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Determination of The Level and Composition of Fatty Acid in Oats: The Case of Ankesha Guagusa District Awi Zone Amhara Region Ethiopia

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DETERMINATION OF THE LEVEL AND COMPOSITION OF FATTY ACID IN OATS: THE CASE OF ANKESHA GUAGUSA DISTRICT AWI ZONE AMHARA REGION ETHIOPIA

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APPROVAL OF THESIS

I hereby certify that i have supervised, read and evaluated this thesis entitled "Determination of the levels and composition of fatty acids in oat: the case of Ankesha Guagusa District Awi Zone Amhara Region Ethiopia" by Gebeyehu Molla prepared under my guidance. I recommend the thesis be submitted for oral defense**.**

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APPROVAL OF THESIS FOR DEFENSE RESULT

This is to certify that the thesis prepared by GEBEYEHU MOLLA NIGATIE entitled "Determination of the levels and composition of fatty acids in oats: the case of Ankesha Guagusa District Awi Zone Amara Region Ethiopia" and submitted to the department of chemistry in partial fulfillment of the requirements for the degree of masters of science in chemistry (Analytical) complies with the regulation of the Universities and meets the accepted standards with respect to originality and quality

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DECLARATION

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Gebeyehu Molla. _____ _____

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List of Abbreviations

ANOVA	Analysis of Variance
DHA	Docosahexaenoic Acid
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
FID	Flame Ionization Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography -Mass Spectrometry
LCFA	Long-Chain Fatty Acid
LDL	Low-Density Lipoproteins
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
RT	Retention Time
SFA	Saturated Fatty Acid
SPSS	Statistical package of social science
TFA	Trans Fatty Acid
UFA	Unsaturated Fatty Acid

ABSTRACT

Oats are considered the cereal of the future due to their unique lipid composition compared to other cereals. Since the production origin and genotype can affect the fatty acid composition of oat lipids, this study evaluated the lipid content and fatty acid composition of three Avena sativa oat samples collected from three different sub-districts of the Awi zone, one of the main oat-producing areas of the Amhara region. The oat samples were collected using a purposive sampling technique during the 2022 crop season from three sub-districts of Ankesha District in the Awi Zone: Den Zuria, Dangula, and Urana. The Folch method was used to extract the lipids, and the acid-catalyzed derivatization method was employed to convert the fatty acids into fatty acid methyl esters (FAME). Gas chromatography-mass spectrometry (GC-MS) was utilized to determine the fatty acids. The results revealed that all oat samples contained a crude fat content of 4.23%, 5.64%, and 4.95% by weight, respectively, in samples collected from Den Zuria, Dangula, and Urana. In addition, in these samples, it was noted that there were a total of thirteen fatty acids, seven of which were unsaturated and six of which were saturated. The most common fatty acid concentrations in the three sub-district samples from Ankesha District were palmitic, linolenic, oleic, stearic, rumenic, gadoleic, and arachidic acids. Palmitic acid was the most abundant fatty acid in the oats, accounting for 43.4-49.6% of the total fatty acid content. The second and third most abundant fatty acids in the oat samples were linoleic and oleic acids, constituting 24.7-28.5% and 8.86-11.6%, respectively. Although the relative abundances of each fatty acid in the three sampling sites are insignificant, nutrient-wide variations are shown in the fatty acid profiles in Den Zuria, Urana, and Dangula oat varieties. The major one was palmitic acid, with as high as 363mg/100g in Dangula, linoleic acid ranged from 129 to 233 mg/100g, and linolenic acid ranged from 32.1 to 56.9 mg/100g. This may indicate that oat is a useful source of nutritionally important fatty acids and hence its consumption is beneficial to cardiovascular health. Further research would be done to find out if these fatty acids have health implications when included in the diet.This study noted that there is a slight variation in crude oil content and minor fatty acids across the sampling districts, but the seeds are richer in fatty acid and crude fat content compared to the common cereals such as barley and wheat reported elsewhere. Thus, oats could be a good source of crude fat for consumers.

Keywords: oats, fatty acids**,** gas chromatography-mass spectrometry (GC-MS), Crude fat

1. Introduction

1.1. Background of the Study

Oat (*Avena Sativa*) is among the important crop of the world and ranks sixth in the cereal production after maize, wheat, rice, barley and sorghum (Gazal *et al*, 2014). Oat groats have the highest lipid concentration among cereal grains (Morrison, 1978; Youngs, 1986). Thus, the lipids and lipid associated components in the groat are important to the functionality of oat products. The high lipid content of oats have an advantage in animal feed, providing high energy as well as being of a good fatty acid composition. But for human food use, high lipid content is less beneficial. It contains substantial lipase complement which is able to act under low moisture conditions (Glennie Holmes *et al*, 1975). Uncontrolled activity of these lipases leads to the development of rancidity and short storage lives for processed products (Hutchinson and Martin, 1955). The majority of the lipids of oats are in the endosperm, not the embryo (Youngs and Püskülcü, 1976). The fatty acid composition of oats and oat groats is a primary consideration and has been reported by several authors (Molteberg, 1995).

The folch method used to extract the lipids with different solvents such as water-saturated buthanol (Forsberg *et al*, 1974) or chloroform-methanol (2:1v/v) (Saastamoinen *et al*, 1989) and direct determination without prior extraction (Welch, 1977). The quantification of FAs in fats and oils by GC involves transforming the analytes into more volatile and nonpolar derivatives after extracting the lipids from the oat before GC analysis (Brondz, 2002). The most important stage for the GC-MS determination of FAs is sample preparation, which usually requires Derivatization of the FAs to increase the volatility of the substances to improve separation and to reduce tailing (Christie, 1993). The main fatty acids in oat are: Palmitic, oleic and linoleic acids represent 90-95% of all fatty acids (FA) (Halima *et al*, 2015), regardless of the place of cultivation and type of extraction (Zhou *et al*, 1999). The environment and genetics can play a major role not only in fat content but also in fatty acid composition (Carla *et al*, 2009). The present study was under taken to determine total lipd content and the fatty acid composition of the lipids of oats and the effect of environmental conditions on both compositions and content.

1.2. Statement of the Problems

Oat (*Avena Sativa*) is one of the less recognized and utilized cereal crops in Ethiopia. Plant (crop) oil characteristics are related to their fatty acid content and composition (Aliena *et al*, 2004). The fatty acid composition depends on the sources of the oils or grain. The fatty acid compositions of plant (crop) oils vary, depending on factors such as the place where the plants are grown, soil condition, and agro-climatic condition (Lawson, 1995).

Oat is commonly cultivated in the Ankesha Guagusa District (Dangula, Den Zuria and Urana sub district) which are selected as sampling site of this study. No more research has been done on determining the total lipid content and fatty acids composition from oat samples in this study area. Now a day's many people suffer from heart diseases because of over concentration and below the limit of essential fatty acids content in their diets. Therefore, this study attempts to determine the total lipid content and fatty acids composition in oat samples collected from in Ankesha Guagusa District Awi Zone (Dangula, Den Zuria and Urana sub district).

1.3. The objectives of the Study

1.3.1. General Objective

The General objective of this study is to determine the total lipid level and fatty acid composition in oats collected from Ankesha Guagusa District, Awl Zone, Amhara Region, Ethiopia by using GC-MS method

1.3.2. Specific Objectives

- To determine the total lipd content of oat
- To determine the individual composition of fatty acids in oats

1.4. Significance of the Study

The significance of conducting this research is:

- To create awareness about the levels and composition of fatty acids which are found in oat cultivated in Ankesha District.
- To make available information on the fatty acid composition of oat cultivated in Ankesha District.

1.5. Scope of the Study

The scope of this study was only to determine the level of fatty acid concentration and composition in oat (*Avena Sativa*). It covered the extraction of fats (lipids) and also the determination of fatty acids content and composition by GC-MS method. This study didn't include the fatty acids of *Avena Sativa* grown in other parts of Ethiopia.

1.6. Limitation of the Study

This study didn't incorporate physico-chemical properties, protein content, and minerals of oats which are grown in Ankesha Guagusa District, Awi Zone, and Amhara Region, Ethiopia.

2. Literature Review

2.1. Origin and Distribution of Oat

Oat is a cereal grain crop belonging to family Gramineae (*Poaceae*) with a haploid chromosome number of seven. The genus Avena has three ploidy levels: diploid, tetraploid and hexaploid. The classification of cultivated and wild species of the genus Avena remains somewhat confusing. Species and subspecies numbers and names vary according to author and criteria used to delineate taxa. A frequently used classification of the genus Avena was provided by (Baum, 1977). The center of origin of oat is unknown, but it may have been domesticated in Asia Minor or the Mediterranean region. The major center of diversity is Asia Minor where most subspecies of cultivated hexaploid oat are found (Gibson and Benson, 2002). The history of the oat crop is little known before the time of Christ. Oat was considered an important crop to man for a long time as it was a weed in other cereals such as wheat and barley for centuries before being domesticated as a crop (Barker, 1985). Domestication most likely occurred outside the major center of diversity. The oldest known oat grains were found in Egypt among remains of the 12th Dynasty and are approximately 4000 years old.

2.2. Oat Agronomy

Oat can be grown in climatically diverse areas but performs best under cool, moist conditions and is likely to be damaged by hot and dry weather, especially during grain fill. It can be grown on different soil types, but medium-textured soils with good water holding characteristics are most suitable. Annual precipitation in oat-growing regions ranges from 38 to 114 cm, but is often 76 cm or less (Sorrels and Simmons, 1992). Growing season precipitation is a key concern for oat production. Grain yield may be reduced greatly if there is water stress during the reproductive stages of plant growth.

2.3. Oat Chemical Composition

Oat grain chemical composition is variable and can be affected by genotype, growing environment and their interaction. Oat grain has a soft kernel (groats) and is considered a high

quality food and feed. The oat groat generally has the highest protein concentration of the cereals (12-20%) depending on genotype and growing environment (Peterson, 1992). Oat amino acid profile is considered nutritionally better than wheat, barely or maize, with higher levels of all essential amino acids. Oat is the only cereal which contains oil distributed throughout the seed. This quality is unique to oat but makes groat milling more difficult than for other cereals (Peterson, 1992). Oat oil percentage normally ranges between 4 and 11%, but values as high as 18% have been reported for an experimental line developed by recurrent selection. Higher oil oat lines are preferred as livestock feed because of the high energy density of oil versus carbohydrate. However, selection for high oil in oat groat appears to reduce potential grain yield.

2.4. Importance of Oat Crop

Oat is a low input cereal crop that is mostly grown in the great plains of United States and many other cooler regions of the world (Doehlert, 2002). Oats are mainly grown for forage, animal feed and human consumption. It was important mostly for human consumption in most of Northern Europe but later used as animal feed, especially a choice for horses. In many parts of the world, oats are grown for grains as well as for fodder and forage, straw for bedding, hay, haylage, silage and chaff (Stevens *et al*, 2004). Oat has a potential of generating threefold green fodder and about double the number of animals per item area can be fed compared to other traditional fodder crops (Husain *et al*, 1993). Oats are important constituents of valuable feed for all classes of livestock such as horses, cows, and poultry, young and breeding animals. Oats are rich in vitamin B1, fats, proteins and minerals such as phosphorus and iron (Stanton, 1953). Thus, it contributes to the considerable need for fodder in terms of quality and nutrition (Dost, 2001). Oats have been a part of the cuisine of the people living in cooler regions of the world. However, there is now a rising interest due to scientific evidence about the importance of oats in view of its high dietary value (Small, 1999; Welch, 2012). The dual purpose of grains owing to having protein and vitamins besides carbohydrates have only increased the biological value of oats compared to wheat, maize and barley.

2.5. Oil Content in Oats

Lipid content in oats is considerably high when compared to other cereals with high level of essential linoleic acid (Mattila *et al*, 2005). Fat content in oats is about 3-12% on a dry weight basis (Brown and Craddock, 1972). The fatty acid composition in oats is important from a nutritional viewpoint. It consists of unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid and saturated fatty acids such as myristic acid, Palmitic acid and stearic acid. Oleic, linoleic and Palmitic acids, however, are found in high levels (Hammond, 1983; Young's, 1986) whereas myristic, palmitoleic, eicosenoic, arachidic and erucic acids are found in very low amount (Saastamoinen *et al*, 1989). Linoleic and linolenic acids are essential fatty acids and are important for human health (Krishnan *et al*, 2000). Various studies have shown that both genetic and environmental conditions affect the amount of fat content in oats (Saastamoinen *et al*, 1989; Humphreys *et al*, 1994a; Welch, 1975). It has been also reported that there is a negative correlation between protein and oil content among different varieties of oats (Brown *et al*, 1966; Forsberg *et al*, 1974) whereas some reported no correlation between protein and oil concentration (Schipper and Prey, 1992; Silva *et al*, 2008).

2.6. Lipid and Fatty Acids

The term lipid refers to a structurally diverse group of molecules generally soluble in organic solvents (e.g. chloroform) and includes a wide variety of fatty acid-derived compounds are related to fatty acids. There is no single definition for lipid although analysts tend to have a firm understanding of the term. The most common class of lipids in nature consists of fatty acids linked to glycerol by ester bonding. Fatty acids are considered the simplest lipids and consist of a monocarboxylic acid at one end of a hydrocarbon chain. Other types of lipids include, but are not limited to, phospholipids, Glycolipids, sphingolipids, steroids, sterols and waxes. Lipids are important for functions in living organisms including serving as a chemical reserve of energy, building blocks of biological membranes and important biological signaling molecules (Dowhan and Bogdanov, 2002).

2.7. Fatty Acids

FAs are composed of carbons, hydrogen and oxygen arranged in a carbon chain skeleton with a carboxyl group (-COOH) at the alpha position. FAs in biological systems usually contain an even number of carbon atoms typically between 8 and 24. The FAs with 16- and 18-carbons are more frequent. Fatty acids differ from each other by the number of carbon atoms and the number and placement of their double bonds. There are three classes of FAs: saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) (Christie, 1989). SFAs have carbon atoms containing all the hydrogen atoms that they can hold. MUFAs have carbon chain containing one double bond. PUFAs contain two or more double bonds. There are two families of polyunsaturated FAs, the omega-3 and omega-6 family. FAs have many physiological roles (Gurr, 1999). Essential FAs (EFA) are those polyunsaturated FAs that are required in the human diet for growth and proper functioning of the body (Erasmus, 1993). They include omega-3 FA such as alpha linolenic acid (ALA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Wijendran and Hayes, 2004). Long- chain FAs (LCFA) are fashaving 20 or more carbons in their chains as the case of arachidonic (20: 4n6) and docosapentaenoic (22: 5n3) acids (Simopoulos, 1998).

2.8. Fatty Acid Composition

Six fatty acids were found in every oat sample: Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and eicosenoic (20:1), acids. Myristic (14:0), palmitoleic **(**16:1**),** arachidic (20:0), and erucic (22:1) acids were found in small concentrations in most of the oat varieties. In most previous studies only myristic, Palmitic, stearic, oleic, linoleic and linolenic acids were found (Forsberg *et al*, 1974; Karow *et al*, 1984; Saastamoinen 1989). Frey and Hammond (1975) found small amounts (<0.1%) of lauric, palmitoleic, and arachidic acids in oil. The separation capability of the packed columns used in previous studies was not as good as that of the modern capillary columns used in this study. Sahasrabudhe (1979) found 20:1 to 20:5 fatty acids in small amounts (0.5-3.0%in all) and traces of behenic, erucic and lignoceric acids, but the amounts of individual fatty acids were not specified.

2.9. Fatty Acid Nomenclature

In chemical nomenclature the carbon of the carboxyl group is carbon number one. Greek numeric prefixes such as di, tri, tetra, penta, hexa.... etc., are used as multiples and describe the length of carbon chains containing more than four atoms. Thus, "9, 12-octadecadienoic acid" indicates that this is an 18-carbon chain (octa-deca) with two double bonds (di-en) located at carbon 9 and 12, with carbon one constituting a carboxyl group (oic acid) (Zamora, 2005). FAs are frequently represented beta notation such as 18:2 that indicates that the FA consists of an 18-carbon chain and 2 double bonds (Beare Rogers *et al*, 2001; Moss, 1976). In biochemical nomenclature the terminal carbon atom is called the omega-carbon atom. The term "omega-3 or omega-6" signifies that their double bond occurs at carbon number 3 or 6, respectively counted from and including the omega carbon. This makes it possible to classify PUFA in families: omega-3 and omega-6. For example the acid Eicosapentaenoic 20:5 omega-3 has 20 carbon atoms and 5 non-saturations (20:5) and the first non-saturation is on carbon 17 (20-3=17).

Table 2.1. Names and descriptions of some fatty acids found in biological materials.

Fatty Acids	Scientific name	No of double bonds	Carbon no & scientific symbol	Reference
Lauric acid	Dodecanoic acid	0	12:0	Beare-Rogers *et al*,2001
Myristic acid	Tetradecanoic acid	0	14:0	Christie,1989
Palmitic acid	Hexadecanoic acid	0	16:0	Beare-Rogers *et al*,2001
Palmitoleic acid	9-Hexadecenoic acid	1	16:1n-7	Beare-Rogers *et al*, 2001
Stearic acid	Octadecanoic acid	0	18:0	Beare-Rogers *et al*, 2001
Vaccenic acid	11-Octadecenoic acid	1	18:1n-7	Christie,1989
Oleic acid	9-Octadecenoic acid	1	18:1n-9	Christie, 1989
Linoleic acid	9,12-Octadecadienoic acid	2	18:2n-6	Christie, 1989

alpha-linoleic acid	9,12,15-Octadecatrienoic acid	3	18:3n-3	Christie, 1989
Arachidic acid	Eicosanoic acid	0	20:0	Zamora, 2005
Gadoleic acid	11-Eicosenoic acid	1	20:1n-9	Zamora, 2005

2.10. Importance of Fatty Acid

In the human body, PUFAs are important for maintaining the membranes of all cells; for making prostaglandins which regulate many body processes which include inflammation and blood clotting. Another requirement for fat in the diet is to enable the fat-soluble vitamins A, D, E and K to be absorbed from food; and for regulating body cholesterol metabolism. Fatty acids are important for living organisms, since they are used as energy stores or hydrophobic parts of biological membranes. The stocks of living organisms can be met either by biosynthesis or by dietary supply. Saturated and monounsaturated fats are not necessary in the diet as they can be made in the human body. Two polyunsaturated FAs (PUFAs) that cannot be made in the body are linoleic acid and alpha-linolenic acid. They must be provided by diet and are known as essential fatty acids. Their metabolism leads to Eicosapentaenoic (EPA) and Docosahexaenoic acid (DHA) (Das and Leukot, 2004).

2.11. Ideal Oil Composition

Oats are an important part of human and other animal's diet, there is ongoing debate regarding the definition of an ideal oil profile. Oil end use is the most important factor to consider when describing an ideal fatty acid composition. Us health claims suggest a higher probability of coronary heart disease from diets high in saturated fats and oil (Food, Fat and Oils, 2006). Saturated fats increase the level of LDL cholesterol in human blood, increasing the risk of heart attack, stroke and other problems. For selecting oil for human health, it is recommended to keep the level of saturated fatty acids (primarily Palmitic and stearic acid) as low as possible. However, commercial food products such as margarine and shortening with desired thermal stability, melting and crystallization properties may require higher levels of saturated fatty acids ranging from 15 to 25% (Wood *et al*, 1993).

The original essential fatty acid linoleic (Tinaco, 1982), nutritionally is one of the two essential fatty acids, the other being linoleic acid, which the human body cannot synthesize on its own. These fatty acids act as substrates for synthesis of long chain polyunsaturated fatty acids (PUFAs) and other regulatory chemicals. A daily intake of 17g of PUFAs for adult males and 12g for adult females is recommended by the National Academy of sciences, the institute of medicines, 1.6g of linoleic acid for adult males and 1.1g for adult females is also recommended (Liu, 1994).

2.12. Extraction of Fatty Acids

Extraction is an important process for determining and characterizing different compounds from different matrixes. Extraction techniques are a powerful tool to extract and purify a wide range of target materials from different samples. They can be either sorbent-based or solvent-based. There are several steps involved in the preparation of a food sample for solvent extraction. It is necessary to dry the samples prior to fat extraction using solvents because many organic solvents are immiscible with water and cannot easily penetrate foods containing much water, and extraction would be inefficient (Hewavitharana *et al*, 2020). Solvent extraction can be used to extract essential oils that are thermally labile. Solvents that are commonly used for extraction are alcohol, hexane, ethanol, petroleum ether, chloroform, and methanol. The main advantage of extraction over distillation is that a lower temperature is used during the process, therefore reducing the risk of chemical changes due to the high temperatures used during distillation. Solvent extraction is inexpensive and relatively fast and is used to extract essential oils from food samples (Stratakos & Koidis, 2015).

2.13. Folch Method

The Folch method is the most well-known fatty acid extraction method proposed by Jordi Folch and the most reliable method for the quantitative extraction of lipids. The Folch extraction method is used as the more accurate method of measuring the amount of whole-body fat. Because the extraction of fat by the usual Folch method and laboratory variations of this method is very time consuming and expensive, but it is more accurate extraction technique used to give accurate result and used for fatty acid extraction (Washburn & Building, 1989).

2.14. Derivatization Methods

The conversion process of fatty acids into fatty acid methyl ester is called derivatization. Fatty acids were converted to the corresponding methyl esters prior to GC-MS analysis. Derivatization of fatty acids for GC analysis is performed to increase the substances' volatility and improve separation. In order to analyze the composition of fatty acids using gas chromatography as the separation method, a derivatization of lipids using esterification and transesterification reactions is needed. The fatty acid composition is determined as the methyl esters of fatty acids by GC-MS. The following reaction is expected during the derivatization process :(Mehari *et al*., 2019)

2.15. Gas Chromatography-Mass Spectrometry

Gas chromatography-Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography which the detection features of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass. The further addition of mass spectrometer in it achieved by single and triple quadruple modes (Sahil *et al*, 2011*).*

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Ankesha District, which is one of the 11th District of Awi Zone, Amhara Region, Ethiopia. Ankesha District is located 140 km far from Bahir Dar town which is the capital city of the Amhara region and 458 km from Addis Ababa. The District is bordered by Guagusa Shikudad District in the east, Guangua District in the west, West Gojjam zone in the south, and Banja District in the north. The District is divided into 29 sub-districts, three sub-districts of which agricultural investments are widely carried out in Dangula, Urana, and Den Zuria sub-districts. Ankesha Guagusa District lies at 10°31'46" to 10°41'32" North latitude and 36°36'18" to 36°59'33" East longitude. The major food crops or cereals grown in the area are maize, sorghum, and teff. Oily crops like that of oat and other crops are also produced in the area. Small-scale irrigation agriculture is practiced in the District. Ankesha District has two agroecological zones, namely dega (53.85%) and woina dega (46.15%). The Woreda is characterized by-modal rainfall pattern, with the main rain occurring during the kiremt season (from June to September) and a short rainy season collected during belg, which falls from February to March.

3.2. Chemicals

All reagents and standards used in the analysis were of analytical grade. Standard fatty acids (Sigma Aldrich) were used to identify the identity of fatty acids. Methanol (99%, ketone free, Alpha Chemika), chloroform (99.8%), and toluene (99%, Blulux Laboratory), n-hexane (99.9% - AR grade), acetone (99.8%, 400974-CARLO ERBA), sulfuric acid, anhydrous sodium sulfate, sodium chloride (Blulux Laboratory), and distilled water were used for the laboratory analysis.

3.3. Instrumentation and Apparatus

The following instruments were used in this study. electrical grinder (FW-100, high-speed universal disintegrator grinder), platform shaker (ZHWY-334), electrical balance, spatula, test tube, measuring cylinder, centrifuge (model 800-1), plastic test tube, micropipette, beaker, oven (universal hot air oven), fume hood, Teflon, aluminium foil, incubator (constant temperature and humidity incubator), vial, GC-MS (Agilent Technologies 7890B-5977A, china).

3.4. Oat Sample Collection

A half kilogram of the oat sample with its chaff (Figure 3.1) was collected from three sub-districts and two farmers were randomly selected, each using plastic bags.

Figure: 3. 1. Sample of oat within chaff

Oat samples collected from each sub districts was used for analysis of fatty acids in this study. Samples of oats were dehulled in a small scale impact dehullers. Contaminants were removed from the grain by shifting, winnowing, and sieving to ensure it free from chaffs, dusts, and other impurities (Figure 3.2). Samples of oat after removed chaff from the three sub districts was kept in plastic bags and transported to laboratory.

Figure: 3. 2. Sample of oat after removed chaff

3.5. Oat Grain Grinding

After cleaning, removing foreign material, oat grains was grounded into powder form with high speed electrical grinder (Figure 3.3), and then packed in polyethylene bags to avoid entrance of air and any other mixing of surrounding material.

Figure: 3. 3. Grinding of oat sample and powder

3.6. Preparation of Reagents

0.73% NaCl Solution:

In a 100 ml volumetric flask containing 50 ml of distilled water, 0.73 gram of NaCl was added and filled up to the mark with distilled water.

5% NaCl Solution:

In a 100 ml of volumetric flask containing 50 ml of distilled water, then 5 gram of NaCl was added and filled up to its mark with distilled water.

1% Methanolic Sulfuric Acid:

1 ml of concentrated sulfuric acid (98%) was pipetted into a 100 ml volumetric flask containing about 50 ml of methanol and filled up to the mark with methanol.

3.7. Sample preparation and Extraction Procedure

3.7.1. Extraction Procedure of Lipids

The folch method is the most well-known fatty acid extraction method proposed by Jordi Folch and the most reliable method for the quantitative extraction of lipids (Liu *et al*., 2018). The folch method was used to extract lipids from oat (*Avena Sativa*). A 1.0 gram (in duplicate) of a powdered sample of oat (Avena Sativa) was extracted with chloroform and methanol (2:1 ratio v/v) by using a Folch extraction method for 36 hours at 280rpm on a platform shaker with the aid of a test tube as shown in Figure: 3.4.

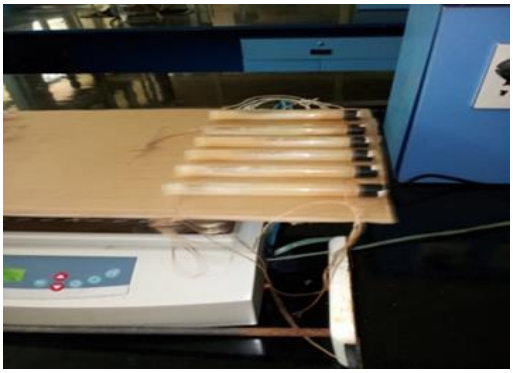

Figure: 3. 4. Extraction of lipids on platform shaker

After shaking with a platform shaker, the extract was decanted and centrifuged for 15 minutes to get a clear extract. Then it was treated with 2 mL of 0.73% aqueous sodium chloride (Figure 3.5) and the upper layer was removed using a micropipette (siphoning), and the lower phase (chloroform) layer containing the lipid was used in analyzing fatty acids (Fernandez *et al*., 2011). The solvent was removed under a hood and the residue was washed with 5.0ml of toluene.

Figure: 3. 5. Extracted lipids from oats

3.7.2. Fatty Acid Derivatization

Fatty acids are commonly derivatized to form fatty acid methyl esters (FAMEs), which are then identified using Gas Chromatography-Mass Spectrometry (Asperger *et al*., 2001). The polarity and inadequate volatility of FA make GC analysis difficult. The polar carboxyl groups must first be changed to more volatile nonpolar derivatives before GC analysis can be performed. For this purpose, various alkylation reagents are available, and fatty acids are typically transformed into fatty acid methyl esters (FAMEs). Acid derivatization methods can be applied to total fatty acids (free and bound). The commonly used derivatization reagent is sulfuric acid (Ichihara and Fukubayashi, 2010). The sulfuric acid derivatization method has also been widely used for the analysis of fatty acids in biological samples. Briefly, a 2 ml sample of the lipid extract in toluene was spiked with 50 µL of 3.48 mg/ml pentadecanoic acid as internal standard and allowed to react for 12hrs with 2.0 ml of 1% Methanolic sulfuric acid solution while being kept at 50℃ in an incubator (Figure 3.6).

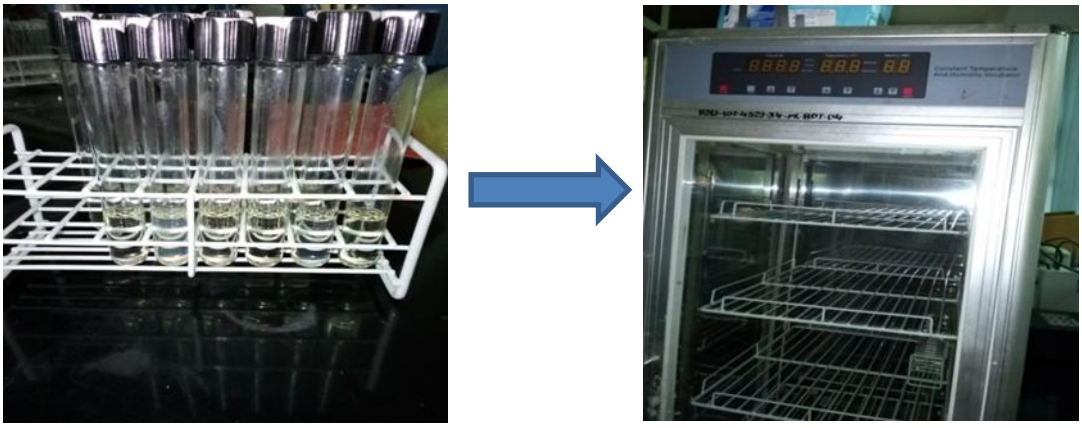

Figure: 3. 6. Derivatization of fatty acid into fatty acid methyl ester

After that, the reaction mixture was treated with 5.0 ml of a 5% aqueous sodium chloride solution and extracted twice with 3 ml of hexane. After phase separation, the upper layer (phase) was taken away into test tube, dried over anhydrous sodium sulfate to absorb water moisture and the upper phase (lipid extract with hexane) was sucked by using a micropipette, and then transferred to the test tube. A 1.5 ml of the sample with hexane was sucked by using micropipette and then added to GC-vial for analysis of GC-MS.

3.8. GC-MS Analysis

An Agilent Technologies 8790A gas chromatographic system equipped with an auto sampler, a split splitless injector, and a mass spectrometer (Agilent Technologies 7890B-5977A) was used for GC-MS analysis of the FAME. The chromatographic conditions were: 1µL sample injection volume (split less), injector temperature 280 ^{o}C, a HP-5MS UI fused silica capillary column (30m x 0.25 mm x 0.25 µm; Agilent Technologies), temperature program conditions of 60°C (held for 3 min), then ramped at 5°C mini-1 to 230°C (held for 20 min), helium was used as carrier gas at a flow rate of 1.0 mL min-1. Conditions used for the MS were: transfer temperature 280 ^{o}C, scan range mz^{-1} 60-550, ionization potential 70eV and electron multiplier voltage 1030 V.

3.9. Quantification of fatty acids

Thirteen fatty acids, with relative percentages of peak area higher than 0.1%, were quantified accurately and used for the geographical origin comparison of the oat sample. The relative composition of the fatty acids was calculated following equation-1. These fatty acids are: Palmitic, linoleic, oleic, linolenic, stearic, rumenic, gadoleic, arachidic, petroselinic, margaric, myristic, palmitoleic and lignoceric acids were determined relative to the internal standard by using the following equation (Karanja *et al*, 2014).

$$relative\ composition\ (\%) = \frac{individual\ peak\ area}{total\ peak\ area} x100\% \ldots\ldots\ldots equation - 1$$

In addition to profiling the relative percentage composition of the fatty acid, we determined the actual concentration of the fatty acid using an internal standard method reported by Mehari et al 2019 following equation 2.

$$\frac{W}{W}\left(\frac{mg}{100g}\right) = \frac{A_{FA}\, x\, m_{IS}}{A_{IS} x\, m_C} \ldots\ldots\ldots\ldots\ldots equation - 2$$

where A_{FA} is the peak area of the fatty acid, A_{IS} is the peak area of the internal standard, m_{IS} is the mass of the internal standard, and m_C is the mass of the oat sample used for the analysis.

3.10. Statistical Analysis

Data analysis was performed using the statistical software packing SPSS 26 (IBM Corp., USA). Each data set consisted of a matrix in which the columns represented the individual oat samples and the rows consisted of the chromatographic peak areas of all the detected fatty acids or the concentrations of fatty acids determined. A one-way ANOVA was used to test the presence of significant differences in the mean content of fatty acids among the different sub-districts of oat. At $p<0.05$, differences were considered significant. Microsoft Excel was used to determine the minimum, maximum, mean, and mean plus or minus (±) standard deviation of the fatty acid concentration of oats for each sub-district.

4. Result and Discussion

4.1. Crude Fat Content

The total fat (oil) content of oat seeds was extracted with chloroform-methanol (2:1 ratio) in folch method for 36 hours and the solvent was removed with fume hood. A specific feature of the oat grain is its high content of oil, which ranges from 3 to 12 % in different cultivars (Brown and Craddock, 1972). The average total lipid contents of oat in Dangula, Den Zuria and Urana sub-districts was found to be 5.64%, 4.95%, and 4.23%, respectively (Table 4.1). The minimum (4.23%) and maximum (5.64%) crude fat content of the oat was observed in the Urana and Dangula sub-districts of the Ankesha district, respectively. Oats from the Dangula sub-district are distinguished from those other sub districts by their total lipid content. According to the results of this study, the mean values of total lipids found in Dangula sub-district oats (an average of 5.64%) are higher than those found in oats from other sub-district. There was no significant differences in lipid contents of oat samples sampled from three sub-districts ($p > 0.05$) (Appendix 4). The absence of significance differences between the samples might be due to the fact that the three sampling districts share some agroecological characteristics.

Table. 4.1. Extracted crude fat content of oat sample.

No	Sub district	Mass of sample (g)	% of crude fat	Standard deviation
1	Den Zuria	1	4.95	2.04
2	Dangula	1	5.64	1.72
3	Urana	1	4.23	0.28

$$\%\ of\ crude\ fat = \frac{W2 - W1}{Ws} x100\%$$

Where W_2 is mass of beaker with sample, W_1 is mass of beaker and W_s is mass of sample.

4.2. Identification of the Fatty Acids in the Oat Samples

The identity of the individual fatty acids was identified using authentic standards and the NIST_MS library. Results of the analysis revealed that the fatty acid composition of oat oil contains unsaturated fatty acids, such as linoleic, oleic, linolenic, palmitoleic, gadoleic, rumenic, and petroselinic acids, and saturated fatty acids such as Palmitic, stearic, myristic, arachidic, margaric and lignoceric acids. The identities of the detected fatty acids were determined by comparing the retention times and mass spectral fragmentation patterns by using the National Institute of Standards and Technology-Mass Spectroscopy (NIST-MS) spectral library as a reference Table (4.2) and analyzing the standard solution of the fatty acids as illustrated in Figure 4.1.

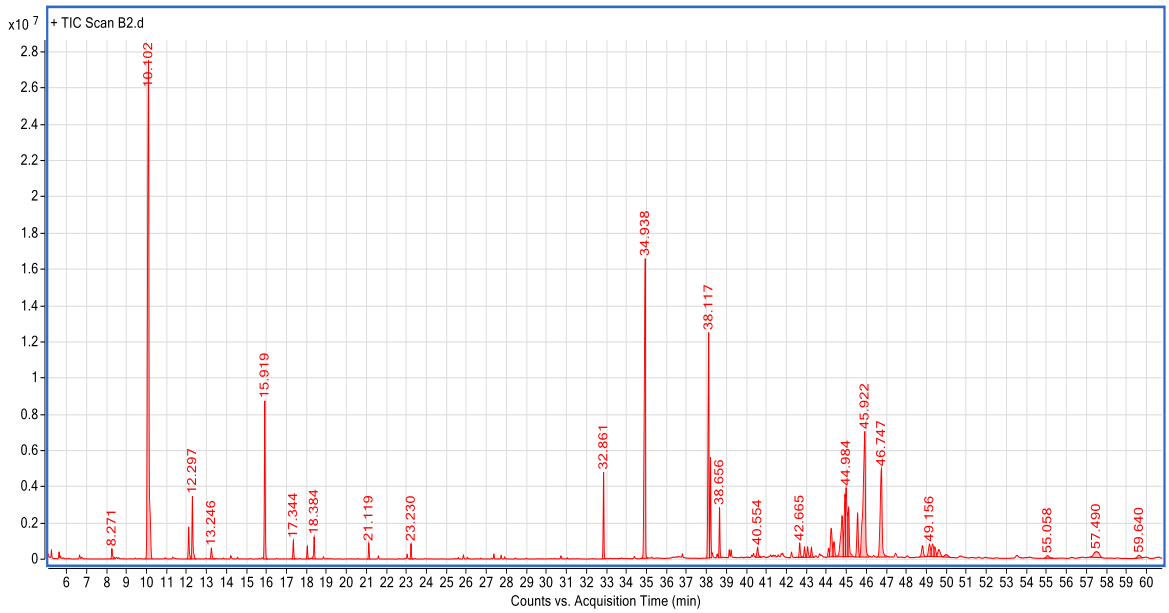

Figure: 4. 1. GC-MS chromatogram of Urana sub district oat sample extract, indicating the 13 detected fatty acids.

Based on the analysis of variance, there was no significant difference between the three sub districts of Ankesha District (Appendix 3). A statistical analysis using one-way ANOVA was performed to test the presence of significant differences in the fatty acid content of oats between the three sub-districts of the Ankesha District (Appendix 3). Appendix 3 showed that the contents of fatty acids in oat samples from three sub-districts of the Ankesha District were not significantly varied between and within groups except the three major fatty acids (Palmitic, Linoleic and rumenic acids). There was no significant difference in remaining fatty acid content,

between and within a group of oats samples sampled from three sub-districts ($p > 0.05$). The absence of significance difference between the samples might be due to the fact that the three sampling districts share some agroecological characteristics.

Table. 4.2. The name, retention time (RT), and means of identification of fatty acid determined in the oat sample

No	CPD name	Common name	Means of identification	RT
1	Tetradecanoic acid	Myristic acid	NIST_MS	30.72346
2	Pentadecanoic acid	Pentadecylic acid	Internal standard	32.861
3	9-Hexadecenoic acid (z)	Palmitoleic acid	NIST_MS	34.39625
4	Hexadecanoic acid	Palmitic acid	NIST_MS	34.9323
5	Heptadecanoic acid	Margaric acid	NIST_MS	36.79666
6	9,12-Octadecadienoic acid (z,z)	Linoleic acid	NIST_MS	38.09727
7	9-Octadecenoic acid (E)	Oleic acid	NIST_MS	38.19642
8	Methyl stearate	Stearic acid	NIST_MS	38.65613
9	Methyl 9-cis, 11-trans-Octadecenoic acid	Rumenic acid	NIST_MS	40.55471
10	6-Octadecenoic acid	Petroselinic acid	NIST_MS	41.58876
11	9-Eicosenoic acid	Gadoleic acid	NIST_MS	41.75628
12	Eicosanoic acid	Arachidic acid	NIST_MS	42.25507
13	6,9,12-Octadecatrienoic acid	Linolenic acid	NIST_MS	45.55122
14	Tetracosanoic acid	Lignoceric acid	NIST_MS	56.27148

4.3. Determinations of Fatty Acid Composition in Oats

Table 4.3 presents the detected fatty acids in the oat samples. Results are expressed in percent relative peak area. The most common fatty acids detected in oats in the study of three sub-districts are Palmitic, linoleic, oleic, linolenic, stearic and rumenic acids. In addition, gadoleic, arachidic, petroselinic, margaric, myristic, palmitoleic and lignoceric acids were present in trace amount. Among the detected fatty acids, some of them were saturated, while others were unsaturated. The detected unsaturated fatty acids were linoleic (18:2), oleic (18:1), linolenic (18:3), rumenic (18:2), gadoleic (20:1), petroselinic (18:) and palmitoleic (16:1) acids, while the saturated fatty acids were Palmitic (16:0), stearic (18:0), arachidic (20:0), margaric (17:0), myristic (14:0) and lignoceric (24:0) acids. Total saturated fatty acid (SFA) contents ranged from 50.34 to 57.24%, whereas total unsaturated fatty acid (UFA) contents ranged from 42.77 to 49.63 % of the total fatty acids. The content of total saturated fatty acids was higher than that of unsaturated fatty acids.

The relative composition of palmitic acid was highest across all three sub districts, being 49.61%, 44.28%, and 43.97% composition in Den Zuria, Dangula, and Urana, respectively. Regarding agronomic aspects, the oat samples were cultivated following the same traditional cultivation process. However,differences might occur due to the variation in means of control and protection applied by various farmers. Farmers with better practices had followed soil preparation, crop fertilization, pest and disease management, and better conditions during storage at harvest (Ahmad & Sharma, 2023).

The contents of palmitic and linoleic acids in the oat samples from the three sub-districts were within the ranges of 43.4 to 49.6% and 24.7 to 28.5% of total fatty acids, respectively. The most abundant fatty acid was palmitic acid, with a range of 43.4 to 49.6%, and the second major component was linoleic acid, with 24.7-28.5%.

Table 4.3 : The Average concentrations (in %) of fatty acids found in oat samples from three sampling areas.

No	Common name of fatty acid	RT	Sampling places		
			Den Zuria	Urana	Dangula
1	Myristic acid	30.72346	0.29±0.02	0.29±0.02	0.29±0.005
2	Pentadecylic acid	32.861	--	--	--
3	Palmitoleic acid	34.39625	0.28±0.005	0.26±0.02	0.26±0.03
4	Palmitic acid	34.9323	49.61±0.004	44.28±0.34	43.97±0.55
5	Margaric acid	36.79666	0.36±0.04	0.34±0.05	0.27±0.007
6	Linoleic acid	38.09727	25.11±0.45	28.47±0.02	27.97±0.03
7	Oleic acid	38.19642	8.89±0.03	10.11±0.14	11.06±0.51
8	Stearic acid	38.65613	6.03±0.10	5.39±0.40	4.86±0.13
9	Rumenic acid	40.55471	1.06±0.001	1.92±0.11	1.71±0.13
10	Petroselinic acid	41.58876	0.41±0.10	0.19±0.04	0.20±0.034
11	Gadoleic acid	41.75628	0.77±0.45	0.70±0.18	0.51±0.08
12	Arachidic acid	42.25507	0.67±0.01	0.64±0.004	0.69±0.04
13	Linolenic acid	45.55122	6.25±0.01	7.09±0.58	7.92±0.35
14	Lignoceric acid	56.27148	0.27±0.09	0.33±0.04	0.30±0.004
	SFA	-	57.24±0.27	51.26±0.85	50.37±0.74
	UFA	-	42.77±1.04	48.74±1.08	49.63±1.15
	MUFA	-	10.35±0.58	11.26±0.38	12.03±0.65
	PUFA	-	32.42±0.46	37.48±0.71	37.60±0.50

The average fatty acid content in percent (%) in oat samples in the study of three different sub districts of Ankesha District is shown in Figure 4.2 below. The following fatty acids were identified using GC-MS analysis: Palmitic, linoleic, oleic, linolenic, stearic, rumenic, gadoleic arachidic, petroselinic, margaric, myristic, palmitoleic and lignoceric acids. Among these Palmitic acid, linoleic acid and oleic acid were distinguished in each of the three different sub districts in the Ankesha District, i.e. the Figure 4.2 bar graph indicates that the content of the average fatty acids in samples differ between sub districts, even though the concentration differences are slight. Petroselinic, margaric, myristic, palmitoleic and lignoceric acids are also present in trace amount.

The observed variations in the fatty acid content between the oats from the three different sub district can be described to several factors, including: genetic factors, growth conditions and climatic factors and analytical procedures (Guil *et al*, 1998).

Other differences may result from harvest conditions, storage and post-harvest treatments or other processes that the crop is subject to before final use. Several investigators have pointed out that genetic properties play a real role in determining the fatty acid content of oat (Caporaso *et al*, 2018).

Figure: 4.2. Average fatty acid content of oat sample in three sub districts.

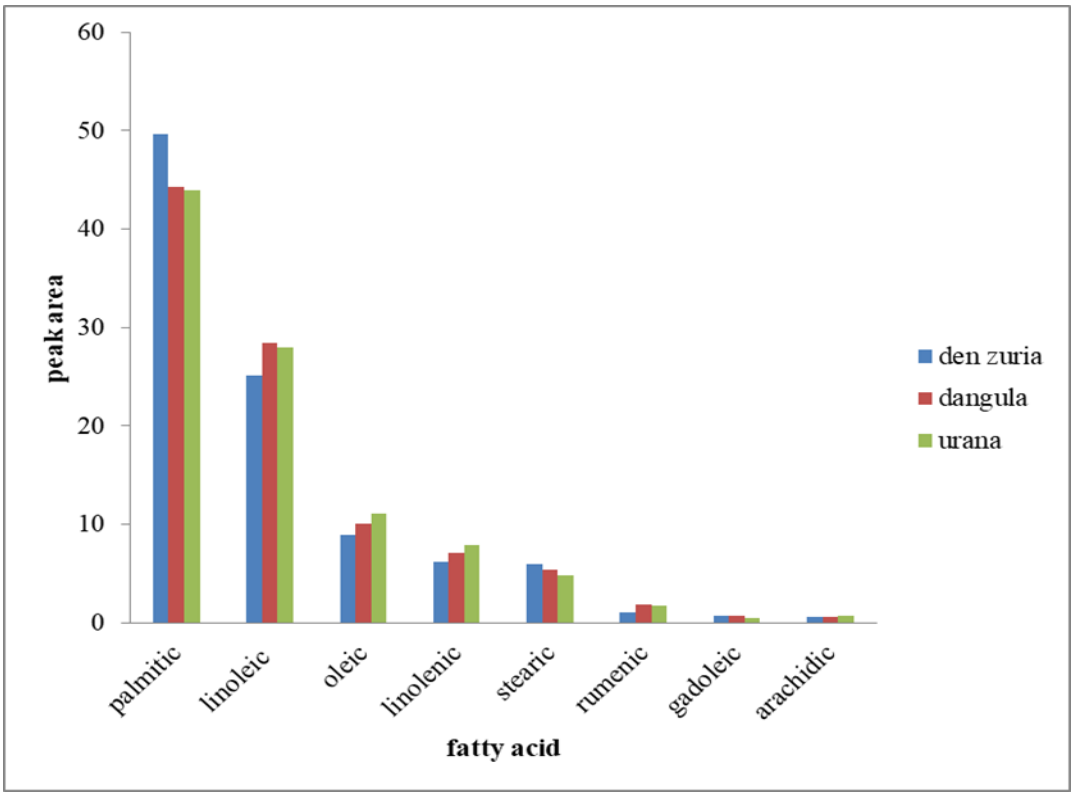

Table 4.4 : The Average concentrations (mg/100g) of fatty acids found in oat samples from three sampling areas.

No	Common name of fatty acid	RT	Sampling places		
			Den Zuria	Urana	Dangula
1	Myristic acid	30.72346	1.51±0.02	1.57±0.04	2.32 ± 0.35
2	Pentadecylic acid	32.861	--	--	--
3	Palmitoleic acid	34.39625	1.44±0.09	1.45±0.26	2.1 ± 0.24
4	Palmitic acid	34.9323	255±19.6	243±8.06	363 ± 96.8
5	Margaric acid	36.79666	1.85±0.06	1.5±0.17	2.85 ± 1.5
6	Linoleic acid	38.09727	129±7.62	154±8.69	233 ± 58.3
7	Oleic acid	38.19642	45.7±3.37	61.3±9.18	83.1±23.1
8	Stearic acid	38.65613	30.9±1.88	26.8±0.14	45±17.7
9	Rumenic acid	40.55471	5.45±0.41	9.38±0.83	15.5±2.18
10	Petroselinic acid	41.58876	2.1±0.68	1.09±0.31	1.46±0.21
11	Gadoleic acid	41.75628	4.04±2.59	2.82±1	5.37±1.57
12	Arachidic acid	42.25507	3.45±0.2	3.79±0.27	5.22±1.25
13	Linolenic acid	45.55122	32.1±2.53	43.8±6.39	56.9±5.16
14	Lignoceric acid	56.27148	1.41±0.59	1.63±0.05	2.79±1.32

The concentration of the individual fatty acids in mg/100g is given in Table 4.4. Fatty acid profile analysis of oat samples taken from the three districts of Den Zuria, Urana, and Dangula of essential nutrients expressed as mean ± standard deviation (mg/100g). Myristic acid was between 1.51 and 2.32 mg/100g, with the highest amount appearing in Dangula; this can be considered beneficial upon consumption in moderation to reduce the risk of heart diseases. Linoleic acid, on the other hand, ranged between 1.44 and 2.1 mg/100g, hence showing that oats may also contribute good monounsaturated fats to the diet that improve lipid profiles. Particularly, palmitic acid was the most abundant of the analyzed acids, especially in Dangula,

with a concentration of 363 mg/100g. Further analysis showed that the margaric acid was low, between 1.5 and 2.85 mg/100g, hence supplementing the fatty acid diversity. Linoleic acid, an essential omega-6 fatty acid, showed tremendous variation from 129 to 233 mg/100g, particularly a lot higher in Dangula. This indicates that oats can be a good source of this nutrient important for general health maintenance. Oleic acid was between 45.7 and 83.1 mg/100g; it showed a high proportion of monounsaturated fatty acids, which are well-known for their cardioprotective characteristics. Stearic acid was at a moderate level of 26.8 to 45 mg/100g and has been said to exert a neutral effect on the levels of cholesterol.

Rumenic acid was found in quite reasonable amount within the range 5.45 to 15.5 mg/100g; it could confer potential health-promoting anti-inflammatory action (Jenkins et al., 2016). Petroselinic acid, present in small amounts of 1.09 to 2.1 mg/100g, furnishes particular health values. The higher levels of gadoleic acid at 2.82 to 5.37 mg/100g enhance the overall positives of oats fatty acids. Linolenic acid was the highest among the rest, ranging between 32.1 and 56.9 mg/100g, marking oats among the key sources of this essential omega-3 fatty acid that is crucial for cardiovascular and neurological health (Mehari et al., 2019). Lastly, lignoceric acid was present in the smallest amount, ranging between 1.41 and 2.79 mg/100 g, and while adding to the overall fatty acid profile, its detailed health implications need further research. Overall, the fatty acid profile of oats across the three districts signifies nutritional variability and health potential. Hence, inclusion of oats in diets may result in increased intake of fatty acids, especially regarding essential fatty acids like linoleic and linolenic acids.

4.4. Comparison of Oat Fatty Acid Composition with Reported Values

Oats (*Avena Sativa*) contain a considerable amount of oil in their seeds compared to other cereal crops, about 3-12% of dry matter (Brown and Craddock, 1972). This research showed that the lipid content of oats ranged from 3.0 to 6.4%. Such results are in good agreement with reported lipid by Banās *et al*, (2007). The lipid content of the Canadian oat genotype was 4.2-11.8%, whereas that of the European one was 3.52-9.5%. The Canadian oats, the dominant fatty acids were linoleic (36.76%), oleic (36.50%) and Palmitic acids (18.57%) whereas, in European oats the content of linoleic (37.25%), oleic (35.67%) and Palmitic acid (18.64%) in average. Among the 3 major fatty acids, significant differences were mostly found in oleic acid rather than in Palmitic and linoleic acids. Lidia (Swedish genotype), Ivore and Dakar (French genotype) oats

contained the highest quantities of Palmitic, oleic and linoleic acids, respectively (Kourimska et al., 2021). In this study Palmitic, linoleic and oleic acids are most predominant, but in other research oleic, linoleic and Palmitic acids are predominant (Hammond *et al*, 1983; Young's, 1986).

The fatty acid composition of oat oil is desirable from both technological and nutritional standpoints. Linoleic and linolenic acids are essential fatty acids in mammalian nutrition. Palmitic acid increases oil stability against peroxidation, whereas linolenic acid causes oil instability (Hammond *et al*, 1972*;* Thro *et al*, 1978). The natural peroxidation of linolenic acid results in hydro peroxides that are toxic to mammals and cause poor oil flavor (Mounts *et al*, 1978). The favorable fatty acid composition of oat oil thus increases the nutritional value of oats for human and animal nutrition.

Table.4.5. Comparison of lipid contents determined in the oat sample with reported values.

Countries	Lipid content (in %)	Reference
Russian oat	<5	Banās *et al*, 2007
Swedish oat	7.4	Åman and Hesselman, 1984
U.S	2.0-11.0	Frey and Hammond, 1975
British	3.0-8.6	Welch, 1977
Canadian	4.2-11.8	Sahasrabudhe, 1979
Australian	4.3-6.5	Karunajeewa *et al*, 1989
European	3.52-9.5	Kourimska *et al*, 2021
Finnish	6.2-7.8	Saastamoinen *et al*, 1989

5. Conclusion and Recommendations

5.1. Conclusion

This study focused on the determination of the relative composition and individual concentration of fatty acids in oat (*Avena Sativa*) collected from three subdistricts of Ankesha District (woreda). Palmitic, linoleic, oleic, linolenic, stearic, rumenic, palmitoleic, myristic, margaric, petroselinic, and gadoleic, arachidic and lignoceric acids were the major fatty acids in oat samples detected by GC-MS analysis. Among the determined fatty acids, Palmitic acid was found to be the major fatty acid in all sub districts. In this study, a slight variations in the relative abundance of the individual fatty acids were noted when results were expressed in the relative percentage composition of the individual fatty acids. However, significant variations were noted when each fatty acids was expressed in mg/100g. The study demonstrated that oat samples under investigation are moderately richer in the studied fatty acids compared to other similar cereal crops.

5.2. Recommendations

Due to the shortage of time, financial shortage, chemical and material, the study only focused on fatty acid determination in Avena Sativa. The undetermined fatty acid of *Avena Sativa* seed oil, physico-chemical properties, protein content, and minerals should be evaluated by researchers.

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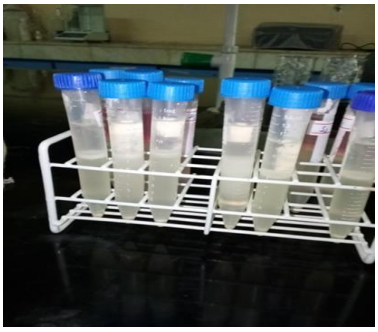
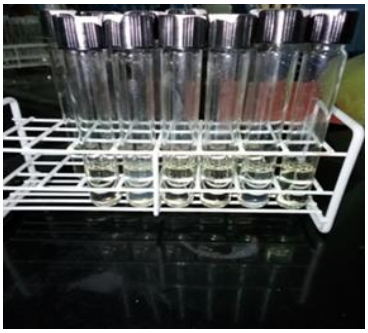
Appendexes

Apendix-1 Sample preparation



Appendix-2: Photos for extracted and derivatization of fatty acids

Appendix-3: The significance of fatty acids between and with groups of oat from three sub districts in Ankesha Woreda.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Palmitc	Between Groups	40.176	2	20.088	36.813	.008
	Within Groups	1.637	3	.546		
	Total	41.813	5			
Linoleic	Between Groups	13.130	2	6.565	94.215	.002
	Within Groups	.209	3	.070		
	Total	13.339	5			
Oleic	Between Groups	351434.380	2	175717.190	1.009	.462
	Within Groups	522456.718	3	174152.239		
	Total	873891.099	5			
Linolenic	Between Groups	2.806	2	1.403	2.310	.247
	Within Groups	1.822	3	.607		
	Total	4.627	5			
Stearic	Between Groups	1.377	2	.689	2.871	.201
	Within Groups	.720	3	.240		
	Total	2.097	5			
Rumenic	Between Groups	.799	2	.400	10.918	.042
	Within Groups	.110	3	.037		
	Total	.909	5			
Gadoleic	Between Groups	.075	2	.037	.312	.753
	Within Groups	.358	3	.119		
	Total	.432	5			
Arechidic	Between Groups	.003	2	.001	.498	.651
	Within Groups	.008	3	.003		
	Total	.011	5			
Petroselinic	Between Groups	.060	2	.030	4.468	.126
	Within Groups	.020	3	.007		
	Total	.080	5			
Margaric	Between Groups	.008	2	.004	1.096	.439
	Within Groups	.012	3	.004		
	Total	.020	5			
Myristic	Between Groups	.000	2	.000	.102	.906
	Within Groups	.001	3	.000		

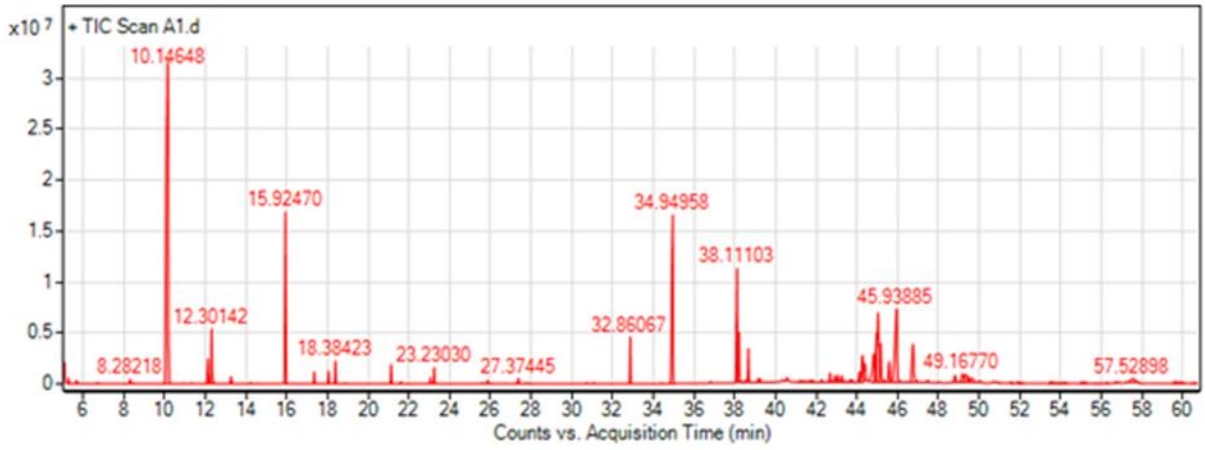
	Total	.001	5			
palmitoleic	Between Groups	.000	2	.000	.140	.875
	Within Groups	.005	3	.002		
	Total	.006	5			
Lignoceric	Between Groups	.004	2	.002	.371	.718
	Within Groups	.015	3	.005		
	Total	.019	5			

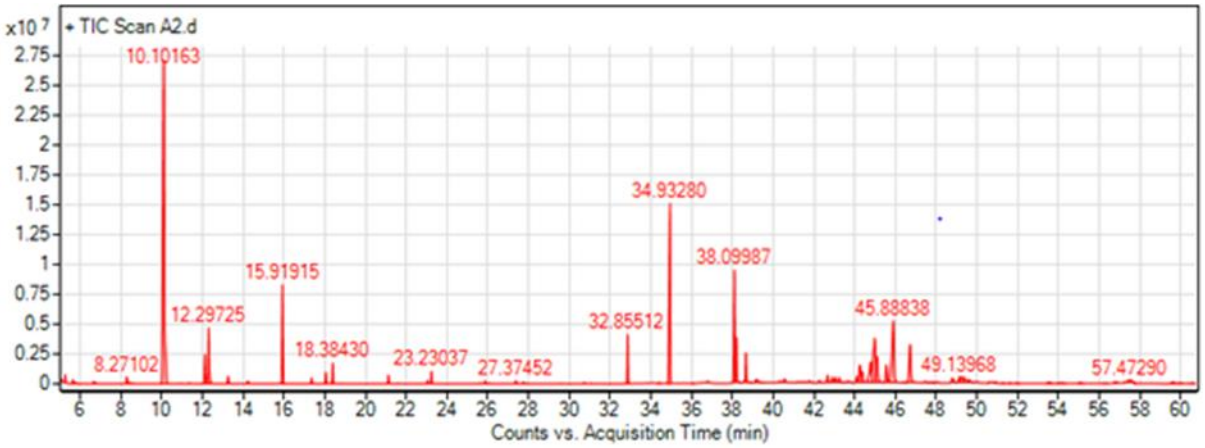
Appendix-4: The significance of lipids of oats from three sub districts in Ankesha Woreda.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	denzurialipid - uranalipid	.72000	3.76181	2.66000	-33.07850	34.51850	.271	1	.832
Pair 2	denzurialipid - dangulalipid	-.69500	2.07182	1.46500	-19.30959	17.91959	-.474	1	.718
Pair 3	uranalipid - dangulalipid	-1.41500	1.68999	1.19500	-16.59891	13.76891	-1.184	1	.446

Appendix-5: GC-MS chromatogram of three sub-district oat sample extract, indicating the 13 detected fatty acids.




x10 7
+ TIC Scan A2.d
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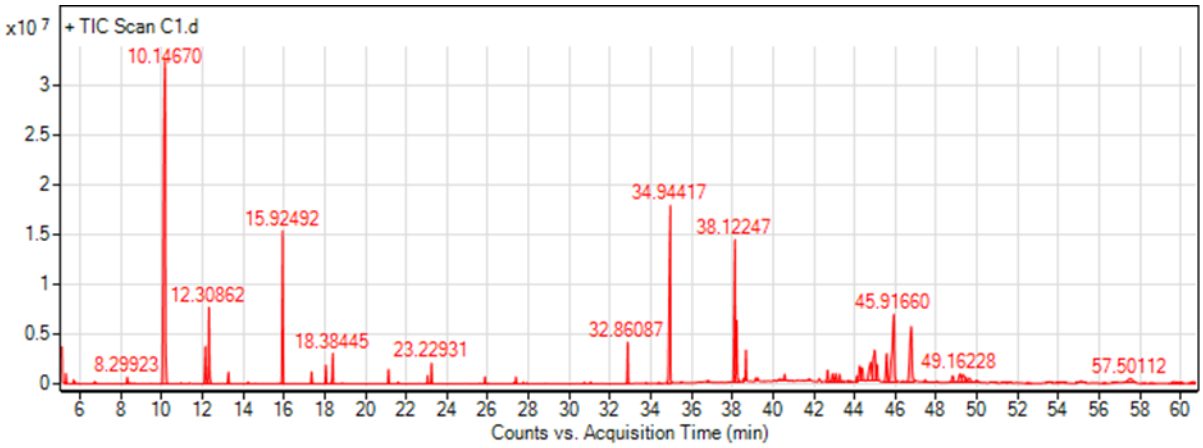
34.93280
38.09987
15.91915
12.29725
45.88838
32.85512
18.38430
8.27102
23.23037
27.37452
49.13968
57.47290
Counts vs. Acquisition Time (min)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+ TIC Scan C1.d
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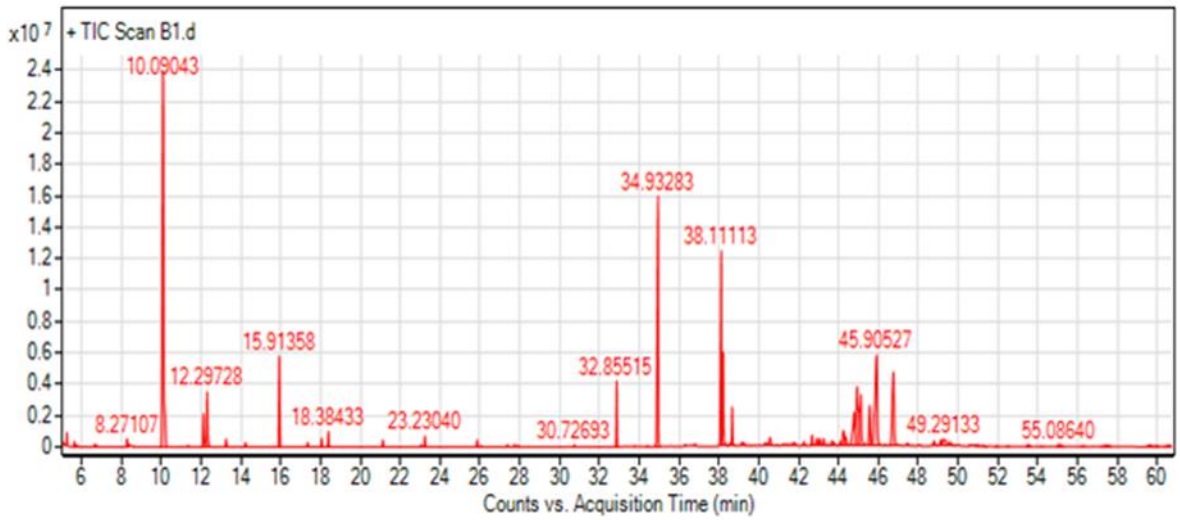
34.94417
15.92492
38.12247
12.30862
45.91660
32.86087
18.38445
23.22931
8.29923
49.16228
57.50112
Counts vs. Acquisition Time (min)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+ TIC Scan B1.d
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34.93283
38.11113
15.91358
45.90527
12.29728
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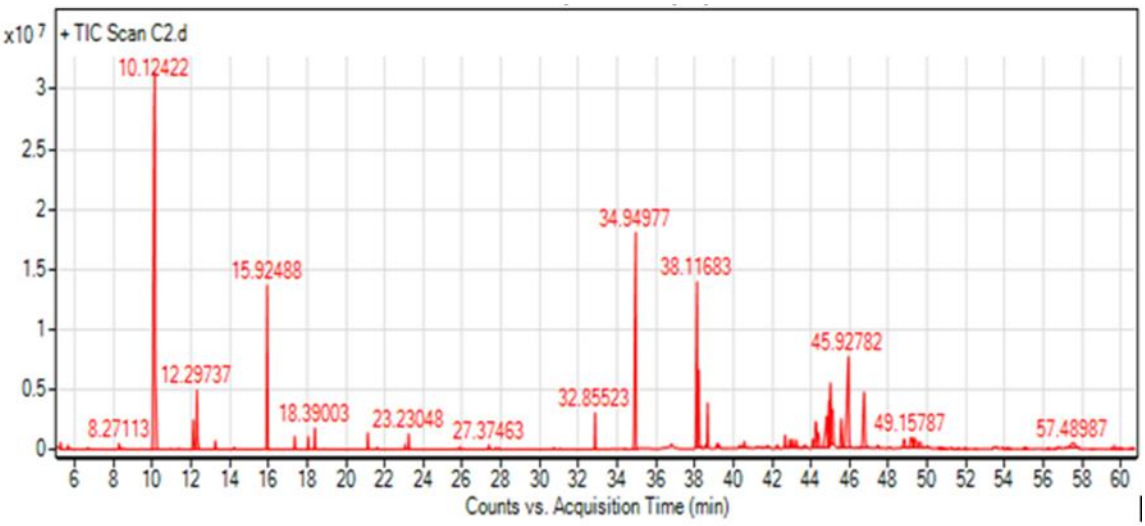
23.23040
30.72693
49.29133
55.08640
Counts vs. Acquisition Time (min)


x10 7
+ TIC Scan C2.d
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34.94977
15.92488
38.11683
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12.29737
32.85523
18.39003
23.23048
8.27113
27.37463
49.15787
57.48987
Counts vs. Acquisition Time (min)