

**EFFECT OF INDUCED AGEING ON VIABILITY AND NUTRIENT LEVEL OF  
SESAME SEED ACCESSIONS**

**BY**

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## ABSTRACT

Sesame seed (*Sesamum indicum* L.) is one of the oldest oil seed plants used by humans, with economic potentials nationally and internationally. The study determined the biochemical changes exerted on the selected sesame seed accessions when subjected to accelerated ageing. A total of twenty (20) sesame seed accessions were screened for oil yield out of which five (5) were selected for accelerated aging at a temperature of 40°C and 80% relative humidity for 0, 3 and 6 days. The Catalase activity, proximate composition, fatty acids and amino acids profiles of the aged seeds were determined at intervals using standard biochemical procedures. The results revealed significant variation in chemical composition and oil yield of the seeds of the sesame accessions. NCRIBEN 203 had significantly higher (54.6%) oil yield ( $p < 0.05$ ) compared to which NCRIBEN106 had the lowest (28.6%) all through the aging period. Percentage moisture, protein, fiber, fat, carbohydrate and ash in the seeds ranged from 1.76% to 3.70%, 21.15% to 25.29%, 3.84% to 4.30%, 44.9% to 52.8%, 8.94% to 18.15% and 5.75% to 6.50%, respectively. Accession NCRIBEN131 had a significantly higher percentage fat (52.817%) and protein (25.295%) than other accessions ( $p < 0.05$ ). The result showed progressive decrease in enzyme activity which was only significant ( $p < 0.05$ ) at day six (6) of ageing. The oil was found to contain high levels of polyunsaturated fatty acid especially oleic acid that ranges from  $34.519 \pm 4.087 \mu\text{g/g}$  to  $25.843 \pm 2.287 \mu\text{g/g}$  and linoleic acid that ranges from  $27.327 \pm 0.234 \mu\text{g/g}$  to  $20.458 \pm 1.969 \mu\text{g/g}$  the dominant saturated fatty acids were stearic acid (up to  $5.056 \pm 1.885 \mu\text{g/g}$  to  $3.900 \pm 0.422 \mu\text{g/g}$  and palmitic acid ( $2.092 \pm 0.277 \mu\text{g/g}$  to  $1.55 \pm 0.149 \mu\text{g/g}$ ) all in accessions NCRIBEN203 and NCRIBEN121 and NCRIBEN106 respectively. whereas the least concentration was observed in lauric acid, cetoleic acid and aracidonic acid the amino acid profile showed that all accessions contained the 20 amino acids. However, there was progressive increase in amino acid concentration with ageing from day 0-3 before decline at day 6. Total amino acid (TNEAA) which ranged from  $6.798 \pm 4.852 \text{ mg/100g}$  to  $5.614 \pm 4.327 \text{ mg/100g}$  was observed to be highest in accession NCRIBEN106 and NCRIBEN 131 when compared to the TarAA  $3.061 \pm 1.812 \text{ mg/100g}$  to  $2.412 \pm 1.529 \text{ mg/100g}$  in accession NCRIBEN 201 and NCRIBEN 121. From the study it can be concluded that accelerated aging affected the nutrient composition of sesame seed negatively by increasing the level of seed deterioration hence reducing the nutrient in the seed.

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## CHAPTER ONE

### 1.0

### INTRODUCTION

#### 1.1 Background of Study

Sesame (*Sesamum indicum*) is a member of the Pedaliaceae plant family. The sesame is one of the world most important and oldest known oil seed crops (Abou-Gharbia *et al.*, 2017). Its cultivation dated as far as 1500 BC in the Middle East, Asia and Africa (Ali *et al.*, 2017). It took the 9th position among the top 13 oilseed crops which make up 90% of the world production of edible oil (Adeola *et al.*, 2016). Sesame is mainly cultivated nationally and internationally for seeds, having 50% oil content. Sesame is a tropical herbaceous annual plant with height up to 2 m, with an unpleasant odor. The leaves vary from ovate to lancelets and are hairy on both sides. The flowers are varied in colour with purple to whitish, resembling foxglove, followed by nearly 3 cm capsules/fruits containing numerous seeds (McCormick, 2018). The number of fruits ranges from 15-20 that can have 70-100 seeds. Sesame matures in 80– 180 days when the stems are cut and hung upside down for the ripe seeds to fall out to be collected on mat.

The sesame crop is adapted and cultivated both in the tropic and temperate zones of the world (Biabani and Pakniyat, 2018). It is grown mostly for the oil extracted from the seed which is edible and use for industrial and pharmaceutical purposes. The oil is used in the production of perfumes moisturizers, hair creams, bath oil, insecticides, paints, vanishes and drugs (Mohammed and Hamza, 2018). The seed is also consumed throughout the world in condiments and as an essential constituent in different recipes (Alyemeni *et al.*, 2016). It is used to add texture and flavor to bread, biscuit, cracks and salad dressing. Sesame seed has been shown to contain about 35 to 60% of oil (Jimoh *et al.*, 2016; Mohammed and Hamza, 2018). The sesame oil has also been reported to show remarkable stability to

oxidation due to the present of lignins (sesamol, sesamolin and sesamin). (Alyemeni *et al.*, 2016; Lee *et al.*, 2018). Nigeria is one of the major producers and exporters of sesame seed and ranked 6th among world sesame producing countries and 2<sup>nd</sup> largest in Africa (FAO, 2015). The production of sesame in Nigeria is concentrated in the Guinea Savannah zone (Middle Belt). Due to the increasing demand for sesame seed as a result of its enormous uses, there is a need to expand the area of cultivation way boosting production and storage of seeds. Various researches have been carried out in areas outside the traditional producing area in Nigeria to determine the growth, yield potential and storage of sesame types and possibility of a viable commercial sesame production in these areas. The quantity and quality of the oil contained in the seed have been shown to depend on ecological factors such as climate and soil type and on cultivars and maturity of plant (Rahman *et al.*, 2017).

The oil inside the seeds will get oxidized easily and deteriorate seed health during storage. These deteriorative changes decline germinability and vigor of seeds. Accelerated ageing is recognized as an accurate indicator of seed vigor and storability; as it correlates with field emergence. The seeds that exposed to accelerated ageing condition generally show a marked reduction in germination (Hampton *et al.*, 2014; McDonough *et al.*, 2014). Accelerated ageing also results in the increase in lipid peroxidation and a decrease in activities of antioxidant and several enzymes which were involved in scavenging free radicals and peroxide (Atici *et al.*, 2017). In order to ensure the availability of seeds for farmers as well as for industrial purposes, it is necessary to develop appropriate storage methods that would prolong the viability of seeds. Accelerated ageing is the commonly used test to predict the storability of seeds, during which seeds were exposed to high temperature and high relative humidity. The temperature, moisture content as well as

storage duration are the most important individual factors which affect viability of stored seeds. Under unfavorable conditions such as the temperature above 30°C and relative air humidity from 80 to 90 per cent, the variation in seed germination rate can be high (Sisman, 2015). Seed aging during storage is an inevitable phenomenon, but the degree and speed of decline in seed quality depend strongly, on storage conditions, on plant species and initial seed quality. The rate at which the seed aging process takes place depends on the ability of seed to resist degradation changes. Seeds of different plant species lose viability to a various degree even when kept under the same storage conditions. Accelerated aging of seed, i.e., seed exposure to high temperature and high relative humidity leads to loss of vigor and finally to a loss of viability which is an outstanding method for determination of changes in vigor during seed storage (Alyemeni *et al.*, 2016). Sometimes, the invigorated seeds will remain unsown for want of proper soil conditions. Under such situations, the seeds need to be stored till next sowing season.

## **1.2 Statement of the Research Problem**

Sesame seeds stored in ambient conditions lose their viability and vigor very fast due to changes in storage conditions of temperature and relative humidity. The ageing of seeds is also influenced by the genetic make-up of the crop. Regular fluctuations in temperature, moisture content and storage time make the processing and storage of sesame seeds troublesome. Report has it that about one third of Nigerian total annual production of sesame is lost due to ageing (Chemonics, 2017).

### **1.3 Aim and Objectives of the Study**

#### **1.3.1 Aim**

The aim of the study is to evaluate the effect of induced ageing on the viability and nutrient composition of selected accessions of sesame seeds.

#### **1.3.2 Objectives:**

The objectives of the study are to determine:

- i. to select sesame seed for this study based on their respective oil yield.
- ii. the proximate composition of selected sesame seeds
- iii. the total fatty acid profile of oils from the selected seeds.
- iv. determine the total amino acids contents from the selected sesame seeds.
- v. the effect of induced ageing on catalase enzyme activities of the selected sesame seeds

### **1.4 Justification for the Study**

Nigeria being the third largest producer of sesame in the world with export value of \$1.5 billion contributing about 0.57% of the country total export value and 36.39% of agricultural export in the year 2018. National Cereals Research Institute (NCRI) Badeggi being the Agency saddled with the responsibility of improving genetics and farming practices that will promote the production of the sesame in the country at large. There is need to select genotypes that tolerates this ambient condition of storage through accelerated ageing or induced ageing. This study was designed to determine the effects of ageing on the viability and nutrient composition of selected sesame seed accessions



## CHAPTER TWO

### 2.0

### LITERATURE REVIEW

#### 2.1 History and Botany of Sesame

Sesame (*Sesamum indicum* L.), a member of the Pedaliaceae family, is an erect annual herb commonly known as sesamum, benniseed, or simsim. It is one of the oldest and most traditional oil seed crops, valued for its high-quality seed oil. According to recent archeological findings, sesame cultivation was derived from wild populations native to South Asia, and its cultivation was established in South Asia from the time of the Harappan civilization and spread west to Mesopotamia before 2000 B.C. (Fuller, 2016). Despite other claims, it was first cultivated in Africa and later taken to India at a very early date (Alegbejo *et al.*, 2016). Tunde-Akintunde *et al.*, (2017) suggested that sesame was the main oil crop grown by the Indus Valley Civilization and was likely transferred to Mesopotamia around 2500 B.C.

The Assyrians used its oil for different purposes such as food, salves (ointments), and medicine, while Hindus believed it to be sacred. Sesame is also known as the “queen of oilseeds,” but it is actually an orphan crop. Little research into sesame has been undertaken and, hence, it is not a crop mandated by any international crop research institute (Bhatia *et al.*, 2019), despite being cultivated in both tropical and temperate zones of Africa, Asia, Latin America, and some parts of the southern United States (Bedigian, 2016).

Sesame is adaptable to a range of soil types, although it performs well in well-drained, fertile medium texture (typically sandy loam) at neutral pH. Generally, sesame is a short-day plant that may grow in long-day areas. Depending upon light intensity and day period in various regions, sesame has produced genotypes with different photoperiod

requirements. Depending upon the cultivar, the crop matures in 75–150 days after sowing (Ashri, 2017).

## **2.2 Nomenclature of Sesame Plant**

The name “sesame” comes from the arabic word *simsim* since early 15c., probably from the siame and directly from the latin *sesamun* from greek *sesamon* meaning “seed or fruit of the sesame plant, ”a very early borrowing via phoenician from late babylonian *shaswash-shammu* (assyrian *shaswash-shammu*, literally meaning “oil-seed also known sesame seed, the sesame plant *sesamum* spp. is spread throughout the tropical and subtropical areas in Asia, Africa, and south America the genus *sesamum* is comprised of about 35 wild species , in addition to the only cultivated species, *sesamum indicum*. The name “sesame” comes from the Arabic word *simsim* since early 15c., probably from the siame and directly from the latin *sesamun* from greek *sesamon* meaning “seed or fruit of the sesame plant, “a very early borrowing via phoenician from late babylonian *shaswash-shammu* (assyrian *shaswash-shammu*, literally meaning “oil-seed also known sesame seed, India was cited as the origin of sesame, but a belief also exists that the actual origin was Africans, where many wild species are found according to FAOSTAT, about 60% of the wild sesame crop is grown in Asia (FAO, 2015). Sesame grows in many tropical and warm temperate areas. However, it worth nothing sesame is typically grown in cultivated beds rather than in the wild which requires a well-drained porous soil. The taxonomy of sesame is as shown below, in 2.2.1

### 2.2.1 Taxonomic tree

**Kingdom;** Plantae

**Subkingdom;** Viridiplantea (green plant)

**Superdivision;** Embryophyta

**Division;** Tracheophyta (vascular plant)

**Subclass;** Spermatophytina (seed plant)

**Family;** Pedaliaceae (pedaliums)

**Genus;** *Sesamum* L.(sesame)

**Species;** *Sesamum indicum* L. (sesame seed)

(Morris, 2017).

### 2.3 General Description of Sesame Seed

Sesame (*Sesamum indicum*) is an introduced annual broadleaf plant that grows 5–6 ft (155–185 cm) tall. It produces a 1–2 in (2.5–5cm) long white, bell-shaped inflorescence growing from the leaf axils (where the leaf stalk joins the stem). The blooms do not open all at once, but gradually, from the base of the stem upwards to the top of the plant. The flowers are both male and female and will self-pollinate, the seed is produced in a 1–1.5 in (2.5–3.8 cm) long, divided seed capsule that opens when the seeds are mature. There are 8 rows of seed within each seed capsule, and seed may be yellow, white, brown, or black (Morris, 2017). Due to the non-uniform, indeterminate nature of the bloom` period, the reproductive, ripening, and drying phases of the seed tend to overlap. Seed lowest on the plant will mature first, even as the upper part of the plant is still flowering or has just formed seed capsules, Sesame varieties have single or multiple stems and the stem is covered with short, soft hairs. which is very leafy with 3–5 in (7.5–12.7 cm) long, somewhat rough, lance-

shaped, entire upper leaves, and tri-lobed lower leaves as shown in figure 2.1. Sesame has been grown in many parts of the world for over 4000 years. First introduced in the southern United States by slaves, the plant's potential as an oil seed was quickly recognized and enthusiastically promoted by Jefferson. Currently, the United States imports more sesame than it grows, so there is potential for increasing production acres in the US, especially as more shatter-resistant varieties are developed. Sesame grows best in well-drained, sandy loam soils, with a pH from 5–8. Sesame cannot survive standing water or high salinity environment. Sesame is notable for its ability to grow under droughty conditions and in extreme heat. It is often grown where cotton can grow, under conditions few other crops can survive, requiring very few inputs. These attributes make sesame an excellent candidate for low-input sustainable food systems. Sesame is deep-rooted and will scavenge nutrients from below most crop root zones (Langham *et al.*, 2018). When grown from East Texas to the Atlantic coast sesame becomes more susceptible to leaf diseases (Langham *et al.*, 2018). Sesame has moderate salt tolerance but will not grow under flooded conditions. Generally, the plant will have a better chance of survival when it is grown in hotter than optimal temperatures rather than lower than optimal temperatures (Langham *et al.*, 2018).



**Figure 2.1.** Mature Sesame Plant. (Source: Langham *et al.*, 2018).

### **2.3.1 Root**

Generally, sesame plants have a strong tap root component and some fibrous roots. However, under differing conditions, the plants may have a stronger tap root or a stronger group of fibrous roots, shown in figure 2.2 below. Sesame is considered a drought resistant species because the root will penetrate the deeper into the soil and find moisture. However, every crop needs moisture, and in a year with little deep moisture, sesame will not do as well. In the US, the optimum situation is to plant sesame into moisture and have no added moisture for about 30 days. Under these conditions the roots will follow the moisture down, and sesame can withstand a lack of rain for the rest of the cycle. In Venezuela, sesame is planted after the rainy season and will produce a crop with zero rain. If there are rains or irrigations soon after planting, there will be more fibrous root development in the upper 30 cm of soil with shorter tap roots. If this condition is followed by a drought, the plants can be in trouble as the moisture in the top 30 cm is depleted, or a heavy rain can waterlog the plants and kill them. Generally, the roots are as deep as the plants are tall, By the end of the

reproductive phase, most of the moisture is being drawn out of the 90-120 cm layer of soil, however it has been shown that when the roots of certain lines hit a rock or a hard pan, the condition develops. Once that root finds a route down or a rain/irrigation often the hardpan, the condition disappears and is difficult to find later in the cycle (Langham *et al.*, 2018).



**Figure 2.2.** Sesame Root. (Source: Langham *et al.*, 2018).

### **2.3.2 Stem**

The main stem will generally have dry capsules before the branches, but the branches will generally dry down before the main stem. The lower capsules dry first, with the top capsules drying and last. Parts of the stems will dry before all of the capsules are dry. The pattern of stem dry down differs in that in some cases, the middle of the stem dries first and then goes in both directions; in others, the top stem dries first and goes down; in others, the bottom stem just below the capsules dries first and goes up and down as shown in figure 2.3 below. In any sequence, the lowest part of the plant between the lowest capsules and the root is the last to dry (Langham *et al.*, 2018).



**Figure 2.3.** Sesame Stem. (Source: Langham *et al.*, 2018).

### **2.3.3 Seed**

Sesame seeds are tiny, flat oval seeds with a nutty taste and a delicate, almost invisible crunch. They come in a host of different colors, depending upon the variety, shown in figure 2.4 below including white, yellow, black and red, Sesame seeds are highly valued for their high content of sesame oil, an oil that is very resistant to rancidity. Sesame seeds are the main ingredients in both tahini and the Middle Eastern sweet treat, halvah. Open sesame the famous phrase from the Arabian Nights reflects the five-distinguishing feature of the sesame seed pod, which bursts open when it reaches maturity (Langham *et al.*, 2018).





**Figure 2.4.** Sesame Seeds. (Source: Langham *et al.*, 2018).

#### **2.3.4 Fruits**

Sesame fruit is a capsule, normally pubescent, rectangular in section, and typically grooved with a short, triangular beak. The length of the fruit capsule varies from 2 to 8 cm, its width varies between 0.5 and 2.0 cm, and the number of loculi varies from four to 12 as shown in figure 2.5 below. The fruit naturally splits open (dehisces) to release the seeds by splitting along the septa from top to bottom or by means of two apical pores, depending on the varietal cultivar. The degree of dehiscence is of importance in breeding for mechanized harvesting, as is the insertion height of the first capsule. Sesame seeds are small. Their sizes



vary with the thousands of varieties known. Typically, the seeds are about 3 to 4 mm long by 2 mm wide and 1 mm thick. The seeds are ovate, slightly flattened, and somewhat thinner at the eye of the seed (hilum) than at the opposite end. The weight of the seeds is between 20 and 40 mg. The seed coat (testa) may be smooth or ribbed (Langham *et al.*, 2018).



**Figure 2.5:** Sesame Fruit. (Source: Morris 2017).

### 2.3.5 Flowers

Sesame (*Sesamum indicum*) is an introduced annual broadleaf plant that grows 5–6 ft (155–185 cm) tall. It produces a 1–2 in (2.5–5cm) long white, bell-shaped inflorescence growing from the leaf axils (where the leaf stalk joins the stem). The blooms do not open all at once, but gradually, from the base of the stem upwards to the top of the plant as shown in figure 2.6 below. The flowers are both male and female and will self-pollinate. The seed is produced in a 1–1.5 in (2.5–3.8 cm) long, divided seed capsule that opens when the seeds are mature. There are 8 rows of seed within each seed capsule, and seed may be yellow, white, brown, or black (Morris, 2017). Due to the non-uniform, indeterminate nature of the bloom period, the reproductive, ripening, and drying phases of the seed tend to overlap. Seed lowest on the plant will mature first, even as the upper part of the plant is still flowering or has just formed seed capsules. Sesame is very leafy; with 3–5 in (7.5–12.7 cm) long, somewhat rough, lance-shaped, entire upper leaves, and tri-lobed lower leaves (Morris, 2017).



**Figure 2.6.** Sesame Seed Flower. (Source: Morris, 2017).

## 2.4 Genetic Variability of Sesame Seed.

Broad-based plant germplasm resources are imperative for sole and successful crop improvement. Genetic diversity has become more important as cropping intensity and monoculture continue to increase in all the major crop-producing regions of the world. A complete array of sesame germplasm consists of the following:

1. Wild relatives, weed races and local races
2. Obsolete lines and cultivars
3. improved varieties.

Proper understanding of genetic variability, heritability and correlation studies of plant traits are vital for effective use of germplasm in any breeding program (Ganesh and Thangavelu, 2015). Germplasm banks are source of genetic variability and are essential for improvement of crop species. Crop variability is characterized by genetic and phenotypic parameters used for identification and selection of desirable parents for breeding program. Despite the high nutritional value, historic and cultural significance of sesame, there has been little focus on sesame research. No international agency (CGIAR, Consultative Group on International Agricultural Research) is assigned work on sesame crop (Bedigian, 2016). Similarly, limited information regarding its genetic diversity is available. Centers for sesame genetic diversity are found in India, China, Central Asia and Abyssinia (Hawkes 2018). Large genetic diversity of sesame should be considered, while planning conservation strategies or exploiting it for breeding programs. Presently, molecular techniques including isozymes, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) are being employed to study genetic variability in sesame (Kim *et al.*, 2017; Abdellatef *et al.*, 2018).

## **2.5 Breeding of Sesame Seeds**

Indeterminate plant growth habit of sesame and seed shattering at maturity results in poor adaptation of plant architecture to modern farming techniques (mechanized harvesting) (Çağırğan, 2016). Due to indeterminate sesame growth habit, flowering continues for long time, this heterogeneous capsule maturation causes harvesting problem and yield losses. Development of sesame varieties with improved architecture and determinate habit can assist sesame yield improvement programs. Sesame yield potential is negatively affected by its early senescence and susceptibility to biotic and abiotic stresses (Rao *et al.*, 2017). Sesame is susceptible to phyllody disease caused by phytoplasma, resulting stunted plant growth and yield losses (Singh *et al.*, 2017). Development of phyllody-resistant varieties is one of the important objectives in sesame breeding program.

### **2.5.1 Breeding objectives**

There are various objectives for sesame breeding.

- High seed yields
- Superior plant architecture (ideotype)
- Indehiscent capsules
- Improved oil quality
- Resistance to diseases and pests

Sesame Crop improvement has resulted in rapid replacement of old races, wild and weedy species and cultivars. These materials are excellent source of genes for adoptability and resistance to biotic and abiotic stresses. The genetic resource management includes collections, conservation, evaluation characterization, classification and cataloging of germplasm. Lack of specific research and understanding of yield-related attributes limited production and extension process of sesame (Ashri, 2017). Yield is an important but

complex parameter of crop that is affected by various factors. Development of high-yielding varieties is the ultimate goal of any plant breeder. For efficient crop breeding and improvement, it is of utmost importance to ascertain the contribution of each yield-related trait toward yield, and to select components maximizing yield. Such studies are helpful in determining the model plant type for species. Sesame wild species possess genes for resistance to biotic and abiotic stresses, which can be introduced into cultivated varieties either through backcrossing or genetic engineering.

### **2.5.2 Breeding methods**

Plant breeding is a combination of both science and art for effective management of available genetic variability and creation of new ones to attain desired goals. It is the process to identify and select plants possessing desirable traits, and/or to develop an ideal type plant by combining these desired traits into single plant. Breeding methods used for sesame genetic improvement are simple varying from plant selection to hybrid development and molecular breeding. Application of biotechnology and molecular breeding methods can boost the breeding process for development of superior sesame varieties.

#### **2.5.2.1 Conventional breeding**

Conventional breeding is under the control of human for choice of parental lines, and selection of their offspring to direct the evolution process for crop production (Najeeb *et al.*, 2017) according to their desires. Although low percentage of cross pollination is reported, the sesame is predominantly regarded as a self-pollinated plant (Ashri, 2017). Development of sesame types with desirable characters is achieved through pedigree selection from segregating generations of different crosses. In conventional plant breeding these traits are manipulated to get desired genetic combination through various procedures.

There are several advantages of conventional breeding, it is technically simple, convenient and need no sophisticated tools. It is suitable for improvement of many traits or polygenic or traits with unidentified genes at one time (Mubashir *et al.*, 2017).

However, there are certain disadvantages of conventional methods including incompatibility in crosses, limitation of genetic variation within crop gene-pool and time consuming. Selection of plants with desirable traits from segregating generations is a time-consuming process, and sexual breeding methods are not useful for improving sexually sterile crops.

#### **2.5.2.2 Pure line and mass selection**

Evaluation and consequent selection of improved lines are the first step in breeding process that largely depends on the knowledge of plant genetic diversity and heritability. Selection is regarded as the most ancient and basic procedure in plant breeding in which desired plants are selected from genetically variable population. These lines are evaluated against existing commercial varieties for yield and other traits for making justified plant selection.

Information about relationship between yield and yield-contributing attributes is very important for a successful breeding program (Ganesh and Sakila, 2019). Plant selection with appropriate type sesame is essential for increasing seed yield and developing novel sesame varieties. It is considered that breeding based on additively controlled characters helps improving sesame yield (Mubashir *et al.*, 2019). Since seed yield is a polygenic character, it is essential to identify yield-contributing attributes for selecting high-yielding sesame cultivars. Various physiological traits are useful for determining selection criteria including higher number of capsules, branching and biomass, harvest index, which exhibit significantly positive correlation with seed yield in sesame (Sarwar and Hussain, 2019).

Large numbers of sesame cultivars and lines have been classified on the basis of diagnostic morphological and genetic traits such as flower characters including phyllotaxis, number of nectar, flower or capsule per axil and carpel number per capsule (Sarwar *et al.*, 2015). These classifications provide foundation for development of high-yielding sesame varieties. High genetic advance and heritability for yield-related parameters including seed yield, capsule number and branches per plant were documented by Sarwar and Haq (2016), who evaluated 106 sesame genotypes from different parts of the world.

They concluded that selection of sesame elite genotypes for seed yield is possible on the basis of these characters. On the basis of these phenotypic and genotypic marker traits, various high-yielding sesame varieties have been selected, and a positive correlation of these traits with seed yield was confirmed (Sarwar *et al.*, 2015). Plant characteristics such as bicarpels, monocapsule, branch and tricapsules, Sesame have been used as marker in pedigree selection method by Baydar (2015) to obtain high-yielding sesame varieties.

High heritability estimates of disease infestation are under additive gene action control, and consequently help in the selection of disease-free sesame plants. El-Bramawy and Abd Al-Wahid (2019) screened 28 sesame genotypes for resistance to *Fusarium oxysporum* under field conditions for two successive seasons. Two genotypes “S2” originated from a selection and “H4” from hybridization demonstrated stable resistant to *Fusarium* wilt throughout the evaluation. Some other genotypes including Mutants-8, A-130, H-1 and S-1 also maintained their resistance classes during the two successive seasons. In another study, Arslan *et al.* (2017) evaluated 29 gamma rays (e.g -rays) induced mutants and selected sesame plants exhibiting high level of resistance to *Fusarium* blight.

### **2.5.2.3 Hybridization**

In conventional plant breeding, hybridization is the most frequently used technique. It helps to combine the desirable traits from different plant lines into a single plant through cross pollination. Desired traits such as disease resistance and improved oil quality can be transferred from wild relatives of a crop species to the cultivated forms. Heritability estimates and combining ability studies assist in predicting genetic improvement of different types and are useful in hybrid selection program.

Production of male sterile lines provides an opportunity to facilitate cross pollination process for hybrid seed production, and to exploit sesame heterotic vigor. Sesame cytoplasmic male sterile (CMS) lines were developed by hybridizing *S. indicum* with its wild relative *S. malabaricum* (Bhuyan *et al.*, 2017). Later using CMS system, Bhuyan and Sarma (2018) obtained 36 hybrid combinations of diverse origin. Out of which many hybrids exhibited high heterosis for seed yield, oil content and capsules number per plant. Heterosis, a phenomenon of increased vigor, is obtained by hybridization of inbred lines. Heterosis breeding is a common technique for developing high-yielding sesame varieties that may exhibit 77–540% heterotic effect (Yadav *et al.*, 2015). Mubashir *et al.* (2019) conducted an experiment comprising of five parental lines and their ten crosses, recording 40.35–255.12% heterosis in yield-contributing components.

#### **(a) Somatic Hybridization**

Sesame is a self-pollinated crop; however, conventional crosses between cultivated sesame and its wild relatives have been attempted, the hybrids were difficult to produce. Use of wild relatives in hybridization program is restricted due to cross incompatibility and low hybrid frequency through embryo culture. Hybrid plants can also be developed through



fusion of somatic plant cells. Protoplast fusion is helpful to overcome sexual incompatibility as distantly related species can be fused. In vitro culturing system can help to multiply F 1 plants in the lab first and then to transfer them into the field (Dasharath *et al.*, 2017a).

In sesame, (Dasharath *et al.*, 2017b) successfully developed inter-specific hybrids between cultivated *S. indicum* and its wild relatives *S. radiatum* and *S. occidentale* through ovary and ovule culture. In another study, a simple and efficient protocol for production of hybrids of a cross between *Sesamum alatum* and *S. indicum* were optimized through ovule culture (Rajeswari *et al.*, 2016). For this purpose, capsule retention without embryo abortion was delayed by spraying mixture of growth regulators 289- m M gibberellic acid (GA 3), 80.6- m M NAA and 23.3- m M Kn. The plants were regenerated through direct organogenesis of 7-day-old capsules by culturing them on MS medium containing 8.8- m M BAP, 2.8- m M IAA and 1,712.3- m M glutamine, the developed hybrids were screened for phyllody resistance which exhibited moderate resistance.

#### **2.5.2.4 Mutation breeding**

Mutation breeding involves induction of new genetic variability through spontaneous or artificial mutagens (chemicals or physical). It minimizes our dependence on the use of wild species or species from other cultivars. Induced mutants are evaluated and selected for desired traits. However, development of large number of mutants with undesirable traits limits its wide application in the breeding programs.

Mutagenic techniques are successfully employed in sesame to induce genetic variability. Applications of appropriate doses of physical mutagen or concentration of chemical mutagen are important to get adequate mutations that could benefit sesame breeding

program. Researchers at FAO/IAEA have initiated coordinated research project for genetic improvement in sesame and developed 142 mutants having agronomically useful characters by using both physical and chemical mutagens and devised method for mutation breeding for sesame (Van Zanten, 2017). Following were the recommendations for mutagen treatment.

Well-adapted, homozygous and uniform varieties should be selected for mutation induction for improvement of one or two characters at a time. Lower dose ranges of mutagens are more suitable for inducing desirable mutations, i.e.,  $\gamma$ -rays 150–800 Gy, fast neutrons' irradiation 30–80 Gy. For chemical mutagenesis, first seeds are pre-soaked in water for 24 h (4°C). Then soaking into chemical mutagen, e.g., in ethyl methane sulfonate (EMS) solution (0.4–1.0% v/v) with phosphate buffer (pH = 7) for 2–4 h or in sodium azide ( $\text{NaN}_3$ ) solution (4–6 mM) with Sörenson phosphate buffer (pH = 3) for 4–6 h at 18–24°C. Sesame mutants have been selected for desirable traits of higher yield and quality, improved plant architecture, seed retention, larger seed size and seed color (Hoballah, 2016). A research program on radiation-induced mutagenesis has been initiated to induce genetic variations and to screen desirable “plant type” (Chowdhury and Datta 2018). Sengupta and Datta (2015) identified a narrow leaf mutant in sesame through nitrous acid and hydrogen peroxide treatments in different doses, and the mutant yielded higher number of capsule per plant on the main axis than control.

Early maturing and high-yielding sesame mutants have been developed by using  $\text{NaN}_3$  and colchicines, Mensah *et al.* (2017) found that 0.0625%  $\text{NaN}_3$  and 0.125% colchicine were the most efficient concentration for inducing mutations in sesame. The  $\gamma$ -ray-induced mutants with improved plant architecture were developed having closed capsule,

determinate growth habit, resistance to *Fusarium* blight, etc. These mutants had improved oil quality with considerably higher oleic acid and low linoleic acid contents (Arslan *et al.*, 2017).

Indeterminate sesame habit is a challenge for sesame breeders, and mutagenic breeding approach is applied to solve this problem (Çağırğan, 2016).

However, due to its low yield and other undesirable side effects it was not used in commercial varieties. The first determinate sesame mutant (dt-45) was selected by Ashri (1989) from an M2 population by irradiating Israeli variety “No-45” with  $\gamma$ -rays (500 Gy). Çağırğan (2016) irradiated seeds of four sesame cultivars with  $\gamma$ -rays (150–750 Gy) and found three true botanical determinate mutants (dt-1, dt-2 and dt-3) of cultivar Muganlı-57 and dt-4, dt-5 and dt-6 of cultivar Çamdibi. They also proved that selection of determinate growth habit mutants depends upon population size, cultivar response to mutagenic treatment and careful screening.

Marker traits are always useful in genetics and breeding as they are easily scorable and selectable in field conditions. Cytogenetical and agronomical aspects of some morphological (leaf and pollen related) marker mutants were induced following different doses of X-rays and  $\gamma$ -rays (Chowdhury *et al.*, 2019). These morphological sesame mutants exhibited distinctive traits viz., narrow, elongated, thick leaf types, ovate, ternate elongated petiole type and white, pigmented flower type. Out of different mutants, thick leaf mutants were the most desirable plant types possessing superior agronomic traits such as plant height, primary and total branches per plant, capsule on main axis, distance from base to first branching, total capsule per plant, seed yield and seed protein content than control.

#### **2.5.2.5 Innovative breeding**

Shortcomings in the conventional breeding (sexual reproduction) are overcome by genetic engineering techniques that introduce desirable genes directly into the target crop making gene pool unbounded. Only desirable traits are improved in this method; therefore, large populations and multiple generations are not required for selection of plants. In addition, there are no limitations for application of this technique to sterile and vegetatively propagated crops.

Likewise, these techniques also have certain drawbacks; only simple and monogenic traits are transferred most of the time, they are relatively expensive and technically demanding and they are controlled by government organizations. Various innovative approaches are used for sesame breeding viz., in vitro culture, genetic transformation and molecular breeding as described below.

##### **(a) In Vitro Culture and Screening**

Somatic plant cells are used for in vitro culturing on nutrient media and new plants are generated from these explants. Plant regeneration through tissue culture is a source of creating genetic variations, heritable variants with desirable agronomic traits are selected, and used in further breeding programs. Plants can also be selected for resistance traits at early stage by exposing cells of calli to pathogens, or isolated pathotoxins by eliminating unwanted plants from the large population. Three factors affect plant regeneration process, viz., genotype, explant source and culture conditions. (Najeeb *et al.*, 2017).

Tissue culture and regeneration through in vitro culturing can speed up breeding process by producing a number of stable regenerants via callus or somatic embryogenesis in a short span of time. In sesame in vitro culturing, cotyledon (Yadav *et al.*, 2019) hypocotyl and

shoot tips (Baskaran and Jayabalan, 2016) have been reported to be more responsive to callus induction and plant regeneration. Appropriate concentrations of plant growth regulators and their combinations are very important to achieve successful plant regeneration from cultured cells and tissues and were optimized in different studies. Application of BAP (benzylaminopurine) in the nutrient media was reported essential and the most effective cytokinin for shoot induction and plant regeneration in *S. indicum*, Yadav *et al.* (2019); Baskaran and Jayabalan (2016) studied the effects of plant growth regulators on callus induction in hypocotyls and cotyledon explants of sesame, and reported callus induction on media containing 2.2–22.6 m M 2, 4-D and 2.6–26.8- m M NAA ( a - naphthalene acetic acid), increased shoot proliferation on BAP and Kn (kinetin), whereas rooting took place on NAA (8.0 m M).

**(b) Genetic transformation**

Sexual incompatibility among plants limits the application of conventional breeding. In genetic engineering techniques, specific genes from any organism (plants, bacteria, fungi, animals and viruses) coding for desired traits are introduced into the genome of any plant. Various techniques are used to obtain transgenic plants viz., DNA transfer through *Agrobacterium* or direct DNA transfer via bombardment, electroporation and polyethylenglycol permeabilization.

The *Agrobacterium* -mediated DNA transformation is the most commonly used techniques in plants (Xu *et al.*, 2019). Desired genes are first transferred to plasmid DNA of *Agrobacterium* and then allowed to transmit into individual plant cells for their expression. This method is suitable for *Agrobacterium* susceptible plants. However, it cannot be used for many economically important plants including cereals; therefore, direct DNA uptake

method is applied. Sesame yield is limited due to different biotic and abiotic stresses (Rao *et al.*, 2017). Some wild sesame species possess resistance genes, but post-fertilization barriers restrict their transfer to cultivated crops through conventional breeding.

Establishment of *in vitro* plant regeneration is a prerequisite of any genetic transformation system that is already optimized (Were *et al.*, 2016). Sesame has been reported as susceptible to *Agrobacterium tumefaciens* infection (Xu *et al.*, 2019). Protocol for genetic transformation and plant regeneration of sesame were optimized by (Were *et al.*, 2016). A significant interaction between hormonal concentration and macronutrients for plant regeneration was recorded, and application of 20-  $\mu$ M TDZ along with 2.5-  $\mu$ M IAA was found the best for successful plant regeneration. (Yadav *et al.*, 2019) optimized an *A. tumefaciens* -mediated transformation protocol to generate fertile transgenic sesame plants. In this method, cotyledon explants were used for plant regeneration via multiple shoot organogenesis. They recovered plants on MS basal medium containing 25.0-  $\mu$ M BAP, 25.0-mg L<sup>-1</sup> kanamycin and 400.0-mg L<sup>-1</sup> cefotaxime.

**(c) *Molecular breeding by; Marker-Assisted Selection***

Marker-assisted selection (MAS) process has revolutionized plant breeding disciplines by increasing selection efficiency at early stages of development and characterization in later generations (Cahill and Schmidt, 2014). The MAS program has been widely applied tool in commercial crop breeding and product development in a variety of agriculturally important economic crops, including cereal, oilseeds, vegetables and ornamentals. (Najeeb *et al.*, 2017).

Various morphological plant traits, their geographical origins and genotype specific bands developed through molecular markers provide useful information about economic

importance of crop, and help in further classification (Ali *et al.*, 2017). Molecular markers have been applied for studying genetic diversity by using various *S. indicum* accessions (Abdellatef *et al.*, 2018) and suggested the usefulness of RAPD technique in sesame breeding and conservation programs, for proper maintenance of germplasm banks and efficient parental line selection.

However, in spite of high economic value, a limited number of reports are available regarding the application of molecular markers for sesame improvement and studying genetic variability viz., isozymes, ISSR, amplified fragment length polymorphism (AFLP) (Ali *et al.*, 2017) and simple sequence repeat (SSR) markers. The application of MAS is generally limited to exploration of genetic variability and germplasm evaluation.

Only few studies are conducted for tagging desired genes to facilitate the process of plant selection for genetic improvement. Construction of genetic linkage maps is a useful technique for tagging of the desired traits in sesame molecular breeding (Wei *et al.*, 2019). Using MAS, Uzun and Çağırğan (2019) tagged *dt* gene, which regulates determinate growth habit in sesame. Development of molecular markers could assist sesame plant identification and selection for breeding programs and facilitate integration of these genes into improved cultivars.

## **2.6 Production of Sesame Seed**

Global production of sesame seed is estimated by FAO at 3.15 mn tonnes per year (FAO, 2012) Having risen from 1.4 mn tonnes in the early 1960's. However only a small proportion of the global sesame harvest enters international trade. For the most part, the oil is expressed locally and used locally for cooking or the seeds themselves are eaten, particularly after being fried.

Sesame is grown in many parts of the world on over 5 million acres (20,000 km<sup>2</sup>). The largest producer of the crop in 2007 was India, China, Myanmar, Sudan, Ethiopia, Uganda and Nigeria. Seventy percent of the world's sesame crop is grown in Asia, with Africa growing 26% (Hansen, 2017).

The largest producers are China and India, each with an annual harvest around 750,000 tonnes followed by Myanmar (425,000 tonnes) and Sudan (300,000 tonnes). These figures are only rough estimates of the situation as sesame is a smallholder crop and much of the harvest is consumed locally, without record of the internal trade and domestic processing. Nigeria has a great market potential for sesame seed production for domestic and export markets noting that the production figures of the commodity has been on a steady increase since 1980, reaching 67000 MT by 1997 and was estimated to reach 139, 000 MT by the year 2010, according to the federal ministry of agriculture and natural resources. This is agreement with the 2008 annual report of the Central Bank of Nigeria which states there has been a rise in production of sesame seed from 98,000,000 to 152,000,000 kg from 2003 to 2007 (CBN, 2019).

Out of the estimated 3.5million hectares of Nigeria's arable land suitable for the growth of sesame seed, only 300,000 is currently used for the crop. However, average yield of crop is about 300kg/ha which is 4 times lower than the average yield of other seed crops e.g. groundnut and soybeans. In major production zones in the country, it is used in traditional food recipes and snacks rather than for export purposes (NAERLS, 2016). Nigeria was the largest supplier to the Japanese market, the world's largest import market for sesame (Chemonics, 2017). Thus, the potentials for beniseed production in Nigeria is high since Japan, as well as Taiwan and Korea, generate global demand and offer opportunity for



Oilseeds Nigerian growers. Nigeria has a 6% share of the \$600 million global market for sesame seed (Nigeria's Harvest, 2019).

Sesame was widely grown in Middle Belt, Northern and Central Nigeria as a minor crop initially in 1974 when it became a major cash crop in many Northern States e.g. Benue, Kogi, Gombe, Jigawa, Kano, Nasarawa, Katsina, Plateau, Yobe and Federal capital Territory (NAERLS, 2016). Sesame is commonly grown by smallholder farmers. The major producing areas in order of priority are Nasarawa, Jigawa and Benue States. Other important areas of production are found in Yobe, Niger, Kano, Katsina, Kogi, Gombe and Plateau States.

There are 2 types of sesame produced in Nigeria

1. White/raw = Food-grade used in bakery industry. 98-100% whitest grade seeds
2. Brown/mixed = Primarily oil-grade

The White (Food Grade) seed is grown around the towns of Keffi, Lafia/Makurdi, Doma, and in Nassarawa, Taraba, and Benue States. It is easier to sort and the Fumani/Denin people consume sesame locally. The Brown/mixed grows in the North, in Kano State and in Jigawa State near Hadejia, and somewhat in the southern part of Katsina State. There is some local consumption of the brown grade, but not much. The brown can be upgraded to Food Grade through bleaching, as discussed earlier (Chemonics, 2017). Several varieties of sesame are cultivated in Nigeria.

**Table 2.1: World Sesame Seed Production – 2018**

<b>Country</b>	<b>Production (tonnes)</b>
Sudan	981,000
Myanmar	768,858
India	746,000
Nigeria	572,761
China	647,893
World	6,015,573

## **2.7 Economic Importance of Sesame Plant**

In 2018, world production of sesame seeds was 6.1 million tonnes, led by Sudan, Myanmar and India as largest producers (FOSFA, 2018). The white and other lighter-coloured sesame seeds are common in Europe, the Americas, West Asia, and the Indian subcontinent. The black and darker-coloured sesame seeds are mostly produced in China and Southeast Asia. (Heuze *et al.*, 2017).

### **2.7.1 Trade**

Japan is the world's largest sesame importer. Sesame oil, particularly from roasted seed, is an important component of Japanese cooking and traditionally the principal use of the seed. China is the second-largest importer of sesame, mostly oil-grade. China exports lower-priced food-grade sesame seeds, particularly to Southeast Asia. Other major importers are the United States, Canada, the Netherlands, Turkey and France. Sesame seed is a high-value cash crop, Prices have ranged between US\$800 and 1700 per metric ton between 2008 and 2010. (Bennet, 2016). Sesame exports sell across a wide price range. Quality

perception, particularly how the seed looks, is a major pricing factor. Most importers who supply ingredient distributors and oil processors only want to purchase scientifically treated, properly cleaned, washed, dried, colour-sorted, size-graded, and impurity-free seeds with a guaranteed minimum oil content (not less than 40%) packed according to international standards. Seeds that do not meet these quality standards are considered unfit for export and are consumed locally. In 2008, by volume, premium prices, and quality, the largest exporter was India, followed by Ethiopia and Myanmar FOSFA of the United Nations (FAO, 2012).

### **2.7.2 Sesame in industry**

In the industry, sesame oil may be used as a solvent in injected drugs or intravenous drip solutions, a cosmetics carrier oil, to coat stored grains to prevent weevil attacks. The oil also has synergy with some insecticides. Lower grade sesame oil can be used locally in soaps, lubricants, and illuminants. Sesame oil can also be used as a raw material in the manufacture of inks (sesame oil yields a top-quality ink after it is burnt), paints, and pharmaceuticals (as healing oil or a vehicle for drug delivery). The oil also has additional use in the industrial preparation of perfumery, cosmetics (skin conditioning agents and moisturizers, hair preparations, bath oils, hand products and make-up), insecticides and paints and varnishes. However, all of these uses are comparatively insignificant in terms of the quantities used.

### **2.7.3 Sesame in biodiesel production**

Biodiesel, a fatty acid-based ester obtained by transesterification of triglycerides and low boiling short chain alcohols is a substitute for fossil fuels. Diesel engines, boilers or other combustion equipment's need not be modified for the use of biodiesel. It is a renewable

source and does not contribute to global warming as CO<sub>2</sub> emission can be reduced by 78% (Saydut *et al.*, 2018) Other advantages include excellent biodegradability, low toxicity, outstanding lubricity and superior combustion efficiency. Present studies indicate that sesame can be used for the production of biodiesel by the use of methanol in the presence of NaOH as catalyst.

Biodiesel produced by this method are under the limits of required standards. Few undesirable properties of triglycerides which result in severe engine deposits, injector coking and piston ring sticking have necessitated chemical alterations thus preventing the use of sesame oil directly.

#### **2.7.4 Nutraceutical and pharmaceutical applications sesame seeds**

Many plant-based nutraceuticals are developed from sesame, the intake of which is related with dietary and non-dietary phytochemicals and health (Reshma *et al.*, 2016). The antioxidant and health promoting property of sesame lignans (sesamin and sesamol) increases both hepatic mitochondrial and peroxisomal fatty acid oxidation rate. Consumption of sesame seed increases plasma gamma-tocopherol and enhances vitamin-E activity which can prevent cancer and heart disease. Sesame seed contains cephalin which has hemostat activity. Fibres from sesame are used as an antidiabetic, antitumor, antiulcer, cancer preventive and cardioprotective. (Noon *et al.*, 2018). For pharmaceutical applications, sesame oil is used as a solvent for intramuscular injections and has nutritive, demulcent, and emollient properties and as a laxative. It was used to cure toothaches and gum diseases in 4th century. It is also used for the treatment of blurred vision, dizziness and headaches. The oil is more efficient than isotonic chloride solution in curing nasal mucosa dryness due to winter. The high polyunsaturated fat content in oil reduces cholesterol.

Sesame oil has been used by Indians as an antibacterial mouthwash, to relieve anxiety and insomnia. Malignant melanoma growth was selectively inhibited due to the presence of large amount of linoleate in triglyceride form in sesame oil (Sanjay *et al.*, 2012).

### **2.7.5 Other uses sesame seed**

Gram negative bacteria causing nosocomial infection is a serious concern in the developing countries. Owing to this problem sesame kernel meals have shown the presence of novel anti-microbial peptides. Through HPLC and mass spectrometric analysis, a major peptide of approximately 5.8 kDa (in both white and black cultivars) has been identified to be an antimicrobial peptide having bactericidal activities against *Klebsiella* species, responsible for human urinary infection (Sanjay *et al.*, 2012).

Thus, it proves to be a potential method for hospital infection control and also to decrease the bacterial resistance to synthetic antibiotics (Abu *et al.*, 2016). Sesamin, a non-fat portion of sesame seed oil, curbs delta-5- desaturase activity and cause compilation of dihomo-gamma-linolenic acid (DGLA), which deracinates arachidonic acid, and subsequently decreases the formation of pro inflammatory mediators (Abu *et al.*,2016) Diets containing sesame seed oil and quila (a spawning that emulsifies fat) exert cumulative effect that decreases the levels of dienoic eicosanoids along with IL-1 beta, elevating the levels of IL-10 with marked increased capacity of endurance.

## **2.8 Sesame and Its Medicinal Uses**

### **2.8.1 Regulating cholesterol**

In recent times it has been important to identify the dietary components that lower or regulate cholesterol levels. Any natural substance interfering in the cholesterol metabolism preventing hypocholesterolemic atherosclerosis has gained therapeutic importance. The

major lignan sesamin, present in sesame seeds is mainly related to lipid metabolism through a series of biochemical actions in both humans and animals (Matsumura *et al.*, 2018).

Dietary sesamin and episesamin has shown significant increase in the gene expression of mitochondrial and peroxisomal fatty acid oxidation enzymes such as carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase thus increasing the hepatic activity of fatty acid oxidation which is due to enhanced ketone body production. This hepatic fatty acid metabolism accounts for lowering the serum lipid level (Kita *et al.*, 2015). Sesamin also increases the activity and gene expression of malic enzyme which has lipogenic activity (Hemalatha and Ghformnissa, 2014). Alpha-tocopherol greatly accentuates the hypo cholesterolemic action of sesamin, although which alone does not affect the concentration of serum cholesterol (Yamada *et al.*, 2018).

### **2.8.2 Neurological role of sesame seed**

A characteristic feature of Alzheimer's disease (AD) i.e. a neurodegenerative disease is seen to be cognitive decline, memory impairment and behavioural abnormalities as a result of neural loss. This can be explained by subtle alterations of synaptic efficacy prior to the neuronal death. Neurotrophic factors such as nerve growth factor (NGF) play a vital role in neuronal differentiation, development and synaptic plasticity (Collinge, 2016). When sesamin and episesamin (sterioisomer of sesamin) are ingested, sesamin is metabolised by cytochrome P40 to SC1 (2-(3,4-methylenedioxyphenyl)- 6-(3, 4-dihydroxyphenyl)-3,7-dioxabicyclo Octane) which is then metabolized to SC2. Similarly, episesamin is metabolized to EC1 and then EC2. These compounds are further metabolized to SC-1m, SC-2m EC-1m and EC-2m by catechol-O-methyl transferase (COMT). The primary

metabolites of this cycle exhibit the most potent neural differentiation activity (Collinge, 2016).

### **2.8.3 Sesame in regulation of blood pressure**

It is impressive to state that sesame oil which is rich in poly unsaturated fatty acids-PUFA, sesamin and vitamin E greatly reduces hypertension when compared to the blood pressure lowering drugs (Costa *et al.*, 2017). Sesamin feeding significantly decreases the wall thickness and area of aorta and superior mesenteric artery. It also decreases histological renal damage such as the thickening of tunica intima and fibrinoid degeneration of the arterial wall, a feature not observed in normal diet (Chavali *et al.*, 2018). Sesamin is valuable for prophylactic treatment to fight the development of cardiac hypertrophy and renal hyper tension (Chavali *et al.*, 2018).

### **2.8.4 Antioxidant properties of sesame seed**

The important antioxidants sesaminol, sesamol, sesamolol and sesamin maintain the fats including Low Density Lipoproteins (LDL) which cause arteriosclerosis and are believed to promote the integrity of body tissues. These antioxidant lignans have shown hypocholesterolemic and immunomodulatory effect (Zhong *et al.*, 2017). Vitamin E, a fat-soluble antioxidant, protects the body from harmful oxidizing compounds. Sesame seed oil contains gamma tocopherols along with sesaminol and sesamin which possess Vitamin E like activity.

### **2.8.5 Dermatological uses of sesame seed**

UV light produces various reactive oxygen species (ROS) in the skin causing skin damage such as sunburns, wrinkles and skin cancer (Balan *et al.*, 2019). The antioxidants present in sesame act as a defence against these ROS. (Chauhan *et al.*, 2017) reported that the

mutation caused by UV irradiation on p53 gene can be prevented by topical application of alpha-tocopherol, dietary intake of  $\alpha$ -tocopherol reduces photocarcinogenesis induced by UVB light (Balan *et al.*, 2019). Application of sesame oil with turmeric powder in milk on the facial skin, makes it smooth, soft removing pimples (Balan *et al.*, 2019).

## **2.9 Nutritional and Chemical Composition of Sesame Seeds**

Sesame seeds have both nutritional and medicinal value because they are rich in fat, protein, carbohydrates, fiber, and essential minerals. They are used in sweets such as sesame bars and halva (dessert) and in bakery products or milled to get high-grade edible oil (Bedigian, 2016). Seeds are chemically composed of 44–57% oil, 18–25% protein, 13–14% carbohydrates (Borchani *et al.*, 2018). Sesame oil is famous for its stability as a result of its resistance to oxidative rancidity after long exposure to air (Global Agri Systems, 2017). Generally, the oil contains 35% monounsaturated fatty acids and 44% polyunsaturated fatty acids (Hansen, 2017).

Sesame oil has significant resistance against oxidation as a result of it containing endogenous antioxidants including lignins and tocopherols (Elleuch *et al.*, 2017; Lee *et al.*, 2018). There are two types of lignins, (i) sesamin and (ii) sesamol, in sesame oil. Sesamol is converted to sesamol after roasting. The molecular structure of sesamol consists of phenolic and benzodioxide groups. The phenolic group is responsible for antioxidant activities in a number of natural products, while the benzodioxide group is involved in anticancer and antioxidant activities.

Recent research into sesame showed that it contains immunoglobulin E (IgE)–mediated food allergens (Agne *et al.*, 2013; Dalal *et al.*, 2017; Pastorello *et al.*, 2018). The



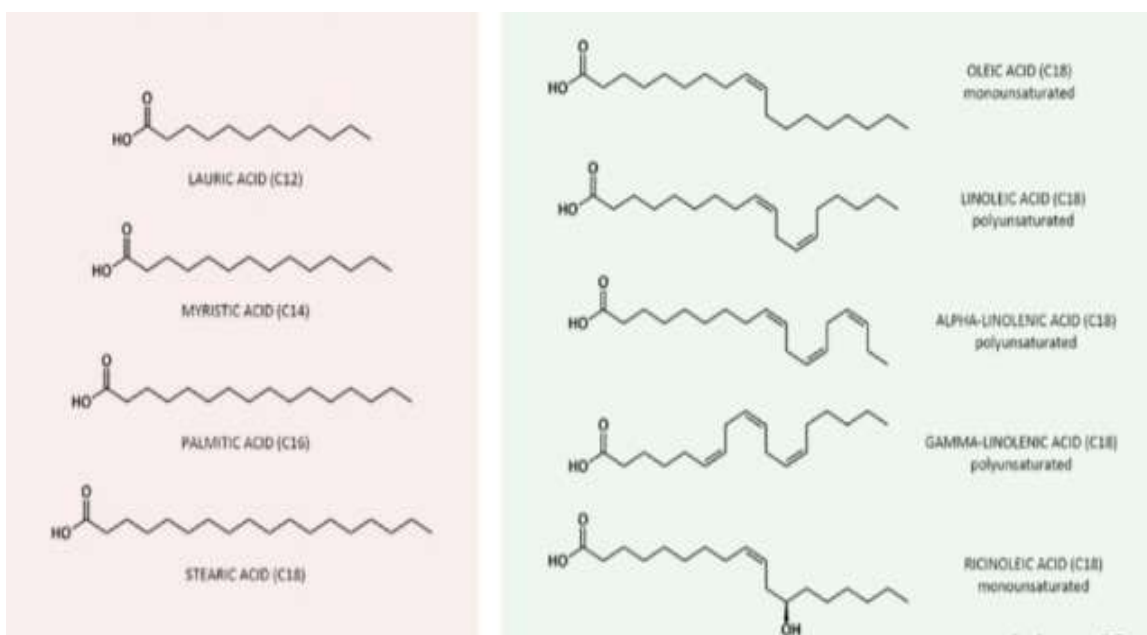
preponderance of allergy to sesame seed is associated with its wider use in baked and fast food products.

### **2.9.1 Oil constituents in sesame**

Sesame seeds are mainly used for its high oil content. They have a high polyunsaturated fatty acid (PUFA) content and rank 4<sup>th</sup> after safflower, soybean and maize for their PUFA content. Sesame oil contains about 47% oleic acid and 39% linoleic acid (Oplinger *et al.*, 2019). It is rich in tocopherols and lignans (notably sesamin and sesamol) that provide exceptional oxidative stability compared to other edible oils (Hwang, 2015). Sesame oil, queen of oils is an ingredient of varieties of food; it is used as a substitute for olive oil, as a salad oil and for cooking fish and vegetables in many parts of the world. Aqua hulled, double washed and dried sesame seeds are used on hamburger buns. Roasted natural sesame seeds are used in the preparation of bread, breadsticks, cookies, chocolates and ice creams. Mechanically hulled sesame seeds are the basis for candies and creamy, sweet wholesome tahini. The antioxidant property of refined sesame oil contributing for its greater shelf life makes it suitable for food industry. Sesame is commercialized in a number of forms. Most sesame is processed directly into oil by the grower or within the producing region but can also be sold in various stages of processing, for various uses, such as meal, paste, confections, and bakery products. Sesame seeds can also be consumed directly as a highly nutritious foodstuff (Naturland, 2017).

**Table 2.2 Average Composition of Sesame Seed Oil/fatty Acid Chains**

Linoleic	39.13-46.38%
Oleic	36.13-43.63%
Linolenic	0.28-0.4%
Palmitic	8.19-10.26%
Stearic	4.63-6.35%
Arachidonic acid	0.66-0.69%



**Figure 2.7:** Structures of Some Fatty Acids Present in Sesame Oil (Ide *et al.*, 2018).

### 2.9.2 Sesame seed and oil as a dietary supplement

Sesame seeds have delicate nutty flavor. Their flavor indeed becomes more pronounced once they are gently roasted under low flame just for few minutes. De-hulled sesame seed is mainly used to add texture, taste and aesthetic value to a variety of bakery products like bread, bread sticks, cookies, sesame bars etc.; and also, as an additive to cereal mixes and crackers. It is also used in the making of tahin or sesame butter - a paste of ground sesame seeds, which is used as an ingredient (in Greece) and halva, placed within breads or sprinkled on the surface of bread and breadsticks as a garnish (Germany and the

Netherlands) and for the preparation of rolls, crackers, cakes and pastry products in commercial bakeries (Nzikou *et al.*, 2018). Ground and processed seeds can also be used in sweet confections, candies are made from sesame mixed with honey or syrup and roasted (in South Asia, middle East and East Asia) while sesame paste and starch are used to make *goma-dofu* (Japan). Sesame seed can also be in the manufacture of margarine, sprinkled over salads and desserts, particularly sundaes and other ice cream-based preparations, preparation of gomshino (a Japanese delicacy) and soybean oil. It can also be used in other food dishes including Mexican and East Asian cuisines. Sesame seed is primarily grown for its oil in Nigeria and the oil is a primary source of cooking oil in Eastern Nigeria. The major portion of sesame seed produced in countries like Nigeria and India is used for extraction of oil. Sesame oil is mostly used as traditional cooking oil in Chinese food items and in Japan. Sesame seed is an excellent source of high quality oil and protein, its oil is odorless and close in quality to olive oil (Tunde-Akintunde and Akintunde, 2017).

Sesame oil has no odor, it is straw-like in colour and has an excellent taste. Sesame seed oil is a natural salad oil, requiring little or no winterization, is one of the few vegetable oils that can be used directly without refining and is used widely as cooking oil. Because of the excellent quality of the edible oil it produces, sesame is often called queen of the oil seed crops. Light sesame oil has a high smoke point and is suitable for deep-frying, while dark sesame oil (from roasted sesame seeds) has a slightly lower smoke point and is unsuitable for deep-frying. Instead it can be used for the stir frying of meats or vegetables, or for the making of an omelet. East Asian cuisines often use roasted sesame oil for seasoning. It is also used widely for production of margarine, shortening, canned sardine and beef as well as in soap and confectionary industries (NAERLS, 2016). Sesame oil has a high

preservative effect though the seeds are prone to rancidity because of its high oil content. The oil prevents rancidity due to a preservative within the oil called sesamol. Sesame oil obtained during the first, cold pressing is one of the costliest produced. The oil is light yellow, does not dry out, and can be used with strong heat. Sesame oil obtained from the second, warm pressing and extraction has a lower quality than cold-pressed.

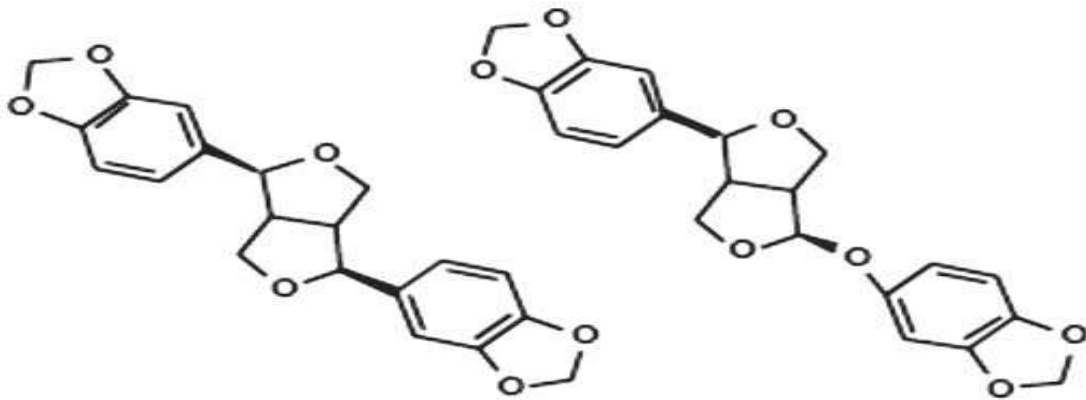
### **2.9.3 Lignans in sesame seeds**

Sesame is rich in sulfur containing amino acids and limited in lysine and contains significant amounts of oxalic (2.5%) and phytic (5%) acids (Kapadia *et al.*, 2017). Because oxalic acid is present in the hulls, decortication can remove most of it. Decorticated sesame seeds have the following composition: 45-63% oil, 19-31% (averaging about 25%) proteins, about 14% carbohydrates and about 3% ash. Unlike many oilseeds, sesame meal is devoid of anti-tryptic compounds. Sesame oil is very rich in polyunsaturated fat used in margarine production and cooking oils. phenolic Sesame seeds contain two unique substances, sesamin and sesamol (Fig. 2.8) hence during refinement the two antioxidants, sesamol and sesaminol, are formed as shown in figure 2.8 below. Both of these substances belong to lignans and have been shown to possess cholesterol-lowering effect in humans (Ogawa *et al.*, 2015) and to prevent high blood pressure and increase vitamin E supplies in animals (Yamashita *et al.*, 2012; Kamal-Eldin *et al.*, 2015). Sesame seeds are an excellent source of copper and calcium. It is also rich in phosphorous, iron, magnesium, manganese, zinc and vitamin B1. A chlorinated red naphthoquinone pigment possessing antifungal activity, named chlorosesamone (2-chloro-5, 8-dihydroxy-3 3methyl-2-butenyl)-1, 4-naphthoquinone), has been reported from sesame root (Hasan *et al.*, 2017).

In another research, three anthraquinones, Anthrasesamones A, B and C, were isolated from the root of sesame (Furumoto *et al.*, 2013). Anthrasesamone C is a rare chlorinated anthraquinone in higher plants. The total phytosterol content in sesame seeds is ~400 mg/100 g, which is higher as compared to English walnuts and Brazil nuts (113 mg/100g and 95 mg/100 g, respectively) (Phillips *et al.*, 2015). Just a quarter-cup of sesame seeds supplies 74.0% of the daily value (DV) for copper, 31.6% of the DV for magnesium and 35.1% of the DV for calcium. This rich assortment of minerals translates into many medicinal properties.

Sesamin is a major lignan compound present in sesame seeds. If the intake of sesamin is around 100 to 150 mg, it can preserve vitamin E in the human body. It also acts as a fatty acid metabolism modifier (Wang *et al.*, 2012).

Sesamolin is another major lignan present in the sesame seeds which is thermally unstable. During sesame oil processing, sesamolin is converted into sesamol and some other products by heat. Sesamol has very strong antioxidant activity. The sesamol content is very high in roasted sesame seed oil than unroasted sesame seed oil. It acts as a scavenger of free radicals (Singh *et al.*, 2017). The present study was carried out to evaluate the  $\alpha$ -linolenic acid (omega 3), sesamin and sesamol content in *Sesamum indicum* L. of Tamil Nadu germplasm for enhancing oil quality and anti-oxidant properties.



**Figure 2.8;** Structure of Lignans (sesamin and sesaminolin)

Source: Ide *et al.*, (2018).

### 2.10 Seed Deterioration Process

Seed deterioration during storage is a complex physiological and biochemical process leading to loss of germination ability. Seed quality could be evaluated by testing seed germination and seed germination index in both laboratory scale and green house. On the other hand, the biochemical changes during seed deterioration such as chromosomal aberrations damage to the DNA, changes in the synthesis of RNA and protein, changes in enzymes, differences in respiratory activity caused by ATP production and membrane alteration are not completely understood (Kerter *et al.*, 2017). Many researchers have reviewed the membrane alteration because the cell membrane was the first part of the cells to interact with the environment and suggested the lipid oxidation of the cell membrane might underlie loss of seed viability (Spano *et al.*, 2016). Many researchers analyzed phospholipids changes and raised the possibility that membrane peroxidation was associated with aging (Kerter *et al.*, 2017).

Seed of oil crops are highly vulnerable to deterioration. They cannot be stored for a long time and are described to have poor storage capacity. The seed quality of peanut and

soybean is difficult to maintain in storage. Sesame seed was found to be a „poor sorer“ compared with soybean and peanut seed. In oil seed crops, the seed quality is significantly affected by unfavorable storage conditions. Changes that occur in seed during aging are significant in terms of seed quality, the feature that, among other things, also implies seed longevity (Spano *et al.*, 2016).

Seeds deteriorate during storage, aging is manifest as a reduction in percentage germination, resulting less and weak seedlings. During the aging process, seeds lose their vigor, ability to germinate and ultimately become less viable (Kerter *et al.*, 2017). Losses in seed quality occur during field weathering, harvesting and storage. The losses are exacerbated if seeds are stored at high temperatures or conditions of high relative humidity. Cottonseed is one of the most sensitive agronomic seeds susceptible to significant deterioration after just one year's storage. Cottonseed like other oil seeds is more prone to deterioration due to its high oil content (Powell *et al.*, 2018).

### **2.10.1 Types of seed deterioration**

Deterioration is evident as a decrease in percentage germination, while those seeds that germinate produce weak seedlings. Losses in seed quality occur during field weathering, harvesting and storage (Mosavi *et al.*, 2017). Harvesting time of several crops depends on its maturity time and on physiological maturity. Harvesting stage influences the quality of seed, germination, vigor, viability and also storability. Physiological maturity attainment is a genotypic character which is influenced by several environmental factors (Mosavi *et al.*, 2017). Deterioration of seed in the field before harvest (field weathering) begins when the seed reaches physiological maturity and it extends till the seeds are harvested. The moisture content of physiological matured seed is approximately 50-55%. Because of the high

moisture content, the seed cannot be harvested commercially and must remain in storage on the plant through a desiccation period till moisture levels are adequately low to permit mechanical harvest without causing undue damage to the seed. This desiccation period varies from a few days to several weeks before the seed attains a harvestable moisture level, about 14%. During this post maturation, pre-harvest period weather conditions have a great influence on the quality of the harvested seed. Field conditions are rarely favourable for such storage. Seed quality is influenced by numerous factors that occur in the field before harvesting and during harvesting, drying, processing and storage. The losses are worsened if seeds are stored at high temperatures and high relative humidity conditions (Mosavi *et al.*, 2017).

#### **2.10.1.1 *Field weathering***

The deterioration of seed quality, vigour and viability, due to high relative humidity and high temperature during the post-maturation and pre-harvest period is referred to as field weathering (Bhatia *et al.*, 2019). Weathering occurs in the period between the attainment of physiological maturity till harvesting in the field. Deterioration caused by weathering is directly related to seed exposure to adverse conditions, so that the physiological quality is depending on the environmental conditions preceding harvesting. Exposure to hot and humid conditions, rainfall, photoperiod after ripening are pre-harvest factors, cause seed quality loss following physiological maturity. Among all these factors, influence of moisture on seeds during ripening appears to exert the major influence on predisposition to weathering. Adverse environmental conditions during seed filling and maturation result in forced seed maturation, which is associated with low yields, leading to a significant decrease in quality and an extensive reduction in the crop productivity (Bhatia *et al.*, 2019).



After physiological maturity if the seeds are retained on mother plant seeds will deteriorate, physiological changes in seed may lead to formation of rigid seeds or off colour seeds in pulse crops. Harvest delays beyond optimum maturity extend field exposure and intensify seed deterioration. Weathering not only lowers seed germination, but also increases susceptibility to mechanical damage and disease infection. Timely harvesting avoids prolonged exposure to moisture and is the best means of avoiding weathering (Bhatia *et al.*, 2019).

#### **2.10.1.2 Harvest and post-harvest deterioration**

Seed quality is highly affected by harvesting and handling methods. Harvest and post-harvest deterioration comprises threshing, processing machinery, seed collection, handling, transporting and drying. Mechanical damage is one of the major causes of seed deterioration during storage. Very dry seeds are prone to mechanical damage and injuries. Such damage may result in physical damage or fracturing of essential seed parts; broken seed coats permit early entry and easy access for microflora, make the seed vulnerable to fungal attack and reduce storage potential (Shelar, 2018). In its severest form, physical seed damage is exhibited by splitting of the cotyledon, shattered and broken seeds. Large seeded varieties are more sensitive to mechanical damage than small seeds.

#### **2.10.1.3 Storage**

Storability of seeds is mainly a genetically regulated character and is influenced by quality of the seed at the time of storage, pre-storage history of seed (environmental factors during pre and post-harvest stages), moisture content of seed or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents (Shelar *et al.*, 2018). Damage of seed during storage is inevitable. These environmental conditions are

very difficult to maintain during storage. The seed storage environment highly influences the period of seed survival. After planting of deteriorate seeds, seedling emergence may be poor and transmission of pathogens to the new crop may occur. Lower temperature and humidity result in delayed seed deteriorative process and thereby leads to prolonged viability period (Mohammadi *et al.*, 2017).

### **2.10.2 Mechanisms of seed deterioration**

Once seed deterioration has happened, this catabolic process cannot be reversed. It is a sequence of events beginning with a chain of biochemical events, predominantly membrane damage and impairment of biosynthetic reactions, and then the resulting losses of various seed performance attributes, starting with reduced germination rate, reduced field emergence, increased numbers of abnormal seedlings and finally seed death. Viability loss results in irreversible chemical and structural changes to cellular constituents (Walters and Sun, 2016).

Structural changes associated with oxidation are reduced membrane fluidity, altered folding of DNA, lost elasticity of proteins and increased brittleness of the cellular matrix. Molecules oxidation leads to either smaller molecules with reactive carbonyl or nitrogen groups that easily diffuse through cells, or adducts between carbohydrates, proteins and nucleic acids that cause intermolecular cross-linking and further degrade into advanced glycation end-products (Walters and Sun, 2016).

### **2.10.3 Biochemical manifestation of seed deterioration**

Seed deterioration is associated with various cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity

(Kibinza *et al.*, 2017). Some of the major physiological and biochemical events of deterioration are presented below.

Major constituents of the seeds are the lipids, carbohydrates, proteins and, of course, the nucleic acids. However, the proportion of each component varies in seeds of various species. Some seeds are rich in lipids or proteins or both while the others are rich in carbohydrates. Any of these components may be damaged during the process of seed deterioration. However, the various reactions leading to this damage are not clearly understood. Several models have been proposed to explain the process of seed deterioration. However, there is no evidence for a single determinant of any one of the symptoms of deterioration. Probably, the exact mechanism is the combination of all these models. Nevertheless, lipid peroxidation model has stimulated the greatest interest of the scientists all over the world and seems to be the major cause of seed deterioration especially in oilseeds. The various models proposed are explained below:

#### **2.10.3.1 *Lipid peroxidation***

The phenomenon of incipient death during germination of aged seeds suggests the presence of destructive element which becomes active only after imbibition. The lipid peroxidation model proposes that this destructive element is oxygenated fatty acids. They can be produced through autoxidation or enzymatically (Wilson and McDonald, 2016). During autoxidation fatty acid hydrocarbon chains spontaneously oxidize in the presence of oxygen and thereby producing reactive free radical intermediates, known as hydroperoxides. The polyunsaturated fatty acids are more susceptible for this reaction, as they contain a methylene group between the two double bonds which is highly reactive and can very lose hydrogen free radical (H·). The rate of this reaction is greatly enhanced by the class of

enzymes called lipoxygenases (LOXs), which are found in many different seeds. The reaction sequence for the non-enzymatic autoxidation is given in Figure 2.9. Once a free radical is produced, a chain reaction is initiated which creates additional reaction cycles and free radicals. The reaction is terminated by combination of two free radicals producing stable end products. Further, the mechanism of lipid peroxidation may differ in seed depending upon their moisture content.

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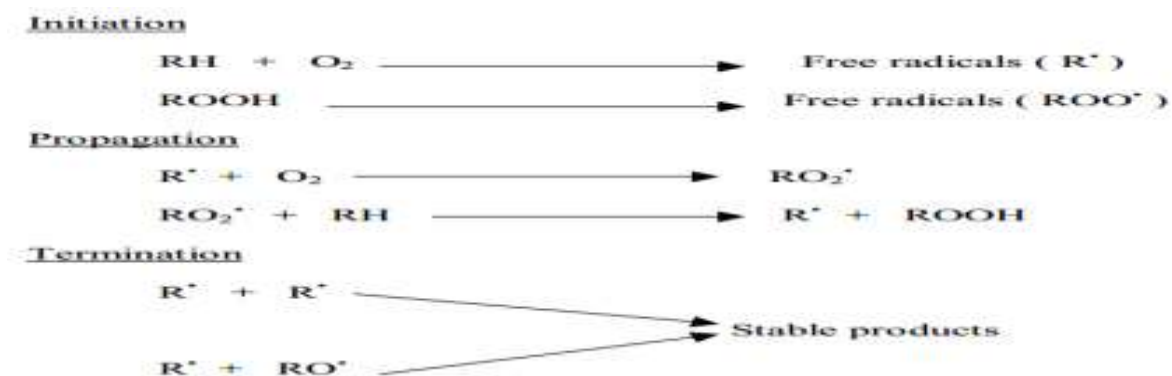
content, lipid peroxidation is likely to be at a minimum because sufficient water is available to serve as a buffer against autoxidative free radical attack but not enough water is present to activate lipoxygenase-mediated free radical production. Thus, the mechanism of lipid peroxidation may be different under accelerated (high relative humidity) and natural (low relative humidity) aging.

Figure 2.9: Free radical chain reaction resulting in autoxidation (Wilson and McDonald, 2016) The free radicals possess a potential for short distance damage due to their short life time. The long distance detrimental effects of lipid peroxidation are due to their conversion into more stable chemical species (Golovina *et al.*, 2018). It has actually been found that the fatty acid hydroperoxides may further be reduced or degraded resulting in the formation of wide array of stable secondary products such as epoxides, hydro-epoxides, hydroxy fatty acids, aldehyde and ketones (Dahuja and Lodha, 2015). The factors triggering hydroperoxide breakdown include heat, enzymes, cytochromes and transition metal ions. Lipid peroxidation is potentially damaging to seeds in three ways.

### **2.11. Membrane degradation**

It is extensively consented that loss in cellular membrane integrity is one of the primary causes for loss of viability. Under harsh storage conditions loss in membrane permeability leads to increased leaching of seed constituents and hence loss in viability. During seed deterioration, membrane degradation increases electrolyte leakage. Decline in seed germination, field emergence and seedling vigour is associated with high level of electrolytes leakage. Membrane deterioration and loss of permeability occur at an early stage during the seed deterioration (Marcos, 2015).

Biomembranes represent a key site of direct injury from lipid peroxidation as they possess an inherently large surface area and usually have more unsaturated fatty acids than storage lipids. Lipid peroxidation of membranes may lead to decline in membrane integrity and to an increase in membrane permeability (Mohan and Knowles, 2018).



**Figure 2.9:** Free Radical Chain Reaction Resulting in Autoxidation (Wilson and McDonald, 2016)

A loss in the integrity of plasma membrane has been demonstrated in aged seeds by the extent of leakage of cytoplasmic components to the external medium (Dahuja and Lodha, 2015). Increased membrane permeability is more common in mitochondrial membranes as they are rich in unsaturated acyl chains. Here, it may have fatal effects as it can lead to a breakdown of the proton gradient necessary to maintain respiratory coupling (Dahuja and Lodha, 2015).

## 2.12 Co-oxidation of Associated Cellular Components by Free Radical Transfer From Oxidized Lipid

Normally stable molecules are incorporated into the autoxidative chain reaction by abstraction of hydrogen atoms. In addition, lipid peroxide and their secondary products can react with terminal groups of amino acids in proteins and enzymes. The lipid peroxidation

has been reported to stimulate the formation of Schiff bases between peroxidised phospholipids and membrane proteins (Spickett *et al.*, 2015). This non-enzymatic reaction may lead to polymerization of proteins.

### **2.13 Formation of Cytotoxic Aldehydes**

It has been reported that deteriorated seeds produce twenty times more volatile aldehydes during imbibition than fresh seeds. The aldehydes formed by hydroperoxide break down produce a variety of cytotoxic effects. These aldehydes react with sulfhydryl groups leading to an inactivation of proteins. For example, they have been shown to inhibit tubulin, the main protein of microtubules which is necessary for mitotic spindle formation (Petry, 2016). In addition, aldehyde strongly inhibit protein and DNA synthesis (Brooks and Zakhari, 2014).

### **2.14 Enzymes Alterations**

Metabolism of the seeds is greatly affected during their storage. This is mainly due to modulation of various enzyme activities present in the seeds. The alterations in the activity of enzymes could be brought about by compositional or configurational changes in their structure which include: Partial folding or unfolding, degradation to subunits, and condensation to form polymers.

There is activation of some enzymes especially the hydrolytic enzymes during storage. But, this is highly dependent upon the moisture level of the seeds. If moisture content reaches higher level, normal germination may occur, however if moisture levels for germination are not attained, the seed deteriorates because of energy expenditure or accumulation of breakdown products. The various hydrolytic enzymes activated by high moisture levels are: lipase, phospholipase, protease, DNase, phosphatase and amylase (Fu *et al.*, 2015).

Reactive oxygen species (ROS) and hydrogen peroxides are produced from several metabolic reactions and could be destroyed by the activity of scavenger and catabolic enzymes like catalase and peroxides (Sharma *et al.*, 2017). Peroxides activity decreases substantially with ageing. Due to this seed become more sensitive to the effects of oxygen and free radicals in membrane unsaturated fatty acids and produce lipid peroxidation products such as monaldehyde and lipid conjugants.

## **2.15 Alterations of Protein Metabolism**

### **(a) Protein synthesis**

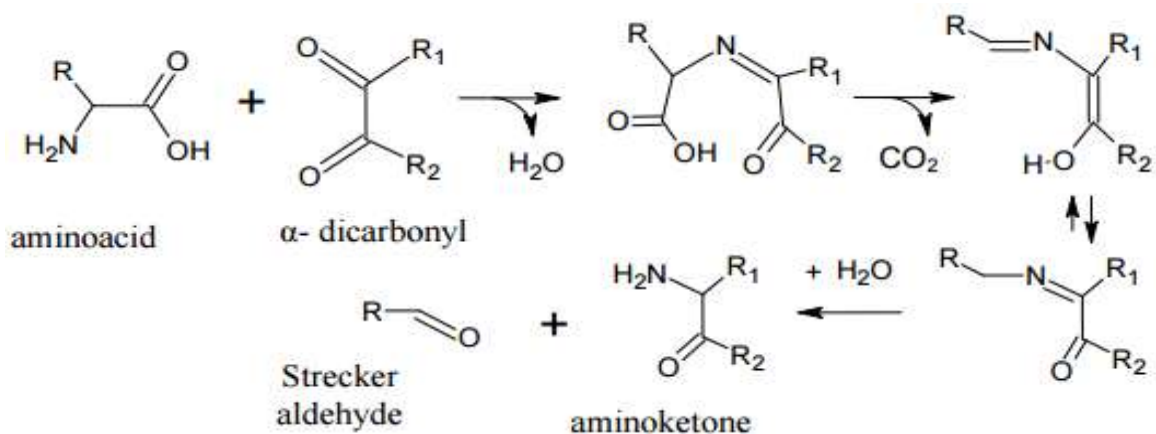
The studies with radioactive amino acids in many seed species have shown that seed deterioration is associated with reduced capacity for protein synthesis (Desai, 2019). The inability to synthesize proteins is accompanied by an excessive loss of capacity to synthesize RNA (Turner and Monzón-Casanova, 2017), which could be a consequence of damage to nuclear DNA. However, protein synthesis is also impaired at the level of translation. It has been found that ribosomes from non-viable seeds have reduced activity. This could result from adverse structural modifications to the ribosomes, or from loss of one or more rRNA species or ribosomal proteins (Polikanov *et al.*, 2015). The retardation of protein synthesis could also be due to failure of the polyribosomes to dissociate. However, the loss of protein synthesis is not associated with changes in tRNA or aminoacyl-tRNA synthetase activity. In addition, reduction of ATP (and possibly GTP) synthesis in non-viable seeds can affect protein and RNA synthesis and it is likely that there is an important link between nucleoside triphosphate levels in seeds and their capacity to carry out essential metabolic functions.



**(b) Protein inactivation**

The inactivation of proteins during seed storage may lead to deterioration as it depresses the metabolic capacity and reduces the ability of metabolic system to repair the damages incurred during storage (Dahuja and Lodha, 2015). The proteins may be inactivated by losing or gaining certain functional groups, by oxidation of sulfhydryl groups or by conversion of amino acids within the protein structure. The spontaneous deamination, isomerization and racemization of L-asparaginyl and L-aspartyl residues of proteins has been observed during cellular aging (Homma, 2017; Sydow *et al.*, 2019). This leads to the accumulation of detrimental residues like L-isoaspartyl in the proteins which have been shown to alter the structure and function of proteins. Changes in the protein structure could also be attributed to attack of free radicals. The soluble proteins may be attacked by different classes of oxidants than membrane proteins. The most reactive amino acids susceptible to oxidative damage appear to be cysteine, histidine, tryptophan, methionine, and phenylalanine, usually in that order (Bhattacharya and Chakraborty, 2015).

Furthermore, proteins may be modified by non-enzymatic glycation through Amadori and Maillard reaction (Popova *et al.*, 2016). The Amadori reaction involves attack on amino groups of protein by reducing sugars to form glycated proteins. The Maillard reaction represents subsequent complex interactions between the glycated Amadori products to form polymeric, brown coloured products, Hence the term "browning reaction". The cytotoxic volatile aldehydes are also produced following Strecker degradation of Maillard products (Dahuja and Lodha, 2015) (Figure 2.10).

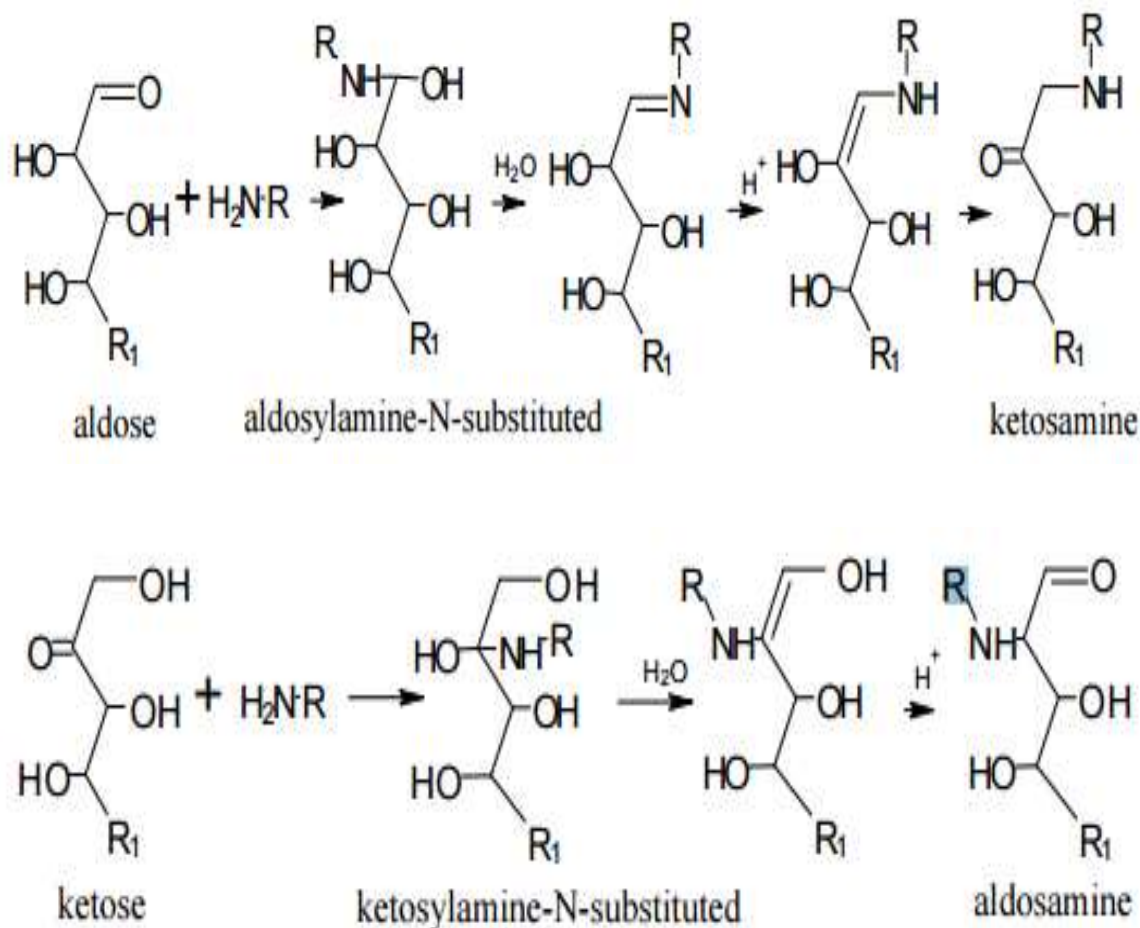


**Figure 2.10:** Strecker Reaction (Whitfield and Mottram, 2019)

These non-enzymatic reactions seem to be the most probable cause for inactivation of proteins during seed deterioration, because dry seeds lack active enzymatic metabolism. As an example, Amadori and Maillard products were found in soybean seeds subjected to accelerated ageing and formed most rapidly in seeds at 40% to 80% relative humidity (Bhatia *et al.*, 2019). A marked increase in reducing sugars such as glucose and galactose has been observed in deteriorating maize seeds (Jyoti and Malik, 2018) that may enhance Amadori and Maillard reactions because of the presence of reactive semialdehyde group in these sugars.

Although the Maillard reaction can occur at refrigeration temperatures, the reaction rate increases significantly when the temperature is increased (Joe, 2015). By contrast, caramelization or the pyrolysis of proteins, needs high temperatures (100–200 °C) to develop. It has also been observed that Maillard reaction is affected by water activity and that any subsequent reaction pathways depend heavily on pH. The nature of the reacting compounds, e.g., type of sugar, amino acid, or protein, the presence of substances that can interfere with the reaction (e.g., carbonyl compounds derived from lipid oxidation), and even the matrix can influence the profile of the resulting aroma compounds.

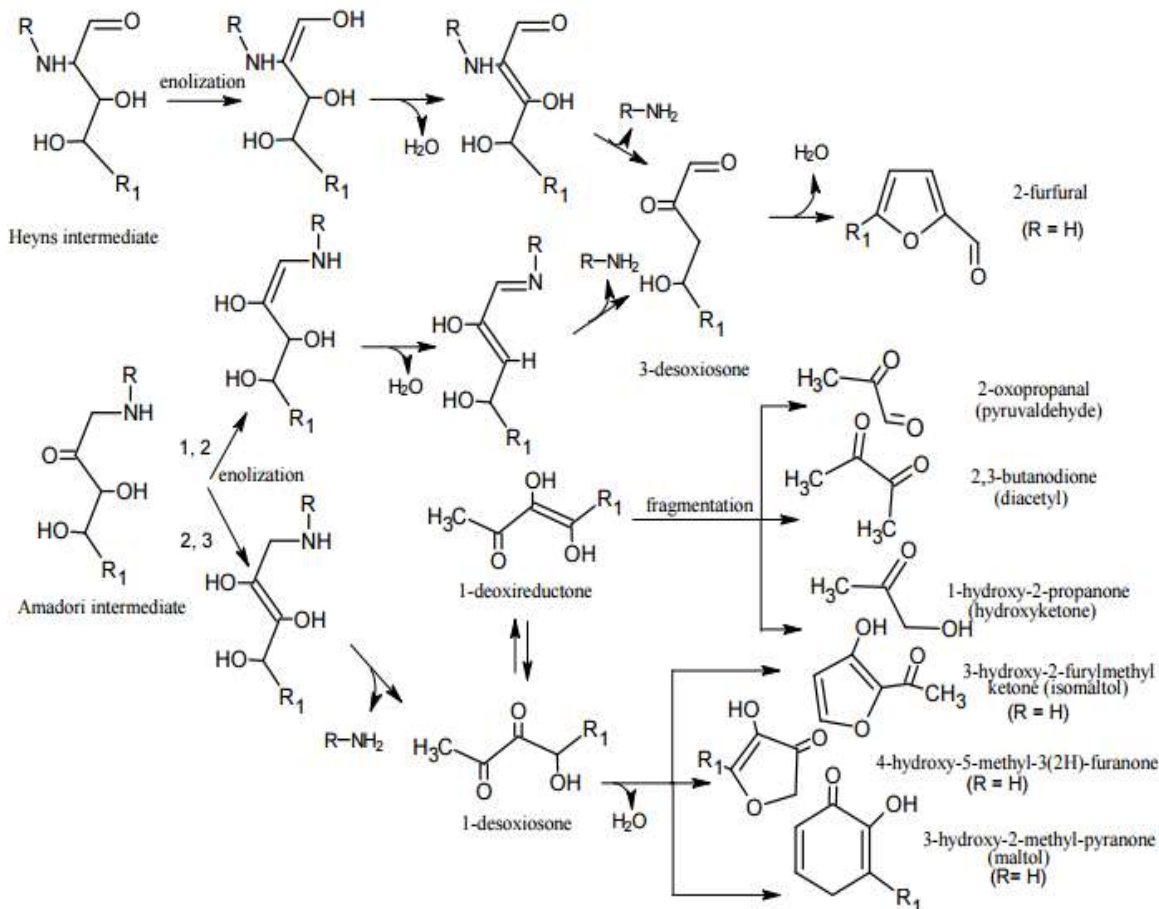
If the initial reducing sugar is an aldose monosaccharide that has an aldehyde group, e.g., ribose or glucose, it forms an aldosylamine-N-substituted, which is rearranged to form an intermediary amadori (Figure 2. 11). If the reducing sugar is a ketose monosaccharide that has a ketone group, e.g., ribulose or fructose, Heyns rearrangement of ketosylamine-N-substituted and the corresponding Heyns intermediary are produced (Figure 2.11).



**Figure 2.11:** Heyns Rearrangement (Fisher and Scott, 1997)

Next, the Amadori/Heyns products are degraded, which leads to dehydrations and deaminations that produce carbonyl compounds such as deoxysones and deoxyreductones. Then, many types of reactions are produced (e.g., dehydrations, fragmentations, cyclizations, and polymerizations) in which the amino groups can participate again, for

example, through the Strecker reaction (Van Boekel, 2016). In Figure 2.12, a general path of some of those reactions is outlined.



**Figure 2.12:** Decomposition of Heyns and Amadori Intermediates (Bailey, 2019)

### (c) Protein hydrolysis

Lower level of protein in non-viable seeds than in viable seeds may be due to the hydrolysis of proteins as is indicated by the fact that there is increase in total free amino acids after aging treatment. Further leaching of the amino acids into the imbibing medium has also been reported. This assumption is further supported by the high protease activity in the seeds exposed to accelerated aging conditions (Calucci *et al.*, 2014).

## 2.16 Impairment of Genetic Functions

One of the changes linked with seed ageing is aberration of chromosomes, sometimes pertained to as mutagenic effects. Some of the chromosome alterations in seeds comprise fragmentation, bridges, fusion, ring formation of chromosomes and variations in nuclear size (Jyoti and Malik, 2018).

Studies by a number of workers on a variety of seeds and grains have shown that almost any combination of time, temperature and moisture content that leads to a loss of viability during storage also leads to genetic damage in the survivors (Bewley and Black, 2012; Milošević *et al.*, 2016). This damage could be in the form of fragmentation of DNA into low molecular weight components, perhaps due to activation of DNase(s). Also, damage to nucleic acids could be a consequence of a build-up of chemical mutagens during storage.

The following observations have been made in the support of above theory:

1. Extracts from aged seeds retard the germination of fresh seeds, and
2. Mutations in seeds are highly correlated with seed age and decline in seed viability.

Free radicals are also suspected of assault on chromosomal DNA. Potential targets for oxidative damage in the DNA chain include the purines and pyrimidine bases as well as the deoxyribose sugar moieties (Vijay *et al.*, 2019; Roldán-Arjona and Ariza, 2019). Specific damage to the bases may leave the strand intact but modification of sugar residues can also lead to strand breakage. This could be one of the explanations for the increased propensity for genetic mutations as seeds age. Further it has been reported that there is an increase in chromosome damage with increase in the period of storage. Extensive chromosomal damage could result in impaired template activity of DNA, and reduced RNA and protein synthesis. However, damage to segments of the DNA containing the genes for repair

enzymes could be more deleterious than damage to less essential DNA fragments. It is well established that DNA degradation occurs during seed deterioration, several factors, however, argue against it (Torres, 2017). First, nuclear DNA is highly conserved against free radical attack. The molecule is enclosed by a protective nuclear membrane and is surrounded by histone proteins. Therefore, it is more likely that free radicals must first penetrate the nuclear membrane and destroy protective histones before causing any damage to nuclear DNA. However, the mitochondrial DNA seems to be more susceptible to free radical attack as it lacks any protective membrane and no histone proteins are associated with it (Shah *et al.*, 2019). Secondly, the integrity of DNA has little effect on transcription of the earliest events of germination though they are the first markers of seed deterioration.

### **2.17 Factor Affecting Seed Deterioration**

The rate of seed deterioration is highly influenced by environmental (temperature, relative humidity and seed moisture content) and biological factors (such as fungi that create their own biological niche) (Ghasemi-Golezani *et al.*, 2016). Seed longevity is determined by seed moisture, temperature and seed attributes that are influenced by genetic and environmental interactions during seed maturation, harvesting and storage (Walters and Sun, 2016). Several other factors such as environmental conditions during seed producing stage, pests, diseases, seed oil content, storage longevity, mechanical damages of seed in processing, fluctuations in moisture (including drought), weathering, nutrient deficiencies, packaging, pesticides, improper handling, drying and biochemical injury of seed tissue can affect vigor of seeds (Walters and Sun, 2016).

### **2.17.1 Kind and variety of the seed**

The seed storability is considerably determined by the kind or variety of seeds. Some seeds are naturally short-lived, e.g., onion, soybeans, peanuts, etc., whereas some seeds like, tall fescue and annual rye grass, appear very similar but differ in storability. Genetic make-up of varieties also influences storability.

### **2.17.2 Genotypic factors**

Some types of seeds are inherently long lived; others are short lived, while others have an intermediate life span owing to their differences on genetic makeup.

### **2.17.3 Initial seed quality**

High initial viability of seeds maintains their quality in storage longer than those with less initial viability. Vigorous and undeteriorated seeds can store longer than deteriorated seeds. Seeds that have been broken, cracked, or bruised due to handling deteriorate more rapidly in storage than undamaged seeds. Cracks in seeds serve as entrance to pathogens causing consequent deterioration. Seeds that have been developed under environmental stress conditions (such as drought, nutrient deficiency and high temperatures) become more susceptible to rapid deterioration (Santhosh, 2015).

### **2.17.4 Temperature**

High temperature hastened the rate of these biochemical processes triggering more rapid deterioration that resulted in rapid losses in seed having high moisture content (Shelar *et al.*, 2018). Seeds sensitivity to high temperatures is strongly dependent on their water content, loss of viability being quicker with increasing moisture content (Kibinza *et al.*, 2015). Temperature is important because it influences the amount of moisture and also enhances the rate of deteriorative reactions occurring in seeds as temperature increases.

### **2.17.5 Moisture content**

Deteriorative reactions occur more readily in seeds at higher moisture content and subsequently, this condition constitute hazard to the longevity of seed survival (Jyoti and Malik, 2018).

Seeds stored at high moisture content demonstrate increased respiration, heating, and fungal invasion resulting in reduced seed vigor and viability. After physiological maturity the rate of seed quality loss depends on the degree of unfavorable environmental conditions surrounding the seed. Environmental moisture, predominantly intermittent or prolonged rainfall, during the post maturation and pre-harvest period, is quite detrimental to seed quality and cause rapid deterioration (Santhosh, 2015). When exposed to humid conditions (heavy rain), dried seeds can absorb enough moisture to reach 27% and subsequently expand in volume. At this moisture level, seed respiration is hastened. Cotyledonary reserves will be consumed, not only by the seed itself, but also by fungi allied with the seed. It has been reported that seed moisture content of about 6-8% is optimum for maximum longevity of most crop species (Jyoti and Malik, 2018). Below 4-6% seed moisture content lipid autoxidation becomes a damaging factor and seeds become more susceptible to mechanical damage. The moisture content of seed during storage is the most persuasive factor affecting the longevity.

Storing seeds at high moisture content enhances the risk of quicker deterioration at shorter time (Santhosh, 2015). Seeds are hygroscopic in nature; they can pick up and releases moisture from and to the surrounding air. They absorb or lose moisture till the vapor pressure of seed moisture and atmospheric moisture reach equilibrium (Shelar *et al.*, 2018). Control of relative humidity is the most important because it directly influences the



moisture content of seeds in storage as they come to equilibrium with the amount of moisture surrounding them; a concept known as equilibrium moisture content. The lower the moisture content, the longer seeds can be stored provided that the moisture level can be controlled all through the storage period (Santhosh, 2015).

#### **2.17.6 Organisms associated with seeds**

Organisms associated with seeds in storage are bacteria, fungi, mites, insects and rodents. The activity of these entire organisms can lead to damage resulting in loss of vigor and viability or, complete loss of seed.

##### **a) Bacteria and fungi**

There are several factors which favor infection fungi and promote their infestation such as moisture content of seed and interspace relative humidity, temperature, prestorage infection and storage pest. Most storage fungi belong to *Penicillium* and *Aspergillus* genera. They induce seed deterioration by producing toxic substances that destroy the cells of seeds. Mechanically damaged seed allow quick and easy access for mycoflora to enter the seed (Shelar *et al.*, 2018). To minimize the risk of fungi invasion, seeds have to be stored at low moisture content, low temperature, and relative humidity.

Researches show that all storage fungi are completely inactive below 62% relative humidity and show very little activity below about 75% relative humidity upwards, the amount of fungi in a seed often shows an exponential relationship with relative humidity. The storage bacteria require at least 90% relative humidity for growth and therefore only become significant under conditions in which fungi are already very active (Santhosh, 2015).

#### **b) Insect and mites**

There is no insect activity at seed moisture contents below 8%, but if grain is infected, increased activity may generally be expected up to about 15% moisture content. The optimum temperature for insect activity of storage insects ranges from 28 to 38°C (My agricultural Information Bank, 2015). The temperatures below 17 to 22°C are considered unsafe for insect activity. Although it is usually preferable to control insect and mite activity by the manipulation of the seed environment, i.e., use of fumigants and insecticides. The main problem of chemical control is the adverse effect of chemicals on seed viability and vigor, and some of them are dangerous to handle (Jyoti and Malik, 2018). However, fumigants which have been used successfully include methyl bromide, hydrogen cyanide, phosphine, ethylene dichloride and carbon tetrachloride in 3:1 mixture, carbon disulphide and naphthalene. Insecticides – used in seed storage include DDT, lindane and Malathion.

#### **c) Oxygen pressure**

Recent researches on the role of a gaseous environment on seed viability indicate that increases in pressure of oxygen incline to decrease the viability period (My agricultural Information Bank, 2015).

#### **d) Other factors**

Factors besides those discussed above that affect seed storage life are the direct sunlight on the seed, number of times and kind of fumigation, effect of seed treatment, etc.

## **2.18 Methods for Testing Seed Deterioration**

### **2.18.1 Germination test**

It is an analytical procedure to evaluate seed germination under standardized, favorable conditions. Standard germination testing includes media, temperature, moisture, light, dormancy breaking and germination counting standard for various crop seeds (Jyoti and Malik, 2018).

### **2.18.2 Tetrazolium (TZ) test**

TZ test is extensively accepted as an accurate mean of estimating seed viability. This method was developed by Professor Georg Lakon in the early 1940s. It is quick method to estimate seed viability (Jyoti and Malik, 2018). This test distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state.

### **2.18.3 Electrical conductivity test**

As seed deterioration progresses, the cell membranes become less rigid and become more water permeable. It allows the cell contents to leakage into solution with the water and increasing electrical conductivity. It provides a rapid indication of seed viability for seed lots (Jyoti and Malik, 2018).

### **2.18.4 Vital colouring test**

The principle of this method is the differential coloration of live against dead tissues when exhibited to certain dyes such as sulfuric acid, indigo carmine and aniline dyes. These dyes stain the dead tissue blue and the live tissue leftovers unstained. This method is particularly useful for determining viability of tree seeds.

### **2.18.5 Enzyme activity test**

These methods measure enzyme activity (such as lipase, amylase, diastase, catalase, peroxidase and dehydrogenase) of imbibed seeds as an indication of their viability (My agricultural Information Bank, 2015).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Materials**

##### **3.1.1 Plant sample**

Sesame seeds were collected from field-grown Nigerian accessions during the 2018 growing season (July – October) at the experimental farm of National Cereal Research Institute (NCRI), Badeggi, Niger State, Nigeria. Approximately, 100 g of sesame seeds harvested at maturity were collected, properly labeled and sun-dried. And taken to the laboratory for further analysis.

##### **3.1.2 Chemicals and reagents**

All the chemicals and reagents used were of analytical grade (AOAC, 2009).

#### **3.2 Methods**

##### **3.2.1 Experimental design**

Twenty accessions of sesame were characterized for oil yield and two accessions that had the highest, median and lowest mean oil yield values of the accessions were then selected for accelerated ageing in a biochemical incubator at a temperature of 40°C and relative humidity of 80% respectively for 0, 3 and 6 days. The aged seeds were then subjected to the various biochemical analyses.

##### **3.2.2 Preparation of sesame seed samples**

The sesame seeds accession were dehulled manually using soaking and the nibs collected. The nibs were then milled with electronic blender and used for oil extraction.

### **3.2.3 Oil extraction**

For Soxhelt extraction, 2 g of the milled sample was placed in a paper thimble and fed into a Soxhlet extractor which was fitted with a 500 mL round bottom flask and a condenser. After extraction, the extra hexane was distilled off under vacuum in a rotary evaporator at 45°C. The extracted oil was weighed and the yield was calculated.

### **3.2.4 Characterization of sesame seeds**

Twenty accessions of sesame were characterized for oil yield and two accessions that had the highest, median and lowest mean oil yield values of the accessions were then selected for accelerated ageing in a biochemical incubator at a temperature and relative humidity of 40°C and 80% respectively.

### **3.2.5 Determination of seed ageing**

Five selected accession of sesame seeds were subjected to accelerated aging in a biochemical incubator at a temperature and relative humidity of 40°C and 100% respectively for 0,3, and 6days. extraction, the extra hexane was distilled off under vacuum in a rotary evaporator at 45°C (Federation of Oils Seeds and Fats Association (FOSFA, 2018). The extracted oil was weighed and the yield was calculated.

### **3.2.6. Proximate Analysis**

A sample of seeds from each accession was taken to the laboratory for proximate analysis for the determination of moisture, protein, fat, carbohydrate, ash and fibre content in the seeds. The method of proximate analyses was according to the standard procedure of AOAC (2009).

### **3.2.6.1 Moisture Determination**

Moisture will be determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. The crucibles were weighed ( $W_1$ ) and 5 grams of the sample was added into the crucible to give a new weight ( $W_2$ ). It was then placed in the oven at  $105^\circ\text{C}$  for 3hrs, after which it was removed, allowed to cool in the desiccator and oven dried again, this was repeated several times until constant weight was noted ( $W_3$ ).

$$\% \text{ Moisture} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where;

$W_1$  = weight of sample

$W_2$  = weight of petri dish + sample before drying

$W_3$  = weight of petri dish + sample after drying

### **3.2.6.2 Determination of Crude Fibre**

The organic residue left after sequential extraction of seeds with petroleum ether can be used to determine the crude fibre. The fat-free material will then be transferred into a flask/beaker and 200 mls of pre-heated 1.25%  $\text{H}_2\text{SO}_4$  will be added and the solution will be gently boiled for about 30 mins, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with whatman filter will be pre-heated by pouring hot water into the funnel. The boiled acid sample mixture will then be filtered hot through the funnel under sufficient suction. The residue will then be washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200mls of pre-heated 1.25%  $\text{Na}_2\text{SO}_4$  will be added and boiled for another 30mins. It will then be Filter under suction and wash thoroughly with hot water and twice with ethanol. The residue will be dried at  $65^\circ\text{C}$  for about 24hrs and weighed. The residue

will be transferred into a crucible and placed in muffle furnace (400-600<sup>0</sup>C) and ash for 4hrs, then it will be cool in desiccator and weigh.

$$\% \text{Crude fibre} = \frac{\text{Dry weight of residue before ashing} - \text{weight of residue after ashing}}{\text{weight of sample}} \times 100$$

### 3.2.6.3 *Determination of Crude Protein*

Crude protein will be determined by measuring the nitrogen content of the seeds and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein will be determined by kjeldahl method. The method involves: Digestion, Distillation and Titration.

**Digestion:** About 2 g of the sample will be weighed into kjeldahl flask and 25mls of concentrated sulphuric acid, 0.5 g of copper sulphate, 5 g of sodium sulphate and a speck of selenium tablet will be added.

Heat will be applied in a fume cupboard slowly at first to prevent undue frothing and continue to digest for 45mins until the digesta become clear pale green. Leave until completely cool and rapidly add 100mls of distilled water. The digestion flask will be rinsed 2-3 times and add the rinsing to the bulk.

**Distillation:** Markham distillation apparatus will be used for distillation. Steam up the distillation apparatus and add about 10mls of the digest into the apparatus via a funnel and allow it to boil.

Add 10mls of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 50 mls of 2% boric acid containing screened methyl red indicator.

**Titration:** the alkaline ammonium borate formed will be titrated directly with 0.1NHCl. The titre value which will be the volume of acid used will be recorded. The volume of acid used will be fitted into the formula which becomes



$$\% \text{Nitrogen} = \frac{14 \times \text{VA} \times 0.1 \times w}{1000 \times 100} \times 100$$

VA = volume of acid used, w= weight of sample, %crude protein = %N x 6.25

#### **3.2.6.4 Determination of Ash content**

Ash is the inorganic residue obtained by burning off the organic matter of the seed at 400-600°C in muffle furnace for 4hrs. Crucible was pre-heated in the oven for 30mins at 105°C, cooled in the desiccator for about 1hr and weighed (W<sub>1</sub>). 2 grams of the sample was added into crucible, given a new weight (W<sub>2</sub>) It was then being placed in the muffle furnace for ashing at 55°C for 3 hrs until the content became whitish in color with no black particles, it was removed and cooled in the desiccator, the weight was noted (W<sub>3</sub>).

$$\% \text{ Ash} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where;

W<sub>1</sub> = weight of crucible

W<sub>2</sub> = weight of crucible + sample before ashing

W<sub>3</sub> = weight of crucible + sample after ashing

#### **3.2.6.5 Determination of crude fat**

A powdered moisture free millet samples will be weighed into 3 different pre-weighed fat – free Filter paper and wrapped separately. Filter paper free from fat was weight (W<sub>1</sub>). About 1 gram of the sample was added into the filter paper, carefully folded and tied to keep the sample intact, the new weight noted (W<sub>2</sub>). A 500ml round bottom flask was filled up to three-quarter with solvent (petroleum-ether). The flask was fitted to Soxhlet extraction with a reflux condenser and placed on an electro-mantle heater. Extraction began as the solvent start refluxing several times. Extraction continued for about 6 hrs after which the condenser was detached, the defatted sample removed, and dried to a constant weight in the oven at

105°C for 2 hrs. The difference between the weight of the defatted sample before and after drying was recorded as the weight of fat ( $W_3$ ).

$$\text{Fat (\%)} = \frac{(W_2 - W_1)}{W} \times 100$$

### **Nitrogen Free Extract (NFE)**

NFE will be determined by mathematical calculation. It will be obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

$$\% \text{NFE} = 100 - (\% \text{moisture} + \% \text{CF} + \% \text{CP} + \% \text{EE} + \% \text{Ash})$$

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in seeds.

### **3.2.7 Determination of fatty acid composition**

The oil obtained by Soxhlet extraction was converted into their fatty acid methyl esters (FAMES). The FAMES were then analyzed on a gas chromatography (Agilent technologies 7890A, USA). A polar capillary column (SP-2330; 30 m × 0.25mm; Supelco Inc., Supelco Park Bellefonte, PA) was used for the separation of fatty acids. Mobile gas phase was nitrogen and was used at a flow rate of 1.5 mL/min. Column oven initial temperature was set at 180 °C and programmed by the linear increment of 5°C/ min to final temperature of 220 °C whereas hold up 2 min before and 10 min after the run were employed. Other conditions were set as follows: injector temperature, 230 °C; detector (FID) temperature, 250 °C. Identification of targeted fatty acid compounds were based upon matching their relative and absolute RT (Retention Times) against those of absolute/pure standards of FAMES. The fatty acid composition was reported as relative percentage of the total peak area.

### 3.2.8 Amino Acid Analysis

Five grams of the samples were weighed into a sterile furnace hydrolysis tube 5nmols nor-leucine was added to the samples and then dried under a vacuum. The tube was placed in a vial containing 10.05N HCl with a small quantity of phenol, thereby hydrolyzing the protein by the HCl vapour under vacuum. This stage of hydrolysis of the sample lasted for between 20-23 hours at 180°C. After the hydrolysis, the samples were dissolved in ultra-pure water (HPLC) grade, containing ethylene diamine tetraacetic acid (EDTA). The EDTA chelates the metal present in the samples. The hydrolyzed samples now were stored in HPLC amino analyser bottles for further analytical operations.

The hydrolysed samples were devitalised automatically on the Water 616/626 HPLC by reacting the five amino acid, under basic situations with phenylisothiocyanate (PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this was 45 minutes per a sample, as calibrated on the instrument.

A set of standard solutions of the amino acids were prepared from Pierce Reference standards H (1000umol) into auto-sampler crops and they were also derivatised. These standards (0.0, 0.5, 1.0, 1.5, 2.0  $\mu$ mol) were used to generate a calibration file that was used to determine the amino acids contents of the samples. After the derivatisation, a methanol solution (1.5N) containing the PTC-amino acids was transferred to a narrow bore Water 616/626HPLC system for separation. The separation and quantitation of the PTC-amino acids were done on a reverse phase (18 silica column and the PTC chromophore were automatically and digitally detected at the wavelength of 254 nm.

The buffer system used for separation was 140 mM sodium acetate pH5.50 as buffer A and 80% acetonitrile as buffer B. The program was run using a gradient of buffer A and

buffer B concentration and ending with a 55% buffer B concentration at the end of the gradient. The elution of the whole amino acids in the samples took 30 minutes.

### **Data Interpretation and Calculations**

The intensity of the chromatographic peaks areas was automatically and digitally identified and quantified using a Dionexchromeleon data analysis system which is attached to the waters 616/626 HPLC System. The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in Au) at the amino acids in 5 standards runs was used. Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by the software that was interphased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample is finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivity of the various amino acids) by the internal standard. (i.e.Pierce) in the chromatogram and multiplying this by the total amount of internal standard added to the original sample.

After the picomole, the intensity of the height of each amino acid has been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the internal standard (Nor leucine), and displays for the-total amount that was pipette into the hydrolysis tube at the beginning of the analysis as below:

### **Calculation**

mg/ml (in Extract) = Dilution factor x Peak height intensity

mg/ml (in sample) =  $\frac{\text{ug/ml in extract} \times \text{sample volume}}{\text{Wheight of sample}}$

### 3.2.9 Determination of catalase activity (CAT)

Two grams (2 g) of the seed was homogenized in extraction buffer (pH 7.6) containing 10 mM EDTA and 10% (w/v) PVPP. Homogenates were centrifuged at 12000 g for 15 min. Catalase activity was spectrophotometrically determined. The decomposition of H<sub>2</sub>O<sub>2</sub> was monitored at 240 nm in a reaction mixture that contained 50mM potassium phosphate buffer (pH 7.0), the sample and 10 mM H<sub>2</sub>O<sub>2</sub>. The assay was performed 25°C in a 3ml cuvette. The protein concentration was measured according to Bradford (1976) using bovine serum albumin as standard.

$$\text{Catalase activity} = \frac{\Delta A_{240}/\text{min}(\text{sample}) - \Delta A_{240}/\text{min}(\text{blank})}{0.0436} \times 1.6$$

Where:  $\Delta A_{240}/\text{min}$  = change in absorbance at 240 nm/min of sample or blank

0.0436 = millimolar extinction coefficient of H<sub>2</sub>O<sub>2</sub> at 240 nm

1.6 = total reaction mixture volume

### 3.3 Data Analysis

Data are represented as Mean  $\pm$  Standard error of mean and were subjected to analysis of variance (ANOVA) using the Statistical Tool for Agricultural Research (STAR). Means were compared with Duncan Multiple Range Test (DMRT) analysis at a confidence level of 0.05

## CHAPTER FOUR

### 4.0

### RESULTS AND DISCUSSION

#### 4.1 Results

##### 4.1.1 Percentage oil yield of selected accessions of sesame seed

The percentage oil yield of sesame seeds accession as shown below in Table 4.1 Accession 5 (NCRIBEN203) and Accession 4 (NCRIBEN201) had the highest oil yields ( $54.6337 \pm 0.4395$  % and  $52.1697 \pm 0.2055$  %) respectively while the least oil %yields ( $28.4220 \pm 1.346$  %) were obtained. for accession 1 (NCRIBEN106). There were significant differences ( $p < 0.05$ ) in oil yield among the sesame accessions.

**Table 4.1: Percentage (%) Oil yield of sesame Seed Accessions**

Accessions	% oil yield
NCRIBEN 201	$52.17 \pm 0.21^d$
NCRIBEN 203	$54.63 \pm 0.44^e$
NCRIBEN 131	$49.79 \pm 0.29^c$
NCRIBEN 121	$37.39 \pm 1.21^b$
NCRIBEN 106	$28.42 \pm 1.35^a$

Values are mean  $\pm$  standard error of mean of three determinations.

Values with different superscript along the column are significantly ( $p < 0.05$ ) different

#### 4.1.2. Effect of induced ageing on mean proximate composition

The result of the proximate analysis carried out on the sesame seeds accessions showed considerable and significant variability in the mean moisture, crude protein, crude fibre, crude fat, carbohydrate and ash content among the accessions.

**Table 4.2: Effect of Induced Ageing on the Mean Proximate Composition**

<b>Accession</b>	<b>Moisture (%)</b>	<b>Ash (%)</b>	<b>Fat (%)</b>	<b>Protein (%)</b>	<b>Crude (%)</b>	<b>CHO (%)</b>
NCRIBEN106	1.86±0.64 <sup>d</sup>	6.51±0.24 <sup>a</sup>	44.92±2.58 <sup>d</sup>	24.02±0.58 <sup>a</sup>	4.31±0.17 <sup>a</sup>	18.16±2.71 <sup>a</sup>
NCRIBEN121	2.73±0.95 <sup>c</sup>	6.11±0.34 <sup>bc</sup>	47.20±1.22 <sup>c</sup>	21.55±0.55 <sup>b</sup>	4.09±0.17 <sup>ab</sup>	17.08±3.45 <sup>a</sup>
NCRIBEN131	3.40±0.41 <sup>b</sup>	5.75±0.21 <sup>c</sup>	52.82±1.44 <sup>a</sup>	25.29±0.26 <sup>a</sup>	3.84±0.10 <sup>c</sup>	8.94±1.85 <sup>c</sup>
NCRIBEN201	2.91±0.83 <sup>c</sup>	6.01±0.42 <sup>bc</sup>	48.41±1.49 <sup>bc</sup>	21.16±3.09 <sup>b</sup>	4.01±0.23 <sup>bc</sup>	17.27±2.03 <sup>a</sup>
NCRIBEN203	3.70±0.91 <sup>a</sup>	6.33±0.81 <sup>ab</sup>	49.17±1.38 <sup>b</sup>	22.56±1.55 <sup>b</sup>	4.16±0.51 <sup>ab</sup>	14.19±2.97 <sup>b</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along the column are significantly ( $p < 0.05$ ) different.

### 4.1.3. Effect of induced ageing on fatty acid profile of sesame seed accessions

#### 4.1.3.1 Mean concentration of saturated fatty acids (SFA) in some sesame seed accessions

The saturated fatty acid (SFA) content of sesame accessions is presented in Table 4.3 below. The fatty acids present include stearic palmitoleic, myristic, behenic, lauric, capric acids. The fatty acid palmitoleic acid had the highest concentration from  $1.55\pm 0.15 \mu\text{g/g}$  in NCRIBEN121 to  $2.09\pm 0.28 \mu\text{g/g}$  in NCRIBEN203 followed by Myristic  $0.68\pm 0.35 \mu\text{g/g}$  in NCRIBEN121 to  $1.09\pm 0.12 \mu\text{g/g}$  in NCRIBEN203 while the fatty acid such as Capric, Behenic, Lauric and Stearic were observed to have the lowest concentration. The accession NCRIBEN203 and NCRIBEN121 had the highest and lowest concentration of each fatty acid respectively. However, there was no significant ( $p > 0.05$ ) difference in the concentrations SFA in other accessions.

**Table 4.3. Mean Concentration of saturated fatty acids (SFA) in some selected sesame seed accessions( $\mu\text{g/g}$ )**

Accession	Stearic	Palmitoleic	Capric	Myristic	Lauric	Behenic
NCRIBEN106	$0.01\pm 0.42^c$	$1.68\pm 0.15^b$	$0.02\pm 0.01^c$	$0.92\pm 0.08^{ab}$	$0.01\pm 0.01^{ab}$	$0.04\pm 0.01^b$
NCRIBEN121	$0.01\pm 0.65^a$	$1.55\pm 0.15^b$	$0.02\pm 0.01^c$	$0.68\pm 0.35^c$	$0.01\pm 0.01^b$	$0.04\pm 0.01^c$
NCRIBEN131	$0.01\pm 0.63^a$	$1.70\pm 0.07^b$	$0.02\pm 0.03^b$	$0.89\pm 0.09^b$	$0.01\pm 0.02^b$	$0.04\pm 0.01^b$
NCRIBEN201	$0.01\pm 0.44^{bc}$	$1.96\pm 0.09^a$	$0.02\pm 0.05^c$	$0.99\pm 0.08^{ab}$	$0.01\pm 0.02^a$	$0.05\pm 0.01^a$
NCRIBEN203	$0.01\pm 1.89^{ab}$	$2.09\pm 0.28^a$	$0.02\pm 0.10^a$	$1.09\pm 0.12^a$	$0.01\pm 0.02^a$	$0.05\pm 0.01^a$

Values are mean  $\pm$  standard error of mean of three determinations Values with different superscript along the column are significantly ( $p < 0.05$ ) different.



#### 4.1.3.2 Mean concentration of mono unsaturated fatty acids (MUFA) in some sesame seed accessions

The mono unsaturated fatty acid (MUFA) content of sesame accessions is presented in Table 4.4 below, The fatty acids present include oleic, petroselenic, vaccenic, cetoleic, erucic and nevononic acids. The fatty acid oleic acid had the highest concentration from 27.33±0.21  $\mu\text{g/g}$  in ACC 5 (NCRIBEN203) seeds to 20.46±1.22 $\mu\text{g/g}$  in ACC 2 (NCRIBEN121) followed by erucic (0.72 ±0.08 $\mu\text{g/g}$  in NCRIBEN203 to 0.54±0.05  $\mu\text{g/g}$  in NCRIBEN121 while the fatty acid observed to have the lowest concentration was cetoleic acid its concentration was 0.02±0.02  $\mu\text{g/g}$  in NCRIBEN203 and NCRIBEN203, while other Accessions has 0.01±01  $\mu\text{g/g}$ . The accession NCRIBEN203 and NCRIBEN121 had the highest and lowest concentration of each fatty acid respectively. However, there was no significant ( $p > 0.05$ ) difference in the concentrations in accessions NCRIBEN 121 NCRIBEN 131 and NCRIBEN 106 except for veccenic acid

**Table 4.4: Concentration of mono un-saturated fatty acids (MUFA) in some selected sesame seed accessions( $\mu\text{g/g}$ )**

Accession	Oleic	Petroselenic	Vaccenic	Cetoleic	erucic	Nevonic
NCRIBEN106	22.26±1.78 <sup>b</sup>	0.04±0.00 <sup>a</sup>	0.11±0.09 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.59±0.05 <sup>c</sup>	0.02±0.02 <sup>a</sup>
NCRIBEN121	20.46±1.22 <sup>a</sup>	0.04±0.05 <sup>a</sup>	0.10±0.00 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.54±0.05 <sup>b</sup>	0.02±0.02 <sup>a</sup>
NCRIBEN131	22.51±0.93 <sup>b</sup>	0.04±0.05 <sup>a</sup>	0.11±0.00 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.59±0.05 <sup>c</sup>	0.02±0.03 <sup>a</sup>
NCRIBEN201	25.90±1.12 <sup>c</sup>	0.05±0.04 <sup>b</sup>	0.12±0.05 <sup>c</sup>	0.02±0.01 <sup>b</sup>	0.02±0.02 <sup>a</sup>	0.03±0.04 <sup>b</sup>
NCRIBEN203	27.33±0.21 <sup>d</sup>	0.05±0.07 <sup>b</sup>	0.13±0.05 <sup>d</sup>	0.02±0.02 <sup>b</sup>	0.72±0.08 <sup>d</sup>	0.03±0.04 <sup>b</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along the column are significantly ( $p < 0.05$ ) different

#### 4.1.3.3 Mean concentration of poly saturated fatty acids (PUFA) in some sesame seed accessions

The polyunsaturated fatty acid content sesame accessions is presented in table 4.5 below. The fatty acids present include arachidonic, linoleic,  $\alpha$ -linolenic, linolenic and total fatty acids. The fatty acid Arachidonic acid had the highest concentration from  $34.52 \pm 1.04 \mu\text{g/g}$  in NCRIBEN203 to  $25.84 \pm 1.23 \mu\text{g/g}$  in NCRIBEN121, followed by  $\alpha$ -linolenic ( $16.70 \pm 0.03 \mu\text{g/g}$  in NCRIBEN203 to  $12.50 \pm 0.02 \mu\text{g/g}$  in NCRIBEN121). While the fatty acid observed to have the lowest concentration was Linolenic acid its concentration was  $0.01 \pm 0.01 \mu\text{g/g}$  was not statistically significantly different ( $p > 0.05$ ) in all the seed accessions. The accession NCRIBEN203 and NCRIBEN121 had the highest and lowest concentration of each fatty acid respectively. However, there was no significant ( $p > 0.05$ ) difference in the concentrations in accessions NCRIBEN 106 and NCRIBEN 1131.

**Table 4.5: Mean Concentration of poly un-saturated fatty acids (PUFA) in some selected sesame seed accessions ( $\mu\text{g/g}$ )**

Accession	Linoleic	Linolenic	Arachidonic	$\alpha$ -linolenic	Total Fatty
NCRIBEN106	$7.37 \pm 0.64^c$	$0.01 \pm 0.07^b$	$28.12 \pm 1.29^b$	$13.61 \pm 0.00^a$	$79.97 \pm 8.35^c$
NCRIBEN121	$6.77 \pm 0.62^c$	$0.01 \pm 0.31^b$	$25.84 \pm 1.23^b$	$12.50 \pm 0.02^b$	$75.02 \pm 6.82^d$
NCRIBEN131	$7.45 \pm 0.32^c$	$0.01 \pm 0.82^b$	$28.00 \pm 0.56^b$	$13.76 \pm 0.01^a$	$81.21 \pm 2.73^c$
NCRIBEN201	$8.59 \pm 0.48^b$	$0.01 \pm 0.43^a$	$32.72 \pm 0.72^a$	$15.83 \pm 0.01^a$	$91.42 \pm 4.00^b$
NCRIBEN203	$9.35 \pm 1.04^{ab}$	$0.01 \pm 0.07^a$	$34.52 \pm 1.04^a$	$16.70 \pm 0.03^c$	$95.87 \pm 11.66^a$

Values are mean  $\pm$  standard error of mean of three determinations. Values with different superscript along a row are significantly ( $p < 0.05$ ) different

#### 4.1.3.4 Comparison of classified fatty acids PUFA, SFA and MUFA concentrations of some selected sesame seed accessions

Table 4.6 shows the comparison of the concentration of polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) in fatty acid content between the selected sesame seed accessions. NCRIBEN203 had highest concentrations of poly unsaturated fatty acid ( $156.45 \pm 0.31 \mu\text{g/g}$ ), mono unsaturated fatty acid ( $28.28 \pm 0.01 \mu\text{g/g}$ ), and saturated fatty acid ( $3.27 \pm 0.15 \mu\text{g/g}$ ) respectively. Followed by NCRIBEN 201 having concentrations of poly unsaturated fatty acid ( $148.56 \pm 0.30 \mu\text{g/g}$ ), mono unsaturated fatty acid ( $26.14 \pm 0.12 \mu\text{g/g}$ ), and saturated fatty acid ( $3.04 \pm 1.00 \mu\text{g/g}$ ) respectively and NCRIBEN121 had the least concentration in all the class of fatty acids.

**Table 4.6: Comparison of Classified Fatty Acids Concentration (PUFA, SFA, MUFA) In Some Selected Sesame Seed Accessions ( $\mu\text{g/g}$ )**

Accession	PUFA	SFA	MUFA
NCRIBEN106	$129.07 \pm 0.21^a$	$2.69 \pm 0.11^b$	$23.04 \pm 0.432^{bc}$
NCRIBEN121	$120.14 \pm 0.01^a$	$2.30 \pm 0.03^a$	$21.17 \pm 0.09^a$
NCRIBEN131	$130.42 \pm 2.16^b$	$2.67 \pm 0.01^b$	$23.20 \pm 0.10^{ab}$
NCRIBEN201	$148.56 \pm 0.30^c$	$3.04 \pm 1.00^c$	$26.14 \pm 0.12^{cd}$
NCRIBEN203	$156.45 \pm 0.31^d$	$3.27 \pm 0.15^c$	$28.28 \pm 0.01^d$

Values are mean  $\pm$  standard error of mean of three determinations. Values with different superscript along a row are significantly ( $p < 0.05$ ) different

PUFA- polyunsaturated fatty acid

SFA- saturated fatty acid

MUFA-mono unsaturated fatty acid

#### 4.1.4 Amino acid concentration of some selected sesame seed accessions

##### 4.1.4.1 Concentration of essential amino acids (EAA) in sesame seed accessions

The essential amino acid content of sesame seed accessions is presented in Table 4.7. The essential amino acids include leucine, lysine, isoleucine, phenylalanine, methionine, tryptophan, valine, histidine and threonine. The result showed that phenylalanine acid had the highest concentration in all the sesame accessions ranging from 14.74±0.11 mg/100g in NCRIBEN201 to 12.22±0.04mg/100g in NCRIBEN121 followed by valine which ranged from 14.59±0.21 mg/100g in NCRIBEN201 to 12.06±0.10mg/100g in NCRIBEN121. Tryptophan was observed to have the lowest concentration in all the accessions analyzed ranging from 3.17±0.26mg/100g in NCRIBEN131 to 3.88±0.01mg/100g in NCRIBEN201. There was significant (P< 0.05) difference in the concentrations of methionine, valine, histidine and threonine amino acids in the accessions of sesame seed sample.

**Table 4.7: Concentration of Essential Amino Acid (EAA) of Selected Some Sesame Accessions mg/100g (%)**

Accession	ISO	PHL	TRP	VAL	MET	HIS	THR
NCRIBEN106	11.18±0.11 <sup>c</sup>	13.18±0.07 <sup>bc</sup>	3.71±0.06 <sup>a</sup>	12.96±0.21 <sup>a</sup>	7.27±0.01 <sup>a</sup>	8.12±0.03 <sup>ab</sup>	9.07±0.04 <sup>ab</sup>
NCRIBEN121	10.70±0.01 <sup>ab</sup>	12.22±0.04 <sup>a</sup>	3.19±1.01 <sup>a</sup>	12.06±0.10 <sup>a</sup>	7.51±0.01 <sup>a</sup>	7.55±0.11 <sup>a</sup>	8.28±0.10 <sup>a</sup>
NCRIBEN131	10.56±0.02 <sup>ab</sup>	12.63±0.42 <sup>a</sup>	3.17±0.26 <sup>a</sup>	12.26±0.11 <sup>a</sup>	7.16±0.02 <sup>a</sup>	7.52±0.04 <sup>a</sup>	8.57±0.01 <sup>a</sup>
NCRIBEN201	11.51±0.02 <sup>a</sup>	14.74±0.11 <sup>c</sup>	3.88±0.01 <sup>a</sup>	14.59±0.21 <sup>b</sup>	8.45±0.25 <sup>b</sup>	8.87±0.01 <sup>b</sup>	10.50±0.14 <sup>b</sup>
NCRIBEN203	11.19±0.01 <sup>a</sup>	14.16±0.12 <sup>c</sup>	3.83±0.20 <sup>a</sup>	14.28±0.24 <sup>b</sup>	8.40±0.10 <sup>b</sup>	8.71±0.02 <sup>b</sup>	9.91±0.11 <sup>ab</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript down the column are significantly (p < 0.05) different.

#### 4.1.4.2 Concentration of non-essential amino acids (NEAA) in some selected sesame seed accessions

The non-essential amino acid content of sesame seed accessions is presented in Table 4.8. The non-essential amino acids include alanine, cystine, glutamine, tyrosine serine, asparagine, arginine, glycine and proline. The result showed that glutamic acid had the highest concentration in all the sesame accessions ranging from 55.83±0.12 mg/100g in NCRIBEN203 to 41.17±0.01 mg/100g in NCRIBEN131, followed by arginine which ranged from 37.89±0.01 mg/100g in NCRIBEN201 to 33.90±0.10 mg/100g in NCRIBEN121. Cystine was observed to have the lowest concentration in all the accessions analyzed ranging from 22.19±1.00 mg/100g in NCRIBEN106 to 4.30±0.08 mg/100g in NCRIBEN121. There was no significant ( $p < 0.05$ ) difference in the concentrations of alanine, glycine, tyrosine, cystine and serine amino acids in the accessions NCRIBEN106, NCRIBEN131 and NCRIBEN121 while NCRIBEN201 and NCRIBEN203 also showed no significant ( $p > 0.05$ ) difference.

**Table 4.8: Concentration of Non-Essential Amino Acid (NEAA) In Some Selected Sesame Accessions mg/100g (%)**

Accession	ALA	CYST	GLY	TY	SE	ARG	ASP	GLU	PRO
NCRIBEN106	12.89±0.01b	22.19±1.00c	13.59±1.14bc	7.28±0.41b	9.63±0.11b	36.35±0.01b	22.89±0.50a	50.33±0.02c	8.36±0.31b
NCRIBEN121	12.17±0.02a	4.30±0.08a	11.98±0.17a	6.30±0.03a	8.54±0.10a	33.90±0.10a	21.64±0.07a	46.18±0.10b	7.67±0.14a
NCRIBEN131	12.61±0.12b	4.41±0.02a	12.72±3.10b	6.81±0.02a	9.21±0.61b	34.16±0.02ab	22.41±1.11b	41.17±0.01a	8.07±0.28b
NCRIBEN201	14.31±0.15c	5.76±0.10b	15.83±0.02c	8.92±0.12a	11.45±0.08c	37.89±0.01c	24.58±0.01d	54.66±0.03d	9.47±0.20c
NCRIBEN203	14.10±0.09c	5.48±0.01b	15.02±1.04c	7.86±0.04b	11.72±0.02c	37.81±0.21c	23.33±0.02c	55.83±0.12e	9.29±0.21c

Values are mean ± standard error of mean of three determinations. Values with different superscript down the row are significantly ( $p < 0.05$ ) different.

#### 4.1.4.3 Concentration of aromatic amino acids (ARAA) in some selected sesame seed accessions

The aromatic amino acid content of sesame seed accessions is presented in Table 4.9. The aromatic amino acids include, phenylalanine, tyrosine and tryptophan. The result showed that phenylalanine acid had the highest concentration in all the sesame accessions ranging from 14.74±0.08 mg/100g in NCRIBEN201 to 12.22±0.03 mg/100g in NCRIBEN121. Tryptophan was observed to have the lowest concentration in all the accessions analyzed ranging from 3.17±0.10mg/100g in NCRIBEN131 to 3.88±0.11 mg/100g in NCRIBEN201. There was no significant ( $p < 0.05$ ) difference in the concentrations of tyrosine, tryptophan and phenylalanine amino acids in the accessions NCRIBEN201, NCRI BEN203 and NCRIBEN106.

**Table 4.9: Mean Concentration of Aromatic Amino Acid (ARAA) In Some Selected Sesame Accessions mg/100g (%)**

Accession	PHL	TRP	TY
NCRIBEN106	13.18±0.17 <sup>bc</sup>	3.71±0.06 <sup>a</sup>	7.28±0.01 <sup>c</sup>
NCRIBEN121	12.22±0.03 <sup>c</sup>	3.19±0.12 <sup>b</sup>	6.30±0.13 <sup>a</sup>
NCRIBEN131	12.63±0.13 <sup>c</sup>	3.17±0.10 <sup>b</sup>	6.81±0.42 <sup>b</sup>
NCRIBEN201	14.74±0.08 <sup>a</sup>	3.88±0.11 <sup>a</sup>	8.92±0.52 <sup>d</sup>
NCRIBEN203	14.16±0.11 <sup>ab</sup>	3.83±0.23 <sup>a</sup>	7.86±0.17 <sup>c</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript down the row are significantly ( $p < 0.05$ ) different.

**4.1.4.4 Concentrations of total essential amino acid, total non-essential amino acid and total aromatic amino acid in sesame seed accessions**

Table 4.10 shows the concentration of three classes of amino acids; total essential amino acid (TEAA), total non-essential amino acid (TNEAA) and total aromatic amino acid (TARAA) in aged sesame accessions. The class TNEAA had the highest concentration of amino acid which ranged from 6.78 ±4.852 mg/100g in NCRIBEN201 to 5.614±4.327 mg/100g in NCRIBEN 106. Total essential amino acid (TEAA) and (TARAA) was observed to be highest in accession NCRIBEN203 (3.805±1.5428 mg/100g) while accession NCRIBEN121 had the lowest concentration (2.412±1.529 mg/100g). respectively.

**Table 4.10: Comparison in Concentration of TEAA, TNEAA and TARAA in Some Selected Sesame Accessions**

<b>Accession</b>	<b>TEAA</b>	<b>TNEAA</b>	<b>TARAA</b>
NCRIBEN106	3.561728±1.4924 <sup>b</sup>	6.797778±4.852 <sup>a</sup>	2.685926±1.593 <sup>b</sup> <sup>c</sup>
NCRIBEN121	3.320123±1.3879 <sup>c</sup>	5.654815±4.774 <sup>b</sup>	2.411852±1.529 <sup>d</sup>
NCRIBEN131	3.369259±1.4606 <sup>c</sup>	5.613827±4.327 <sup>b</sup>	2.511481±1.590 <sup>cd</sup>
NCRIBEN201	3.910741±1.5940 <sup>a</sup>	6.77284±5.401 <sup>a</sup>	3.060741±1.812 <sup>a</sup>
NCRIBEN203	3.805432±1.5428 <sup>c</sup>	6.68321±5.544 <sup>a</sup>	2.872222±1.735 <sup>ab</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript down the row are significantly (p < 0.05) different

#### 4.1.5. Effect of Induced Ageing on Catalase Activity of Sesame Seed Accessions

The effect of induced ageing on the catalase activity of sesame seed accessions is as shown in Table 4.11. The accession NCRIBEN 201 had the highest catalase activity all through the ageing period with values ranging from 31.96±0.21 mg/mL/min on Day 0 to 22.18±1.03 mg/mL/min, on the Day 6. While NCRIBEN 131 had the lowest catalase activity of 22.18 ±1.03 mg/mL/min on Day 0 and NCRIBEN121 had the lowest on Day 6 (14.21±0.81 mg/mL/min). The catalase activity of the sesame accessions was observed to decrease on third day with subsequent decrease on the sixth day in all the accessions. The decrease in catalase activity became significantly ( $p < 0.05$ ) different on the sixth day of ageing. However, the rate of reduction was highest (46.00 %) in accession NCRIBEN 121 whereas accession NCRIBEN131 had the least % reduction (8.90 %).

**Table 4.11: Effect of Induced Ageing on Catalase Activity (mg/mL/min) of Sesame Seed Accessions**

Accessions	0	3	6	% Reduction
NCRIBEN 106	31.96±0.21 <sup>a</sup>	33.05±0.02 <sup>b</sup>	29.10±0.03 <sup>b</sup>	9.20
NCRIBEN 121	26.33±0.22 <sup>a</sup>	14.56±0.54 <sup>b</sup>	14.21±0.81 <sup>b</sup>	46.00
NCRIBEN131	29.36±0.35 <sup>a</sup>	27.02±0.28 <sup>b</sup>	26.66±0.24 <sup>c</sup>	8.90
NCRIBEN201	22.18±1.03 <sup>a</sup>	21.42±0.47 <sup>ab</sup>	15.16±0.61 <sup>b</sup>	11.90
NCRIBEN 203	20.21±0.76 <sup>a</sup>	19.19±0.55 <sup>a</sup>	18.40±0.47 <sup>b</sup>	31.70

Values are mean ± standard error of mean of five determinations. Values with different superscript along a row are significantly ( $p < 0.05$ ) different



## 4.2 Discussion

### 4.2.1 Percentage oil yield of sesame seed accessions

In the present study, the highest values of oil yields ( $54.63 \pm 0.44$  % to  $28.42 \pm 1.35$  %) obtained for different sesame seed accessions respectively are in agreement with the findings of Nadeem *et al.* (2015) who evaluated the physico-chemical properties of sunflower seed oil. Sesame oil has been reported to be about 62-47 % yield (Borchani *et al.*, 2018). Uzun *et al.* (2018) reported that oil content of 103 turkish sesame cultivars ranged from 41.2-62.7%. A similar result was reported by Asgar and Majeed (2018) and Nzikou *et al.* (2018) who reported that oil content of different sesame cultivars ranged from 50 to 69.03 %, with the average of 59.5%. Baydar *et al.* (2019) also observed higher average oil content (63.25%) in Turkish sesame cultivars. Variation in oil content can be attributed to either varietal factor, environmental factor or interaction of both factors. It is reported that Moroccan sesame cultivars contained higher oil percentages (over 50%) which is a desirable trait for breeding programs to improve sesame cultivars (Arslan *et al.*, 2017).

The observed variations from the work of others may be due to difference in variety of seeds. Variations in seeds may be due to slight changes in some of the genes that code for certain enzymes (e.g. fatty acids synthesizing enzymes) as such the extent to which the products (e.g. fatty acids) are made differ from one variety to another. Geographical location could also be another factor that may be responsible for variations observed in the yield of oil. Climatic conditions and soil composition amongst others may cause differences in the content of seeds from one region to another geographical location. Another reason for oil yield variation may be the extraction protocol as well as the solvent of extraction employed (Arslan *et al.*, 2017).

However, the involvement of extracting solvent as well as high temperature employed in Soxhlet extraction method may be responsible for the higher oil yield observed with Soxhlet extraction as observed in the present study. The polarity of the extracting solvent has a major role to play in the extraction protocol. Therefore, the selective permeability of solvents may enhance the rate of oil recovery as non-polar solvents permeate the sample (sesame seed flour) more compared to polar solvents and much more than that without any extraction solvent. This non-significant ( $p < 0.05$ ) difference in oil yield (%) obtained using Soxhlet extraction method irrespective of the type of accession, may be attributed to the fact that all accession may probably have very close genetic makeup. However, the accession NCRIBEN 203 which had the highest oil yield ( $54.63 \pm 0.44$  %) than others accessions may hence be given more attention to improve on its breeding technique for commercial production so as to become readily available to farmers for planting as well as for sesame oil producing industries.

#### **4.2.2 Effect of induced ageing on proximate constituents of sesame seed accessions**

The variability of chemical composition and oil quality recorded among the accessions in the present study agrees with reports of other researchers. At day 3, the range of moisture content in accession NCRIBEN203 (4.03% to 2.03% in NCRIBEN106 obtained in this study is lower than 4.16 to 4.62 and 2.7 to 4.7% reported by Tokusoglu *et al.*, (2014) and El Khier *et al.*, (2018), respectively. The mean moisture content of 3.4% was however less than the mean of 8.5% recorded by Jimoh *et al.*, (2016) in other accessions of sesame. It has been shown that high moisture content encourages the growth of microorganisms thereby causing the deterioration of stored seeds (Afolabi, 2018). The high moisture content therefore implies that the seeds may not store well and to avoid this, effort should be made to reduce the moisture content before storage of the seed.

The crude protein content of the accessions was in agreement with what was obtained elsewhere. At day 0 the range of 25.62% to 21.04% recorded among the accessions NCRIBEN131 and NCRIBEN121 respectively is less than 32 to 40 reported by El Khier *et al.*, (2018). The mean crude protein of 21.155% is also less than the mean values of 24.63, and 21.78 reported by Borchani *et al.*, (2018), Jimoh *et al.*, (2016). The highest value of 25.62% recorded from accession NCRIBEN131 was however close to these means. Protein is very important in human nutrition and is one of the nutrients that are frequently low in plant products. Although, the mean crude fibre 4.54% recorded in this study may appear to be in agreement with the reports of (Jimoh *et al.*, 2016) in all accessions. At day 6 the range of 4.77 % in NCRIBEN203 to 3.92% in NCRIBEN131 which have high crude fibre contents if compared with other reports (Jimoh *et al.*, 2016, Nzikou *et al.*,2018) similar findings reported by Hahm *et al.*, (2019). Fibre in diet is important as it helps to maintain human health by reducing cholesterol level in the body (Belury *et al.*, 2019). The sesame accessions showed appreciable crude fat content. At day 6 the range of 53.55% to 46.29% in accessions NCRIBEN131 and NCRIBEN121 respectively can be considered to be favorable when compared with results obtained by other researchers (Borchani *et al.*, 2018; Mohammed and Hamza, 2018). The economic important of sesame is determined by the quantity of oil it contains. Accessions such as NCRIBEN131 that yielded high crude fat should be selected for production in this area, though seed yield will also be a major determinant in making a choice. (El Khier *et al.*, 2018).

The carbohydrate content of 7.75% to 21.63% in the accessions NCRIBEN131 to NCRIBEN106 is also low when compared with that of other legume seed ranging from 23% in groundnut to 66% in bambara groundnut (El Khier *et al.*, 2018). The ash content

(6.37% to 5.72 %) in accessions NCRIBEN106 to NCRIBEN131 obtained in this study is comparable with that of other legume seed which have been reported to range between 3.0 to 5.8% (Elegbede, 1998) this was also within the range reported by Alyemini *et al.* (2016). High level of ash makes the oilseed a good source of mineral nutrition to the consumer (Afolabi, 2018).

#### **4.2.3 Fatty acid profile**

In general, seed oil contains predominantly unsaturated fatty acids and significant amounts of saturated fatty acids. Oleic ( $27.33 \pm 0.21 \mu\text{g/g}$ ) and linoleic ( $9.35 \pm 1.04 \mu\text{g/g}$ ) acids are the major fatty acids of sesame oil and they were found to be present in large amounts in the oil of sesame accessions (Crews *et al.*, 2016). The percentage of oleic acid in sesame seed oil ranged from  $27.33 \pm 0.21 \mu\text{g/g}$  to  $20.46 \pm 1.22 \mu\text{g/g}$ , in with an average value of  $23.69 \pm 0.61 \mu\text{g/g}$  (Table 4.6). While, linoleic acid varied from  $9.35 \pm 1.04 \mu\text{g/g}$  to  $6.77 \pm 0.62 \mu\text{g/g}$ , with an average content of  $7.91 \pm 0.62 \mu\text{g/g}$ .

However, the content of Linolenic acid and Cetoleic acid were found to be very low in all accessions tested ( $0.01 \pm 0.07$  to  $0.01 \pm 0.82$  and  $0.01 \pm 0.01$  to  $0.02 \pm 0.02$ , respectively). The highest content of oleic acid ( $27.33 \pm 0.21 \mu\text{g/g}$ ) was found in NCRIBEN203, whilst the lowest one ( $20.46 \pm 1.22 \mu\text{g/g}$ ) was observed in NCRIBEN121. Conversely, the highest contents of linoleic and  $\alpha$ -linolenic acids ( $9.35 \pm 1.04 \mu\text{g/g}$  and  $16.70 \pm 0.03 \mu\text{g/g}$  respectively) were found in the accession NCRIBEN 203. Palmitoleic and Myristic acids were the predominant saturated fatty acids of sesame oil with an average content of  $1.80 \mu\text{g/g}$  and  $0.91 \mu\text{g/g}$  respectively. Palmitic acid content varied from  $1.55 \pm 0.15 \mu\text{g/g}$  to  $2.09 \pm 0.28 \mu\text{g/g}$  for the accessions NCRIBEN121 and NCRIBEN203, respectively. Stearic acid content varied from  $0.01 \pm 0.42 \mu\text{g/g}$  to  $0.01 \pm 1.89 \mu\text{g/g}$  for the accessions

NCRIBEN106 and NCRIBEN203 respectively. Lauric, Behenic and Linolenic acids were minor constituents of sesame seed oil, with mean value of 0.01, 0.05 and 0.01  $\mu\text{g/g}$  respectively. For each oilseed species, it is well known that Fatty Acid composition is genotype-dependent. Also, it has demonstrated that unsaturated fatty acids are influenced by the environmental conditions, mainly air temperature during seed ageing and oil biosynthesis. Thus, under low temperature conditions, there is an increase of unsaturation acid of seed oil, which leads to a higher proportion of linoleic and linolenic acids. Contrarily, under high temperature conditions, there is a low proportion of these acids and a high proportion of oleic acid in seed oil. In addition to that, amplitude of maximum and minimum temperatures as well as duration of plant exposure to these temperatures, during seed ageing effect significantly fatty acids composition.

In the present study, it was observed that oleic acid content was higher than linoleic acid content for all accessions and with this characteristic sesame oil appeared to be different from other seed oils. On the other hand, for most of the studied accessions, the total of these two unsaturated fatty acids accounted for more than 80% of the total fatty acid composition.

However, with the respect to their higher Arachidonic acid content, sesame seed accessions have proved to be considered as a source of high Arachidonic acid content. Unlike other SFA, Palmitoleic, Myristic and Lauric acids, which increase blood cholesterol levels, stearic acid has been proved to lower LDL cholesterol (Mensink, 2015). In addition, contrary to food rich in other saturated fatty acids, those having increased level of stearic acid, such as dark chocolate, does not pose a problem. Also, oils with high stearic acid content are developed to allow the production of solid fats without the need of

hydrogenation (Liu *et al.*, 2012). In this context, seed oils of accessions NCRIBEN121 could be interesting for food industry.

For a better classification of fatty acid compositions, Variations occur in saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) are presented in Table 4.10. The average content was, 2.79, 24.38 and 136.93  $\mu\text{g/g}$  respectively. The accession NCRIBEN203 exhibited the highest PUFA content (156.45 $\pm$ 0.31  $\mu\text{g/g}$ ) followed by MUFA in accession NCRIBEN203 (28.28 $\pm$ 0.01  $\mu\text{g/g}$ ) and the lowest SFA (2.30 $\pm$ 0.03  $\mu\text{g/g}$ ) in accession NCRIBEN121. A high level of PUFA increases the oil quality, making this oil suitable for human consumption. The highest PUFA content (156.45 $\pm$ 0.31  $\mu\text{g/g}$ ) was found in accession NCRIBEN203.

All knowing that polyunsaturated fatty acids are qualified as essential for our organism that cannot synthesized them, so they must be incorporated in the daily diet. It was reported that, conjugated linoleic acid, a new therapeutic nutrient with promising antioxidant and antitumor properties, is produced from linoleic acid-rich oils (Belury, 2019). The fatty acid is also an important component of skin care products (Darmstadt *et al.*, 2017).

#### **4.2.4 Amino acid content of sesame seed.**

Sesame seed is a rich source of both essential and non-essential amino acids as shown in the result. It is majorly rich in glycine, leucine, methionine, phenylalanine, arginine, aspartic acid glutamic acid. Sesame seed is comparatively richer in some essential amino acids than the melon seed such as lysine, methionine, serine, leucine, glycine and valine (Belury, 2019). In the present study glutamic acid have the highest amounts in all the accessions and the result was similar to that of Darmstadt *et al.*, (2017) that aspartic acid and glutamic acid were the most abundant amino acids in legumes and nuts.

The concentration of total essential amino acid (TEAA), total non-essential amino acid (TNEAA) and total aromatic amino acid (TARAA) of sesame seeds in the present work agree with the findings of Darmstadt *et al.* (2017) who evaluated the amino acid composition of cowpea (*Vigna unguiculata* L. Walp) seed flour and its protein isolates.

This implies that the amino acids in the seed of sesame have a high biological value and could contribute meaningfully in meeting the human requirements for these essential amino acids. The nutritive value of plant protein quality is usually assessed by comparing its essential amino acids content with reference standards for ideal protein quality set by the World Health Organization (FAO/WHO/UNU, 2019), which is based on the amino acids requirement for children aged 2- 5 years. The amino acid profile of sesame seed investigated in this study suggests that sesame seeds are an excellent source of both essential and non-essential amino acids, notably glutamic acid, arginine, the other amino acids are present in moderate amounts while tryptophan, asparagine, and glycine. Significant variation ( $P < 0.05$ ) exists among the individual amino acids amongst the accessions especially glutamic acid and aspartic acid. These differences in amount of amino acids could be attributed to genetic differences among the sesame accessions (Tashiro *et al.*, 2017). No differences were found in the profile of amino acids between the essential and non-essential amino acids. Therefore, our results showed that sesame seed contain enough protein ( $22.92 \pm 0.03$  mg/100g) and almost all the essential amino acids needed, with some above the 25 mg/100g relative chemical score.

The finding of Igwe *et al.* (2017) showed a higher concentration of TEAA, TNEAA and TARAA in both raw and locally processed seeds of *Ricinus communis* than that obtained in the present study. However, there was similarity in the percentage TEAA, TNEAA and

TARAA obtained for *sesamum indicum* L. seed with that obtained in the present study. Grieshop and Fahey, (2016) and Karr-Lilienthal *et al.* (2015) also reported a lower TEAA and TNEAA concentrations for soybean meal from Northern zones of the United States was in agreement with the results obtained for accessions NCRIBEN 106 (Table 4.16). The concentration of TEAA reported for soya beans by Belury (2019), were higher than that reported for accessions NCRIBEN 201 in the present study. A higher concentration of TNEAA and TARAA in kidney beans and Cowpea (*Vigna unguiculata* L. Walp) seed was reported by Goel *et al.* (2012). Crew *et al.* (2016) also reported a higher concentration of TNEAA and TARAA than that obtained in this study. The accessions of sesame seed (66.37±0.16 mg/100g) TEAA is higher than that of egg (50.12±0.16 mg/100g) as reported by the Food and Agricultural Organization (FAO/WHO, 2019).

Essential amino or indispensable amino acids cannot be synthesized by the human body and must therefore be supplied in food for optimal growth. In this present work, the TNEAA of sesame seed (6.798±4.852 mg/100g) is higher than that obtained for defatted *Tectona grandis* seed meal as reported by Oyewusi *et al.* (2016) while the authors' report for TEAA and TARAA concentrations were higher than those of the present study. These variations may be attributed to differences in the genetic makeup of the seeds. Similarly, environmental factors such as temperature from different geographical regions could lead to the observed differences. High temperature as well as the availability of water, could create a dilution effect, contributing to the decline of protein concentration. This in turn may result to the accumulation of relatively higher oil content in seeds. Hence, favoring a highly negative correlation between oil and protein concentration and thereby affecting the amino acid concentration. The significant variation in the concentrations of TEAA and



TNEAA sesame seed accessions as observed in the present study may be due to the certain genetic, growth and environmental factors to which both seeds were subjected.

The present study showed that the accessions NCRIBEN201 had higher concentrations of total essential amino and total non-essential amino acid ( $6.798 \pm 4.852$  mg/100g and  $3.06 \pm 1.82$  mg/100g respectively) than other accessions. This points to the fact that they could be used in formulating weaning food to improve the nutritional qualities of local staples like cereals, roots and tubers flours which have lower values of protein. Hence, more attention is needed to improve on the breeding technique for commercial production so that high quality seeds become readily available to farmers for planting.

#### **4.2.5 Effect of induced ageing on the activities of some enzymes**

Certain anabolic enzymes aid in maintenance of seed viability while some catabolic enzymes decrease seed viability (Begum *et al.*, 2018). Several studies have suggested that decrease occur in the activity of enzymes present in aged seeds (Goel *et al.*, 2012; Bailly, 2014). The concentration of some seed enzymes are markers of ageing in stored seeds. Rao *et al.* (2017).

##### **4.2.5.1 Catalase activity**

The decrease in catalase enzyme activity of aged sesame seed accessions as observed in the present study agrees with the report of Kabinza *et al.* (2016) for aged soya bean seeds. The study of Nadeem *et al.* (2015) has also shown a significant decrease in the activity of catalase enzyme of soybean seeds under induced aging time. The decreased catalase activity observed in the present study is also in agreement with the reports of Rao *et al.*, (2017) who found out that the activity of catalase decreased in onion seed after prolonged storage. Chauhan *et al.* (2017) reported a decreased catalase activity in their study of

changes of storage enzymes activities in natural and accelerated aged seeds in wheat. All these reports agree with the findings of the present study. Decrease in catalase activity with increasing ageing time may be due to formation of cracks as well as bruises in seeds under high temperature therefore, exposing the enzyme to leakages. The antioxidant activity of catalase in plant seeds play a role of aiding the plant tolerate stress as well as assist in the physiology of germination. Catalase activity in seeds is an index of seed germination as well as deterioration (Ayse *et al.*, 2017). Similar % decline in catalase activity of sesame seed accessions NCRIBEN2106 (9.20%) points to the fact that it may have stress tolerating ability and as such attention should be given to the accession NCRIBEN106 for proper breeding and availability for local farmers use.

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

Differences observed for oil content, fatty acid profile, enzymes, proximate composition and amino acid content between the studied accessions may be due to genotypic effect, climatic conditions and cropping practices, some accessions were found to have very high oil content, which make them viable for commercial extraction. Regarding fatty acids profile, the main components in seed oil were oleic and linoleic acids, which have important nutritional and industrial applications.

Furthermore, the accessions studied were characterized by decreased enzyme activities in all the accessions from the 3<sup>rd</sup> day of accelerated ageing. The rich content of nutrient coupled with high amino acid profile suggest that sesame seeds could meaningfully contribute to human and livestock nutrition.

#### 5.2 Recommendations

It is recommended that:

- (1) Breeding techniques should be enhanced on sesame seed accessions for varietal release which will increase local utilization of sesame to create more jobs increase the income of sesame farmers.
- (2) The seed could be further explored to develop a high oil yielding variety that would be of immense nutritional and economic advantage to the Nigerian economy.

### **5.3 Contribution of the Research to Knowledge**

In the present study glutamic acid has the highest concentration in all the sesame accessions ranging from  $55.8 \pm 0.12$  mg/100g in NERIBEN 203 to  $41.17 \pm 0.01$  mg/100g in NCRIBEN 131, this inherent variability could be usefully exploited in plant breeding program to achieve genetic gain for nutrient composition through selection and hybridization..

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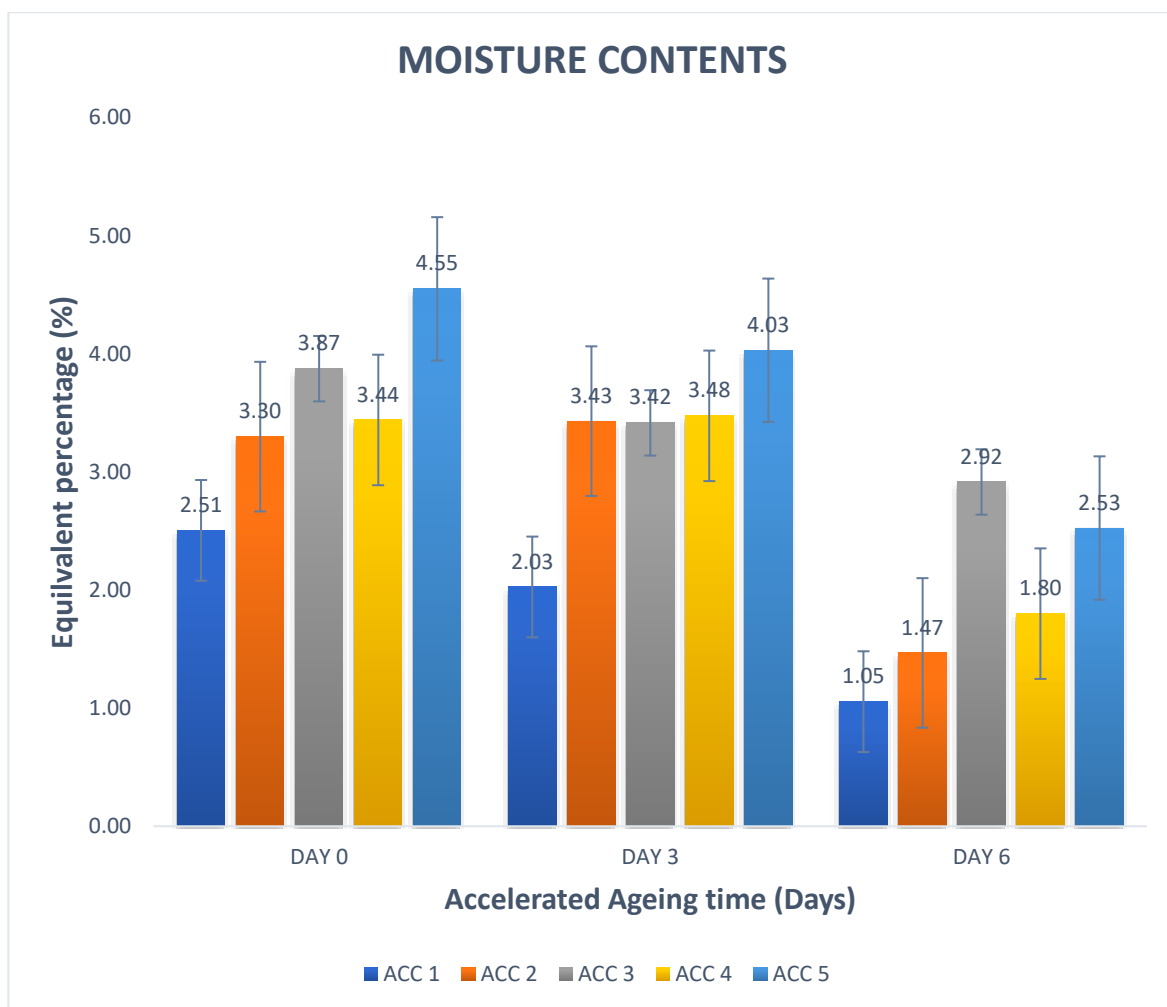


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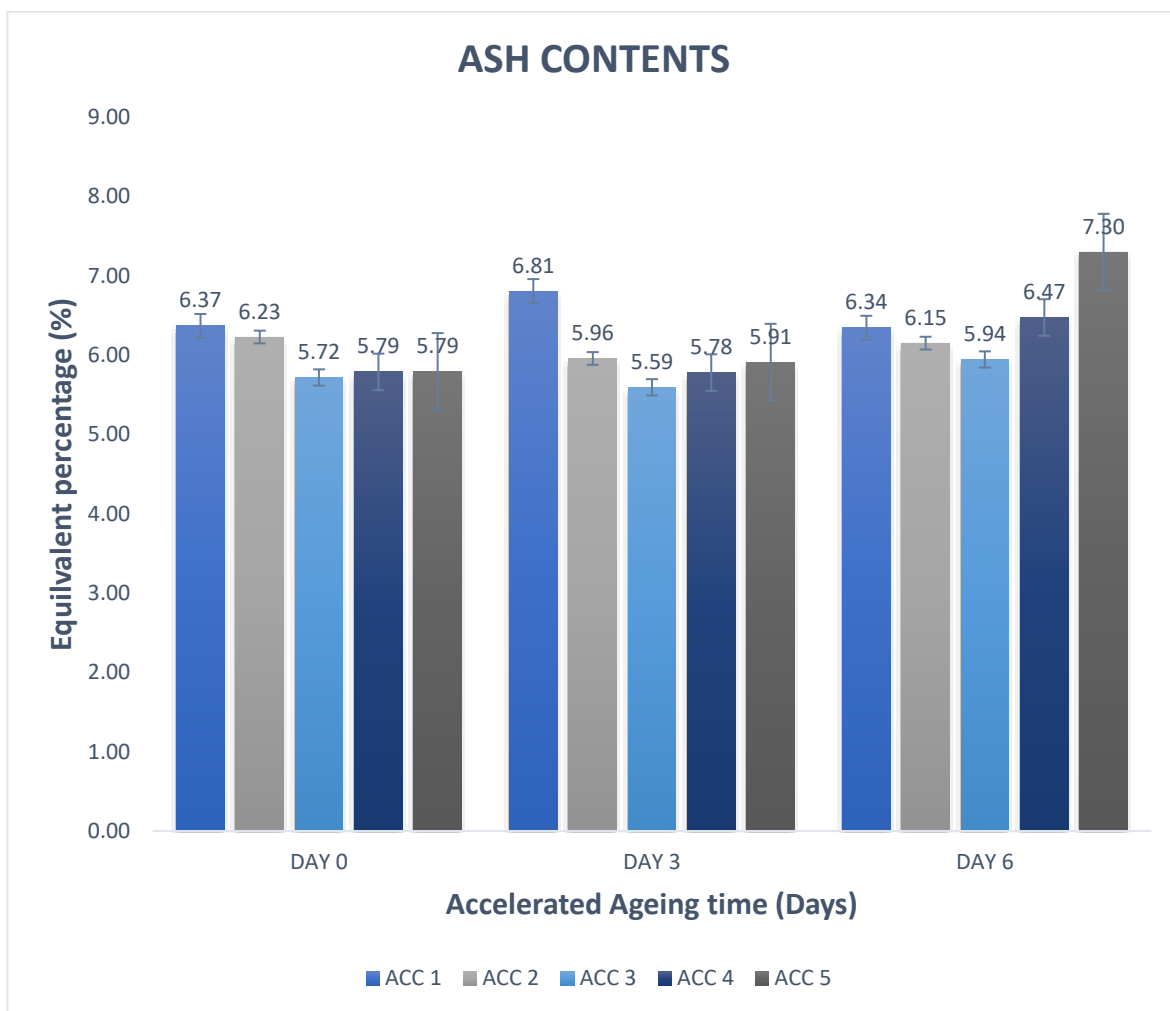
## APPENDIXES

### Appendix I. Proximate Composition of Aged Selected Sesame Seeds Accessions



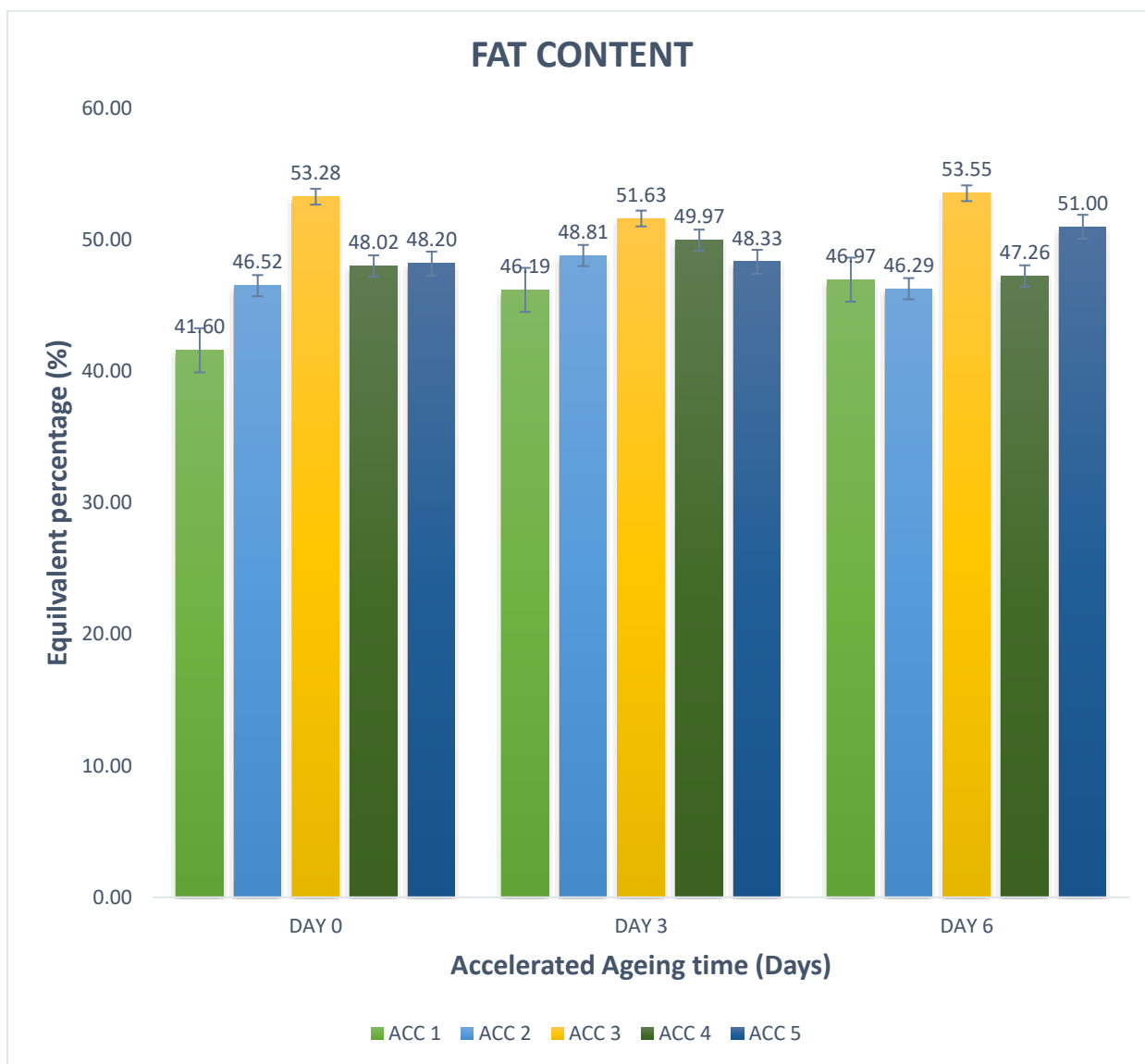
**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix II. Ash Composition in Sesame Seed Accessions



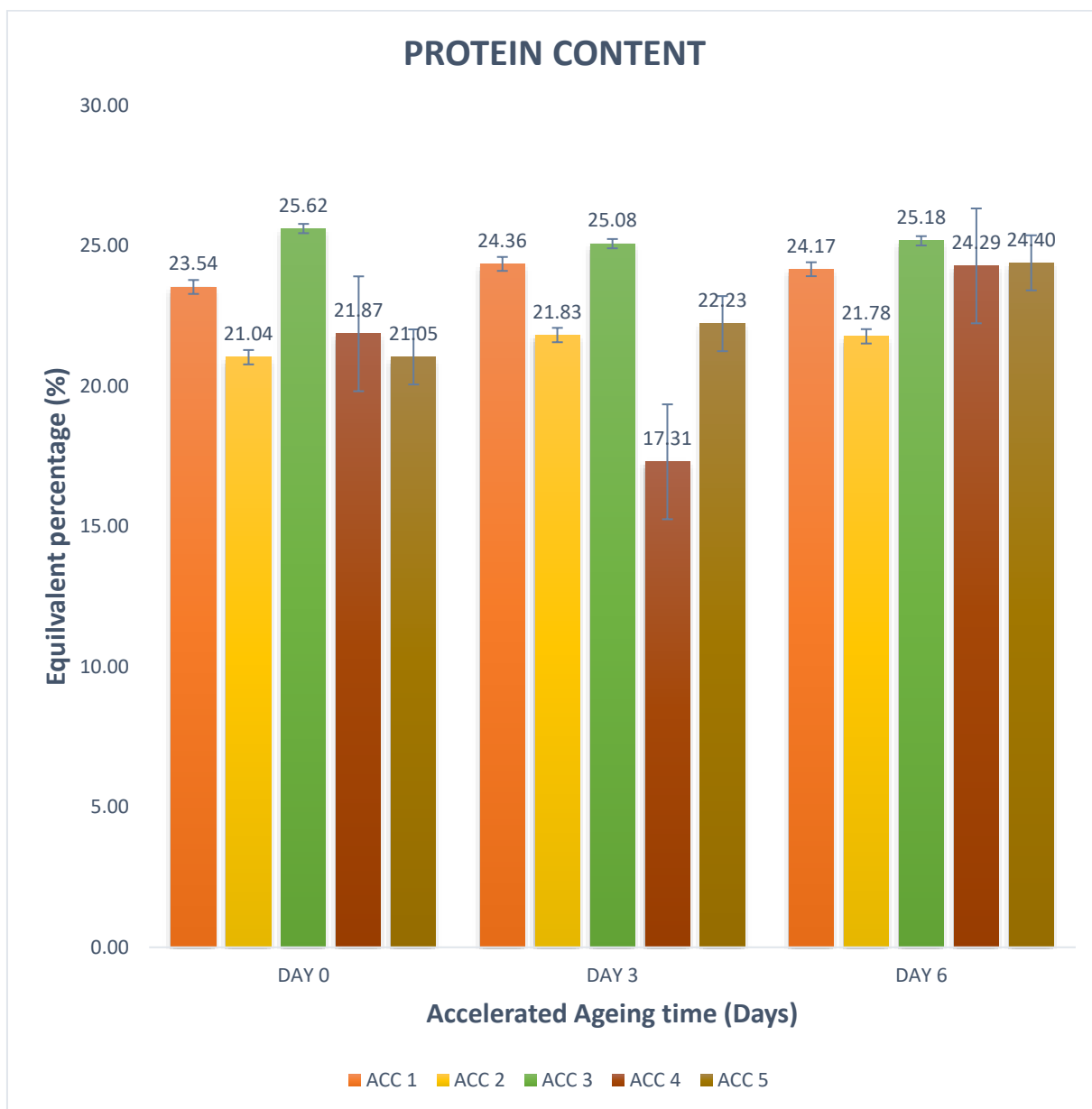
**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

### Appendix III. Fat Composition in Sesame Seed Accessions



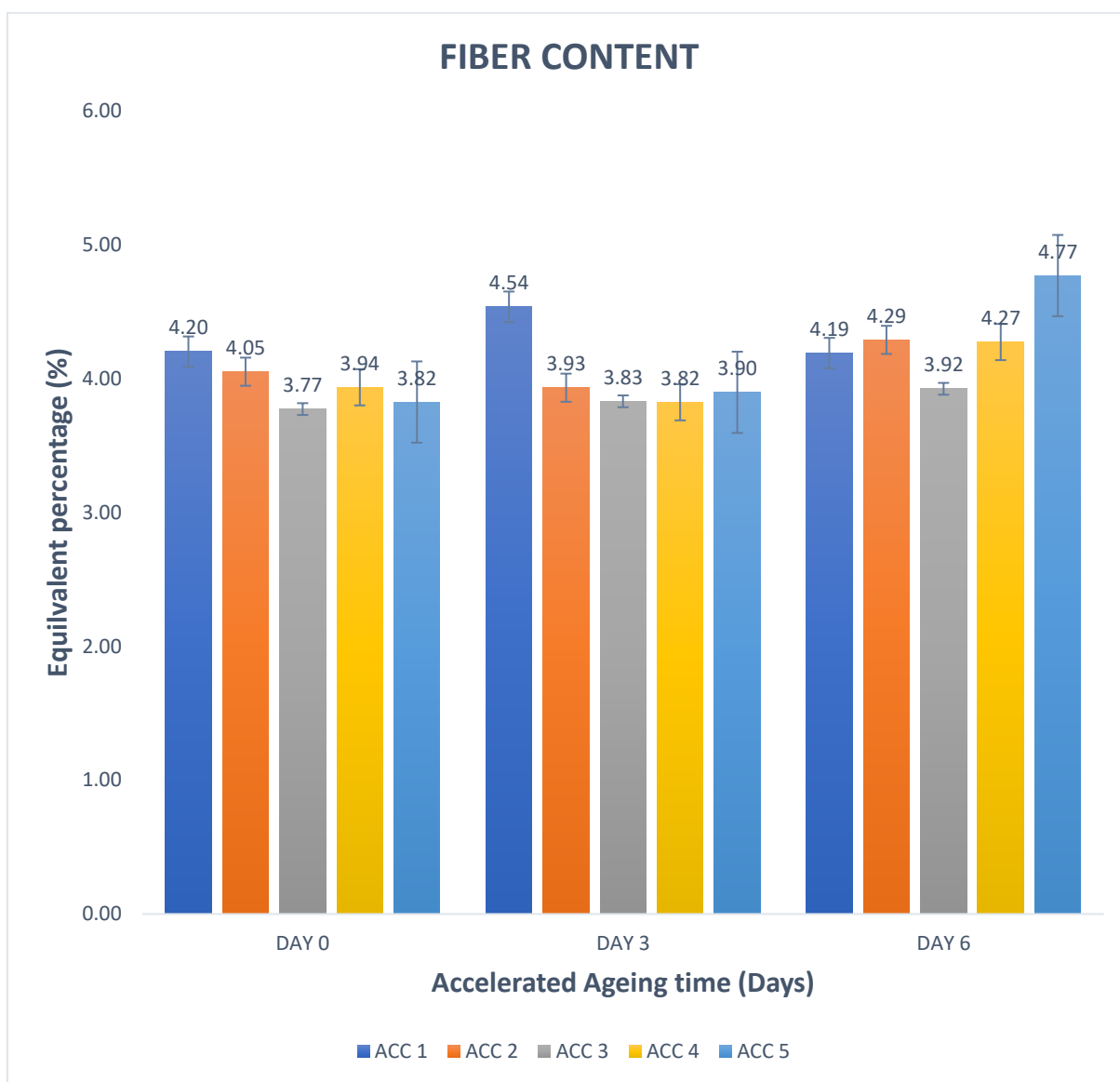
Key; ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

**Appendix (IV). Crude Protein Composition in Sesame Seed Accessions**



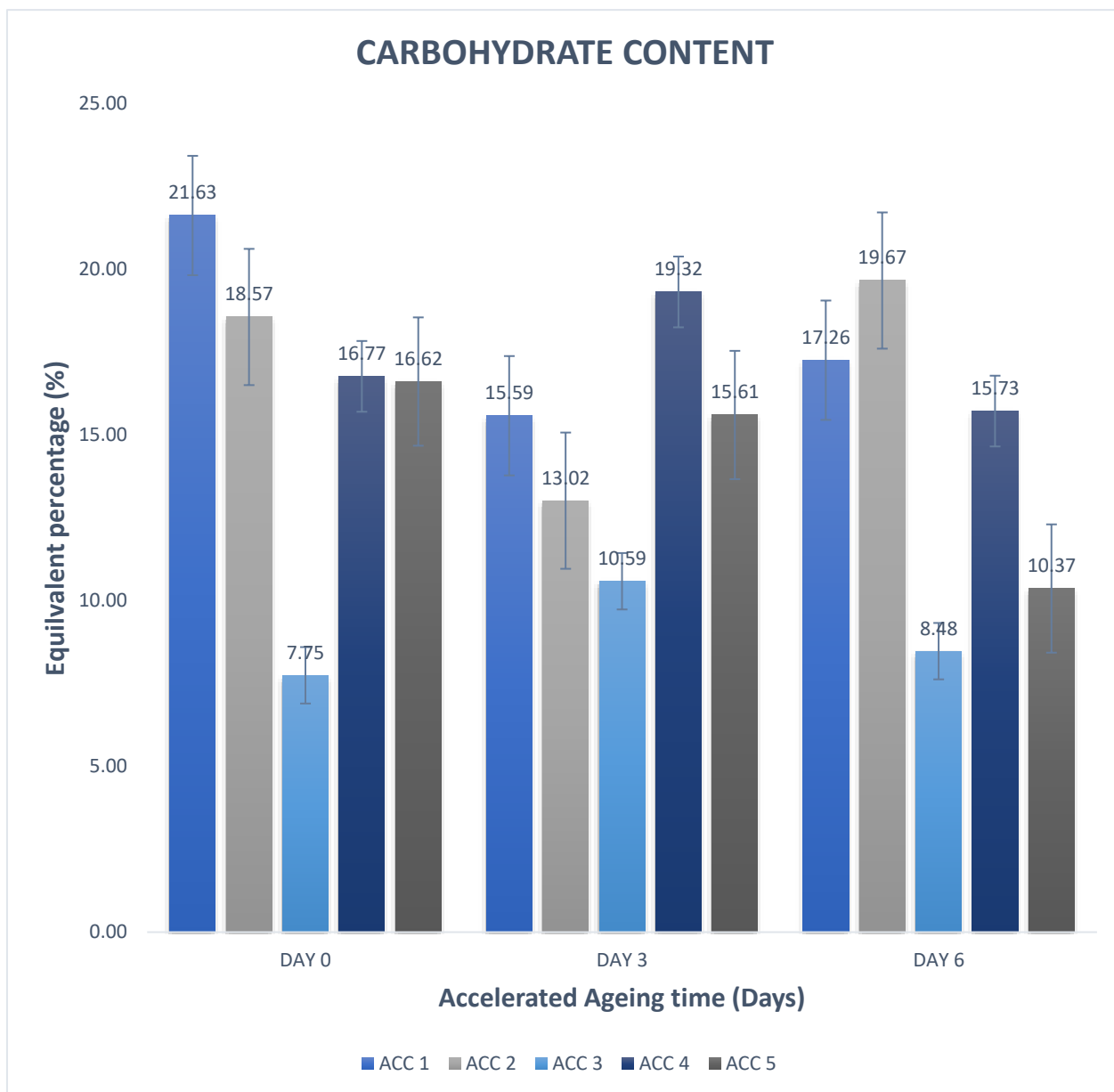
**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix V. Crude Fiber Composition in sesame seed Accessions



**sKey;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

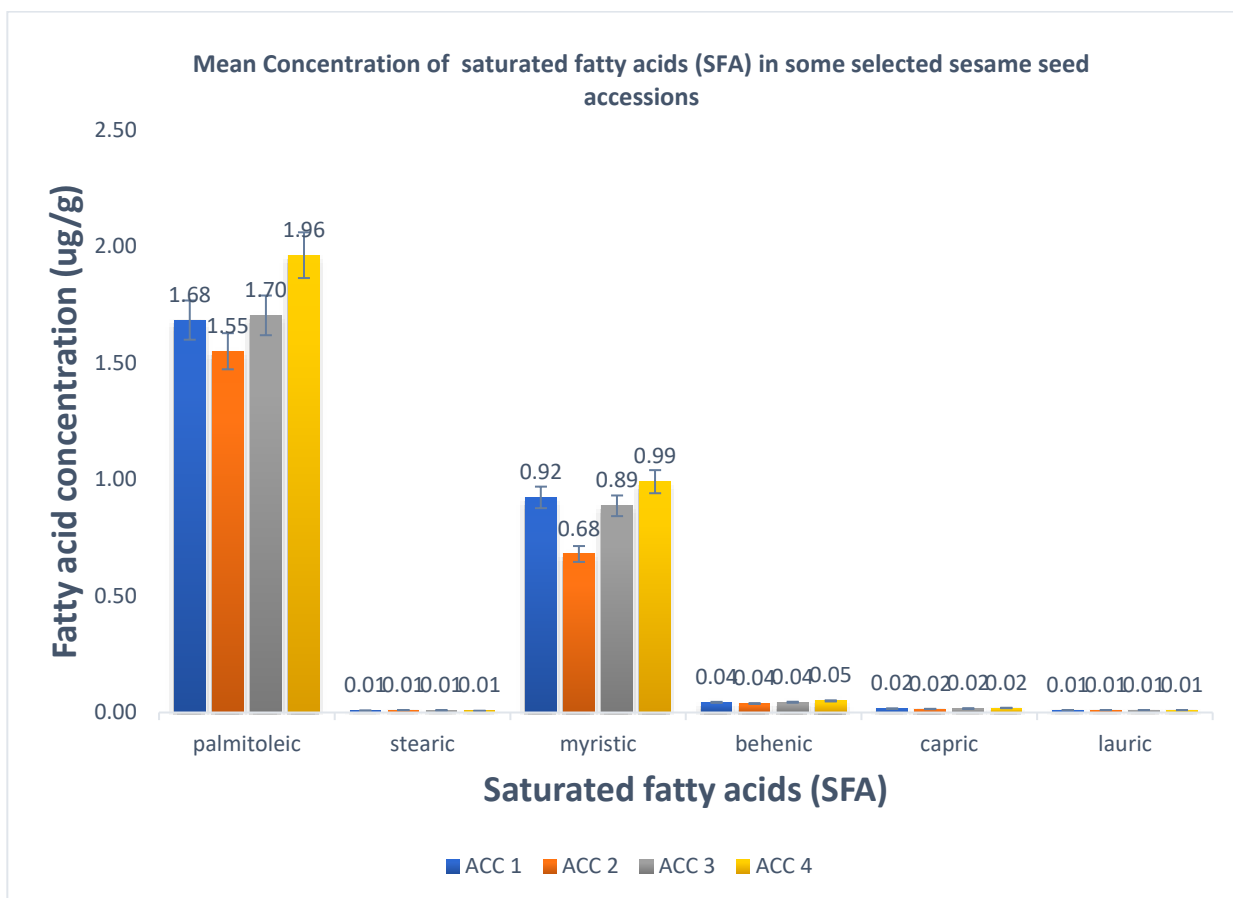
**Appendix (VI). Carbohydrate composition Sesame Seed Accessions**



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

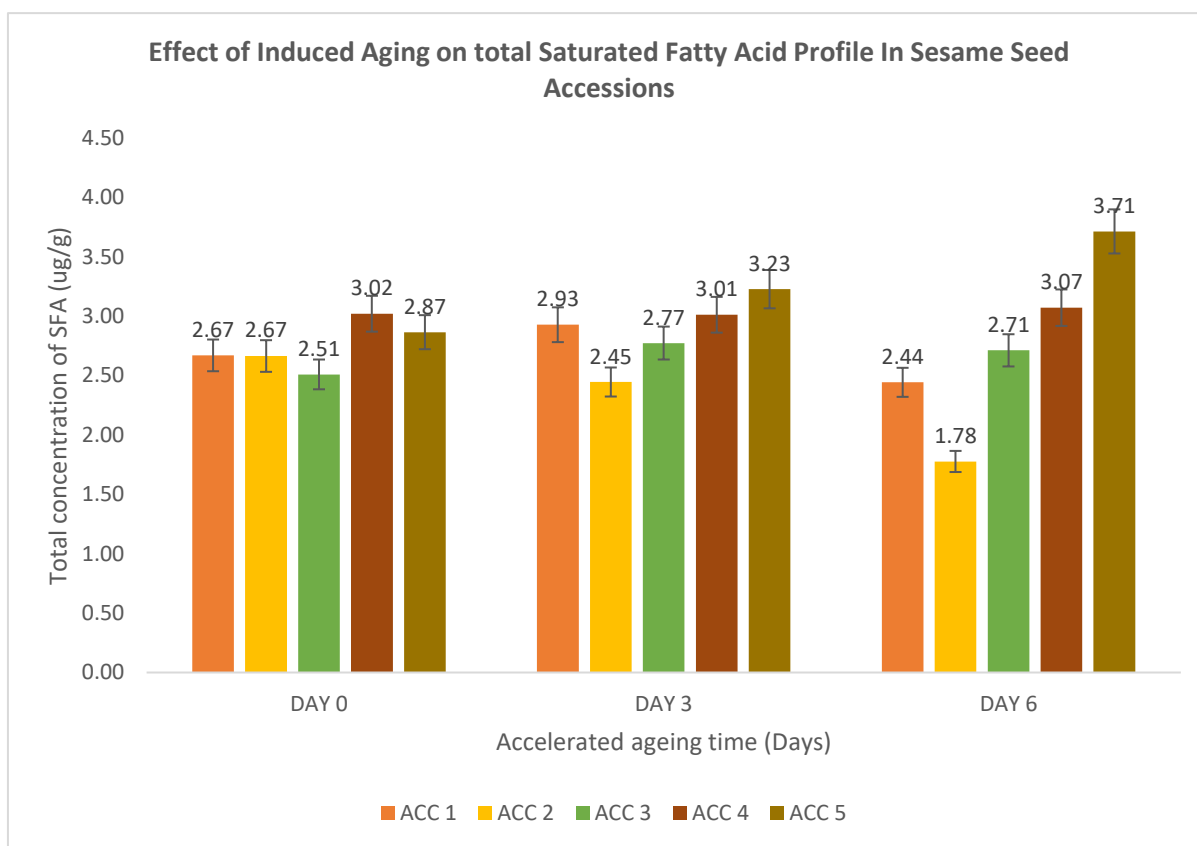


**Appendix VII. Mean Concentration (SFA) in selected sesame seed accessions (µg/g)**



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

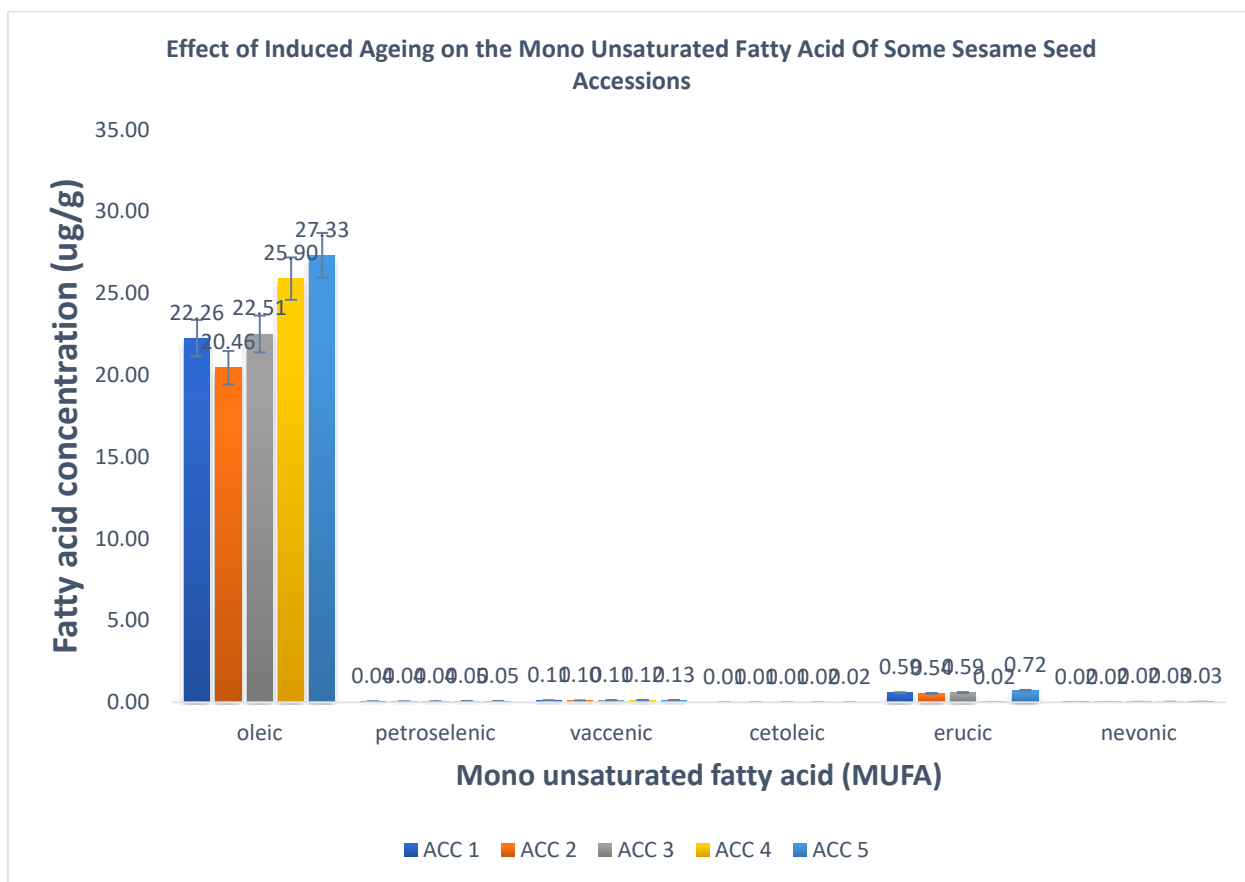
## Appendix VIII. Total Saturated Fatty Acid (SFA) Profile in Sesame Seed Accessions



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix IX. Mean Mono Unsaturated Fatty Acids (MUFA) of Some Sesame Seed

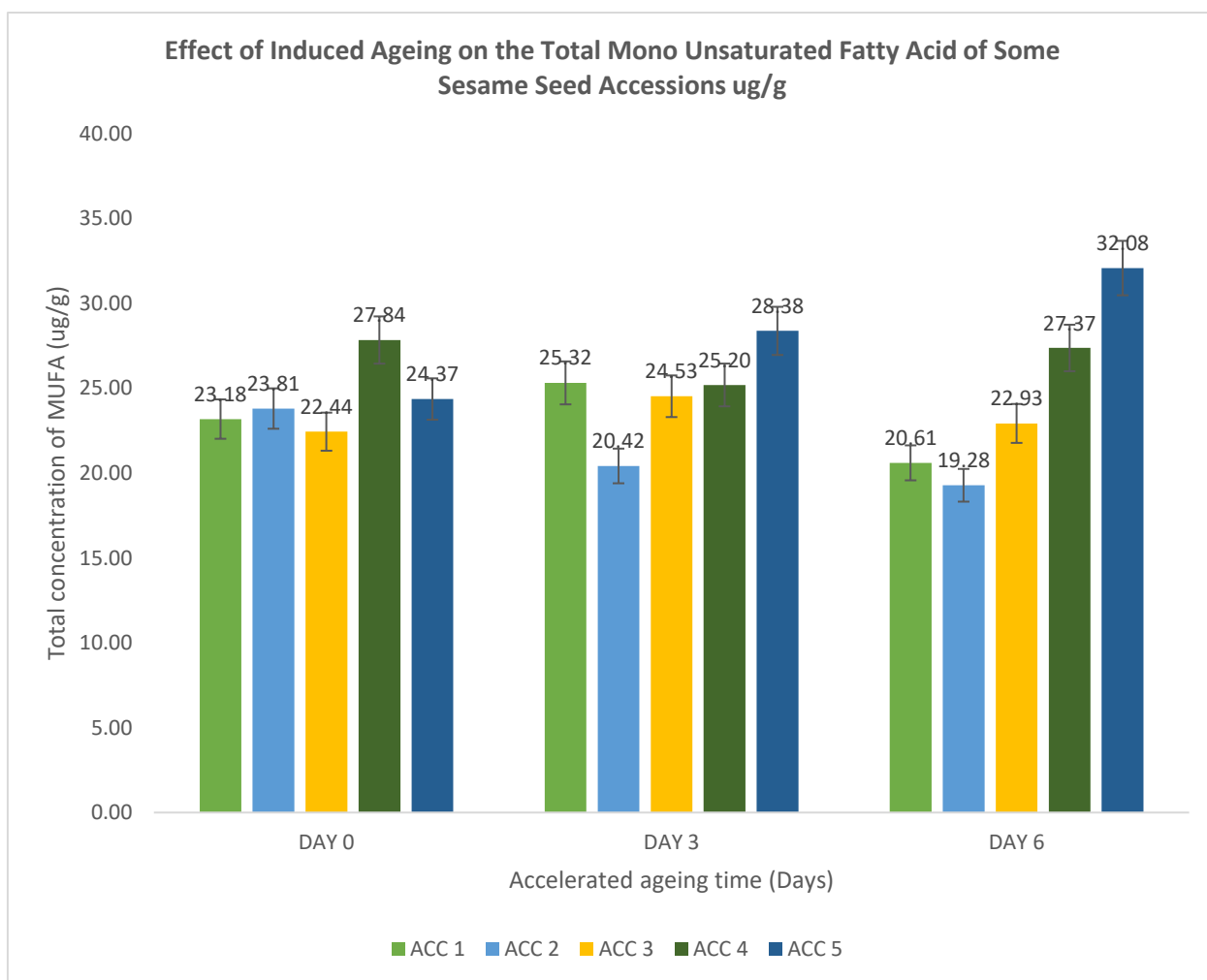
### Accessions



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

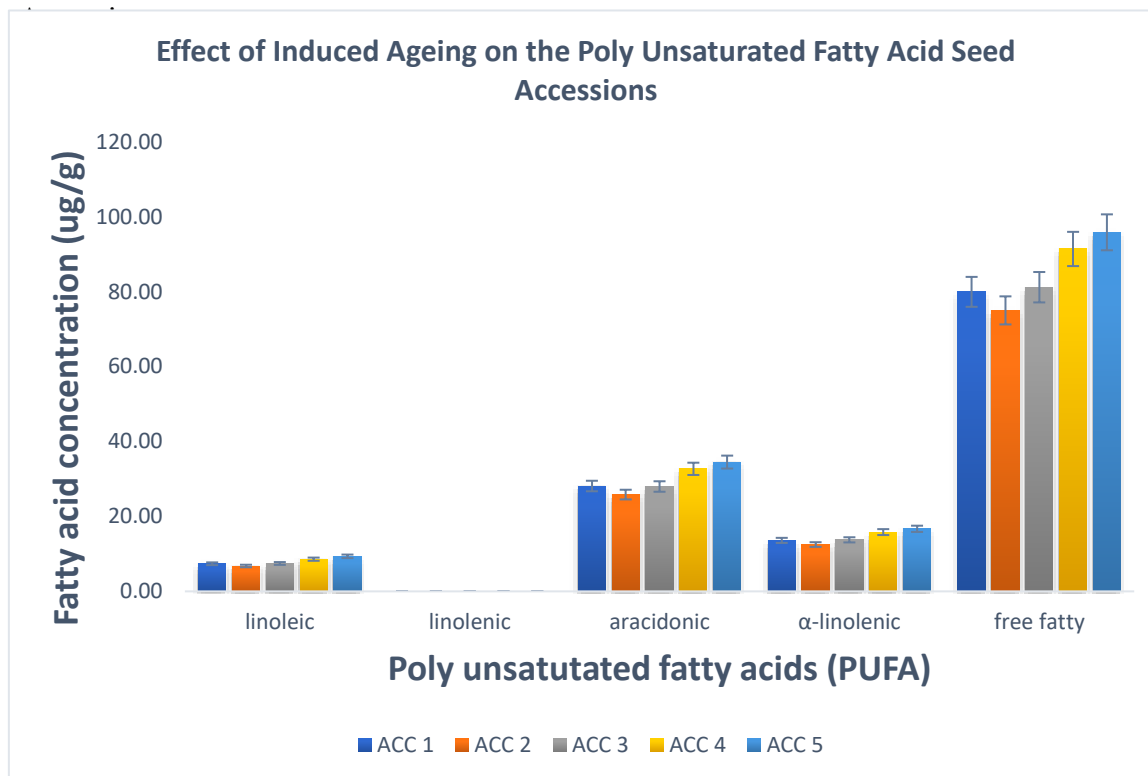
## Appendix X. Total Mono Unsaturated Fatty Acid (MUFA) Profile in Sesame Seed

### Accessions



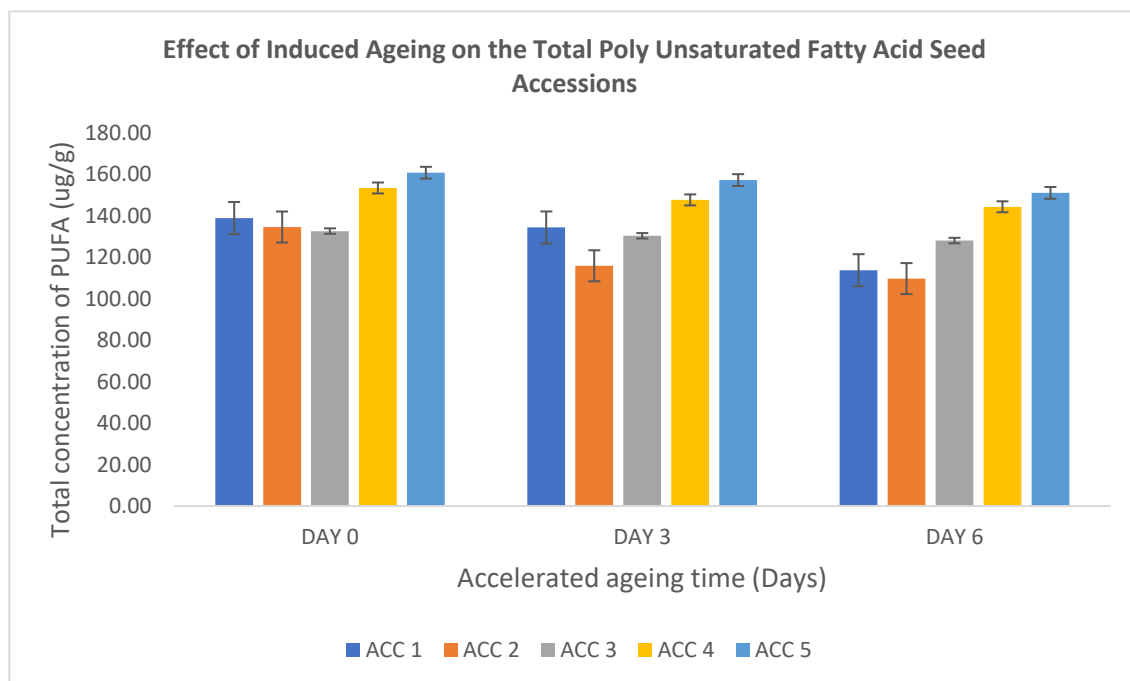
**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix XI. Mean Poly Unsaturated Fatty Acids (PUFA) of Some Sesame Seed



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

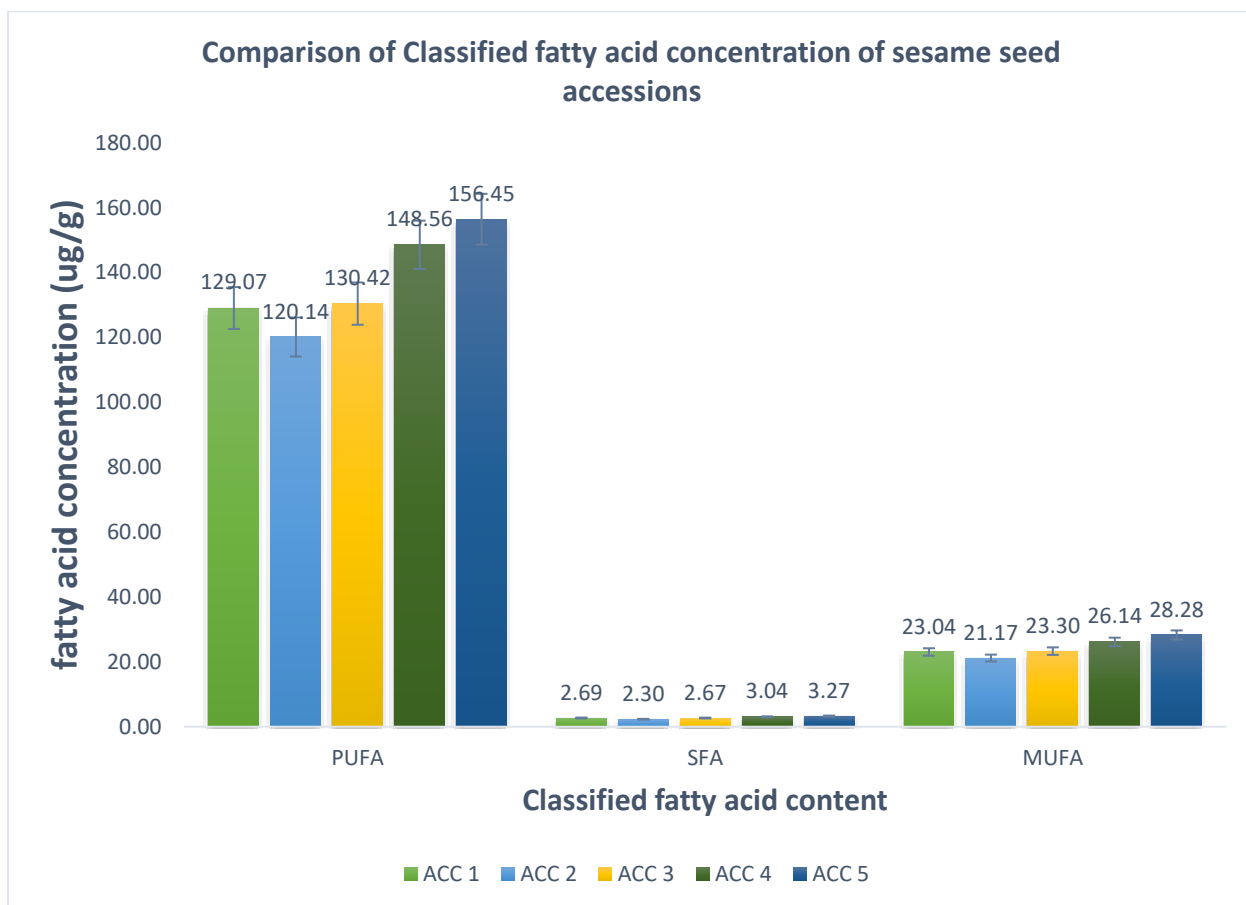
## Appendix XII. Total Poly Unsaturated Fatty Acids (PUFA) of Some Sesame Seed Accessions



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## Appendix XIII. Comparison of Classified Fatty Acid Concentration of Sesame Seed

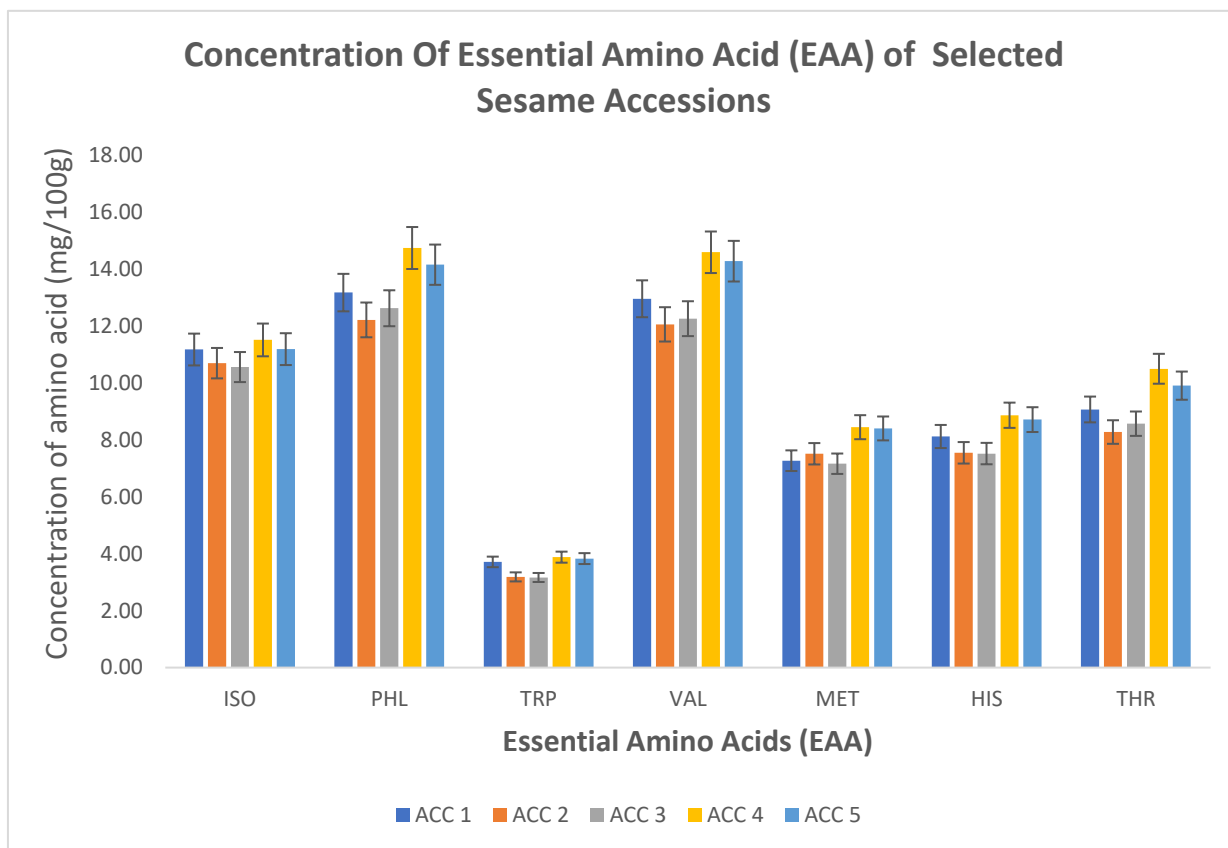
### Accessions



Key; ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix XIV. Concentration of Essential Amino Acid (EAA) of Selected Sesame

### Accessions

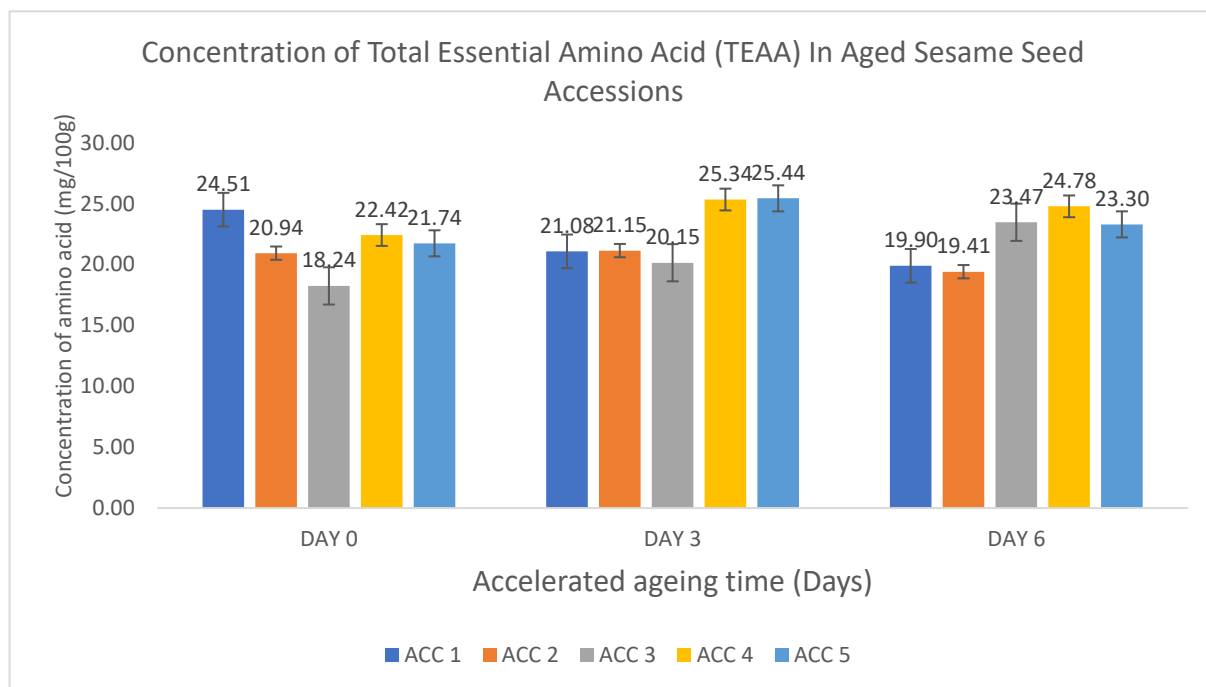


**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203



## Appendix XV. Concentration of Total Essential Amino Acid (TEAA) In Aged Sesame

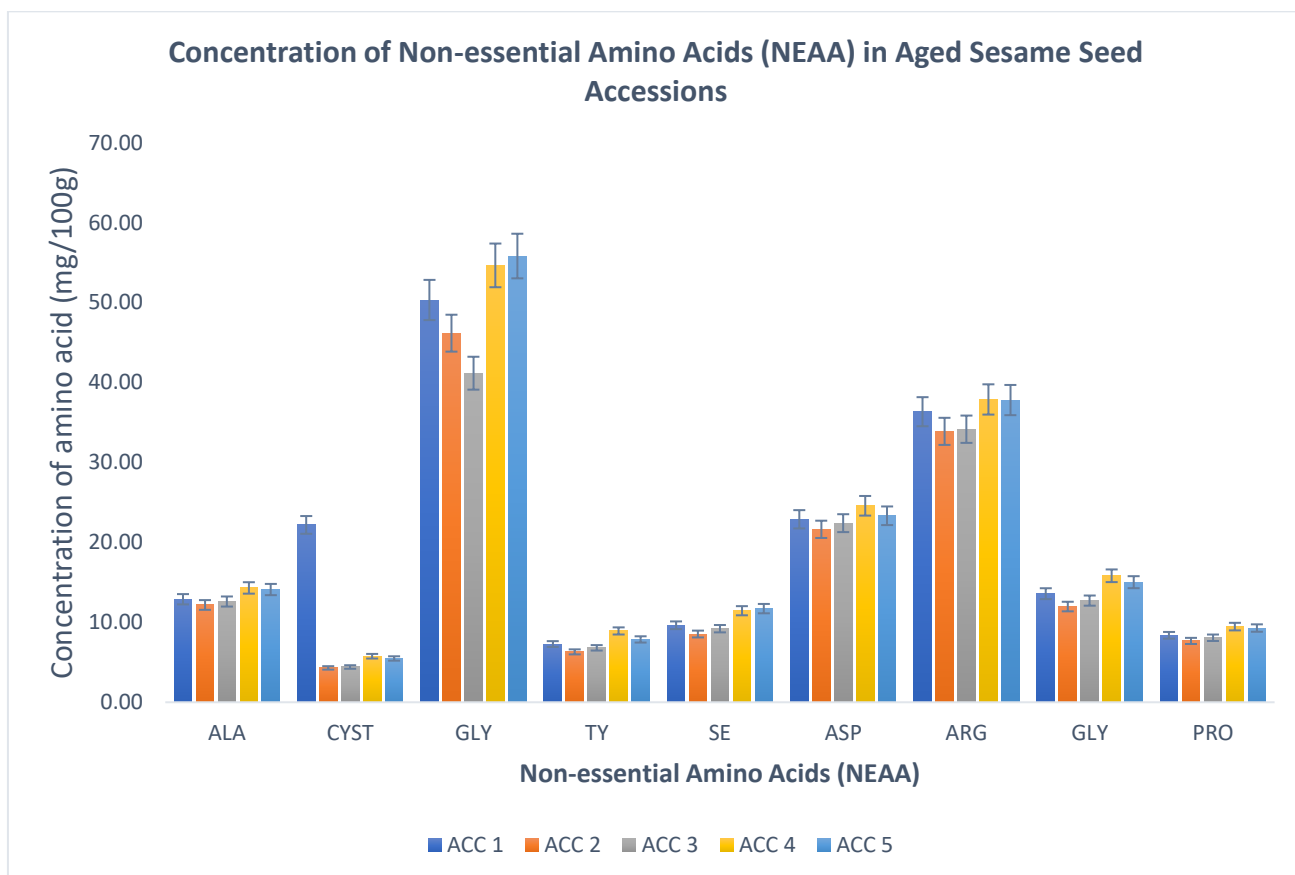
### Seed Accessions



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

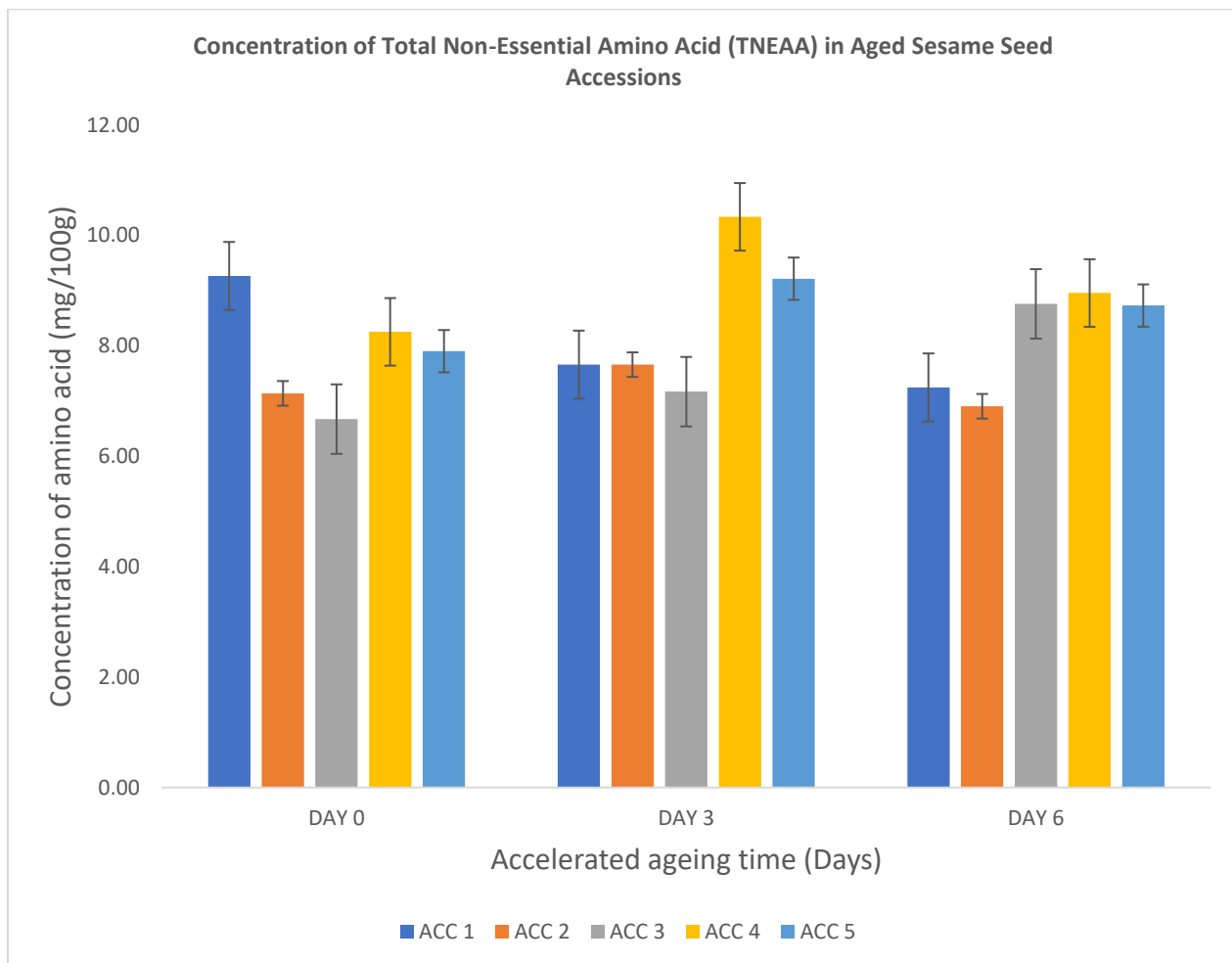
## Appendix XVI. Concentration of Non-Essential Amino Acids (NEAA) In Aged Sesame

### Seed Accessions



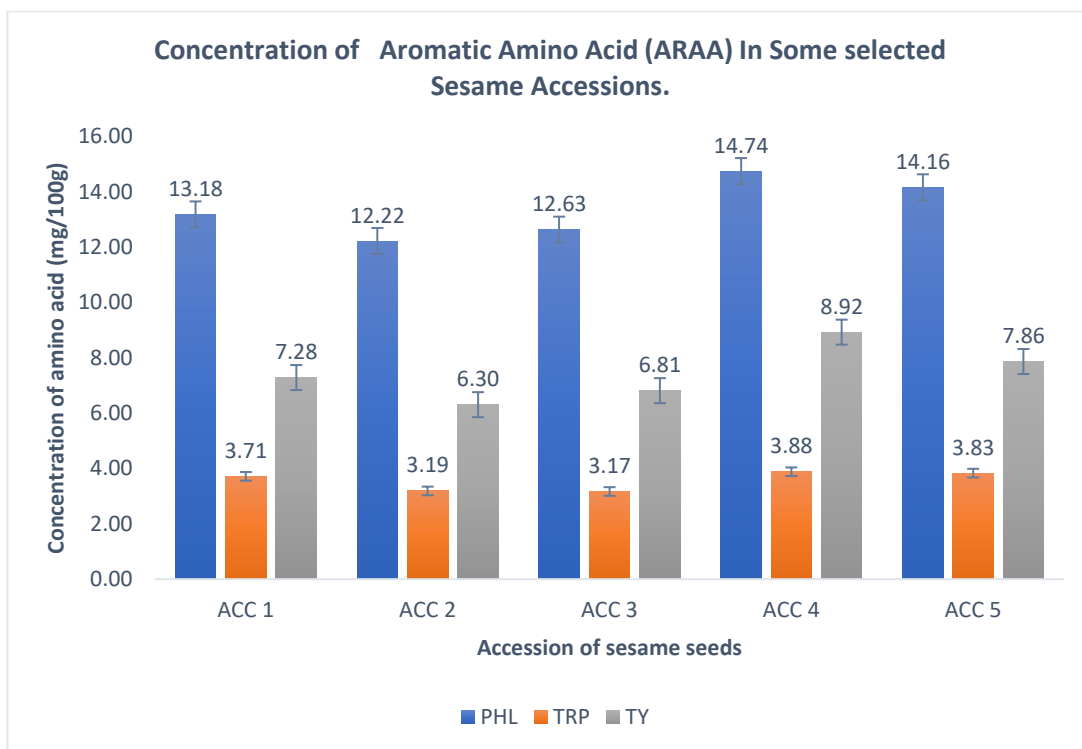
**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

**Appendix XVII. Concentration of Total Non-Essential Amino Acids (NEAA) In Aged Sesame Seed Accessions.**

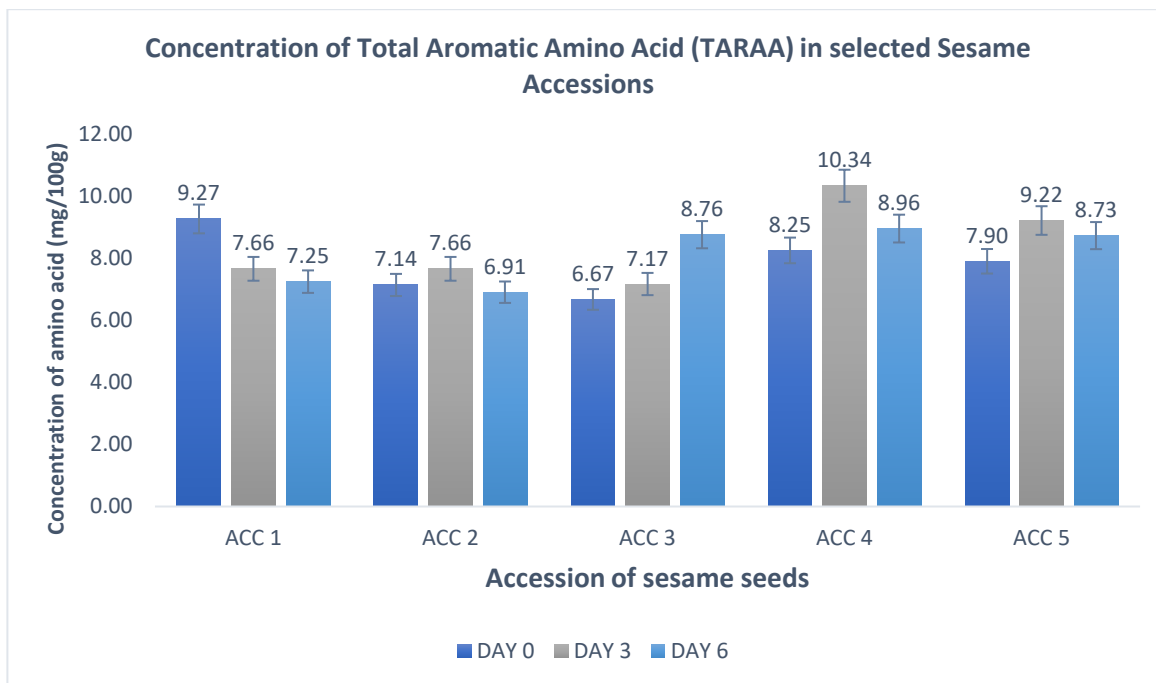


**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

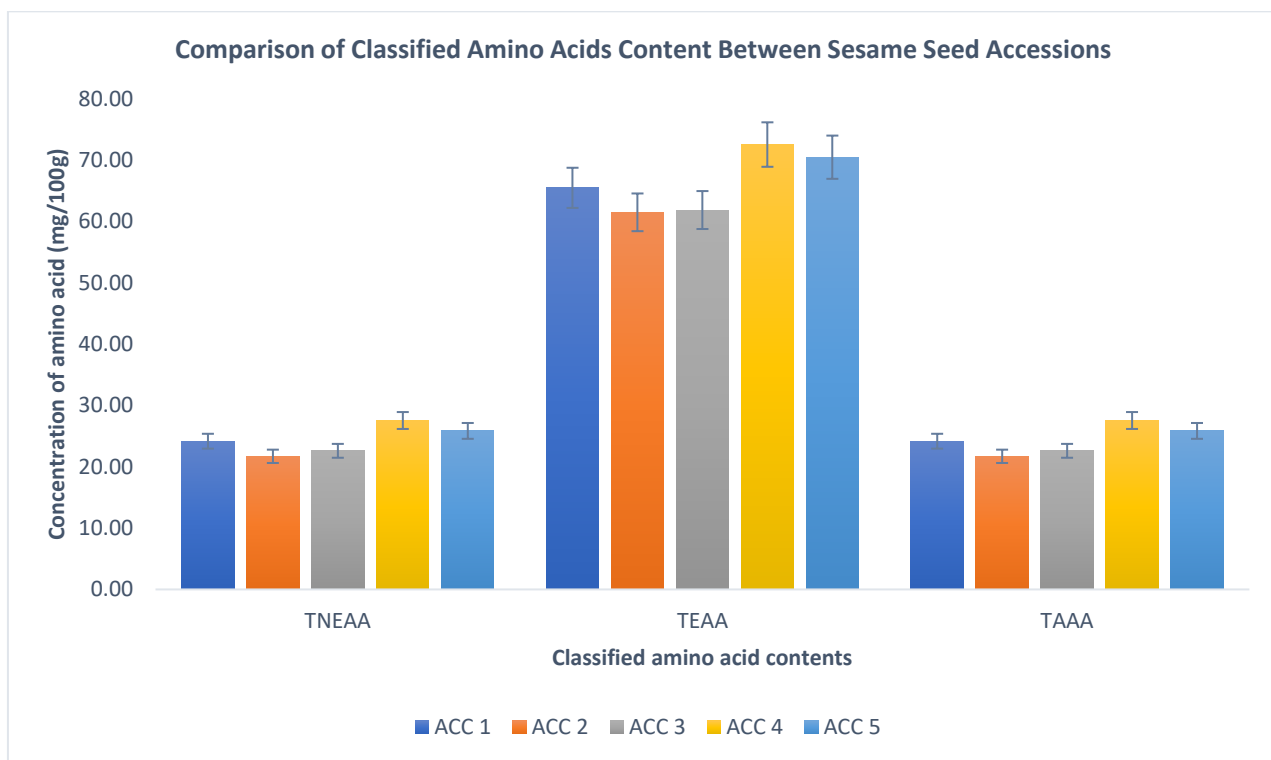
**Appendix XVIII. Concentration of Aromatic Amino Acid (ARAA) In Some Selected Sesame Accessions**



## Appendix XIX. Concentration of Total Aromatic Amino Acid (TARAA) in Selected Sesame Accessions



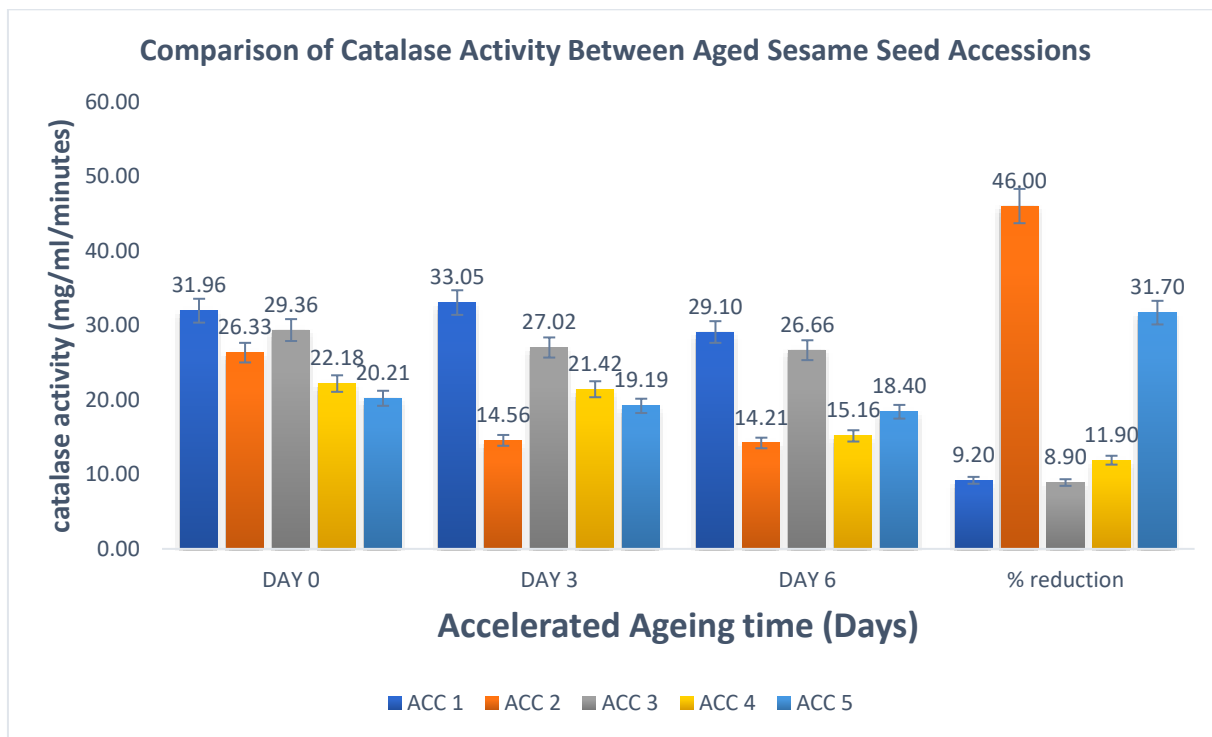
## Appendix XX. Comparison of Classified Amino Acids Composition Between Sesame Seed Accessions



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

**TEAA:** Total Essential Amino Acids, **TNEAA:** Total Nonessential Amino Acids, **TARAA:** Total Aromatic Amino Acids

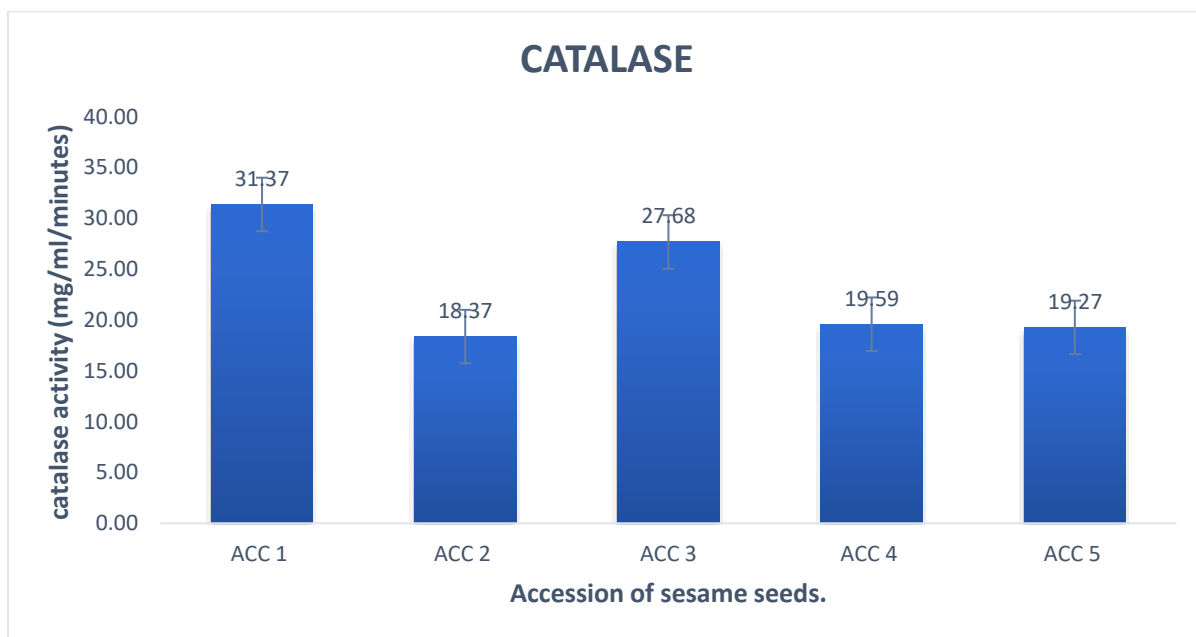
**Appendix XXI. Concentration of Induced Ageing on Catalase Activity (mg/mL/min) of Sesame Seed**



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203

## Appendix XXII. Comparison of Catalase Activity Between Aged Sesame Seed

### Accessions



**Key;** ACC 1= NCRIBEN106, ACC 2= NCRIBEN121, ACC 3= NCRIBEN131, ACC 4= NCRIBEN201 and ACC 5= NCRIBEN203



**Appendix XXIII. Effect of Induced Ageing on the Total Saturated Fatty Acid (SFA) of Some Sesame Seed Accessions ug/g**

Accessions	Days of induced ageing		
	0	3	6
NCRI-BEN106	2.67±0.10 <sup>a</sup>	2.93±0.20 <sup>b</sup>	2.44±0.12 <sup>b</sup>
NCRI-BEN121	2.67±0.02 <sup>a</sup>	2.45±0.01 <sup>a</sup>	1.78±0.02 <sup>a</sup>
NCRI-BEN131	2.51±0.30 <sup>a</sup>	2.77±0.02 <sup>b</sup>	2.71±0.06 <sup>c</sup>
NCRI-BEN201	3.02±0.20 <sup>c</sup>	3.01±0.12 <sup>c</sup>	3.07±0.03 <sup>d</sup>
NCRI-BEN203	2.87±0.14 <sup>b</sup>	3.23±0.10 <sup>c</sup>	3.71±0.01 <sup>e</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along a column are significantly ( $p < 0.05$ ) different.

**Appendix XXIV. Effect of Induced Ageing on the Total Mono Unsaturated Fatty Acid (MUFA) of Some Sesame Seed Accessions ug/g**

Accessions	Days of induced ageing		
	0	3	6
NCRI-BEN106	23.18±0.02 <sup>a</sup>	25.32±0.21 <sup>b</sup>	20.61±0.12 <sup>b</sup>
NCRI-BEN121	23.81±0.01 <sup>a</sup>	20.42±0.22 <sup>a</sup>	19.28±0.02 <sup>a</sup>
NCRI-BEN131	22.44±0.03 <sup>a</sup>	24.53±0.41 <sup>b</sup>	22.93±0.26 <sup>c</sup>
NCRI-BEN201	27.84±0.20 <sup>c</sup>	25.20±0.10 <sup>c</sup>	27.37±0.11 <sup>d</sup>
NCRI-BEN203	24.37±0.11 <sup>b</sup>	28.38±0.01 <sup>c</sup>	32.08±0.21 <sup>e</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along a column are significantly ( $p < 0.05$ ) different.

**Appendix XXV. Effect of Induced Ageing on the Total Poly Unsaturated Fatty Acid (PUFA) of Some Sesame Seed Accessions ug/g**

Accessions	Days of induced ageing		
	0	3	6
NCRI-BEN106	23.18±0.02 <sup>a</sup>	25.32±0.21 <sup>b</sup>	20.61±0.12 <sup>b</sup>
NCRI-BEN121	23.81±0.01 <sup>a</sup>	20.42±0.22 <sup>a</sup>	19.28±0.02 <sup>a</sup>
NCRI-BEN131	22.44±0.03 <sup>a</sup>	24.53±0.41 <sup>b</sup>	22.93±0.26 <sup>c</sup>
NCRI-BEN201	27.84±0.20 <sup>c</sup>	25.20±0.10 <sup>c</sup>	27.37±0.11 <sup>d</sup>
NCRI-BEN203	24.37±0.11 <sup>b</sup>	28.38±0.01 <sup>c</sup>	32.08±0.21 <sup>e</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along a row are significantly ( $p < 0.05$ ) different

**Appendix XXVI. Effect of Induced Ageing on the total Essential Amino Acid (EAA) of Selected Some Sesame Accessions mg/100g (%)**

Accessions	Days of induced ageing		
	0	3	6
NCRI-BEN106	24.51±0.02 <sup>a</sup>	21.08±0.21 <sup>b</sup>	19.90±0.12 <sup>b</sup>
NCRI-BEN121	20.94±0.01 <sup>a</sup>	21.15±0.22 <sup>a</sup>	19.41±0.02 <sup>a</sup>
NCRI-BEN131	18.24±0.03 <sup>a</sup>	20.15±0.41 <sup>b</sup>	23.47±0.26 <sup>c</sup>
NCRI-BEN201	22.42±0.20 <sup>c</sup>	25.34±0.10 <sup>c</sup>	24.78±0.11 <sup>d</sup>
NCRI-BEN203	21.74±0.11 <sup>b</sup>	25.44±0.01 <sup>c</sup>	23.30±0.21 <sup>e</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along a column are significantly ( $p < 0.05$ ) different.

**Appendix XXVII. Effect of Induced Ageing on the Total Non-essential Amino Acid (TNEAA) of Selected Some Sesame Accessions mg/100g (%)**

Accessions	Days of induced ageing		
	0	3	6
NCRI-BEN106	9.27±0.11 <sup>d</sup>	7.66±0.01 <sup>b</sup>	7.25±0.11 <sup>b</sup>
NCRI-BEN121	7.14±0.21 <sup>b</sup>	7.66±0.32 <sup>b</sup>	6.91±0.40 <sup>a</sup>
NCRI-BEN131	6.67±0.01 <sup>a</sup>	7.17±0.61 <sup>a</sup>	8.76±0.36 <sup>c</sup>
NCRI-BEN201	8.25±0.02 <sup>c</sup>	10.34±0.52 <sup>d</sup>	8.96±0.21 <sup>d</sup>
NCRI-BEN203	7.90±0.55 <sup>b</sup>	9.22±0.14 <sup>c</sup>	8.73±0.46 <sup>c</sup>

Values are mean ± standard error of mean of three determinations. Values with different superscript along a column are significantly ( $p < 0.05$ ) different.