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Determination of Fatty Acid in Barley By Using Gc-Ms: in Dangila District, Awi Zone, Northwest Ethiopia

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BAHRDAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

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BY:

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The Thesis Submitted to the Department of Chemistry Presented in Partial Fulfillment of the Requirement for the Degree of Masters of Science in Chemistry

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October, 2024

Bahir Dar,Ethioipia

APPROVAL OF THESIS

I hereby certify that I have supervised, read, and evaluated this thesis titled "Determination of Fatty Acids in barley by Using GC-MS" by Emebet Ayaleneh prepared under my guidance. I recommend the thesis be submitted for oral defense.

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APPROVAL OF THESIS FOR DEFENSE RESULT

As members of the board of examiners, we examined this thesis entitled "Determination of Fatty Acids in Barley by Using GC-MS" by Emebet Ayaleneh.

We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Science in Chemistry.

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DECLARATION

This is to certify that the thesis entitled "Determination of Fatty Acids in Barley by Using GC-MS" is submitted in partial fulfillment of the requirements for the degree of Master of Science in Analytical Chemistry to the postgraduate Program of the College of Science, Bahir Dar University is an authentic work conducted by Emebet Ayaleneh Adamu.

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ABBREVIATION

TFAS	Trans fatty acids
MUFAS	Mono unsaturated fatty acids
SFAS	saturated fatty acids
GC-MS	Gas chromatography with mass spectroscopy
FFAS	free fatty acids
FAS	fatty acids
PUFAS	poly unsaturated fatty acids
FAMES	fatty acid methyl est

ABSTRACT

Barley, a fundamental cereal grain, is extensively grown for its nutritional advantages and adaptability in food and beverage manufacturing. Hence, this study aimed at profiling the fatty acid composition and quantifying the crude fat content and the individual fatty acid constituents in barley samples taken from three sub-districts of Dangla district. the barley samples were collected from Debaca, Chara, and Sahara sub-district and the crude fat and fatty acid constituents were determined following a standard procedure. Results revieweld that the crude fat contet of three samples ranged from 4.85-5.95% by mass. In addition, atotal of twelve fatty acids, Seven saturated and five unsaturated fatty acids were detected in all barley samples. The highest fatty acid content of barley in three sub-district were myristic, Linolenic ,Palmitic, oleic ,linoleic, Arachidic, gondoic, behenic, Palmitoleic, Margaric ,stearic, and lignoceric acid. Palmitic acid was the most abundant fatty acid in the barley and accounted for 44.2–49.9% of the total fatty acid content. The level of Linoleic acid found in this study ranged from28.47% - 32.22% of the total fatty acid. Furthermore, the total content of saturated fatty acids fell between 266 and 311 mg/100g, at its pure content of 210-266 mg/100 g, with palmitic acid being the most predominant contributor. Myristic and stearic acids were other members of saturated fatty acids contributing to its total value. On the other hand, unsaturated fatty acids are between 166-227mg/100g in terms of total concentration, with linoleic acid, between 121-169mg/100g, being the biggest contributor, and included in this class are the dominating Oleic and gondoic acids, ranging from 44-60 mg/100g and 2-4 mg/100 respectively. It could, therefore, be inferred from the data presented that the saturated fats were higher in number compared to their unsaturated counterparts in the barley samples. There is no significant difference in the mean cocnetration of the fatty acid between the sampling sub districts.

Keywords: Barley; Fatty acids; Gas Chromatography Mass Spectrometry

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Barley (*HordeumvulgareL.*) ranks fourth in global cereal production after maize, wheat, and rice (Iftikhar, et al; 2019).In Ethiopia barley is the third most important cereal crop next to teff and maize. It is commercially used for making local beer, Injera (Ethiopian flat bread), Kolo (roasted barley), for animal feed, and in a brew factories as a raw material. Barley breakfast foods and snacks are increasingly available, driven by recent research findings, which show that barley fiber contains beta-glucans and tocotrienols, chemical agents known to lower serum cholesterol levels in the blood. Barley grains have a hull of adherent pales, which are removable only with difficulty in hulled forms. In naked forms, the hull is readily lost during threshing. The hull amounts to about 13% of the grain (by weight) on average, the proportion ranging from 7 to 25% according to type, variety, grain size, and latitude where the barley is grown. The factors which regulate the growth of plant are depending on number of biochemical process involving various inorganic elements present in the soil. Large variation in mineral content of different crops depends upon plants species, nutrient availability, soil and climate (Bekele and Madhu 2019).

Barley is rich in several health-boosting components, such as dietary fiber, essential fatty acids, vitamins, minerals and phytochemicals, including several phenolic compounds (Badea,A et al;2021). Barley is an economically important cereal that used in the production of malt, human food and animal feed (Ighwelaetal.2011).

Spring barley is the main raw material for production of malt and subsequently beer in the territory of the Czech Republic from the late 19^{th} century. Requirements for the quality of malting barley have gradually been increased and specified (Svoboda et al; 2009). Increased awareness of the health benefits of soluble dietary fiber and other whole grain constituents, gives barley (*Hordeum vulgare L*) foods a good opportunity of regaining an important place in the human diet (Sonia et al; 2021). In addition, barley is an important source of other bioactive substances including phenolic compounds, natural antioxidants and vitamin E with antiradical and antiproliferative potentials (Gangopadhyay et al.2015; Shen et al. 2016).

Lipid fractions in cereals are mainly concentrated in the grains germ; and they determined its greatest extent energy value and had a significant impact on its nutritional attributes through the fatty acid content (Gangopadhyay et al. 2015). Fatty acids in Barely and other cerals are family of lipid molecules, including omega-3and omega-6 that prevent chronic diseases and have positive antioxidant effects (Cozzolino& Degner2016). Fatty acids also plays an important role in the transport of substances in and out of the cell because of their impact on the fluidity of the cellmembrane(Petrović et al., 2010). Fruits, vegetable oils, seeds, nuts, animal fats, and fish oils are just a few examples of diverse sources of fatty acids(Hewavitharana et al., 2020).

Fatty acids are the primary component of lipids and play a crucial role in biological systems.

There are many biological functions associated with the fatty acids, including them act as primary constituents of cell membranes, an energy source, and regulating the activity of enzymes, a precursor for many different molecules and inflammatory processes (Christinat et al., 2016).

Moreover, fatty acids are organic and bioactive compounds which can be efficiently used as functional ingredients in bakery products and natural foods. In addition, barley flour can easily be incorporated into cereal based products (bread, cakes, cookies, noodles and extruded snack foods), and also could be used for the development of accepted dietary and functional food products (Baik and Ullrich 2008). Lipids, along with fatty acids, are important constituents of the barley grain and can influence the storage and processing of the grain. For the analysis of fatty acids composition, sample preparation is a significant task. It can be done by converting the fatty acids into the methyl esters followed by gas chromatography (GC) (Chiu and Kuo, 2019). Different metabolomics platforms, such as mass spectrometry coupled (MS) with gas chromatography (GC), and various bioinformatics tools have greatly facilitated the identification, isolation, structural characterization, and interpretation of biologically active compounds in nutrition research (Kumar et al; 2018). The fatty acid composition of barley can be substantially affected by environmental factors like soil quality, climatic circumstances, and farming techniques. Consequently, there is significant variance in crude fat and fatty acid composition in barley cultivated across diverse production regions in various countries. However, there is a paucity of information regarding the crude fat and fatty acid content of barley cultivated in the Awi Zone, where barley is extensively grown.

1.2 Statement of the problem

In Ethiopia, barley is produced mainly for human consumption and is one of the most important food crops (Bantayehu, 2009). Among Traditional foods such as bread, injera, kitta, atmit (soup) or muk can be prepared only with barley or blended with other cereal flours (Grando, and macpherson, 2005). In general almost all of the important cereal based traditional recipes, with the exception of nifro, can be prepared with barley (Grando and macpherson, 2005; Shewayrga and Sopade, 2011). Food produced from barley is a good source for many nutrients such as protein, fiber, minerals, fatty acids and vitamins (Newman andWalter2008). However, to the best of our knowledge the total lipid content and the fatty acid compostion in barely samples from Awi zone, in Dangila district has not been reported yet. This motivated me to determine the total lipid content and the fatty acid compostion of the barley samples collected from Awi zone, Dangila district.

1.3 Objectives of the study

1.3.1 General Objectives

The main objective of the study was to determine the total lipid content and the fatty acid compostion in barely grown from Sahara, Debaca and Chara sub district of Dangila district , Awi Zone, using GC-MS.

1.3.2Specific Objective

The specific objectives of this study was:

- To detrmine the total lipid content of barely samples collected from Sahara, Debaca and Chara sub district of Dangila district , Awi Zone
- To determine the individual fatty acid compostion of barely samples collected from Sahara, Debaca and Chara sub district of Dangila district, Awi Zone
- To compare the total lipid content in barely samples collected from the three different sampling sites

1.4 Significance of the study

This research was expected to give the following benefits:

- Give information for consumers and concerned bodies about the total lipid content

- Give information for consumers that feed the barely grown in this study area about the individual fatty acid compostion
- As the study was new in this study area, it may serve as the base line data especially for the local agriculchur extention workers in Dangila district and other resreachers in this field

CHAPTER TWO

2. LITRATURE REVIEW

2.1 Major crops

Cereal and oil seed crops are the dominant crops of the Canadian agriculture, followed by forages, potatoes, pulses and fruits. The major oilseed crops include canola, soybeans and flax, whereas wheat, barley, corn, oats and rye are the most common cereals. During the 2007 to 2009 time period, wheat, barley, canola, soybean and oats contributed 45%, 24%, 10%, 8%, and 5% respectively to the Canadian agricultural production (Statistics Canada, 2009). Since the last 75 years, cereal production on the Canadian Prairies occupies 75-80% of the cultivated acreage and is dominated by wheat, barley and oats. Barley is mainly destined for animal feed or production of malt for domestic or export markets. Canadian barley constitutes 8.1% of global trade and is valued at $1.9 billion per year (FAOSTAT, 2009).

2.2 Barley classification

Barley is classified by the number of kernel rows in a spike. Two forms are cultivated; Two - row and six- row barley. The number of rows on the ear is controlled by two alleles (Powell et al., 1990). Only one spikelet per node is fertile in two - row barley, whereas all three spikelets produce seeds in six - row forms source (www.barleyworld.org).The grains of two-row are more circular and have a higher test and grain weight than six - row kernels (Tanno and Takeda 2004). In addition, two - row barley produces grain with higher starch and protein concentrations (Marquez- Cedillo et al., 2001), and thus, the two – row grain has a higher feed conversion (>4.6%) than six-row grain (Fox et al., 2009). Two –rowgenotypes also have the advantage of being more resistant to lodging than six-row types (Berryet al., 2006). The higher number of kernels per spike developed in six-row varieties translated to 20 to 27% higher yield than two - row types. The fiber and beta -glucan content in six - row grain is relatively high, which is of advantage for production of fiber - rich barley food products.Another barley classification is based on the kernel being hulled or naked (www.barleyworld.org).The naked trait is controlled by a single recessive gene on chromosome arm 7HL (Pourkheirandish and Komatsuda 2007). Wild barley and some of the cultivated forms are hulled with paleas fused with kernel after

threshing. Paleas of hulless or "naked" barley (HordeumvulgareL.) are easily removed by threshing. The naked barley is mostly used for food and feed whereas the hulled barley is preferred for malting.

2.3 Origin and Domestication

Archeological excavations have identified barley in many pre-agricultural and incipientsites in the Near East, Southeast Asia and Ethiopia where it was one of the first crops cultivated for more than 10,000 years ago (Zohary and Hopf 2000). The Fertile Crescent which stretches from Israel and Jordan to south Turkey, Iraq, Kurdistan, and south-western Iran still remains the centre of origin and is the primary habitat for barley‘s wild progenitor ,H. spontaneum(Harlan and Zohary 1966). The Mediterranean marquis, abandoned fields, and roadsides are considered as secondary habitats for H.spontaneum. H. spontaneum is also present in the marginal habitats in the Aegean region, south- eastern Iran, and central Asia, including Afghanistan and the Himalayan region (Zohary and Hopf 2000). The discovery of H. spontaneum in several locations suggests that barley (H. vulgare) is an oligocentric crop based on its evolutionary pattern (Aberg, 1938; Bekele, 1983; Molina-Cano et al., 1987). This hypothesis is supported by a study of 317 wild and 57 cultivated barley which indicated that barley was brought into cultivation in the Israel - Jordan area and that the Himalayas is another region of domesticated barley diversification (Badr et al.,2000).

2.4 Barley Growth

Barley is a Crop three plant after maize and teff and prefers climates with cool temperatures (15 - 30^{o}C) and an annual precipitation of 500 - 1,000 mm. Adaptation to soil salinity and alkalinity is good, but barley is less tolerant to acidic and wet soils (Bhatty, 1999). Barley grows rapidly, out-competes many weeds and reaches maturity earlier than wheat and oats. Most of the wild barley have winter growth habit (Karsai et al., 2005), which demands a period of low temperatures (around 10^{o}C) to become flowering competent. Cultivated barley with winter habit is grown in tropical areas, whereas spring types dominate in temperate regions.

2.5 Barley Distribution

Barley is cultivated over a wide range of environments from 70^{o}N in Norway to 46^{o}S in Chile. As barley is a relatively drought tolerant crop, it can be grown in many countries with dry climate such as Afghanistan, Pakistan, Eritrea and Yemen and areas in Northern Africa and

Western Asia. Barley is also widely cultivated on high altitude mountain slopes in Tibet, Ethiopia and the Andes. Europe has the largest area under barley cultivation (ca. 27.3 million hectares) followed by Asia (ca. 12.2 million hectares), North and South Americas (ca. 6.6 million hectares), Africa (ca. 4.9 million hectares) and the Oceania (ca. 4.5 million hectares) by Spain (9%), Canada (8%) and Germany (8%) and France (7%) (FAOSTAT,2009).

2.6 Uses of Barley

The most important uses of barley are in the malting and brewing industry for beer and Whiskey production, animal feed and human food. In Canada, about 83% of produced barley is used as livestock feed whereas 12% and 5% are destined for malting and other purposes, respectively. There has been a higher demand for food and malting barley in the last five to ten years as a result of increased health awareness and favorable market prices (Baik and Ullrich 2008). Novel uses of high β- glucan barley in the nutraceutical industry has emerged lately (Delaney et al., 2003) and industrial applications of high-amylose barley starches are under investigation to diversify barley utilization (Ganeshan et al., 2008).

2.6.1 Animal Feed

Barley is often used for animal feed although the nutritive value is lower than corn or wheat. Both two-and six-row hulless barleys are grown to produce animal feed. In addition, a considerable amount of hulled malting barley with inadequate malting quality enters the animal feed market. This makes feed barley a non-homogeneous commodity with varying nutritive value. The two-row and six-row hulless barleys produced in Canada for feed are relatively high in protein (14-15%),but the two-row grain is preferred as it has a comparatively higher carbohydrate (starch) content (Fregeau -Reid et al., 2001) and is more digestible by mono gastric farm animals such as poultry and swine.

To aid digestion of the fibrous hulls of hulled barley, the rations of mono gastric animals are often supplemented with beta- glucanase (Mathlouthi et al., 2003). Besides hulls, phytate is another seed component that negatively affects barley utilization as animal feed. Phytic acid efficiently chelates multivalent cations such as zinc, calcium, copper, iron, magnesium, and aluminum making the minerals unavailable for absorption (Adams et al;2002). Among the minerals, zinc is the most susceptible to phytate complexation. A reduction of Phytate production in barley cultivar HB379 (Roslinskyet al., 2007) has doubled the availability of phosphorous and

zinc for broilers (Linares et al., 2007). Although barley is popular as animal feed, grain with a high starch concentration is not suitable for ruminants. A rapid starch fermentation in rumen results in a pH drop, which reduces fiber digestion and causes digestive disorders. Depression in milk fat content is seen when high-starch barley is used as feed for lactating cows (Larsen et al., 2009). It is therefore important to consider seed composition when selecting barley grain for ruminants and non-ruminants, respectively.

2.6.2 Malting and Brewing

About 10% of barley produced worldwide is used to make malt for brewing beer. The malting cultivars include hulled, hulless, two-row and six-row varieties, but the hulled barley is preferred as hulls contributes to flavor and aids filtering during the brewing process (Gunkel et al., 2002). Malting barley varieties are generally developed for a specific market e.g. domestic brewing or for export. The physical, chemical and biochemical properties of barley grain can have a large impact on the malting process and quality of beer. Kernel physical characteristics such as germination percentage, germ growth, kernel maturity, size, amount of seed-borne diseases and frost damage are factors that affect malting.

The amount of grain starch, protein, β-glucan and their interactions during grain filling affect grain hardiness with effects on the yield of malt extract (Psota et al.,2007). The alpha amylase level is another factor that determines the amount of malt extract. Preferred malting barley varieties are generally soft (Gupta et al., 2010) with protein levels ranging from 10.5% to 13.0% for six-row types and 10.5% to 12.5% for two-rowvarieties (Dusabenyagasani , 2003).

Barley with high protein concentration (> 15%) is not used for malting as it requires a long steeping time, has erratic germination and produces low malt extract (Swanston and Molina-Cano , 2001). Discolored barley grain is also unsuitable for malting due to undesirable flavors produced in beer by the breakdown of phenolic (Mussatto et al., 2006). A successful sustenance of malting barley export market demands proper selection of cultivars with appropriate malting characteristics.

2.6.3 Nutrition and Human Health

About 2% of the global barley production is used for food (Baik and Ullrich 2008). The preferred barley for food use is clean, thin-hulled, bright yellow-white, plump, medium-hard and

uniform in size. A few two-row and six-row hulless genotypes with a minimal cleaning requirement meet these specifications.

Barley is nutritionally rich because it is has a high carbohydrate concentration, moderate protein concentration, high dietary fiber content and is a good source of selenium, phosphorus, copper and manganese (Ames et al., 2006).De hulled, polished and milled barley is often used in porridge and soups, and as a substitute for rice in certain Asian countries e.g. Iran. Also a substantial amount of barley is used in baked foods such as breads, grits, noodle and pilaf in India and surrounding countries. Barley-based foods provide several positive effects on the human digestive system. Consumption of barley increases bulk and reduces transit time of fecal matter, which is associated with a lower frequency of hemorrhoids and colon cancer (Tsai et al., 2004).Fermentation of barley's insoluble dietary fiber in large intestine produces short-chain fatty acids such as butyric acid that help to maintain a healthy colon (Behall et al., 2004). Other fermentation products such as propionic and acetic acids provide fuel for liver and muscle cells (Liu, 2004). Propionic acid is also known to inhibit HMG-CoA reductase involved in cholesterol biosynthesis in liver (Erkkila et al., 2005), thus lowering blood cholesterol levels.One of the important dietary fibers produced by barley is the soluble glucan polymer β -glucan (1→3, 1→4)-β-D- glucan).The concentration of β – glucan in barley kernels is normally < 5% (Izydorczyk et al., 2000), but genotypes producing waxy or high- amylose starch generally have a higher concentration of β-glucanand dietary fiber (Izydorczyk et al., 2000). The presenceof β-glucan in diets increases the viscosity of foods during digestion leading to lower glucose absorption and reduced blood glucose level measured as glycemic index (GI) (Jenkins et al., 1981). Foods with a low GI is preferred to decrease the risk of diabetes in humans. Beta-glucan also has a positive effect on cholesterol levels, as the fiber absorbs and removes bile acids produced from cholesterol in the liver.

The absorption of bile acids triggers the liver to produce more bile acids from cholesterol (Brennan, 2005) and the net effect is a reduction in blood cholesterol levels (Behall, 2004). Barley fiber is also a good source of niacin, a B-vitamin that reduces platelet aggregations that cause blood clots and lowers the levels of total cholesterol, lipoprotein and free radicals which oxidize low-density lipoprotein cholesterol. Thus, niacin protects against cardiovascular diseases (Jood and Kalra, 2001). Consumption of food with 21 g fiber per day have been suggested to lower the chances of coronary heart and cardiovascular diseases by 12-15% and 10-11%,

respectively (Jensen et al., 2004).As various health claims are associated with barley grain consumption, future barley based food products are aimed at regulation of blood sugar levels in diabetics, reducing cholesterol and lowering the incidence of heart disease. Besides low GI foods being desirable for diabetics, they are also beneficial for athletes requiring a slow release of glucose into the blood.

2.7 Chemical Composition and modes of consumption of barley

Barley grain is rich in starch and sugars relatively poor in protein and very low in fat. The husk is mostly composed of Legnin, Pentosans, Mannan, Uronic acids, Hemicelluloses and Cellulose Fiber. Silica is present in the outer walls of the husk and the awns contain large amounts of silica. Polyphenols which may complex with proteins are abundant in the paricarp, testa and aleuroue layre. The strategy endosperm is composed of about 85-89 % starch enclosed in cell walls. B-Glucan makes up 75% of the cell wall, and the rust arabinoxylans. The embryo consists of about 7% cellulose, 14-17% lipids, 14-15% sucrose 5-10 ash and 34% proteins. The ash content of barley (2-3%) is influenced by the growing season, soil zone, and soil type and soil fertility. The distribution of minerals is uneven throughout the kernel and the rachis (5-14%) and awns (17-38%) contain large portion of the minerals. The ash content of barley ranges from 2.2 to 3.9% .In recent years barley is gaining renewed interest as a food component because of its high soluble dietary fiber andβ-glucan content compared with other cereals. Barley grain contains about 20% of dietary fiber, β-glucan, is an important dietary fiber in barley, varies between 3 and 7% have shown that a significant blood cholesterol lowering effects. Moreover, barley β-glucan increases the viscosity of digestion in the intestine, slowing down the rate of starch digestion and absorption, which is beneficial to diabetics. There is, however, a large variation in the chemical composition between different barley types. Hull-less barley contains less ash and dietary fiber and more starch, protein and fat than covered barley (Bekele and Madhu; 2019).

2.8. Barley Lipids

Lipids are nutritionally important because they contain two and one-fourth times the energy per weight of carbohydrates and proteins and add flavor. The lipids of barley account for 2.0 to 3.6 percent of the total dry weight of the grain. The amount of lipid obtained was highly variable and depended on the method used for extraction. The average values for glycolipids, phospholipids and neutral lipids were 9, 20, and 71 percent. Lipids were determined by use of preparative thin

layer chromatography (TLC). The neutral lipid class consists of a complex group of compounds containing free fatty acids (FFA), glycerides, free sterols, and sterol esters. There is intensive degradation and oxidation of barley lipids when drying grain at 55°C. They determined barley lipid composition by analytical TLC and GLC. Tri acyl glyceride and sterol ester content decreased whereas the content of FFA and sterols increased. These researchers found that the drying temperature had no effect on the content of saturated fatty acids but it did decrease the content of unsaturated fatty acids. The decrease in unsaturated fatty acids may be indicative of fatty acid oxidation leading to oxidative rancidity during processing. The initial substrate in lipid oxidation (autoxidation) is almost always unsaturated lipids which are quite plentiful in barley (Irene Susan; 1984).

2.9 Fatty acids in barley

Fatty acids are long chain hydrocarbons that can be divided into two categories: Based on the absences or presences of double bond saturated and unsaturated. Unsaturated fatty acids are; monounsaturated, polyunsaturated, and Tran's fatty acids based on the number of double bonds. Each carbon in saturated fatty acids is bonded to two hydrogen atoms, with single bonds between the carbons. Unsaturated fatty acids have one or more points of unsaturation, or double bonds between the carbon atoms (Wanders et al., 2017). Saturated and Trans fatty acids have been linked to an increased risk of coronary heart disease. Monounsaturated and polyunsaturated fatty acids have been linked to a lower risk of coronary heart disease (White, 2009). Fatty acids can exist as free forms and bound forms, such as cholesterol and phospholipids, free fatty acids refer to fatty acids that are not chemically bound to other molecules and are able to move freely in the body. They are typically found in the blood and can be used as a source of energy by cells and they are lipid species released from the adipose tissue and several cell types upon lipolysis. Bound fatty acids are chemically linked to other molecules, such as triglycerides or phospholipids, and are not as readily available for use as energy. They are mostly stored in the adipose tissue .Fatty acids are divided into categories for a variety of reasons, including: Based on chain length, they are classified as short-chain fatty acids (C6), medium-chain fatty acids (C6–C12), long-chain fatty acids (C13–C21), and very-long-chain fatty acids (>C22), (Hewavitharana et al., 2020).Oleic acid (C18:1), linoleic acid with two double bonds (C18:2), linolenic acid with three double bonds (C18:3), and, arachidonic acid, which contains four double bonds (C20:4), are all examples of unsaturated Fatty acid.

The term "essential fatty acid" refers to a fatty acid that the body cannot produce and must acquire from food. Dietary sources of essential fatty acids (linoleic and a-linolenic) are required(Glick & Fischer, 2013).Polyunsaturated fatty acids (PUFAs) are especially significant since they can help prevent cardiovascular disease, psychological problems, and a variety of other ailments like atherosclerosis, thrombogenesis, high blood pressure, cancer, and skin diseases(Robert, Mfilinge, Limbu, & Mwita, 2014).The fact that the two polyunsaturated fatty acids, linoleic and linolenic acids, are essential and unobtainable in the human body unless through dietary means emphasizes the importance of unsaturated fatty acids. Linoleic acid, an unsaturated omega-6 fatty acid present in a variety of plants, including pumpkin seeds, canola oil, soy beans, and flaxseeds, plays a critical role in a variety of human biological functions, including the neurological, skeletal, and reproductive systems, allowing them to work properly(Doan et al., 2019). Saturated fatty acids predominate in animal-based foods, while polyunsaturated and monounsaturated fatty acids predominate in plant-based foods. Tran's fatty acids are produced in food processing by partial hydrogenation of polyunsaturated oils, and they also occur naturally in animal foods. When hydrogen atoms are on the opposite side of a carbon-carbon double bond, trans-fatty acids are formed. Fruits, vegetable oils, seeds, nuts, animal fats, and fish oils are all good sources of fatty acids. Many plant foods, such as safflower, sunflower, soybean, pine nuts, pecans, Brazil nuts, and corn oils, contain the fatty acid (White, 2009).

In addition to these, A- linolenic acid is an essential fatty acid because it is part of the essential foods that are not synthesized by mammals. It is also the main fatty acid that composes the thylakoid membranes of green leaves of plants. So, green plants and animals eating them are sources of this fatty acid. Some seeds and systematically derived oils are rich in ALFA, especially seeds of flax, camelina, rapeseed, hemp, soybeans and nuts. However, these oils and seeds equally contain omega-6 that competes with omega-3 at the cellular level, while their physiological effects are compromised. So increassed use of ALFA reduces the risk of cardio vascular and chronic diseases (Cozzolino&Degner 2016).

Linoleic acid was the major fatty acid in all cultivars (42.51%– 46.16%). It is a polyunsaturated fatty acid corresponding to the unique essential fatty acid of the omega 6 family, involved in the manufacture of the cell membrane. Due to this fatty acid, the body can produce all other lipids of the omega 6 family. Linoleic acid is present in almost all vegetable oils, especially linseed oil

(80%). Other cereal oils (corn, sunflower and soya) and plants (evening primrose, borage and grape seed) also contain this omega 6 fatty acid (Gangopadhyay et al., 2015; Cozzolino and Degner 2016). Inside our skin, linoleic acid enters in the composition of ceramides, which in turn, are part of the lipid cement (true protective barrier of the epidermis). A lack of omega 6 causes an intense dryness of the skin, a less dazzling complexion, brittle and dull hair, etc (Zarrouk et al. 2015; Cozzolino et al. 2014). Linoleic and palmitic acids are the most abundant fatty acids in Barley (Sonia et al; 2021). Palmitic acid was the most common saturated fatty acid, of animal origin or present in certain vegetable oils such as vegetable oil of palm or coconut. It is an important component of the skin barrier and the acidic layer of the epidermis; it has emollient, emulsifying and cleansing powers. It also comes in the composition of certain perfumes (Cozzolino et al. 2014, 2015).

2.10 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).

2.11 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).

2.12 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 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).

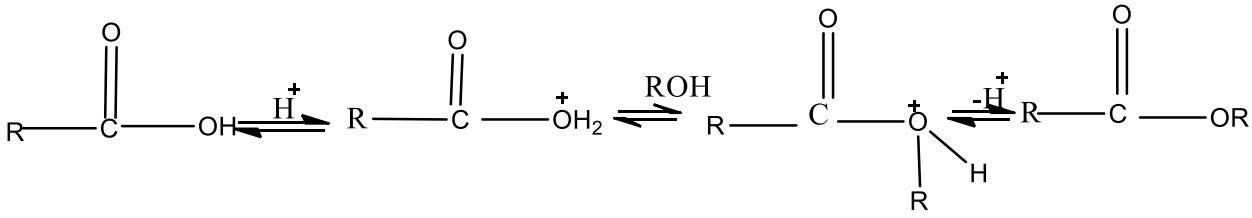


Figure 1 General mechanism of acid-catalyzed esterification of fatty acids

2.13 PRINCIPLE OF GC-MS AND ITS USE FOR FATTY ACIDS

GC/MS has long been used for the selective analysis of non-polar compounds. The carboxylic fatty acids require prior hydrolysis from their glycerolipid sources and derivatization to a respective ester form for separation on capillary chromatographic columns. The detection of structural molecular ions generated from the MS source provides more sensitive and selective assay of varied arrays of fatty acids present in lipid samples. Fast quadrapole technology present in most modern mass spectrometers facilitate for selective ion monitoring with simultaneous full scan capabilities (Johnston & Sobhi, 2017)

CHAPTER THREE

3 METHODOLOGY

3.1 Description of the Study Area

The study area is located in Amhara regional state in Awi zone Dangila district. The district is situated in northwestern highlands of Ethiopia. Dangila is located between $11^{0}04'48''$-$11^{0}24'36''$N latitude and $36^{0}34'48''$-$37^{0}00'37''$E longitude. It is one of the Woreda in the Amhara Regional State of Ethiopia, part of Agewawi zone. Dangila is bordered on the south by Faggetalekoma , on the southwest by Guangua, on the Northwest by Jawi, and on the Northeast by West Gojjam Zone. Dangila town is 485 Kilometers far from Addis Ababa along the Addis Ababa-Bahir Dar road at a distance of 76.9 Kms south West of Bahir Dar (DWRDAO,2017).

The topography of the Dangila district consists of various landforms, mountains (11 %), plains (58 %), valleys and caves (1%), swamp areas (0.05%), and others (29.95%). It has an altitude ranging from 1700 meters to 2370 meters above sea level (DWRDAO,2017). The annual average temperature of the Dangila district ranges between 16% to 30%, and it receives about 1700 mm of mean annual rainfall. This shows that it is entirely located in sub –tropical climatic zones (Dangila Woreda Agricultural and Rural development office and Dangila branch Meteorology Agency, 2017). Based on Dangila Woreda agriculture and rural development office (2017), the district's total area is 73,613,387 hectares. The total population of Dangila district is estimated to be 231,210, of which 112 619 are males and 118,591 are females. Among the total of population 37,603 males and 40,000 females are urban residents and 75,016 males and 78,591 females are rural inhabitants (DWCSO, 2018). Sahara, Debaca and Chara kebele are found in the direction of northwest from Dangila town. These areas are the major barley-producing areas in the district.

3.2 Sample Collection and preparation

The sampling site was selected according to the area in which barley is highly cultivated, namely, the Debaka, Sahara, and Chara sub-districts. From each sub-district, three farmers were selected, and from each farmer, 500 g of barley was collected during March, 2023. Samples from different farmers but from the sub–district were mixed to get a representative sample. Then, the collected samples were cleaned and placed in a polyethylene plastic box. The samples were dried

to remove any moisture and ground using an electric grinder. The flour was sealed in a plastic bag for crude fat and fatty acid analysis (Sonia et al., 2021).

Figure 2; represents a representative photo of one of the collected samples.

3.3 Instrument and Apparatus

Electrical grinder (FW-100 high-speed universal disintegrator grinder), platform shaker (ZHWY-334), measuring cylinder, flasks, beaker, Balance (electronic balance), Oven(Universal hot air oven (New Delhi-110020(INDIA), fume hood(Neuberger, type AZ 150), centrifuge(model 800-1), vial, micropipette, incubator (constant temperature and humidity incubator) ,(GC-MS (Agilent Technologies 7890B-5977A, China), acrodisc syringe filter were used for laboratory analysis.

3.4 Chemicals Used

All reagents and standards used in the analysis were of analytical quality. Standard fatty acids (Sigma Aldrich) were utilized to identify the identity of fatty acids. Pentadecanoic acid (C15:0) was used as an internal standard. Methanol (99%, ketone free, Alpha Chemika), chloroform (99.8%), and toluene (99%, Blulux Laboratory), n-hexane (99.9% - AR grade), sulfuric acid, anhydrous sodium sulfate, sodium chloride (Blulux Laboratory), and distilled water were used for the laboratory analysis.

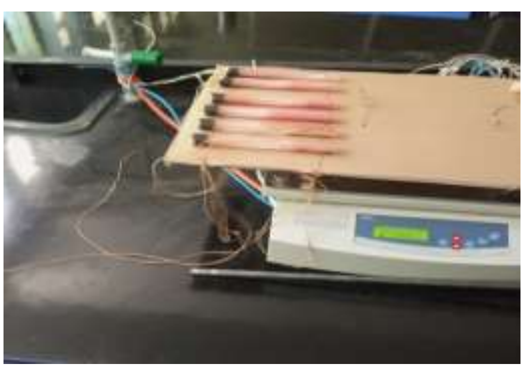
3.5 Extraction of Lipid

The barley samples' lipid content was extracted using the Folch method following the procedure given by Mehari et al. (2019) and Agidew et al. (2021). Briefly, a 1 g quantity of powder sample was taken and placed in a test tube and combined with 10 mL chloroform and 5 mL methanol (a 2:1 ratio v/v). The mixture was extracted for 36 hours on a platform shaker at 280 rpm (Figure 3A). The filtrate was obtained after centrifuging the extract. The lipid phase was separated with the use of 2 ml of 0.73% aqueous sodium chloride, then the upper phase was removed by using a micropipette, and the lower phase (chloroform) layer containing the lipid was recovered. The solvent was removed by a fume hood for one day. After the mass of the lipid was tabulated following the following equation 1, the residue (the crude fat) was re-dissolved with 5.0 mL of toluene.

$$\%Crude\ fat\ = \frac{(W2 - W1)}{ws}\ x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ equation\ 1$$

where, $W2 =$ mass of the beaker with Lipid, $W1 =$ mass of empty beaker and, $WS\ =$ mass of sample used

Figure 3 : Extraction of lipids on platform shaker (A) and extracted lipids (B) from Barley Sample



A

B

3.6 Derivatization

Since fatty acids have a high boiling point, analysis of the samples should be preceded by the derivatization of the fatty acid to the corresponding methyl ester (Mehari et al.,2019). Hence, the lipid extracts were derivatized following the method reported by Mehari et al., 2019. Briefly, a

2.0 mL portion of the lipid extract in toluene was spiked with 50µL of 3.48 mg/ml of Penta decanoic acid (internal standard) in an air-tight glass tube and 2.0 mL of 1% sulfuric acid in a methanol solution was added to the mixture as a derivatizing agent. Then, the mixture was kept in an incubator at 50°C for 12 hrs. 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 phase was taken away by using a micropipette (siphoning) and dried over anhydrous sodium sulfate, filtered with an acrodisc syringe filter, transferred into the vial, and analyzed and submitted to GC-MS analysis (Figure 4).

Figure 4 :Derivatized sample transferred to (the vial by an acrodisc syringe)

3.7 GC-MS ANALYSIS

An Agilent Technologies 7890B gas chromatographic system equipped with an autosampler, A split splitless injector and a mass spectrometer (Agilent Technologies 7890B-5977A) wasused for GC-MS analysis of the essential oils. 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 min^{-1} to 230° C (held for 20 min), the total run time was 60.8 min. Helium was used as carrier gas at a flow rate of 1.0 mLmin^{-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.

Quantification of the Fatty Acids

Twelve fatty acids, with relative percentages of peak area higher than 0.1%, were taken for reporting, although there were some other minor fatty acids whose relative peak area was less than 0.1%. Identification of the fatty acids was made using the NIST-MS library and authentic standards for some of the fatty acids. These twelve fatty acids were taken, quantified accurately, and used to compare three barley samples. In order to see the distribution of the fatty acids in the three samples, the percent relative abundance of each fatty acid was calculated following equqation 2. In addition, the concentration of the individual fatty acids in mg/100g was calculated following equation 3 which was recommended by Mahri et al., 2019.

$$\text{Relative composition (in \%)} = \frac{Peak\ area\ of\ individual\ fatty\ acid}{Total\ peak\ area\ of\ the\ idintified\ fatty\ acids}\ x\ 100\% \ldots equation\ 2$$

$$\frac{W}{W}\left(\frac{mg}{100g}\right) = \frac{A_{FA}\ x\ m_{IS}}{A_{IS}x\ m_C} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots equation\ 3$$

where AFA 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 barley sample used for the analysis.

3.8 Statistical analysis

Descriptive statistical analysis was done, and the results were expressed as mean values and standard deviation SD) of duplicate measurements. Significant differences of the data among the parameters were done by one-way ANOVA) was used to test the presence of significant differences in the mean content of fatty acids among the different sub district of barley. At p 0.05, differences were considered significant. Samples were extracted and analyzed in duplicate, and average values were used for statistical calculations.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Lipid content in barley samples

Table 1 shows the mass of the crude oil and its per cent by mass from one gram of barely flour sample used in this study. The result of this study revealed that average total lipid content in the three samples was found to be 5.95%,5.18%, and 4.85%, respectively, in samples from Sahara, Chara, and Debaca sub districts. Barley from the Sahara are distinguished from those from other district by their total lipid content. According to the results of this study, the mean values of total lipids found in Sahara Barley (an average of 5.95 %) are significantly higher than those found in Barley from other sub districts. The observed variations in the lipid content between the Barley from the three different sub districts can be ascribed to several factors, including soil fertility, harvesting conditions, agricultural practices, and environmental growing conditions (Fedak et al;1977).

Table 1. Lipid Content of Barley

No	Sub-district	Mass of sample(g)	Mass of lipid oil (g)	% of lipid(mean±standared devation), n=2
1	Debaca	1	0.0485	4.85±0.004
2	Chara	1	0.0518	5.18±0.014
3	Sahara	1	0.0595	5.95±0.006

The lipid content of barley has been quoted by various authors as between 2.1-2.6%, 3.15-4.25%, 2.5-3.1%, 2.0-4.0%,4.0-7.4%, respectively on a dry weight basis (Price 1972, Johansson 1976, Fedak and De La Roche 1977, Bhatty 1974and1975, Bhatt and Rossnagel 1980). Though both variety and environment undoubtedly have detectable effects on the lipid content of barley, Fedak et al;1977 this wide range was almost certainly due to the different methodology or extraction conditions, solvent system and degree of purification employed for estimation of the total lipid value(Osman et al;2000).

4.2 Identification of the fatty acids in the barley samples

A total of twelve fatty acids, seven saturated and five unsaturated fatty acids, were detected in all of the Barely samples(Fig 5).The identities of the detected fatty acids were determined by comparing the retention times and mass spectral fragmentation patterns by using the NIST spectral library as a reference (table 2) and reference standards for some of the fatty acids

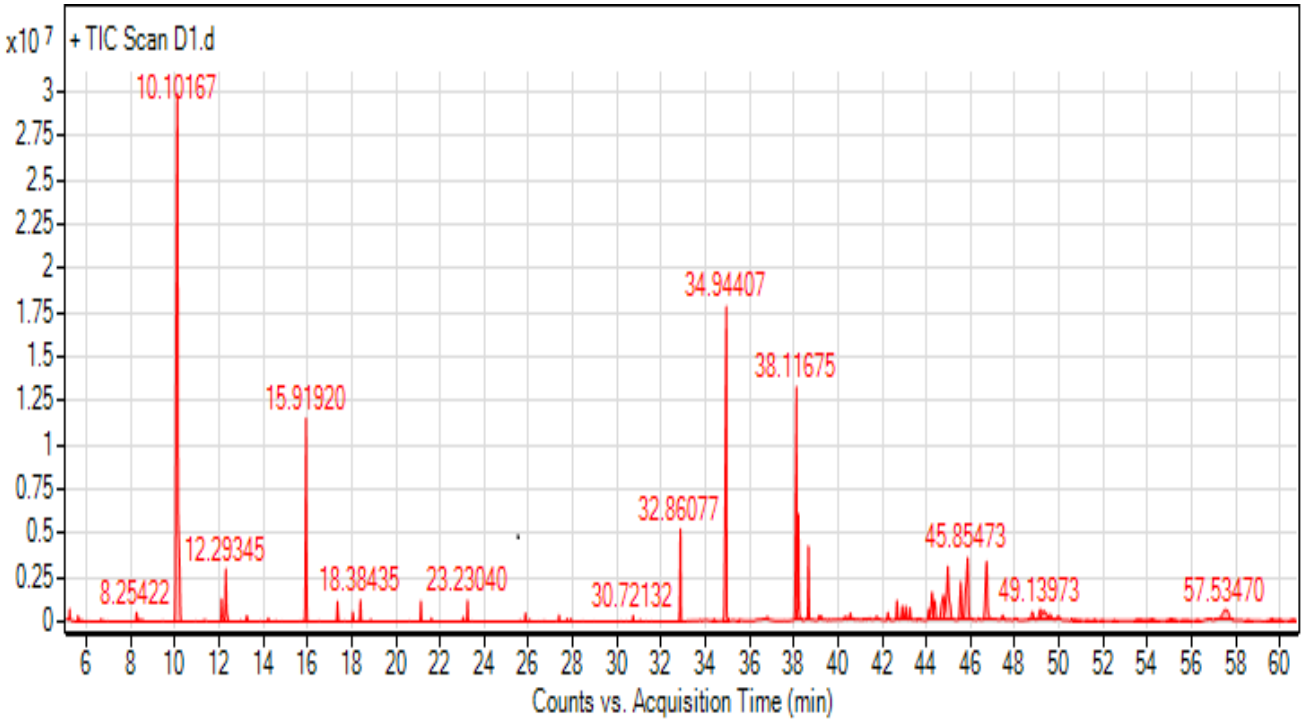


Figure 5 A typical GC-MS chromatogram of Dangila Woreda Barley extract, indicating the twelve detected fatty acids.

Table *2:* The name, retention time (RT), and means of identification of fatty acids determined in the Barley.

SN	Name of the fatty acid	Common name of the corresponding fatty acid	Formula	RT	Means of Identification
1	Tetra decanoic acid	Myristic acid	$C_{14}H_{28}O_2$	30.72268	NIST-MS
2	Pentadecanoic acid (internal standard)	Pentadecylic acid(IS)	$C_{15}H_{30}O_2$	32.86023	NIST-MS
3	9-Hexadecenoic acid(Z)	Palmitoleic acid	$C_{16}H_{30}O_2$	34.39621	Standard & NIST-MS
4	Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	34.94148	Standard & NIST-MS
5	Heptadecanoic acid	Margaric acid	$C_{17}H_{34}O_2$	36.79435	NIST-MS
6	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	$C_{18}H_{32}O_2$	38.11543	Standard, NIST-MS
7	9-Octadecenoic acid	Oleic acid	$C_{18}H_{34}O_2$	38.20476	Standard& NIST-MS
8	stearic acid	Stearic acid	$C_{18}H_{36}O_2$	38.65816	Standard &NIST-MS
9	6,9,12-Octadecatrienoic acid	Linolenic acid	$C_{19}H_{32}O_2$	41.43544	NIST-MS
10	cis-11-Eicosenoic acid	Gondoic acid	$C_{20}H_{38}O_2$	41.7448	NIST-MS
11	Eicosanoic acid	Arachidic acid	$C_{20}H_{40}O_2$	42.25147	Standard & NIST-MS
12	Docosanoic acid	Behenic acid	$C_{22}H_{44}O_2$	47.44345	NIST-MS
13	Tetracosanoic acid	Lignoceric acid	$C_{24}H_{48}O_2$	56.28268	NIST-MS

NIST-MS = National Institute of Standards and Technology-Mass Spectroscopy

4.3 Fatty acid profile of barley at district level

The identified compounds in the studied samples are: palmitoleic acid, palmitic acid, margaric acid, linoleic acid, Oleic acid, linolenic acid, stearic acid, arachidic acid, and behenic acid, gondoic acid, myristic acid, lignoceric acid (Table 3). From the twelve identified FAs, myristic acid(C14:0) palmitic (C16:0), margaric acid (C17:0), stearic acid(C18:0), arachidic acid (C20:0) ,and behenic acid (C22:0), lignoceric acid(C24:0) were saturated fatty acids while palmitoleic acid (C16:1), , oleic acid (C18:1), and linoleic acid (C18:2), linolenic acid(18:3),gondoic acid(C20:1) were unsaturated fatty acids. And among the twelve fatty acids Palmitoleic, Margaric, Gondoic, Linolenic, Myristic, and lignoceric acids are present in trace amounts or

modest abundance of the total fatty acids and were identified in each sub-district in concentrations less than 1% of the total fatty acids.

The relative composition of fatty acids in barley samples taken from three sub-districts of Debaca, Chara, and Sahara allows the variation in the profile of fatty acids among different regions. The most common saturated fatty acid is palmitic acid followed by stearic acid, and it comprises about 58% of the total fatty acids. In contrast, unsaturated fatty acids make up about 42% of the total fatty acid; the main contributor here is linoleic acid.

Saturated Fatty Acids: The concentration for palmitic acid (C16:0) was very high, ranging from 44.2 to 49.9% with a mean of 47.23% across all three sub districts. Palmitic acid is known generally to be one of the major constituents in most agricultural produce. Its concentration is highest among samples from Debaca and lowest in the Sahara sub district. These values are highly above those reported by Sonia et al. (2021), who showed the palmitic acid composition to lie in the range of 18.54% to 20.18%. This difference would therefore suggest that the contribution of genetic varietal or environmental factors, such as soil fertility, different climatic conditions, or other agricultural practices, is very important to the synthesis of these fatty acids in barley. The second major saturated fatty acid, stearic acid, C18:0, presents reasonable consistency in its concentration across the sub districts, as shown by values ranging from 7.23% to 7.80% (mean 7.59%). Other minor saturated fatty acids detected in trace amounts each below 1.5% were myristic, arachidic, behenic, and lignoceric acids.

On the other hand, unsaturated fatty acids comprised a smaller yet significant percentage of the total fatty acid composition in barley from these sub-districts. The most abundant unsaturated fatty acid was linoleic acid (C18:2), with a variation between 28.47 and 32.22% of the total fatty acid composition. Among them, the Sahara sub district barley samples contain the highest relative abundance, 32.22%, followed by 29.8% for Chara and 28.47% for Debaca. This variation of concentration may point out, from the agroecology perspective, that environmental conditions, such as differences in soil composition and local climate, may be influential over the synthesis of linoleic acid in barley. These values are significantly lower than those by Sonia et al. 2021, who recorded a linoleic acid level of 42.51%-46.16%. Another major unsaturated fatty acid is oleic acid (C18:1); values reported in the sub districts are more stable, ranging from 10.33% to 11.16%, and averaging 10.68%. This uniformity supports the fact that oleic acid

levels in barley are less altered by regional environmental factors. On the other hand, the oleic acid content realized is also lower compared to that reported by Sonia et al. (2021), which was between 21.86 and 25.45%.

The differences in the fatty acid composition in the barley samples from the three sub-districts are linked to a variety of aspects. These differences, according to Osman et al. (2000), could be due to regional changes in soil fertility, climatic factors, harvesting methods, and general agricultural management. For instance, the relatively high concentration of linoleic acid in Sahara district barley can be an indication of conditions favoring its biosynthesis. Similarly, the somewhat higher levels of palmitic acid in Debaca could suggest regional conditions favoring accumulation of saturated fatty acids.

In general, the higher levels of saturated fatty acid in barley samples from the three sub-districts may indicate that this barley is different in its nutritional or industrial uses from that with a higher content of unsaturated fatty acids. The low levels of linoleic and oleic acids obtained in the present work, when compared to previously reported values, further underscore the influence of environmental and genetic factors on the pattern of fatty acid content in barley. Variations observed form the basis of further studies in ascertaining how different environmental and agronomic conditions influence biochemical composition in barley, with a view to further exploitation.

Table3: Relative percentage composition (%) of fatty acids detected in barley sample collected from three different districts (debaca, chara and sahara sub district in Dangila district awi zone) Ethiopia.

Fatty acid	Fatty acid composition (%) (mean ± standard deviation; n=2			
	Debaca	Chara	Sahara	Mean of average
Palmitoleic acid	0.25±0.04	0.16±0.07		0.21
Palmitic acid	49.9±2.56	47.59±2.19	44.2±1.32	47.23
Margaric acid	0.28±0.03	0.36±0.06	0.56±0.31	0.4
Linoleic acid	28.47±1.72	29.80±1.60	32.22±0.75	31.16
Oleic acid	10.33±0.97	10.55±0.36	11.16±0.43	10.68
Stearic acid	7.23±0.1	7.76±0.31	7.8±0.14	7.59
Arachidic acid	0.94±0.05	1.01±0.11	1.13±0.09	1.02
Gondoic acid	0.51±0.08	0.68±0.22	0.74±0.32	0.64
Linolenic acid	0.16±0.01	0.15±0.05	0.11±0	0.14
Myristic acid	0.61±0.03	0.62±0.05	0.65±0.06	0.63
Lignoceric acid	0.3±0.05	0.33±0.06	0.43±0.02	0.35
Behenic acid	0.97±0.09	0.96±0.02	1.07±0.03	1

The fatty acid composition of barley seed varieties was compared with other reported seed oils from Ethiopia and other countries (Table 4). The fatty acid profiles obtained from the present study conformed to the trend reported in the literature for barley seed oils. Unlike the other trace fatty acids, palmitic acid and linoleic acids have shown broader ranges of variation between the present result and reported data (Table 3). This indicates the existence of some genetic variations with the studied, variation with extraction methodology and reported barley seeds and/or environmental conditions between the cultivation district (Fedak et.al; 1977,Osman et al; 2000 and Sonia et al 2021) .

Table 4: comparisons of fatty acid composition in barley seed varieties reported from different country

Fatty acid composition	Country					
	Egypt	Tunisian	Turkey	US	Canada	Ethiopia
Linoleic acid	39.49-53.4%	42.5-46%	52.2-53%	53.6-58.5%	42.5-46%	28.5-32.2%
Palmitic acid	17.9-23.8%	18.5-20.2%	22.2-22.8%	19-28.4%	24-28%	44.2-49.9%
Oleic acid	13.9-22.4%	21.9-25.5%	14.6-16.3%	9.2-16%	11%	10.3-11.2%
Linolenic acid	4.6-11.2%	-	4.6-5.5%	4.5-7.1%	4%	Trace
Stearic acid	1.5-3.3%	1.7-3.1%	-	0.6-2%	-	7.2-7.8%
Palmitoleic acid	1.5-3.6%	0.2-0.4%	-	-	-	Trace
Myrestic acid	0.1-2.3%	0.3-0.4%	-	-	-	0.6-0.7%
Archidic acid	-	0.3-0.9%	-	-	-	0.9-1.1%
Margaric acid	-	0.1-0.2%	-	-	-	0.3-0.6%
Gondoic acid	-	1.5-2.2%	-	-	-	0.5-0.7%
Behenic acid	-	-	-	-	-	0.9-1.1%
Lignoceric acid	-	-	-	-	-	0.3-0.4%
Reference	Osman et al; 2000	Sonia et al; 2021	Minyal et al;2010	Fedaketal;1977	Wijekone tal;2022	This study

Fatty acid composition of Barley samples in mg/100g

Alongside detailing the relative composition of fatty acids in the barley samples from the three sub-districts, we assessed the fatty acid content in mg/100g. data are presented in Table 6, while ANOVA data are provided in Appendix Table A1. Despite visible variations in mean concentration, statistical examination among these sub-districts reveals no significant variances. This may indicate consistent environmental and agricultural circumstances.

The primary aim of the study was to quantify and evaluate the fatty acid composition in barley samples from three sub-districts, while assessing variances at sub-district levels. The average concentrations of the fatty acids differed among the sub-districts. The concentration of myristic acid was measured at 2.58 mg/100g in Debeka, 3.44 mg/100g in Chara, and 3.19 mg/100g in Sahara. The palmitic acid readings were 210 mg/100g for Debeka, 266 mg/100g for Chara, and 216 mg/100g for Sahara. The linoleic acid concentrations were 121 mg/100g for Debeka, 169 mg/100g for Chara, and 158 mg/100g for Sahara. Despite the fluctuation in mean values, the ANOVA findings indicated no statistical difference among sub-districts for each of these fatty

acids. This would imply that the soil composition, climate, and farming practices remain somewhat stable in these places.

Table 5 : The Average concentrations (mg/100g) of fatty acids found in barley samples from three sampling sub-districts (Debeka, Chara, Sahara) n=2

			Debeka sub-districts		Chara sub-district		Sahara-sub districts	
SN	Common name of the fatty acids	RT	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
1	Myristic acid	30.72268	2.58	0.43	3.44	0.59	3.19	0.2
2	Palmitoleic acid	34.39621	1.12	0.435	0.96	0.63	ND	ND
3	Palmitic acid	34.94148	210	36.27	266	55.8	216	0.6
4	Margaric acid	36.79435	1.23	0.396	2.1	0.86	2.77	1.65
5	Linoleic acid	38.11543	121	34.13	169	51.6	158	8.88
6	Oleic acid	38.20476	44.2	13.88	59.5	17.1	54.8	3.91
7	Stearic acid	38.65816	30.6	6.406	43.4	9.37	38.3	1.97
8	Linolenic	41.43544	0.7	0.209	0.9	0.51	0.53	0
9	Gondoic acid	41.7448	2.13	0.132	4.02	2.2	3.61	1.47
10	Arachidic acid	42.25147	4.02	1.131	5.73	2.05	5.55	0.62
11	Behenic acid	47.44345	4.07	0.535	5.42	1.48	5.28	0.31
12	Lignoceric acid	56.28268	1.3	0.502	1.93	0.84	2.13	0.17

CHAPTER FIVE

5 CONCLUSION AND RECOMMENDATION

The total Lipid content and fatty acids in barley samples representing selected districts of the Awi zone in Dangila District were reported in this study. Among the fatty acids detected in this study, the following were found to be significant in all of the barley samples and were taken into account in a comparative analysis of the barley samples with respect to the sampling sub-districts: Myristic (C14:0) , palmitolic (C16:1) ,Palmitic (C16:0), linoleic (C18:2), margaric (C17:0),behenic acid(C22:0), Lignoceric acid(C24:0), gondoic acid(C20:1), linolenic acid(18:3) , arachidic (C20:0), Oleic acid (C18:1) and Stearic acid (C18:0) were detected by GC-MS analysis. Among the determined fatty acids, Palmitic acid was found to be the major dominant fatty acid in all sub -districts. Palmitic acid was discovered to be the main fatty acid in all sub-districts among the identified fatty acids. From three sub-districts, Debaca sub-district contains a higher concentration of Palmitic acid than the other sub-districts. Linoleic and Oleic acids were the second and third most prevalent fatty acids in the barley sample, respectively. Based on this result, barley sample could be a good source of fatty acids, and it must be promoted to other parts of the Amhara region.

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Appendi x

Table A1 ANOVA result of Fatty acids in barley Samples

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
Myristic acid	Between Groups	.480	2	.240	2.571	.224
	Within Groups	.280	3	.093		
	Total	.760	5			
Palmitoleic acid	Between Groups	.023	1	.023	.077	.808
	Within Groups	.585	2	.292		
	Total	.607	3			
Palmitic acid	Between Groups	3692.923	2	1846.462	1.255	.402
	Within Groups	4413.510	3	1471.170		
	Total	8106.433	5			
Margaric acid	Between Groups	2.263	2	1.132	.973	.472
	Within Groups	3.490	3	1.163		
	Total	5.753	5			
Linoleicacid	Between Groups	2432.243	2	1216.122	.929	.485
	Within Groups	3927.225	3	1309.075		
	Total	6359.468	5			
Oleicacid	Between Groups	244.173	2	122.087	.735	.550
	Within Groups	498.420	3	166.140		
	Total	742.593	5			
Stearic acid	Between Groups	166.093	2	83.047	1.894	.294
	Within Groups	131.540	3	43.847		
	Total	297.633	5			
linolenicacid	Between Groups	.160	2	.080	.706	.561
	Within Groups	.340	3	.113		
	Total	.500	5			
Gondoic acid	Between Groups	4.243	2	2.122	.905	.492
	Within Groups	7.030	3	2.343		
	Total	11.273	5			
Arachidic acid	Between Groups	3.670	2	1.835	.935	.484
	Within Groups	5.890	3	1.963		
	Total	9.560	5			
Behenic acid	Between Groups	1.563	2	.782	.992	.467
	Within Groups	2.365	3	.788		
	Total	3.928	5			
lignocericacid	Between Groups	.670	2	.335	.995	.466
	Within Groups	1.010	3	.337		
	Total	1.680	5			