 2000). Research findings by Leudeu et al. (2009) revealed about 33% of α -ESA in Njangsa seed oil. According to Dhar et al. (1999), α -ESA was observed to significantly reduce plasma lipid peroxidation, lipoprotein peroxidation, and erythrocyte membrane lipid peroxidation in both diabetic and non-diabetic individuals. Pal and Ghosh (2012) also reported that α -ESA reversed oxidative stress induced by methyl mercury in rat liver and kidneys. In two separate studies, α -ESA exhibited selective and considerable

inhibition of tumor growth by inducing apoptosis (Suzuki et al., 2001). Furthermore, α -ESA inhibited the growth of human breast cancer cells and thus may be a good candidate for a chemotherapeutic agent against breast cancer (Grossmann et al., 2009). The protective effect of α -ESA on cardiovascular function was further studied by Leudeu et al. (2009) who reported that a diet formulated with *Ricinodendron heudelotti* seed oil, which contained 52.6% α -ESA, significantly reduced cholesterol and triglyceride levels in rats compared to the control group, which were fed with a standard diet. Also, the beneficial effect of α -ESA on obesity has been reported. The seed is also a major source of tocopherol and phytosterol and also contain an appreciable amount of omega-3 fatty acid (Pal and Ghosh, 2012; Yeboah et al., 2011; Lumor et al., 2007). The beneficial effects on serum lipid levels, and improvement of anti-inflammatory and anti-mutagenic activities of Njangsa seed oil have been documented (Saha et al., 2012 Leuden et al., 2009). This is due to the positive effect of α -ESA on serum cholesterol and the fatty acid profile which results from its ability to reduce low density lipoprotein (LDL) cholesterol and triglycerides. The anti-proliferative effect of α -ESA against certain cancers has been reviewed by Hennessy et al. (2011). In addition to its food uses, Njangsa oil also has a wide industrial application such as pharmaceuticals, cosmetics, soaps, and varnish production (Esekwe et al., 2014; Anjah et al., 2009).

Seed oils are mainly extracted by convectional pressing or solvent extraction. During convectional processing, severe heat treatment during the process not only affect oil quality but also denatures the proteins in the seed (Latif and Anwar, 2008; Mani et al., 2007). A recent study on Njangsa seed extraction by pressing documented an extraction of 70% of the total seed oil (Tchiegang et al., 2007). Solvents generally used in oil extraction include ethanol, isopropanol, methylene chloride, acetone, and hexane. The most commonly used solvent in the last few decades

is hexane with a higher efficiency of about 90 - 98% oil yield (Kalia et al., 2001). However, this method has disadvantages because of poor quality oil it produces, high cost and significant energy demand.

Enzymatic extractions are receiving considerable interest in oil industry due to their high specificity and low operating temperatures. Studies have shown that they are environmentally friendly and safe (Latif and Anwar, 2009). Dobozi et al. (2009) reported that the treatment of mustard seeds with cellulolytic enzymes resulted in an increased (20 -30%) oil yield. Najafian et al. (1999) observed that oil extraction from olive can be enhanced by enzyme hydrolysis and demonstrated that pre-extraction enzyme digestion increases cellular degradation and significantly increases oil recovery upon extraction. Similarly, Dominguez et al. (1995) reported an increase in soy bean extractability by 8 - 10% of the extractable oil and up to 4% in the case of sunflower oil.

The main goal of this study is to investigate the use of four enzymes (hemicellulase, amylase, pectinase and protease) for the extraction of oil from Njangsa seeds and evaluate the quality of the oil obtained in comparison to solvent-extracted oils.

1.2. Statement of Problem and Hypothesis

Hexane is listed among hazardous air pollutants associated with neurological and respiratory disorders on prolonged exposure. The International Standard Organization permits only 50 ppm residual hexane in oil seed meal (Sharma et al., 2001). Yet, hexane is widely used for oil extraction, resulting in a growing concern that hexane residues in oil, no matter how insignificant, could pose health problem in the long run. Therefore, alternative safe and efficient oil extraction processes must be developed.

Additionally, it is important to identify a secondary oxidative product of α -ESA, which will lead to the identification of a compound that can be a reliable indicator of α -ESA deterioration. The detection of this compound will make the product suitable for shelf-life modeling of α -ESA containing foods. Finally, it is important to compare the quality of Njangsa seed oil extracted using solvent and aqueous enzymatic methods. I hypothesized that the use of enzymes to extract the oil from Njangsa seed will degrade the cell wall in the oil seed, loosen oil sacs embedded in the structures and improve oil yield and quality.

1.3. Specific Objectives

- Use selected enzymes (hemicellulase, protease, pectinase and amylase) to extract oil from Njangsa (*Ricinidendron hedeulotii*) seeds.
- Assess the quality of Njangsa seed oil (NSO) extracted through solvent and enzyme-assisted techniques.

CHAPTER 2: LITERATURE REVIEW

2.1 *Ricinodendron heudelotti* (Njangsa)

Central and West Africa forests contain a vast number of oil bearing seeds which are unexplored with respect to their economic potential (Tchiegang et al., 2007). Among these *Ricinodendron heudelotti*, the tree that produces Njangsa seeds is semi-deciduous and belong to the family of Euphorbiaceae, genus: *Ricinodendron* and species *R. heudelotti*. The habitat of this species stretches along the coastal countries of West Africa including Guinea-Bissau, Liberia, Ivory Coast, Ghana and Nigeria, and also spreads to Cameroon, Equatorial Guinea and Uganda (Plenderleith, 1997). In Cameroon, it is common throughout the south west in forests, cocoa and other farms. The tree is valued for its distinctively- flavored seeds, locally known as Njangsa, which are dried, ground and used as a flavoring and thickening agent in a wide array of foods (Ouya, 2015). The seeds are obtained from the natural forest, a trade largely driven by women in the rural sector. Traditional processing of the fruits from the tree requires between two and six months but with the introduction of mechanical kernel extractors, some improvements have been observed in the overall processing speed (Mbosso et al., 2013). According to Ruffo et al. (2002), it is a multifunction tree, providing food, medicine and broad commodities to the local population, and having potential for use as a prime species. Njangsa seeds are sold in local markets, or exported to other countries. According to Burkil (2003), this tree grows best in areas where the mean minimum and maximum temperatures which fall within range of 20 - 30°C, but it tolerates 14 - 34°C variation. Ecologically, the roots of Njangsa helps to improve soil quality due to its mycorrhizae association and because of its natural leaf litter (Hogberg, 1982). Anigbogu (1996) found that the leaves were a good source of protein (16%) for livestock. Udo and Epidi (2009)

reported repellent activity of ethanolic extracts of *R. heudelotti* leaves against common pests of corn and cowpea grains. It was revealed by Anjah and Oyun (2009), that extract from the bark of the tree and leaves have anti-inflammatory and aphrodisiac characteristics and also contain lupeol which is a pharmacologically active triterpenoid used by traditional doctors as antidote against poison. It can also be used to treat various diseases such as diarrhea, yellow fever, rheumatism, toothache, headache, etc. (Anigboru, 1996)

To extract the lipid reserves stored in cells, it is necessary to be able to cross several barriers. The cell wall of plants consists mainly of pectin, cellulose, hemicellulose, lignin and protein, whereas lipid bodies are enveloped in a lipoprotein layer (Dominguez et al., 1995). Hydrolytic enzymes like cellulase, hemicellulase, amylase, protease, and pectinase break down the cellwall and facilitate oil release. For a better understanding of the possible role of enzymes, it is essential to consider the structure of the seed.

2.2. Cell Wall Constituents of Oil Seeds

Typically, there are two types of cell walls; type I and type II. Type I primary cell walls, which contain pectin and xyloglucan are found in dicotyledons (for example oil seeds), non-graminaceous monocotyledons, and gymnosperms (Voragen et al., 1995). Type II primary cell walls present in poaceae or grains, are rich in arabinoxylan, and contain less than 10% pectin (Pauly et al., 1999). Type II plants have thicker secondary cell walls than type I plants. Generally, in the primary cell wall, cellulose microfibrils are interlinked with xyloglucan (Type I) or xylan (Type II) via hydrogen bonds forming a stiff network (Mcbride et al., 2009; Voragen et al., 1995). Pectins or xylans together with structural proteins are physically entangled within this network. In the secondary cell wall of monocotyledous plants, the main carbohydrates are

glucuronoarabinoxylan and cellulose, while in dicotyledonous plants (oil seeds.), the main carbohydrates are 4-O-methylglucuronoxylan, xyloglucan, and cellulose (Issacson et al., 2012).

2.2.1. Hemicellulose

Hemicelluloses form a compound concrete structure around plants cells. They are linear or branched polysaccharides bound to the cellulose microfibrils by hydrogen bonds or connected to lignin by covalent bonds. Hemicellulose structures present in plants cell walls are xylans, xyloglucans, β -glucans and mannans. Xyloglucans are the main hemicellulose in dicotyledonous plants (Cai et al., 2004). Xyloglucans are composed of a β -1-4-glucosyl backbone, which can be substituted with xylosyl residues at the O6 - position. Xyloglucan is present in free loops and cross-links, which are enzyme accessible and is also entrapped in the amorphous cellulose fibrils, which are not extractable by concentrated alkali and only accessible for enzymes when cellulose is degraded (Greenhalf et al., 2012). Since enzymes are mostly specific, the complete degradation of hemicellulose, requires the action of hemicellulase (Cai et al., 2004; Pauly et al., 1999).

2.2.2. Cellulose

Cellulose, the most abundant carbohydrate, is a water-insoluble, linear chain of several hundred to over nine thousand of β -(1-4)- linked D-glucose units. In the cell wall, cellulose molecules are grouped alongside in bundles, held together by hydrogen bonds between the hydroxyl groups of the nearby glucose residues. Therefore, the enzymatic action of cellulase is required to hydrolyze the intermolecular β -(1-4)-glycosidic bond and the cellulose from the extremities of glycosidic chains (Annemieke, 2013). In general, plants cell walls are built from

different kinds of polymers like lignin, polysaccharides and protein, of which polysaccharides contribute the most to the cell wall composition.

Protein bodies contain 60 - 70% of the protein in oil seeds. Lipid bodies are the main sites of lipid reserves in oilseeds. Dominguez et al. (1995) reported that the secondary cell walls of rapeseeds are composed of 39% pectin, 29% hemicelluloses, 22% cellulose, 8% of arabinogalactans and the primary cell wall contains 10% of glycoprotein. According to Kalia et al. (2001) most of the oil from oilseed is found within the vegetative cells, joined with other macromolecules, such that partial hydrolysis of the structural polysaccharide, cell wall constituents, and lipid body by enzymes make the cell structure more permeable. Cellular disruption is important because the cell wall is the primary barrier to extraction, and it must be ruptured for oil release. As in most plants, the Njangsa seed primary cell wall is constructed of pectins, hemicelluloses, and microfibrils of cellulose cross-linked with protein. About 80% of the total protein in oilseeds is stored in protein bodies, which occupy most of the cotyledon cell volume (Dominguez et al., 1995). Therefore, their removal is important to allow release of oil. To be able to cross the cellular walls, specific enzymes such as hemicellulase, amylase, and pectinase are needed to degrade the complex structure of the cell.

2.3. Properties and Sources of Enzymes Used in the Study

2.3.1. Hemicellulase

A thermally stable food grade enzyme characterized by beta-mannanase containing some cellulase and hemicellulase activities is derived from *Aspergillus niger*. This enzyme has an optimum pH of 4.0 with a pH stability of 3.0 - 6.0. The temperature range of the enzyme is 50 - 60°C. Thermal stability diminishes rapidly above 60°C (**Figure 2-1**). The enzyme is used for the

removal of the sediment from coffee extract in the manufacture of instant coffee, for enzymatic peeling of soy bean or as an anti-staling agent (especially in the presence of small amounts of glucomannan or galactomannan like guar gum).

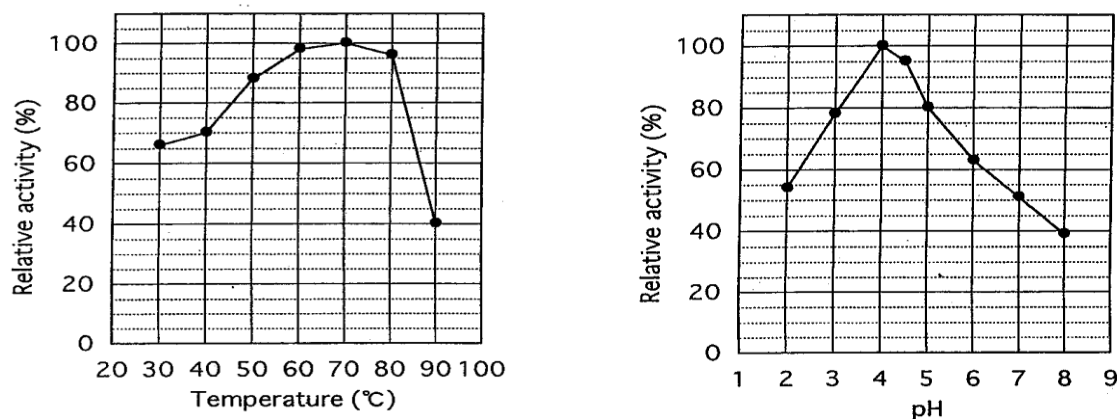


Figure 2-1. Effect of pH and temperature on hemicellase activity (Adapted from Enzyme Development Corporation, New York City, NY)

2.3.2. Protease

This enzyme is produced by *Aspergillus oryzae* fermentation, and is developed for protein hydrolysates rich in amino acids. The enzyme has high peptidase activity and also high proteinase activity. The proteolytic combination system helps hydrolyze various proteins at high level. It has a brown granular color. The optimum conditions of this enzyme are about pH 5.0 - 7.0, and 37°C. Proteases are protein hydrolysis and debittering hydrolysates. The amount of enzyme used vary with processing conditions (substrate concentration, degree of hydrolysis desired, pH, temperature and time) (**Fig 2-2**). Initially, it is evaluated at 0.05 - 0.15% based on the weight of the protein substrate dry matter.

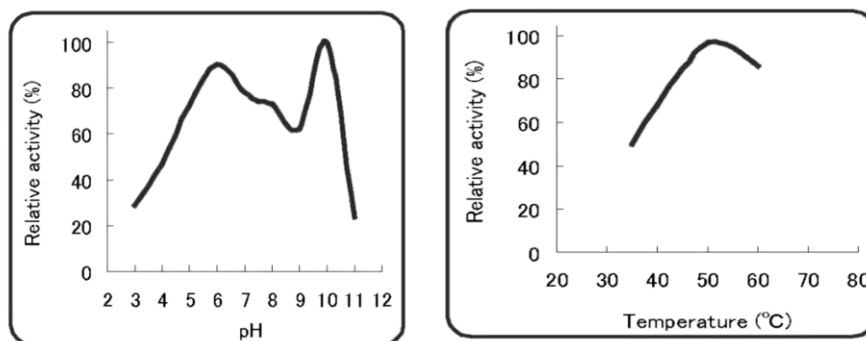


Figure 2-2. Effect of pH and temperature on protease activity (Adapted from Enzyme Development Corporation, New York City NY)

2.3.3. Amylase

Amylase-2LC is a liquefying amylase derived from *Bacillus amyloliquefaciens*. It exhibits a strong characteristic ability to liquefy starch and has intermediate heat stability. This product is able to hydrolyze at random the α -D (1-4) glucosidic bonds found in starch and other glucosyl oligosaccharides. It is a brown liquid with a density of approximately 1.2 g/ml. The temperature optimum range for this enzyme is from 70 - 90°C, with an optimum pH range of 4.5 - 7.0 (**Figure 2-3**). The enzyme may be inactivated after application by heating the reaction mixture to approximately 95 - 100°C and maintaining it at that temperature for 10 - 20 min.

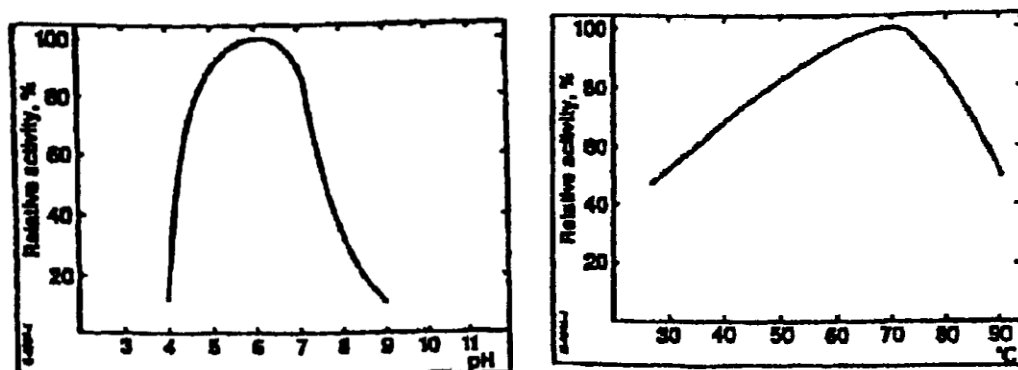


Figure 2-3. Effect of pH and temperature on amylase activity (Adapted from Enzyme Development Corporation, New York City, NY)

2.3.4. Pectinase

These pectolytic food grade enzyme preparations for use in fruit juice and wine making. They are derived from the *Aspergillus* species and are an amber to brown colored liquid, with an approximate specific gravity of 1.18. These enzymes are versatile broad-spectrum depectinizer for use in fruit and vegetable processing. The preparations contain all three types of the component pectinases: (pectin lyase (PL), polygalacturonase (PG) and pectin methyl esterase (PME) for partial or thorough pectin degradation. They also contain arabanase to further reduce cloudiness in clarified juices and can prevent excessive long-term thickening of tomato based products.

2.4. Physical and Chemical Properties of Seed Oil

Seed oils are important sources of nutritional oils, which are vital constituents of our daily diet, provide energy, essential fatty acids and serve as a carrier of fat soluble vitamins. Also, they find application as industrial raw materials and nutraceuticals. The characteristics of oils from

different sources depend mainly on their compositions; no oil from a single source can be suitable for all purposes. Thus, the study of their constituents is important corn oil which is obtained from seeds of *Zea mays* is an important component usually used as food and as a vehicle in certain pharmaceutical formulations such as suspensions and emulsions (Alvarez and Rodriguez, 2000). Mustard oil from *Brassica nigra* has 30% protein, calcium, phytins, phenolics and natural antioxidants. Mustard oil contains a high amount of monounsaturated fatty acids and a good ratio of polyunsaturated fatty acids, which is good for the heart. It contains the least amount of saturated fatty acids, making it safe for heart patients (Alvarez and Rodriguez, 2000).

Many consumers are looking for variety in their diets and are aware of the health benefits of fresh fruits and vegetables. Of special interest are food sources rich in antioxidants (Toscano et al., 2012). Omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes mellitus, arthritis, other inflammatory and autoimmune disorders, and cancer (Gaikwad and Swamy, 2008). Edible plants provide alpha-linolenic acid and other polyunsaturated fatty acids (John et al., 1997).

Atmospheric oxygen reacts instantly with lipids and other organic compounds of the oil and cause structural degradation through oxidation which leads to loss of quality and is harmful to human health (Bhattacharya et al., 2008). Therefore, it is essential to monitor the quality of oil to avoid the use of oxidized oil due to the health concerns of consuming foods fried in degraded oil. To maintain the quality of fried foods and to minimize the production costs associated with early disposal of the frying medium (Vijayan et al., 1996). Therefore, the incorporation of antioxidants has proven useful in retarding lipid oxidation of commercial oils with high polyunsaturated fatty acid (PUFA) contents (Aluyor and Ori-Jesu, 2008; Pokorný, 2007).

With a PUFA content of about 70% the oil from Njangsa seeds is expected to have a low resistance to oxidation (Assanvo et al., 2015). Moreover, α -ESA, with its conjugated triene system further exacerbates its susceptibility to lipid oxidation. Various studies have demonstrated this through reaction kinetics by comparing activation energies of conjugated fatty acids with their non-conjugated counterparts (Yang et al., 2009; Zhang and Chen, 1997). The stability of NSO has not been the subject of any publication so far. However, according a to recent study conducted in the Delaware state university food chemistry Laboratory by Abaidoo-Ayin et al. (unpublished results 2015) on the oxidative stability and shelf-life modeling of Njangsa seeds, the effectiveness of the antioxidants was recorded in the following order tert-butylhydroquinone(TBHQ) > butylated hydroxyanisole (BHA) > natural rosemary extracts, Herbalox® Seasoning Types O (HBTO)= Type W (HBTW). Their results also revealed that, at a concentration of 200 ppm, TBHQ showed the greatest activity in NSO. The activity of primary antioxidants is related to their ability to donate hydrogens to peroxy and alkyl radicals. Thus, the presence of two –OH groups in TBHQ implies that there are more available hydrogen atoms to quench lipid radicals.

2.4.1. Lipid Oxidation

Lipid oxidation is undoubtedly one of the most important topics when the quality of oil is of interest. In vegetable oils and other lipid-based foods, it results in the production of volatile compounds causing a decline in shelf-life and quality. These volatile compounds are responsible for off-flavors, which render the oil rancid. The loss in quality occurs as the chemistry of fatty acids in the food is altered, thus compromising its physiological benefits (Frankel, 2005). Generally, the rate of lipid oxidation is related to the degree of unsaturation of fatty acids present in an oil or food system (Erickson, 2008; Frankel, 2005).

2.4.1.1. Mechanism of Lipid Oxidation

Lipid oxidation employs a free-radical chain reaction mechanism. Although lipid oxidation is more complex in food matrices, it is easier to study in bulk oils where it is invariably proportional to the degree of unsaturation of fatty acids (Wagner et al., 1994). The process has been extensively reviewed elsewhere (Frankel, 2005; Labuza, 1979). As with every free-radical reaction, it begins with an initiation phase and ends by termination of the free radicals (**Figure 2-4**). Initiation begins with the abstraction of a hydrogen atom from an unsaturated fatty acid (LH) which generates an alkyl radical (L•). A conjugated diene system is formed which is stabilized by delocalization of electrons across the methylene bridge (Shahidi and Zhong, 2009). This radical interacts with oxygen to produce a peroxy radical (LOO•). Hydroperoxides (LOOH), the primary products of oxidation, are formed when the peroxy radical abstracts a hydrogen atom from another unsaturated bond, which liberates another free radical to propagate the chain reaction. This step has been described as the rate-limiting step as it occurs much slower than the initial hydrogen atom abstraction (Frankel, 2005). The propagation phase employs any of the five observed reactions during autoxidation – radical coupling, atom transfer, fragmentation, rearrangement and cyclization (Porter et al., 1995). The mechanism portrayed in (**Figure 2-4**) occurs via atom transfer. Termination eventually occurs as free radicals accumulate and combine to form non-radical compounds. As Johnson and Decker (2015) have pointed out, rancidity occurs after hydroperoxides start to give off-flavors during decomposition into low molecular weight aldehydes, ketones, alcohols, and esters.

Initiation: $\text{LH} \rightarrow \text{L}^\bullet + \text{H}^\bullet$ Propagation: $\text{L}^\bullet + \text{O}_2 \rightarrow \text{LOO}^\bullet$ and $\text{LOO}^\bullet + \text{LH} \rightarrow \text{LOOH} + \text{L}^\bullet$

Termination: $\text{LOO}^\bullet + \text{L}^\bullet \rightarrow \text{LOOL}$ and $\text{L}^\bullet + \text{L}^\bullet \rightarrow \text{LL}$

Figure 2-4. Free radical oxidation sequence in lipids (Adapted from Frankel, 2005).

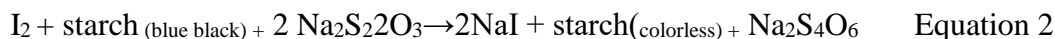
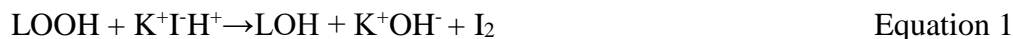
When oil becomes oxidized, considerable changes in its physical, chemical and organoleptic parameters occur (Shahidi and Wanasundara, 2008). Several analytical methods exist to assess the degree of oxidation. Lipid oxidation products are characterized as either primary or secondary; thus, it has become a conventional practice by most researchers to combine at least one of each to gain a better understanding of oxidative stability. Hydroperoxides are the major primary products and can be determined via iodine value (IV), peroxide value (PV), weight gain or oxygen uptake and conjugated diene or triene value. Secondary products include aldehydes, ketones, malondialdehyde, and volatile compounds depending on the fatty acid chain length. Analytical methods such as *p*-anisidine (*p*-AV) value, thiobarbituric acid assay (TBA) and headspace gas chromatography measure these secondary products. These indices and methods have been adapted by the American Oil Chemists' Society (AOCS) in addition to spectroscopic measurements such as ultraviolet (UV), nuclear magnetic resonance (NMR) and infrared (IR) measurements for the determination of the composition and stability of fats and oils ('Otunola et al., 2009; Yookyung et al., 2007; Barbara et al., 2005). Triacylglycerols are degraded as consequence of high temperature, loss of water, and exposure to oxygen (Morreto and Fett, 1998). Besides the formation of oxidized products and the resulting disagreeable flavor and taste, polymeric compounds can be produced which present unhealthy properties as they can promote arteriosclerosis and cancer (Jorge et al., 2005; Naz et al., 2004). Therefore, it is very important to know if the oil or a fat is appropriate for human intake.

2.5. Oxidative Stability Study of Njangsa Seed Oil (NSO)

2.5.1. Peroxide Value (PV)

Peroxide value is a measure of oxidation during storage and of the freshness of the lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild condition and it is a measure of the primary lipid oxidation products. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acids. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. High PV is an indicator of oxidation level. The greater the PV, the more oxidized the oil becomes. The maximum Codex standard peroxide for vegetable oil deterioration is (10 meq O₂/Kg). PV estimates the milliequivalent of peroxides in a given amount of fat or oil (O'Keefe and Pike, 2010). The standard method involves reacting oil previously dissolved in glacial acetic acid–isooctane with potassium iodide (K⁺I⁻) to liberate iodine (eq.1) which is further titrated against sodium thiosulfate (Na₂S₂O₃) using starch as indicator to a colorless endpoint (eq.2) (AOCS Cd 8-53, 1997). Study by Mohamed and JorgThomas (2002), showed that niger seed k-16 has significantly high (3.80 ± 0.28 meq O₂/kg) PV and hence a high degree of unsaturation. This observation suggests that niger seed oil has high content of unsaturated fatty acids, linoleic (C_{18:2}) and oleic acid (C_{18:1}), which are responsible for oxidative rancidity (Mohamed and JörgThomas, 2002). A significantly lower value was observed in cottonseed oil (0.60 ± 0.85 meqO₂/kg). Since peroxides are transient products of lipid oxidation, caution should be taken when interpreting PVs, as low values could indicate both initial and advanced stages (Frankel, 2005). Successive determinations are used to monitor the progress of peroxide formation over time in many studies (Cerretani et al., 2009; Naz et al., 2004; Rudnik et al., 2001). Although the titrimetric method has been critized due to low sensitivity, evasive endpoint and large sample requirement, it is still considered a good

determinant of primary oxidation. The alternative method which replaces the titration step with an electrochemical procedure is able to detect values as low as 0.06 mEq per kg of oil (Shahidi and Wansundara, 2008). Oils with PVs above 10 meq are of poor quality (O’Keefe and Pike, 2010). PV is a widely-used indicator of primary oxidation. Chu and Kung (1998) determined the quality of different vegetable oil blends using PV. Rudnik et al (2001) assessed the effect of antioxidants on the oxidative stability of linseed oil by monitoring PV for nine months. Peroxide values of olive, corn and soybean oil were found to correlate with the rate of oxidation at room temperature (20°C) for 30 days and up to 90 minutes at frying temperatures (Naz et al., 2004). Another study observed a good correlation between decreasing PVs and rapid decomposition of peroxides into secondary products determined by the p-AV value (Cerratani et al., 2009).

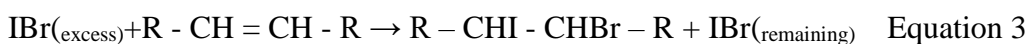


Where LOOH is hydroperoxide, $\text{Na}_2\text{S}_2\text{O}_3$ is sodium thiosulfate, NaI is sodium iodide, $\text{Na}_2\text{S}_4\text{O}_6$ is sodium tetrathionate, K^+OH^- is potassium hydroxide.

2.5.2. Acid Value (AV)

Acid value is a measure of the free fatty acids in oil. Normally, fatty acids are found attached to the glycerol backbone. During decomposition FFA, peroxides, low molecular weight aldehydes and ketones are produced. This would result in distinctive smell and affect the quality of the oil (Dimberu et al., 2011). In view of this, AV defined as the number of mg of KOH required to neutralize the free acid in 1g of fat, or other related substances is determined to assess the rancidity of the oil samples.

This method is applicable to crude and refined animal, vegetable, and marine fats and oils, and various products derived from them. The higher the acid value measured, the higher the level of FFAs which translates into decreased oil quality. Acceptable levels for all oil samples should be below 0.6 mg KOH/g (AOCS Official Method Cd 853, 2003). The usual method for its determination is the AOCS Cd 3d-63 procedure (AOCS, 1973), in which the sample is dissolved in toluene and isopropyl alcohol and a visual titration is performed with a 0.1N KOH solution prepared in this alcohol. The detection of the end point is done with phenolphthalein.



Where IBr is iodine monobromide, KI is potassium iodide, KCl is potassium chloride, R is an alkyl group, I₂ is iodide, CH=CH is an unsaturated hydrocarbon.

The presence of free fatty acids is undesirable in oils and fats as it suggests poor nutritional quality of the product. The quantity of these acids indicates how the oil was treated during industrial processing and during storage. Therefore, the determination of their concentrations throughout the refining process and during the storage is important for monitoring the occurrence of degradation reactions (Jorge et al., 2005; Naz et al., 2004; Rukunudin et al., 1998).

2.5.3. Saponification Value (SV)

Saponification value is defined as the number of mg of KOH required to neutralize the FFA and saponify the esters in 1 g of test substance. It is an indicator of the molecular weights of triglycerides in oil with the Codex standard permissibility level of SV is (168 - 181 mg KOH/g) (CODEX-STAN210-1999). The shorter the average chain length (C4 - C12) the higher the SV (Tamzid et al, 2007). Higher SV indicates high proportion of lower fatty acids since saponification

value is inversely proportional to the average molecular weight or chain length of the fatty acids (Muhammad et al., 2011). High SV indicate that oils are useful in production of liquid soap and shampoo. According to Tamzid et al. (2007), the significantly ($p < 0.05$) high proportion of saponification value in palm oil (260.22 ± 1.38 mg KOH/g) suggests this oil as a good raw material for soap industries, because it contains high amounts of short chain fatty acids ($< C_{12}$), which is greater than the regulation of Codex standard permissibility level (CODEX-STAN210-1999) ($190 - 209$ mg KOH/g). SV is determined according to titrimetric method discussed by Pearson (1981), which involves adding 2 g of oil to 25 ml of ethanoic KOH in a conical flask. The solution is refluxed for 2 h with intermittent shaking, and titrated with 0.5N HCl using phenolphthalein as indicator.

2.5.4. Thiobarbituric Acid (TBA) Value

The most common method for measuring oxidative changes in biological samples and food products is the TBA test based on a spectrophotometric quantitation of a red-violet complex formed with malonaldehyde (MDA). This method determines both the MDA already formed naturally from hydroperoxide cleavage, and the secondary release due to the heating step in the TBA reaction. The structure of MDA consists of carbonyl groups at the C1 and C3 positions of a three-carbon dialdehyde (Fernandez et al., 1997). Formation of MDA is highly dependent on the presence of α and β unsaturated peroxides.

Other substances known as TBA-reactive substances (TBARS) can potentially react with the TBA reagent. According to Fernandez et al. (1997), the adduct formed between TBARS and TBA has a spectrum comparable to that of the MDA standard and the characteristic yellow to amber color, which has a maximum UV absorption between 530 - 532 nm (Shahidi and

Wanasundara, 2008). The TBA assay is widely used for evaluating secondary oxidation in lipids. Shyamala et al. (2005) assessed the effect of antioxidants on the rate of oxidation of groundnut and sunflower oils at frying temperatures. They reported that TBA values accurately reflected the MDA concentration due to linoleic acid peroxidation. TBA value has also been used to characterize the effect of natural antioxidants on the oxidative stability of salmon oil (King et al., 1992) and soybean oil (Taghvaei et al., 2014; Shimada et al., 1992). Results supporting the correlation of TBA tests to sensory analysis seem conflicting. While some studies have reported positive correlations (Kulshrestha and Rhee, 1996), others have described a poor correlation between TBA values and sensory analysis (Stevenson et al., 1984). According to Kolanawski et al. (2007) TBA values may not fully reflect rancidity in bulk oils because aldehydes may not have been formed yet or may have already been lost as a result of processing. However, trained panelists in a sensory score test assigned higher odor intensity scores for regular canola oil than low-linolenic acid canola oil (Eskin et al., 1989). Incidentally, TBA values for regular and low-linolenic acid canola oils were 0.49 and 0.31, respectively. Kerrihard et al. (2015), reported a higher correlation between IV and TBA ($R^2 = 0.758$) than the *p*-AV value ($R^2 = 0.661$) in vegetable oil blends.

2.5.5. Para-Anisidine Value (*p*-AV)

The *p*-AV test also measures the amount of α and β unsaturated aldehydes that are formed during the breakdown of peroxides (O'Keefe and Pike, 2010). The product that arises from the reaction of aldehydes with *p*-methoxyaniline (anisidine) absorbs at 350 nm under acidic conditions. The *p*-AV represents the absorbance of 1 g of fat dissolved in 100 ml solution of 0.25% anisidine reagent in isooctane (Moigradean et al., 2012). In previous studies where *p*-AV was used

to monitor secondary oxidation products, a good reflection of corresponding changes in PV was observed (Guillén and Cabo, 2002). Others have also investigated the effect of antioxidants on the stability of rice bran oil (Mishra et al., 2012), walnut oil (Martínez et al., 2013) and palm oil (İnanc et al., 2014) using *p*-AV. The latter demonstrated the consistency of their results even at frying temperatures. This test is more frequently used in Europe compare to North America.

2.6. Gas Chromatography/Fatty Acid Composition and Analysis

Fatty acid profiling (also known as the analysis of fatty acid methyl esters (FAME)) determines the quality of oil seeds and processed oil by identifying and quantifying the fatty acids present in a sample. Gas chromatography is commonly used to determine fatty acid composition. Established methods from AOCS and AOAC (such as AOAC Fat in foods 966.06 2005; AOCS Official Methods Ce 1h-05 2005; Ce 1j-07 2007) can be used to determine the fatty acid composition of oil seeds. FAME are separated on a capillary gas chromatography column having a highly polar stationary phase, according to their chain length, degree of unsaturation, geometry and position of the double bonds. This method is specially designed to evaluate, by a single capillary gas chromatography procedure, the levels of *trans* isomers, saturated fatty acids (SFA), *cis*- and *trans*-monounsaturated fatty acids, and *cis*- and *trans*-polyunsaturated fatty acid levels. For nutritional labeling purposes, the total fat, saturated, *cis*-monounsaturated (MUFA), *cis*-polyunsaturated and *trans* fatty acid contents are to be determined. This method may also determine *cis*-polyunsaturated fatty acids, including arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). This method utilizes a triacylglycerol (13:0 TAG) internal standard for determining the concentration of the individual fatty acids in the oil samples after methylation. Fatty acid profiling benefits oil-seed production and distribution by establishing

a quality assurance program for oil-seed crops (AOCS Official Methods Ce 1h-05 2005; Ce 1j-07 2007).

2.7. Differential Scanning Calorimetry (DSC) Melting and Crystallization Behavior

DSC is a thermal analysis technique to measure the temperature and heat flows associated with phase transitions in materials as a function of time and temperature. It is the most widely used thermo analytical technique in oil and fat research (Cebula and Smith, 1998). Some of the physical phenomena studied with a DSC are glass transitions, melting profiles, heats of fusion, amount of crystallinity, oxidative stability, curing kinetics, crystallization kinetics and other phase transitions. Such measurements can provide both quantitative and qualitative information concerning physical and chemical changes that involve endothermic (energy consuming) and exothermic (energy producing) processes, or changes in heat capacity. DSC offers a sensitive, rapid and reproducible fingerprint method for the identification of vegetable oils and fats. Solid fat content (SFC) is responsible for many important characters of fat like physical appearance, organoleptic properties, and spreadability. SFC also influences the melting properties indicating the behavior of a fat at different temperatures. Plasticity or consistency of an edible oil product depends on the amount of solid present (Brekke, 1980). The variation of SFC with temperature and the sharpness of melting range determine the range within which a fat could be considered plastic (Seriburi and Akoh, 1988).

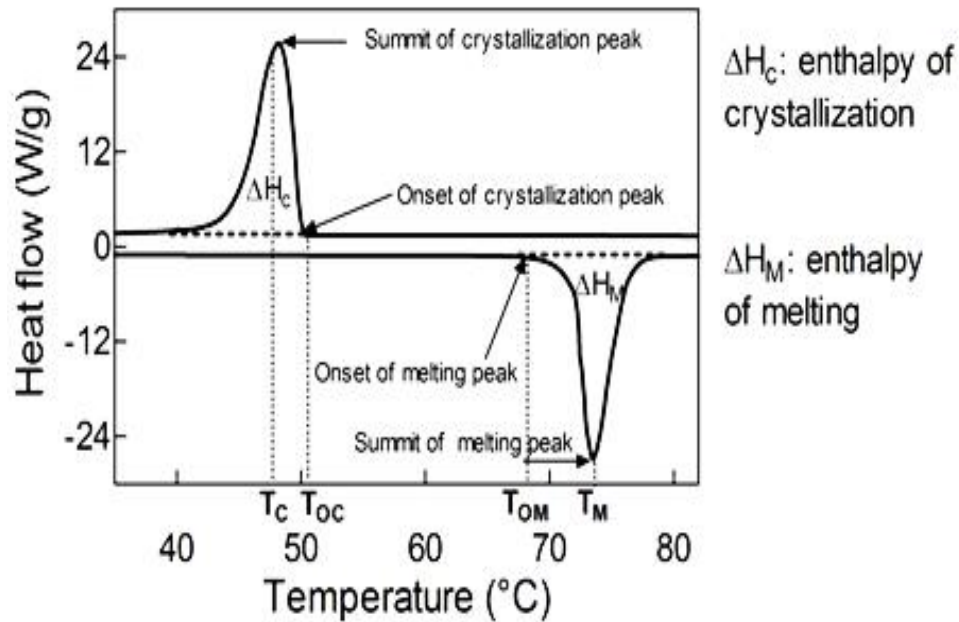


Figure 2-5. Schematic diagram of a thermogram, with heat flow as a function of temperature, showing the parameters typically measured for fats (Seriburi and Akoh, 1988)

2.8. Factors Affecting Oil Yield

2.8.1. Size Reduction

This is mostly done by grinding or flaking. Grinding is considered one of the most important steps in the oil extraction process that directly affects oil yield. According to Njam et al. (2001), smaller particle size allows not only for the easier diffusion of water-soluble components, thereby disintegrating the original structure and facilitating oil release, but also enhances enzyme diffusion rates which can then more easily act on the substrates. Kalia et al. (2001) also revealed that excessive grinding favored cell rupture and increased the efficiency of oil extraction, producing smaller oil globules, which made de-emulsification more difficult. Insufficient grinding, on the other hand, resulted in loss of oil in the residue.

2.8.2. Solid: Water Ratio

The highest possible solid: water ratios were desirable in the extraction step to obtain a less stable emulsion and generate less effluent (Kalia et al., 2001). However, to obtain the highest extraction rate and extraction yield, the use of large quantities of water was recommended with a ratio of solid to water of 1:5 - 1:12 for peanut, 1:10 for sunflower seeds, 1:2.5 - 1:3.5 for rapeseeds (Kalia et al., 2001).

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials

Njangsa seeds were obtained from the South West region of Cameroon. All solvents and chemicals used were of analytical grade obtained from Sigma-Aldrich, Inc. (PA, USA) and Fisher Scientific, Inc. (St. Louis). Fatty acid methyl ester (FAME) standard were purchased from Supelco (Bellanfonte, PA). All the enzymes were donated by Enzymes Development Corporation (New York City, NY).

3.2. Enzymes

The source and activities of enzymes used in this study are shown in **Table 3-1**.

Table 3-1. Manufacturer Specifications for Enzymes Used in the Extraction

Enzyme	Source	pH	Temp (°C)	Activity
Hemicellulase	<i>Aspergillus niger</i>	5.0	55	19,000 -23000 ACH/g
Protease	<i>Aspergillus oryzae</i>	5.0	37	45,000 -70,000 CFA/g
Pectinase	<i>Aspergillus species</i>	4.0	40	1100 PE/g
Amylase	<i>Bacillus</i> <i>Amylolyque faciens</i>	5.0	70	25,000 SKB/g

3.3. Sample Preparation

Njangsa seeds were dried, and ground to a particle size of less than 0.4mm using a blended at room temperature (22 -25°C).

3.3.1. Enzymatic Extraction

The experiments were conducted to compare oil yield from enzyme assisted extractions with solvent (hexane) extracted samples and the control, a reaction mixture without any enzyme as described by Tchiégang et al. (2007). Enzymes in **Table 3-1** were used separately. Twenty-five gram of Njangsa seed powder were mixed with distilled water at a ratio of 1:6 (w/v). The pH was adjusted with 0.5 N NaOH or 0.5 N HCl to the optimal pH of the various enzymes. The enzymes(2%w/v) were added in various amount and the mixture was then incubated at various temperatures for 24 h in a water bath (Amerex Instruments, Inc. Concord, CA) with constant shaking at 120 rpm. The oil was recovered from the aqueous mixture at 4000 rpm and 20°C for 20 min by using an Eppendorf centrifuge 5810 (Hauppauge NY). The emulsion was then decanted into a separating funnel and allowed to separate into oil and water layers. The oil was passed through anhydrous sodium sulfate to remove any residual moisture, stored at -20°C until analysis. All experiments were carried out in triplicate.

3.3.2. Solvent Extraction

Two hundred ml hexane was added to 25 g of Njangsa seed powder in a beaker followed by stirring at 400 rpm for 1 h. The organic solvent was then filtered and passed through a column of anhydrous sodium sulfate to remove any residual moisture. The oil was separated from the organic phase by means of a rotary evaporator under vacuum. Then it was transferred into amber vials and stored in a freezer (-20°C) until further analysis. Percent yield was calculated as:

$$\text{Extraction yield of seed oil} = \frac{\text{mass of extracted oil}}{\text{mass of dried material}} \times 100 \quad \text{Equation 5}$$

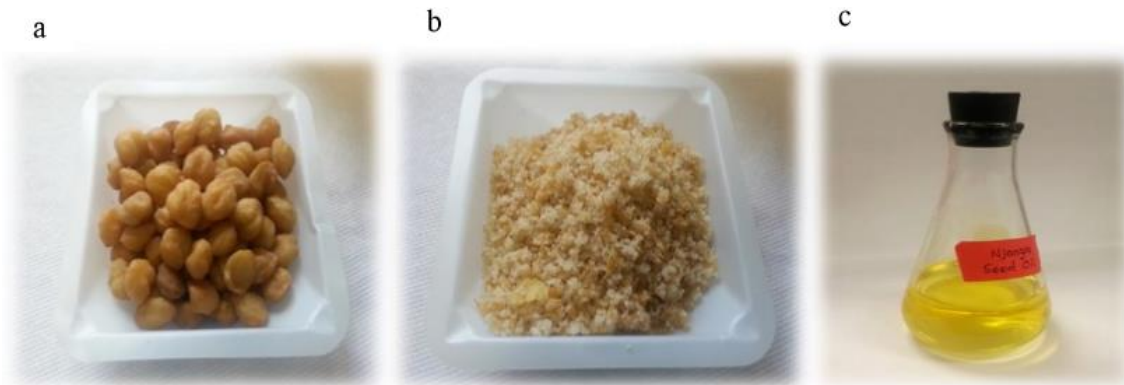


Figure 3-1. Seeds (a), homogenized seeds (b) and oil (c) from Njangsa

3.4. Physical and Chemical Parameters of Oil

3.4.1. Peroxide Value (PV)

Peroxide value was evaluated according to AOCS Official Method Cd 8-53 (2003). The 2.5 g of NSO samples were weighed into a conical flask; then 15 ml of solvent mixture of glacial acetic acid-chloroform in the ratio of 3:2 was added to the oil samples. After which 0.25 ml saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 min, 30 ml of distilled water were added and titrated with 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$ solution using starch as indicator until the yellow color was discharged. A blank was prepared alongside the oil samples.

The peroxide value was calculated as:

$$PV = \frac{10 (V_1 - V_2)}{m} \quad \text{Equation 6}$$

Where: V_1 is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ for determination of test sample in ml, V_2 is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ for determination of blank solution in ml, and m is mass of test portion in g (2.5 g).

3.4.2. Acid Value (AV)

One gram of NSO was weighed and dissolved with 50 ml ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated with 0.1 N KOH solution to a pink end point (which persisted for 15 min). Acid value was calculated according to the equation below (Okpuzor et al., 2009)

$$AV = \frac{56.1 \times V \times C}{m} \quad \text{Equation 7}$$

Where 56.1 is equivalent weight of KOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact concentration in KOH solution used (0.1 N), and m is the mass in grams of the test portion (1.0 g).

3.4.3. Saponification Value (SV)

Saponification value was determined according to titrimetric method discussed by Pearson (1981). Two grams of oil samples were weighed into a conical flask and 25 ml ethanoic KOH (0.5 N) was added. The solution was refluxed for 2 h with intermittent shaking, and titrated with 0.5 N HCl using phenolphthalein as indicator. A blank was run in tandem. The value was calculated as

$$SV = \frac{(V_0 - V_1) \times C \times 56.1}{m} \quad \text{Equation 8}$$

Where 56.1 is equivalent weight of KOH, V_0 is the volume in ml of standard HCl solution used for the blank test, V_1 is the volume in ml of the standard HCl solution used for sample, C is the exact concentration of the standard HCl (0.5 N) solution, and m is the mass in gram of the test portion (2 g).

3.5. Lipid Hydroperoxide Determination

3.5.1. Para-Anisidine value (*p*-AV)

The amount of non-volatile aldehydes (principally 2-alkenals) in the oils was measured using *p*-anisidine test, the AOCS official method for determining levels of the aldehydes (AOCS, 2004). NSO (0.5 g) was accurately weighed in a 25 ml volumetric flask and mixed with 10 ml isooctane for dissolution. The solution was brought to volume with 25 ml additional isooctane, and its absorbance (A_b) at 350 nm was measured with the isooctane as a reagent blank using a UV spectrophotometer (Beckman Coulter DU 720, Sharon Hill, PA). Then, 5 ml of the solution was pipetted into a test tube and 5 ml of isooctane into another test tube. One ml of *p*-anisidine solution (0.25 g *p*-anisidine in 100 ml glacial acetic acid) was added to both test tubes and the solutions were mixed. After 10 min of incubation at room temperature (23 - 25°C), the absorbance (A_s) of the sample solution was read with the test tube containing isooctane and *p*-anisidine as blank. The test was conducted in triplicate for all samples. The (*p*-AV) was calculated as:

$$p - AV = \frac{25 (A_s - A_b)}{m} \quad \text{Equation 9}$$

Where; A_s is the absorbance of the oil solution after reaction with the *p*-anisidine reagent, A_b is the absorbance of the oil solution, and m is the mass (g) of the test portion.

3.5.2. Thiobarbituric Acid Value

Malondaldehyde (MDA) a secondary oxidation product was measured according to AOCS Cd 18-90 method (AOCS, 2004). The assay is based on the reaction between 2 molecules of TBA and one molecule of MDA during heating at acidic pH to produce a red pigment with a maximum absorbance at 532 nm. NSO (0.1 g) was accurately weighed into a 25 ml volumetric flask, then 5

ml of 1-butanol was added and vortexed thoroughly. Five milliliter of TBA (0.2%) in 1-butanol was added to the sample solution. After vertexing, the mixture was incubated at 95°C in a water bath for 2 h. The resulting solution was allowed to stand at room temperature for 10 min to stabilize the chromogenic MDA-TBA complex, and then its absorbance was measured using a UV-spectrophotometer (Beckman Coulter DU 720, Sharon Hill, PA) at 532 nm. TBA value was calculated as:

$$TBA = \frac{10 (A_1 - A_0)}{m} \quad \text{Equation 10}$$

A_1 is the absorbance of the test solution, A_0 the absorbance of the reagent blank, m the mass(mg) of the test portion and “10” is the dilution factor and width of the spectrophotometer cell used. TBA values were expressed as milligrams of malonaldehyde per kilogram of oil.

3.6. Determination of Fatty Acid (FA) Composition

3.6.1. Preparation of FAME by Base Catalysis

The method described by Yurawecz et al. (1997) was followed. Fifty milligram NSO was weighed into screw-capped test tubes followed by the addition of 50 µL of internal standard (heptadecanoic acid, Sigma Aldrich) and 2 ml of 0.5 N solution of sodium methoxide in methanol. Incubation was carried out at 50°C for 30 min. One milliliter water and 2 ml hexane were added and vortexed. After separation, the hexane layer containing FAME was passed through a column of anhydrous sodium sulfate followed by GC analysis.

3.6.2. Fatty Acid Profile Analysis

FAME was analyzed on a ZB-5 capillary column (30 m × 0.25 mm ID, 0.25 Km film thickness) in parallel with a FAME standard (Supelco 37 component FAME mix, Supelco®,

Bellafonte, PA). Using a 6850 series GC- MS (Agilent Technologies, Newark) interfaced to a 5973 MSD. A gradient temperature program comprising an initial temperature of 70°C, increased to 190°C at 30°C/min after injection, then 35 ramped to 210°C at a rate of 1°C/min followed by holding for 10 min provided separation of the major fatty acids in 45 min. FAMES were identified by comparing their relative and absolute retention times to those of the standard, and the FAs composition were reported as relative percentage of the total peak area.

3.7. Determination of the melting profile

A Perkin-Elmer model DSC 4000 (Norwalk, CT) equipped with an intra-cooler was used to assess the melting profiles of NSO. Analyses were performed using a modification of the AOCS recommended procedure Cj 1-94. The samples were held for 2 min at 25°C, followed by rapid heating to 80°C at 10°C increments and held for 10 min. The samples were then cooled to -80°C at 5°C increments and held for another 10 min. In the final step, samples were heated to 80°C at 5°C increments Normal standardization was performed with cyclohexane (2 thermal transitions; 1 at -87.06°C and 1 at 6.54°C) and indium (thermal transition at 156.6°C). The melting points and enthalpy changes of the samples were recorded.

3.8. Statistical Analysis

Analysis were performed using the Statistical Package for Social Science (SPSS) version 24.0 (SPSS Inc., Chicago, Il). Difference between the means were analyzed using one-way analysis of variance. Post-hoc comparisons to evaluate pairwise differences among group means were conducted with the use of Tukey HSD tests. Alpha value was set at ($p \leq 0.05$).

CHAPTER 4: RESULT AND DISCUSSION

4.1. Oil Extraction

My research goal was to investigate application of four enzymes (hemicellulase, amylase, pectinase and protease) for the extraction of oil from Njangsa seeds and evaluate the quality of the oil obtained in comparison with those of solvent-extraction. The percentage of oil recovery (28.57 - 36.12%) of the enzyme-assisted extraction of Njangsa seed oil (NSO) was significantly ($p < 0.05$) higher than that of the control (19.24%) but lower than the hexane extraction (46.43%) (**Table 4-1**). The highest oil yield of 36.12% was obtained from hemicellulase while the lowest oil yield of 28.12% was observed with pectinase. The increase in oil yield in the enzyme-assisted extraction of NSO as compared to the control can be ascribed to the enzyme activity, due to the hydrolysis of proteins which causes a breakdown in the protein network of the cotyledon cells, and in the oleosin based membranes that surround the lipid bodies (Rosenthal et al., 2001). It can also be attributed to the fact that oils are fastened within the matrix of interacting macromolecules. Therefore, the complete interruption of the organization by enzymes to digest the different components results in the release of more oil contained within the matrix (Latif, 2008).

Studies reported by other researchers have also shown that the recovery of oil using enzyme extraction is usually lower than that of organic solvent extraction (Latif, 2008). Sharma et al. (2001) reported that the yield of rice bran oil using mixtures of ProtizymeTM and cellulase gave an oil recovery of 77.0%. Hanmoungjai et al. (2001) observed an oil recovery of rice bran oil 79.0% with a commercial Alcalase. The advantage of enzymatic extraction is lower temperature, no need for explosive solvents and no production of harmful solvents solvents (Hanmoungjai et al., 2001). Enzymatic extraction facilitates separation of the extracted vegetable components as the

mild condition of the process ensures that extracted components remain unchanged, producing and only few by-products that may affect taste and smell of the final product (Ksenija et al., 1997). As an enzymatic extraction process is carried out in an aqueous medium, phospholipids are separated from the oil so that there is no need for degumming, thereby reducing the overall cost of processing of the oil and of the final product (Christensen, 1991). The disadvantage of using enzymes is that they are expensive.

4.2. Quality Parameters of Njangsa Seed Oil

Table 4-2 shows the data for various physicochemical parameters of the enzyme-assisted extraction, solvent extraction, and the control of NSO. The saponification value (SV) of the enzyme-assisted extraction (198.42 - 203.47 mg KOH/g) was comparable to the control with 204.54 mg KOH/g but lower than the solvent extract with 208.60 mg KOH/g. The value obtained for NSO during this study show that it contains high amounts of long chain fatty acids (>C12), which is lower than the regulation of the codex standard permissibility level (CODEX-STAN210 - 1999) (190 - 209 mg KOH/g). Puangsri et al. (2005) reported that some significant differences in the SV were observed between solvent extracted and aqueous enzymatic extracted papaya seed oils. Dimberu et al. (2011) revealed that among nine oil samples studied, cottonseed oil (228.55 ± 0.00 mg KOH/g) and olive oil (228.39 ± 0.23 mg KOH/g) have higher SV than the regulation of codex standard. Typically, these oils have low molecular weight fatty acids.

Table 4-1. Effect of Enzyme Type on Oil Recovery from Njangsa Seed

Parameter (%)	Solvent- extraction	Enzyme-Assisted Extraction				Control	F-value	Significant
		Hemicellulase	Protease	Pectinase	Amylase			
Yield	46.43±0.05 ^f	36.12 ± 0.07 ^e	31.28± 0.04 ^c	28.58± 0.38 ^b	34.51± 0.23 ^d	19.24 ±0.27 ^a	5572.288	.000

Results are means ± standard deviation, values followed by different superscript are significantly different

Table 4-2. Chemical Properties of Njangsa Seed Oil

Parameter	Solvent- extraction	Enzyme-Assisted Extraction				Control	F-value	Significant
		Hemicellulase	Protease	Pectinase	Amylase			
SV	208.60 ± 0.19 ^f	199.58 ± 0.12 ^b	198.42 ±0.12 ^a	200.63±0.30 ^c	203.47±0.12 ^d	204.54±0.20 ^e	1222.813	.000
AV	10.17 ± 0.02 ^e	8.26 ± 0.02 ^c	8.22 ± 0.02 ^c	6.17±0.02 ^a	6.36±0.02 ^b	8.59 ± 0.01 ^d	23782.984	.000
PV	11.04 ± 0.01 ^c	10.08 ± 0.01 ^{ab}	10.00±1.00 ^a	10.14±0.01 ^b	10.12 ± 0.02 ^b	9.99 ± 0.02 ^a	275.658	.000
FFA	5.09 ± 0.06 ^d	4.14 ± 0.01 ^b	4.13 ± 0.02 ^b	3.09 ± 0.01 ^a	3.09 ± 0.00 ^a	4.29 ± 0.01 ^c	41707.270	.000

Values are means ±SD, mean values in the same row followed by different superscript are significantly different. Saponification Value (SV; mg KOH/g of oil), Acid Value (AV; mg KOH/g of oil), Peroxide Value (mEq/kg of oil)

On the other hand, sunflower oil (65.27 ± 0.04 mg KOH/g), and niger seed oils both k-7 (34.12 ± 2.08 mg KOH/g) and k-16 (162.76 ± 0.69 mg KOH/g) have low SV compared to the codex values. This indicates that they both have high content of long chain FA.

The peroxide value (PV) of the enzyme-assisted extraction of NSO (10.00 - 10.14 mEq/kg of oil) were also found to be comparable to the control (9.99 mEq/kg of oil), but significantly ($p < 0.05$) lower than the solvent-extracted oil (11.04 mEq/kg of oil). The operational temperature during conventional hexane oilseed extraction might affect the oil quality, especially the oxidation of the oil (Latif, 2008). PV is the most common parameter used to characterize oils and fats. Therefore, a product with a PV of 1 - 5 mEq/kg is classified as having low oxidation state, 5 and 10 mEq/kg and >10 mEq/kg as moderate and high oxidation state, respectively (O'Brien, 1998). Codex standard cites a PV limit of a 15 mEq/kg for virgin oils in general. In terms of these criteria, NSO with a PV of 9.99 - 11.03 mEq/kg of oil showed moderate oxidation.

As seen in **Table 4.2**, the acid value (AV) of the enzyme-assisted oils (6.17 - 8.27 mg KOH/g) were comparable with the control (8.59 mg KOH/g), but significantly ($p < 0.05$) lower than solvent extracted oil (10.17 mg KOH/g). No previous data were available on the AV of NSO for comparison. Acceptable levels for all oil samples should be below 0.6 mg KOH/g (AOCS Official Method Cd 8-53, 2003). During processing the fatty acids may have reacted with water and hydrolyzed forming free fatty acids (FFA). According oil Tamzid et al. (2007) hydrolysis may result from the presence of moisture in oil. The previous study by Rajko et al. (2010) unrefined vegetable oils had higher AV than refined oils. Dimberu et al. (2011) also showed an increase in the AV of olive oil (7.29 mg KOH/g), rapeseed (10.38 mg KOH/g) and niger seed K-16 (8.69 mg

KOH/g). Dominguez et al. (1995) also observed an increase in soya oil acid value after the treatment of the beans with Celluclast.

4.3. Oxidative Stability study of Njangsa Seed Oil

The oxidative stability parameters of NSO are presented in **Table 4-3**. The specific extinctions at 530 - 532 nm (TBA) revealed the oxidative deterioration and purity of the oil. It resulted in 0.20 - 0.26 mg of malonaldehyde/kg for the enzyme-assisted extracted oil, significantly ($p < 0.05$) lower than 0.78 and 0.56 mg of malonaldehyde/kg for the control and solvent-extracted oil, respectively. *p*-AV value test measures the secondary oxidation, which referred to high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerol. The para-anisidine value (*p*-AV) for NSO ranged from 0.63 - 0.74 for the enzyme-assisted extraction, 0.99 for hexane-extract and 0.91 for the control. There were significant differences in the *p*-AV among the NSO samples. According to Marina et al. (2009) oils with an anisidine value below 10 are considered good quality, while Subramaniam et al. (2000) considered good quality oils as having an anisidine value of less than 2.

Tables 4-4 and 4-5 and **Fig. 4-1a-c** show the fatty acid (FAs) profile and composition of control, enzyme-, and solvent-extracted NSO. There was a significant difference in the major FA between control, enzyme-, and solvent-extracted NSO. The FA composition was affected by the type of enzyme used. Alpha-eleostearic acid (α -ESA), which belong to the class of fatty acid known as conjugated fatty acids ($C_{18:3}$) was the most prominent fatty acid ranging from 40.86 - 41.08% for enzyme-extracted samples, at 40.67 and 40.92 % for the control and solvent-extracted oil, respectively.

Table 4-3. Oxidative State of Njangsa Seed Oil

Parameter	Solvent-extraction	Enzyme-Assisted Extraction				Control	F-value	Significant
		Hemicellulase	Protease	Pectinase	Amylase			
TBA	0.56 ± 0.01 ^e	0.25 ± 0.01 ^c	0.24 ± 0.01 ^b	0.20 ± 0.01 ^a	0.26 ± 0.01 ^c	0.78 ± 0.10 ^d	3686.000	.000
<i>p</i>-AV	0.99 ± 0.01 ^e	0.74 ± 0.01 ^c	0.68 ± 0.1 ^b	0.63 ± 0.01 ^a	0.72 ± 0.01 ^c	0.91 ± 0.01 ^d	400.583	.000

Results are means ± standard deviation, values in the same row followed by different superscript are significantly different. Thiobarbituric acid value (TBA), Para-anisidine (*p*-AV).

Table 4-4. Fatty Acid Composition of Njangsa Seed Oil

FA (%)	Solvent-extraction	Enzyme-Assisted Extraction				Control	F- value	Significant
		Hemicellulase	Protease	Pectinase	Amylase			
C_{10:0} (Capric)	0.11 ± 0.01 ^a	0.10 ± 0.01 ^a	0.11 ± 0.01 ^a	0.10 ± 0.01 ^a	0.10 ± 0.01 ^a	0.11 ± 0.00 ^a	0.369	.860
C_{14:0} (Myristic)	0.84 ± 0.01 ^c	0.86 ± 0.01 ^c	0.76 ± 0.01 ^a	0.77 ± 0.01 ^a	0.84 ± 0.01 ^c	0.80 ± 0.01 ^b	68.967	.000
C_{16:0} (Palmitic)	9.24 ± 0.01 ^c	9.04 ± 0.01 ^a	9.41 ± 0.01 ^c	9.27 ± 0.01 ^c	9.16 ± 0.01 ^b	9.35 ± 0.01 ^d	236.903	.000
C_{18:0} (Stearic)	8.10 ± 0.01 ^a	8.57 ± 0.25 ^c	8.69 ± 0.01 ^d	8.41 ± 0.01 ^b	8.37 ± 0.01 ^b	8.41 ± 0.01 ^b	619.818	.000
C_{18:1} (Oleic)	8.70 ± 0.01 ^d	8.72 ± 0.02 ^d	8.13 ± 0.02 ^a	8.83 ± 0.01 ^e	8.55 ± 0.01 ^c	8.45 ± 0.01 ^b	1418.100	.000
C_{18:2} (Linoleic)	31.78 ± 0.01 ^e	31.43 ± 0.01 ^a	31.55 ± 0.01 ^c	31.46 ± 0.01 ^b	31.64 ± 0.01 ^d	32.18 ± 0.01 ^f	5304.125	.000
C_{18:3} (α-eleostearic)	40.92 ± 0.01 ^c	41.08 ± 0.01 ^d	41.06 ± 0.01 ^d	40.86 ± 0.01 ^b	41.06 ± 0.01 ^d	40.67 ± 0.01 ^a	1749.632	.000
C_{20:0} (Arachidic)	0.27 ± 0.01 ^b	0.30 ± 0.01 ^{cd}	0.31 ± 0.01 ^d	0.31 ± 0.01 ^d	0.29 ± 0.01 ^b	0.03 ± 0.01 ^a	766.697	.000

Values are means ± standard deviation of three NSO analyzed individually in triplicate. Mean values in the same row are not significantly different.

The prominent FAs were linoleic (31.43 - 32.78%), stearic (8.10 - 8.69%), oleic (8.13 - 8.83%), and palmitic acids (9.04 - 9.41%). The significant α -ESA content makes oil from *Ricinodendron heudelotii* a potential commercial product with important health benefits. Several studies suggested that α -ESA is effective in alleviating cancer (Yasui et al., 2005; Grossman et al., 2009), reducing oxidative stress in the body (Saha et al., 2012), and improving overall cardiovascular health (Leudeu et al., 2009). NSO also contains moderate amounts of linoleic acid, which has been demonstrated to have positive effects on plasma cholesterol levels in human trials (Siguel, 1996; Mensink et al., 2003) and a possible reduction in the risk of type 2 diabetes (Salmeron et al., 2001).

Our results support earlier studies on NSO where Leudeu et al. (2009) quantified approximately 52% of α -ESA. Also, 39.19 - 52.72% of α ESA was quantified from Njangsa seed oil according to recent study conducted in the Delaware state university food chemistry Laboratory by Abaidoo-Ayin et al. (unpublished results 2015). Compared to commercial vegetable oils such as canola and soybean, NSO is superior in terms of PUFA content (72 -73%). Warner and Mounts (1993) reported average PUFA contents of 30 and 60% for canola and soybean oil respectively. On the other hand, oil from canola and soybean contain about 60 and 24% oleic acid, respectively, which are significantly higher than those of NSO.

Interest in the PUFA as health-promoting nutrients has expanded dramatically in recent years, and a rapidly growing literature illustrates their benefits (Riemersma, 2001). The oil from Njangsa seed can be used as a rich source of polyunsaturated fatty acids like other vegetable oils.

Table 4-5. Fatty Acid Group of Njangsa Seed Oil

FA Group	Solvent- extraction	Enzyme-Assisted Extraction				Control
		Hemicellulase	Protease	Pectinase	Amylase	
SFA (%)	18.56	18.8	19.26	18.86	18.76	18.70
MUFA (%)	8.70	8.72	8.13	8.83	8.55	8.45
PUFA (%)	72.70	72.02	72.61	72.31	72.69	72.85

Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA)

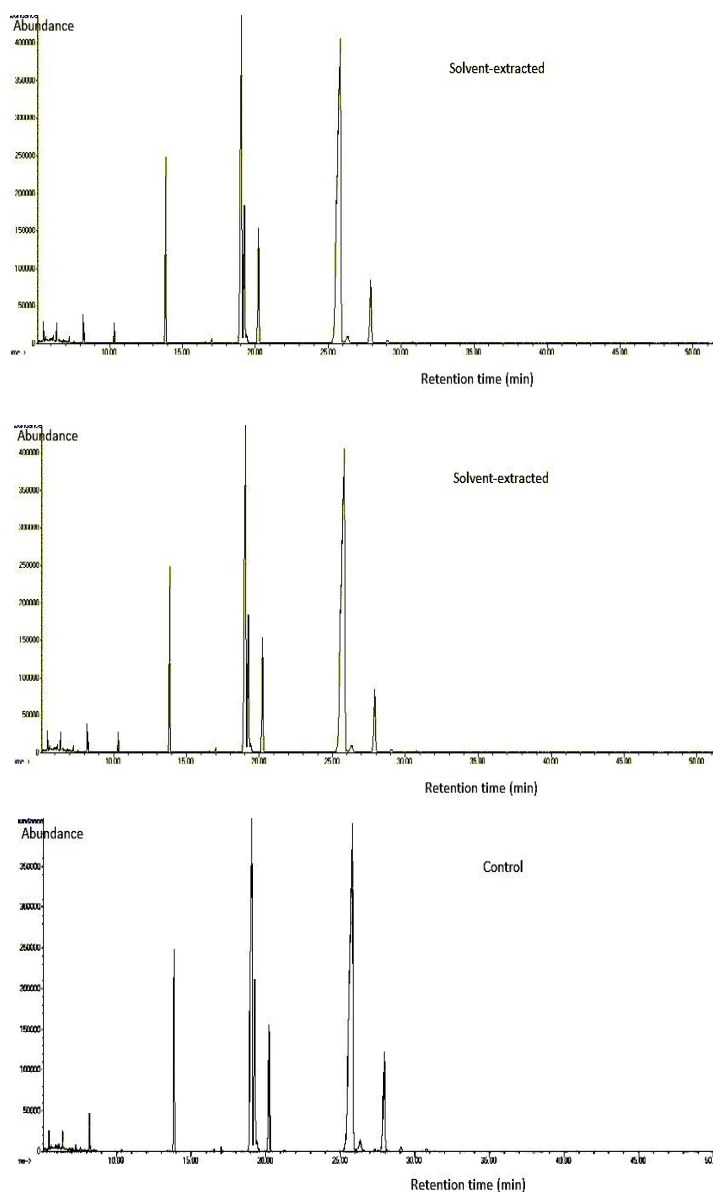


Figure 4-1a-c. GC-MS chromatogram of the FA profile of enzyme-assisted, solvent-extracted and control of NSO

4.7. Thermal Behavior of Njangsa Seed Oil

The thermal properties of the oil samples include information on the temperature at which the melting process starts and when it is completed. It requires intake or release of thermal enthalpy. The complete melting points as derived from differential scanning calorimetry (DSC) measurements of NSO extracted using solvent, enzymes and control were not significantly different. The heating profile showed that, there are two major peaks b and c and one trough (a) for the solvent, enzymes, and control oil samples. (**Fig 4-2**). The trough a represented the melting temperature of polyunsaturated fatty acid. The more stable low melting unsaturated fatty acids melted at peak b. The higher melting, more saturated fatty acids melted at higher temperatures (peak c). Puangsri et al. (2005) showed that there was no significant difference in the melting points of solvent and enzyme-extracted oils when extracting oil from papaya seeds using solvent and enzymes.

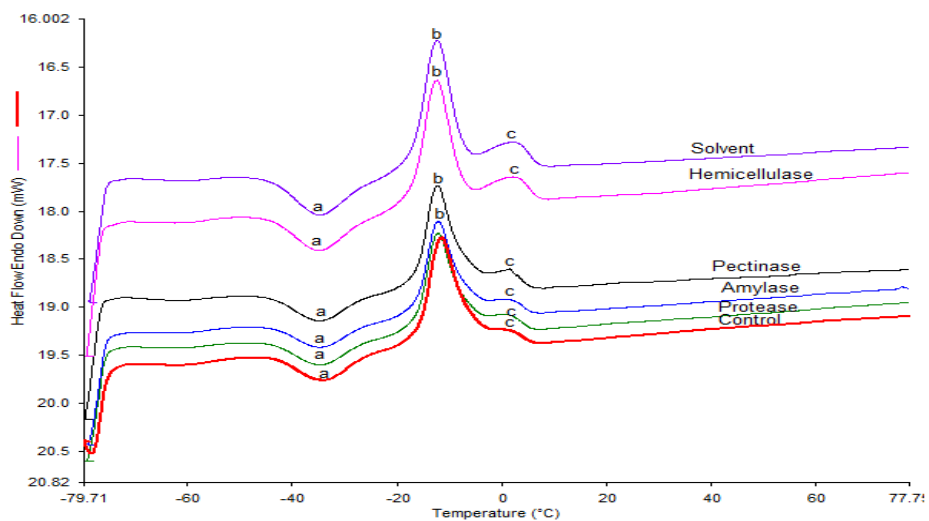


Fig 4-2. Melting profiles of enzyme-assisted, solvent-extracted and control of Njangsa seed oil

Table 4-6. Thermal Behavior of Njangsa Seed Oil

Peak No	Solvent- extraction	Enzyme-Assisted Extraction				Control	F-value	Significant
		Hemicellulase	Protease	Pectinase	Amylase			
a	-74.49±0.59 ^b	-74.44±0.01 ^c	-73.22±0.02 ^d	-74.49±0.01 ^b	-74.64±0.01 ^a	-74.49±0.01 ^b	7305.676	.000
b	-12.46±0.01 ^b	- 12.30±0.01 ^d	-11.84±0.01 ^e	-12.32±0.01 ^d	-12.95±0.01 ^a	-12.37±0.01 ^c	5655.150	.000
c	2.69±0.01 ^f	1.84±0.01 ^b	2.11±0.01 ^c	1.54±0.01 ^a	2.31±0.02 ^d	2.60±0.01 ^e	8212.982	.000
ΔHm	-234.73±0.01 ^c	-221.57±0.01 ^e	271.74±0.01 ^a	214.83±0.01 ^f	213.83±0.02 ^d	-243.74±0.01 ^b	13577365.11	.000

Values are means ± standard deviation, Mean values in the same row are not significantly different Thermal Enthalpy (ΔHm; J/g)

CHAPTER 5: CONCLUSIONS & RECOMMENDATION

In this study, 28.58 - 36.12%, 46.43%, and 19.24% was obtained from the enzyme assisted extraction (using; hemicellulase, protease, pectinase and amylase), solvent extraction (using; hexane) and control (water), respectively to the enzymes hydrolyzed and degraded the cell wall which enhanced oil extraction.

Oil extracted by the enzyme-assisted method showed lower peroxide, saponification, *p*-anisidine, and thiobarbituric acid values. The peroxide, saponification, *p*-anisidine, free fatty acid and thiobarbituric acid values of NSO were; 10.00 - 10.14, 11.04, and 9.99 mEq/kg of oil, 198.42 - 203.47, 208.60, and 204.54 mg KOH/g of oil, The *p*-AV of 0.63 - 0.74, 0.99, 0.91, FFA of 3.09 - 4.14, 5.09, 4.29 and 0.20 - 0.26, 0.56, and 0.78 mg of malonaldehyde per kg of oil, for the enzyme-assisted extraction, solvent extraction and the control, respectively. These quality indices are within the acceptable limits for edible vegetable oils and indicative of its good quality, minimal secondary oxidation and the absence of any off-flavors, high content of long chain fatty acids (>C12) and lower amount of high molecular weight saturated and unsaturated carbonyl compounds.

Njangsa seed oil contains significant amounts of α -ESA (~41%), linoleic (~30%), oleic (~8%), stearic (~8%), palmitic (~9%), myristic (~0.8%), capric (~0.1%) and arachidic acid (~0.2%). The high amount of α -ESA in NSO may have potential anticancer and cardiovascular health benefits, whilst the high PUFA (~73%) content may have nutritional advantages. MUFA

(~8%) and SFA (~18%). In addition to the health and nutritional potentials of NSO, NSO may find useful industrial application in pharmaceuticals, cosmetics, soaps, and varnish production.

The PUFA, MUFA and SFA of NSO melted between -74 - -73°C, -12°C - -11°C, 1.53 - 2.69°C, respectively, indicating that NSO is liquid at room temperature.

Although solvent extraction resulted in higher oil yield, enzymes are safer to use, non-hazardous and possesses little to no environmental, toxicological and health threats.

Future studies on NSO, could include;

1. The combine use of enzymes.
2. Optimization of extraction conditions such as; the pH, temperature and solid/water ratio.
3. In order to reduce the cost of the process, the use of lower enzyme concentration for longer time is also recommended for future studies.
4. Further, Njangsa seed quality under different growing conditions can be compared to obtain the maximum seed yield

REFERENCES

- Aluyor, E.O. and Ori-Jesu, M. (2008). The use of antioxidants in vegetable oils - A review. *Afr. J. Biotechnol.* 7(25): 4836-4842.
- Alvarez, A.M.B. and Rodríguez, M.L.G. (2000). Lipids in pharmaceutical and cosmetic preparations. 51: 74-96.
- Andersson, B., Caroline, W.P., Francinete, R.C., Fabio, S., Cesar, A. and Antonio, G.F. (2010). A simple methodology for the determination of fatty acid composition in edible oils through ^1H NMR spectroscopy. *Magn. Reson. Chem.* 48: 642-650.
- Anigbogu, N.M. (1996). Nature's gifts. Improving trees and shrubs around the world: *Ricinodendron heudelotii* in Nigeria. *Agro. Today* 8(2): 18.
- Anjah, G.M and Oyun, M.B. (2009). Autoecological study of *Ricinodendron heudelotii* (Baill) pierre et pax in Agulli forest. *Int. J. Biol. Chem. Sci.* 39: 1057-1064.
- Annemieke, M.P. (2013). Fate of rapeseed meal polysaccharides during digestion in pigs and poultry Effect of processing and enzyme addition. Wageningen University, Wageningen, NL ISBN: 978-94-6173-660-4.
- AOCS (2003). Official Method Cd 8-53. American Oil Chemists Society, Champaign, IL. www.bioriginal.com (Accessed on: 1/20/2017).
- AOCS (1973). Official Method Cd 8d-63: In Official Methods and Recommended Practices of the AOCS; Firestone, D. eds. AOCS: Champaign, USA.
- AOCS (1997). Official methods and recommended practices of the American Oil Chemists' Society. Champaign, IL
- AOCS (2005). Official Method Ce 1h-05. Determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC <https://aocs.personifycloud.com/PersonifyEbusiness/Store/ProductDetails>. (Accessed on: 2/15/2017)

AOCS (2009). Official Method Ce 1k-09. Direct methylation of lipids in foods for the determination of total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated and trans fatty acids by gas chromatography.

AOCS (2004). Official Methods and Recommended Practices. Method Additions and Revisions.

Assanvo, E.F., Gogoi, P., Dolui, S. and Baruah, S.D. (2015). Synthesis, characterization, and performance characteristics of alkyd resins based on *Riciodendron heudelotii* oil and their blending with epoxy resins. Ind. Crop Prod. 65: 293-302.

Atef, M.A. (2010). Physiological effects of some plant oils supplementation on streptozotocin-induced diabetic rats. Res. J. Med. Sci. 5: 55-71.

Barbara, M., Bernhard, L., Antonio, M. and Maria, J.A. (2005). Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy. Chem. Phys. Lipids 134: 173-182.

Bhattacharya, A.B., Sajilata, M.G., Tiwari, S.R. and Singhal, R. (2008). Regeneration of thermally polymerized frying oils with adsorbents Food Chem. 110: 562-570.

Brekke, O.L, Monts, T.L. and Falb, R.A. (1980). Handbook of soy oil processing. American Soybean Association and AOCS, Champaign, USA. (eds). David Erickson. 396-440.

Burkil, H.M. (1985). *Riciodendron heudelotti*, the useful plants of West Tropical Africa. Royal Botanic; Kew. Gardens; <https://plants.jstor.org/compilation/Riciodendron.africanum> (Accessed on: 12/13/2017)

Cai, J., Zhang, L., Zhou, J., Li, H., Chen, H. and Jin, H. (2004). Novel fibers prepared from cellulose in NaOH/urea aqueous solution. Macromolec. Rap. Commun. 25: 1558-1562.

Cebula, D.J. and Smith, K.W. (1992.). Differential scanning calorimetry of confectionery fats. Part II - Effects of blends and minor components. J. Am Oil Chem. Soc. 69: 992-998.

Cerretani, L., Bendini, A., Rodriguez-Estrada, M.T., Vittadini, E. and Chiavaro, E. (2009). Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds. Food Chem. 115: 1381-1388.

Christensen, F.M. (1991). Extraction by aqueous enzymatic process. Inform. 2: 984-987.

Chu, Y. and Kung, Y. (1998). A study on vegetable oil blends. *Food Chem.* 62(2): 191-195.

CODEX (1989). Normes codex pour les huiles d'olive vierges et raffinées et pour l'huile de grignons d'olive raffinée. CODEX STAN. 33-1981 (Rév. 1-1989), 6p.

CODEX (1999). Norme codex pour graisses et les huiles comestibles non-visées par des normes individuelles. Codex STAN 19-1981 (Rév. 2-1999), 5p.

Atinafu, D.G. and Bedemo, B. (2011). Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir DAR. *J. Cereals Oilseeds* 2(6): 71-76.

Dobozi, S., Halasz, A and Kovacs, K.E. (1988). Enhancement of mustard oil yield by cellulolytic pretreatment. *Appl Microbial Biotechnol.* 29: 39-43

Dominguez, H., Nunez, M.J. and Lema, J.M. (1995). Aqueous processing of sunflower kernels with enzymatic technology. *Food Chem.* 53: 427-434.

Erickson, M.C. (2008). Lipid oxidation of muscle foods. In: Akoh, C.C. and Min, D.B. (eds). *Food lipids chemistry, nutrition and biotechnology*. 3rd ed. Boca Raton: CRC Press. 321-364.

Eskin, N.A.M., Vaisey-Genser, M., Durance-Todd, S. and Przybylski, R (1989). Stability of low linolenic acid canola oil to frying temperatures. *J. Am. Oil Chem. Soc.* 66(8): 1081-1084.

Ezekwe, M.O., Besong, S.A. and Johnson, R. (2014). Nutritive composition of omega-3 fatty acids-rich *Ricinodendron heudelotii* and its potential for nutrition. *Int. J. Nutr. Metab.* 6(6): 56-62.

FAO (1997). Committee Report on Commodity Problems, Biotechnology Development in the Oil Seeds Sector. Rome, Italy:

<http://www.fao.org/unfao/bodies/ccp/ccp62/W7581E.htm>. (Accessed on: 2/12/2017).

Fernandez, J., Perez-Alvarez, J.A. and Fernandez-Lopez, J.A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chem.* 59(3): 345-353.

Frankel, E.N., Huang, S.W., Aeschbach, R. and Prior, E. (1996). Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk and oil-in-water emulsion. *J Agric Food Chem.* 44: 131-135.

Frankel, E.N. (2005). *Lipid Oxidation*. 2nd ed. Bridgewater: The Oily Press. 15-23.

Gaikwad, R.D. and Swamy, P. (2008). *Physico-Chemical and Viscosity Studies*

in Some Seed Oils from Wild and Cultivated Plants. *Acta Chim. Slov.* 55: 683.

Greenhalf, C.E., Nowakowski, D.J., Bridgwate, A.V., Titiloye, J., Yates, N., Riche, A. and Shield, I. (2012). Thermochemical characterisation of straws and high yielding perennial grasses. *Ind. Crops Prod.* 36: 449-459.

Grossman, M.E., Mizuno, N.K., Dammen, M.L., Schuster, T., Ray, A. and Cleary M.P. (2009). Alpha-eleostearic acid inhibits breast cancer proliferation by means of an oxidation dependent mechanism. *Cancer Prev. Res.* 2: 879-886.

Guillén, M.D. and Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chem.* 77: 503-510.

Hanmoungjai, P., Pyle, D.L. and Niranjana, K. (2001). Enzymatic process for extracting oil and protein from rice bran. *J. Am. Oil Chem. Soc.* 78: 817-821.

Hennessy, A.A., Ross, R.P., Devery, R. and Stanton, C. (2011). The health promoting properties of the conjugated isomers of α -linolenic acid. *Lipids.* 46: 105-119.

Högberg, P. (1982). Mycorrhizal associations in some woodland and forest trees and shrubs in Tanzania. *New Phytol.* 92: 407-415.

İnanc, T. and Maskan, M. (2014). Effect of carvacrol on the oxidative stability of palm oil during frying. *Grasas Aceites.* 65(4): 1-8.

Isaacson, R. and Kim, H.B. (2012). The intestinal microbiome of the pig. *Anim. Health Res. Rev.* 13: 100-109.

John, N., Coupland, D. and McClements, J. (1997). *J. Am. Oil Chem Soc.* 74: 1559-1564.

Johnson, D.R. and Decker, E.A. (2015). The role of oxygen in lipid oxidation reactions: A review. *Annu Rev Food Sci Technol* 6: 171-190.

Jorge, N., Soares, B.B., Lunardi, V.M, and Malacrida, C.R. (2005). Behavior of sunflower, soybean and corn oils in fried frozen pre-fried breaded meat products *Quim Nova.* 28: 947.

Kalia, V.C., Rashmi, and Sadhana Lal. (2001). Using enzymes for oil recovery from edible seeds. Centre for Biochemical Technology (CSIR), Delhi University. *J Sci Ind Res.* 60: 298-310.

Kapseu, C. and Tchiegang, C. (1995). Chemical properties of *Ricinodendron heudelotii*(Baill) seed oil. *Food Lipids* 2(2): 87-98.

Kerrihard, A.L., Nagy, K., Craft, B.D., Beggio, M. and Pegg, R.B. (2015). Oxidative stability of commodity fats and oils: modeling based on fatty acid composition. *J. Agric. Food Chem.* 92: 1153-1163.

King, M.F., Boyd, L.C. and Sheldon, B.W. (1992). Effects of phospholipids on lipid oxidation of a salmon oil model system. *J. Am. Oil Chem. Soc.* 69(3): 237-242.

Kolanowski, W., Jaworska, D. and Weißbrodt, J. (2007). Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 longchain polyunsaturated fatty acid-rich foods. *J Sci Food Agric.* 87: 181-191.

Kramer, J.K.G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.M, and Yurawecz, M.P. (1999). Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* 32: 1219-1228.

Ksenija, P.J., Zarko, V. and Mirjana, M. (1997). Aqueous-enzymatic extraction of palm kernel oil. 99: 433-435.

Kulshrestha, S.A. and Rhee, K.S. (1996). Precooked reduced-fat beef patties chemical and sensory quality as affected by sodium ascorbate, lactate and phosphate. *J. Food Sci.* 61(5): 1052-1057.

Labuza, T.P. (1979). A theoretical comparison of the losses in foods under fluctuating temperature sequences. *J. Food Sci.* 44: 1162-1168.

Latif, S. and Anwar, F. (2008). Quality assessment of *Moringa concanensis* seed oil extracted through solvent and aqueous enzymatic techniques. *Grasas Aceites.* 59: 67-73.

Leudeu, B.C.T., Tchiégang, C., Barbé, F., Nicolas, B. and Guéant, J.L. (2009). *Ricinodendron heudelotti* (Baill) or *Tetracarpidium conophorum* Müll oils fed to male rats lower blood lipids. *Nutr Res.* 29: 503-509.

Lumor, S.E., Jones, K.C., Ashby, R., Strahan, G.D., Kim, B.H., Lee, G.C., Shaw, J.F., Kays, S.E, Chang, S.W., Foglia, T.A. and Akoh, C.C. (2007). Synthesis and characterization of canola oil-stearic acid-based structured lipids for possible food applications. *J. Agric. Food Chem.* 55: 10692-10702.

Manga, T.T., Fondoun, J.M., Kengue, J. and Thiengang, C. (2000). Chemical composition of *Ricinodendron heudelotii*: AHN indigenous fruit tree in southern Cameroon. *Afr. Crop Sci. J.* 8(2): 195-201.

Mani, S., Jaya, S. and Vadivambal, R. (2007). Optimization of solvent extraction of moringa (*Moringa Oleifera*) Seed Kernel Oil using response surface methodology. Food and Bio products Processing. 85: 328-335

Marina, A. M., Che Man, Y.B., Nazimah, S.A.H. and Amin, I. (2009). Chemical Properties of Virgin Coconut Oil. J Am Oil Chem Soc. 86(4): 301-307.

Martínez, M.L., Pencì, M.C., Ixtaina, V., Ribotta, P.D. and Maestri, D. (2013). Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions. LWT-Food Sci Technol. 51: 44-50.

Mapongmetsem, P.M., Duguma, B. and Nkongmeneck, B.A. (1999). Domestication of *Ricinodendron heudelotii* (Baill.) pierre ex pax in the humid lowlands of Cameroon. Ghana J Sci 39: 3-8.

Mbosso, C., Degrande, A., Tabougue, P., Franzel, S., Van Noordwijk, M., Van Damme, P., Tchoundjeu, Z. and Foundjem-Tita, D. (2013). Gender differences in knowledge, perception and use of the *Ricinodendron heudelotii* (Baill. Pierre ex pax) kernel extraction machine. Afr. J. Agric. Res. 8(46): 5741-5751.

McBride, M.J., Xie, G., Martens E.C., Lapidus, A., Henrissat, B., Rhodes, R.G., Goltsman, E., Wang, W., Xu, J., Hunnicutt, D., Staroscik, A.M., Hoover, T.R., Cheng, Y.Q. and stein, J.L. (2009). Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. Appl. Environ. Microbiol. 75: 6864-6875.

Mensink, R.Q., Zock, P.L., Arnold, D.M. K. and Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. Am. J. Clin. Nutr. 77: 1146-1155.

Mishra, R., Sharma, H.K., Sarkar, B.C. and Singh, C. (2012). Thermal oxidation of rice bran oil during oven test and microwave heating. J. Food Sci. Technol. 49(2): 221-227.

Mohamed, F.R. and Jörg-Thomas. M. (2002). Proximate Neutral Lipid Composition of Niger (*Guizotia abyssinica* Cass.) Seed. Czech. J. Food Sci. 20: 98 -104.

Moigradean, D., Poiana, M.A. and Gogoasa, I. (2012). Quality characteristics and oxidative stability of coconut oil during storage. JAPT.18(4): 272-276.

Morreto, E. and Fett, R. (1998). Technology of oils and vegetable fats, in the food industry. Varela: São Paulo, Brasil. (date:1/11/2017).

- Muhammad, N., Bamishaiye, E., Bamishaiye, O.L., Usman, L.M., Salawu, M.M., Nafiu, M.O. and Oloyede, O. (2011). Physicochemical properties and fatty acid Composition of *Cyperus esculentus* (tiger nut) Tuber oil. Biores. Bull. 5: 51-54.
- Najafian, L. (2009). Aqueous extraction of virgin olive oil using industrialenzymes. Food Res Int. 42: 171-175.
- Naz, S., Sheikh, H., Siddiqi, R. and Sayeed, S.A. (2004). Oxidative stability of olive, corn, and soybean under different conditions. Food Chem. 88: 253-259.
- Nyam, L.K., Tan, P.C., Lai, O.M., Long, K., Yaakob, B. and Man, C. (2001). Enzyme-assisted aqueous extraction of kalahari melon seed oil: Optimization using response surface methodology. J. Am. Oil Chem. Soc. 86: 1235-1240.
- O'Brien, R.D. (1998). Fats and oils, technomic publishing Co., Inc., United States of America.181-250.
- O'Keefe, S. and Pike, O.A. (2010). Fat Characterization. In: Nielsen, S.S editor. Food analysis. 4th ed. New York: Springer Science+Business Media, LLC. 239-260.
- Okpuzor, J., Okochi, V.I., Ogbunugafor, H.A., Ogbonnia, S., Fagbayi, T. and Obidiegwu, C. (2009). Estimation of cholesterol level in different brands of vegetable oils. Pak. J. Nutr. 8: 57-62.
- 'Otunola, G.A., Adebayo, G.B. and Olufemi, O.G. (2009). Evaluation of some physicochemical parameters of selected brands of vegetable oils sold in Ilorin metropolis. Int. J. Phys. Sci. 4: 327-329.
- Ouya, D. (2015). Easier and faster processing of njansang heralds opportunities for local. <http://blog.worldagroforestry.org/index.php/2015/04/30/easier-and-faster-processing-of-njansang-heralds-opportunities-for-local-development/>(Accessed on:1/23/2017).
- Pal, M. and Ghosh, M. (2012). Studies on comparative efficacy of α -linolenic acid and alpha eleostearic acid on prevention of organic mercury-induced oxidative stress in kidney and liver of rat. Food Chem Toxicol. 50: 1066-1072.
- Pauly, M., Andersen, L.N., Kauppinen, S., Kofod, L.V., York, W.S., Albersheim, P. and Darvill, A. (1999). A xyloglucan-specific endo- β -1,4-glucanase from *Aspergillus aculeatus*: Expression cloning in yeast, purification and characterization of the recombinant enzyme. Glycobiol. 9: 93-100.

Pearson, D. (1981). The chemical analysis of food. Churchill publishing, London, 580-581.

Plenderleith, K. (1997). *Ricinodendron heudelotti*, a state of knowledge study undertaken for the central African regional program for the environment. Oxford Forestry Institute, UK. <http://carpe.umd.edu/Documents/2000/report-plenderleith.pdf>.

(Accessed on: 12/1/2016.)

Pokorný, J. (2009). Are natural antioxidants better and safer than synthetic antioxidants? Eur. J. Lipid Sci. Technol. 109: 629-642.

Porter, N.A., Caldwell, S.E. and Mills, K.A. (1995). Mechanism of free radical oxidation of unsaturated lipids. Lipids 30(4): 277-290.

Puangsri, T., Abdulkarim, S.M. and Ghazali, H.M. (2005). Properties of papaya seed oil. J. Food Lipids 12: 62-76.

Rajko, V., Sergeja, V. and Helena, A. (2010). Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. Czech J. Food Sci. 28: 376-384.

Riemersma, R.A. (2001). The demise of the n-6 to n-3 fatty acid ratio? A. dossier. EUR J. Lipid Sci. Technol. 103: 372-373.

Rosenthal, A., Pyle, D.L., Niranjana, K., Gilmour, S. and Trinca, L. (2001). Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. Enzyme Microb. Technol. 28: 499-509.

Rossell, J.B., Measurement in rancidity. In: Allen, J.C and Hamilton, R.J (eds) Rancidity in food. (1989). Elsevier, London. 22-51.

Rudnik, E., Szczucinska, A., Gwardiak, H., Szulc, A and Winiarska, A. (2001). Comparative studies of oxidative stability of linseed oil. Thermo Acta. 370: 135-140.

Ruffo, C. K., Birnie, A. and Tengnas, B. (2002). *Ricinodendron heudelotti*, edible wild plants of Tanzania. regional land management unit; Nairobi.

<http://www.worldagroforestry.org/downloads/Publications/PDFS/B11913.pdf>.

(Accessed on: 12/02/2016).

Rukunudin, I.H., White, P.J., Bern, C.J. and Bailey, T.B. (1998). A Modified Method for Determining Free Fatty Acids from Small Soybean Oil Sample Sizes. *J. Am. Oil Chem. Soc.* 75: 563.

Saha, S.S., Dasgupta, P., Sengupta, S. and Ghosh, M. (2012). Synergistic effect of conjugated linolenic acid isomers against induced oxidative stress, inflammation and erythrocyte membrane disintegrity in rat model. *Biochim Biophys Acta.* 1820: 1951-1970.

Saha, S.S. and Ghosh, M. (2009). Comparative study of antioxidant activity of α -eleostearic acid and punicic acid against oxidative stress generated by sodium arsenite. *Food Chem Toxicol.* 47: 2551-2556.

Salmerón, J., Hu, F.B., Manson, J.E., Stampfer, M.J., Colditz, G.A., Rimm, E.B., Willett, W.C. (2001). Dietary fat intake and risk of type 2 diabetes in women. *Am. J. Clin. Nutr.* 73: 1019-1026.

Seriburi, V. and Akoh, C.C. (1988). Study of melting behaviour of hydrogenated vegetable oils in supercritical propane. *J. Biol. Chem.* 263: 8017-8021.

Shahidi, F. and Wanasundara, U.N. (2008). Methods for measuring oxidative rancidity in fats and oils. In: Akoh, C.C and Min, D.B. (eds). *Food lipids chemistry, nutrition and biotechnology*. 3rd ed. Boca Raton: CRC Press. 387-408.

Shahidi, F. and Zhong, Y. (2009). Lipid oxidation and improving stability. *Chem. Soc. Rev.* 39: 4067-4079.

Sharma, A., Khare, S.K. and Gupta, M.N. (2001). Enzyme-assisted aqueous extraction of rice bran oil. *J. Am. Oil Chem. Soc.* 78: 949-951.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Am. Oil Chem. Soc.* 40: 945-948.

Shyamala, B. N., Gupta, S., Lakshmi, A. J., Prakash. (2005). Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oils. *Innov. Food Sci. Emerg. Technol.* 6: 239-245.

Siguel, E. (1996). A new relationship between total/high density lipoprotein cholesterol and polyunsaturated fatty acids. *Lipids* 31: 51-56.

Stevenson, S.G., Vaisey-Genser, M. and Eskin N.A.M. (1984). Quality control in the use of deep frying oils. J. Am. Oil Chem. Soc. 61(6): 1102-1108.

Subramaniam, R., Nandinim, K.E., Sheila, P.M., Gopalakrishna, A.G., Raghavarao, K.S.M.S., Nakajima, M., Kimura, T. and Maekawa, T. (2000). Membrane processing of used frying oil. J. Am. Oil Chem. Soc. 77(3): 323-328.

Suzuki, R., Noguchi, R., Ota, T., Abe, M., Miyashita, K. and Kawada, T. (2001). Cytotoxic effect of conjugated trienoic fatty acids on mouse tumor and human monocytic leukemia cells. Lipids 36: 477-482.

Taghvaei, M., Jafari, S.M., Mahoonak, A.S., Nikoo, A.M., Rahmanian, N., Hajibatar, J. and Meshginfar, N. (2014). The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. LWT-Food Sci. Technol. 56: 124-130.

Tamzid, H.M., Alam, M.T. and Islam, M. (2007). Physico-chemical and nutritional studies of *Terminalia belerica roxb* seed oil and seed kernel. J. bio.sci. 15: 117-126.

Tchiégang, C., Barbe, F., Nicolas, B., Parmentier, M. and Gueant, J.L. (2007). Effect of two dietary oils from: *Ricinodendron heudelotii* (Baill.) and *Tetracarpidium conophorum* müll. nuts on some biochemical blood parameters of rats. Ngaoundere, Cameroon.

Tchiégang, C., Kapseu, C., Ndjouenkeu, R. and Ngassoum, M.B. (1997). Amandes de *Ricinodendron heudelotii* (Baill): Matière première potentielle pour les industries Agroalimentaires tropicales. J. Food Eng. 32: 1-10.

Toscano, G., Riva, G., Poppa, E. and Duca, D. (2012). A physico-chemical study for some edible oils properties. Biomass and Bioenergy. 46: 511-516.

Udo, I.O. and Epidi, T.T. (2009). Biological effect of ethanolic fractions of *Ricinodendron heudelotii* (Baill) pierre ex pax against *Sitophilus zeamais* Motschulsky and *Callosobruchus maculatus* Fabricius on stored grains. Afr. J. Agric. Res. 4(10): 1080-1085.

Vijayan, J., Slaughter, D.C. and Paul, S.R. (1996). Optical properties of corn oil during frying. Int. J. Food Sci. Technol. 31: 353-358.

Voragen, A.G., Pilnik, W., Thibault, J.F., Axelos, M.A. and Renard, C.M. (1995). Pectins. In Food polysaccharides and their applications, Stephen, A.M.(eds) Marcel Dekker: New York, USA. 287-339.

Wagner, B.A., Buettner, G.R. and Burns, C.P. (1994). Free radical-mediated lipid peroxidation in cells: Oxidizability is a function of cell lipid bis-allylic hydrogen content. *Biochemistry*. 33(15): 4449-4453.

Warner, K. and Mounts, T.L. (1993). Frying stability of soybean and canola oils with modified fatty acid compositions. *J. Am. Oil Chem. Soc.* 70(10): 983-988.

Yang, L., Cao, Y., Chen, J.N. and Chen, Z.Y. (2009). Oxidative stability of conjugated linolenic acids. *J. Agric. Food Chem.* 57: 4212-4217.

Yasui, Y., Hosokawa, M., Sahara, T., Suzuki, R., Ohgiya, S., Kohno, H., Tanaka, T. and Miyashita, K. (2005). Bitter gourd seed fatty acid rich in 9c,11t,13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPAR γ in human colon cancer Caco-2 cells. *Prostaglandins Leukot. Essent. Fatty Acids*. 73: 113-119.

Yeboah, S.O., Mitei Y.C., Ngila, J.C, Wessjohann, L. and Schmidt, J. (2011). Compositional and structural studies of the major and minor components in three Cameroonian seed oils by GC-MS, ESI-FTICR-MS and HPLC. *J. Am. Oil Chem. Soc.* 88: 1539-1549.

Yookyung, K., David, S.H. and Sandra, E.K. (2007). ATR-Fourier transform mid-infrared spectroscopy for determination of trans fatty acids in ground cereal products without oil extraction. *J. Agric. Food Chem.* 55: 4327-4333.

Yurawecz, M.P., Sehat, N., Mossoba, M.M., Roach, J.A.G. and Ku, Y. (1997). Oxidation products of conjugated linoleic acid and furan fatty acids. In: McDonald, R.E., Mossoba, M.M. (eds.) *New techniques and applications in lipid analysis*. Champaign: AOCS Press. 183-215.

Zhang, A. and Chen, Z.Y. (1997). Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* 74: 1611-1613.