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Development and Evaluation of physicochemical, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia

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MSc THESIS

Degree in Food Engineering

(Specialization in Food Safety and Quality)

Development and Evaluation of *physicochemical*, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia

By

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February,2023

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Development and Evaluation of *physicochemical*, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia

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In Partial Fulfillment of
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(specialization in Food Safety and Quality)

By

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Approval of Thesis for Defense

I here by certify that I have supervised, read, and evaluated the above-mentioned work "Development and Evaluation of *physicochemical*, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia"prepared by Teshager Mebrat under my guidance. I recommend that the thesis be submitted for oral defense.

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Approval of thesis for defense result

I hereby confirm that the changes required by the examiners have been carried out and incorporated in the final thesis.

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As members of the board of examiners, we examined this thesis entitled "Development and Evaluation of *physicochemical*, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia" by Teshager Mebrat. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Masters of Science in "Food Engineering (specialization in food safety and quality)".

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Declaration

This is to certify that the thesis entitled "Development and Evaluation of *physicochemical*, Nutritional and Sensory Properties of Bread Prepared from a Blend of Wheat and Amaranth Flour in Ethiopia", A Thesis Presented to the School of Research and Graduate Studies of Bahir Dar University In Partial Fulfillment of the Requirements for the Degree of Master of Science Degree in Food Engineering (specialization in Food Safety and Quality), I declare that this thesis presented for the degree of Master of Science in Food Safety and Quality was written entirely by myself and has not been submitted elsewhere, in whole or in part, in any previous application for a degree. The work presented is entirely my own, unless it specifically states otherwise by reference or acknowledgment.

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This thesis has been submitted for examination with my approval and done under my supervision as university advisor.

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Abstract

Amaranth grain is an underutilized pseudocereal that can be used to improve the nutritional quality of foods made of different staple grains. One nutritionally poor staple food is bread made of white wheat flour (WWF). Hence, supplementing WWF with amaranthus flour (AMF) to produce bread was targeted. WWF was mixed with AMF at a 5% interval from 0 to 25%. Each treatment level combination was run in triplicate, with a completely randomized design (CRD) arrangement. Data regarding the physicochemical and sensory properties of flour and bread were collected using standard procedures. The water absorption and degree of softening quality of the dough was ranged from 52.2 to 49.8% ,and 56.0 to 67.0%,respectively. It AF supplementation increased the moisture (33.9 to 36.2%), ash (2.28 to 2.91%), protein (12.6 to 13.16.3%), fat (2.31 to 4.15%), crude fibre content (0.56 to 5.04%).Mineral analysis showed that the amaranth flour has 137.7mg/kg Fe and 33.4mg/kg Zn. Total digestable carbohydrate decreased (82.2to 71.6%). Results showed that adding AMF to WWF significantly (P<0.05) improved many of the nutritional, rheological and some of the sensory qualities. The protein, fat, fiber and ash contents of bread made by mixing WWF with AMF increased significantly (P<0.05). Among the sensory parameters tested, aroma, taste, and the overall acceptability of bread improved significantly due to the addition of AMF at a level of 10-15%. It can be concluded that amaranthus grain is an economical and nutritious part replacer of wheat, especially nowadays, when food and nutritional insecurity is a notorious issue in the developing world.

Chapter 1

Introduction

1.1 Background

Bread is an ancient food that humans consumed in many different forms. Wheat grains contain unique gluten proteins that give leavened bread dough viscoelastic properties (Wang & Jian, 2022). The standard raw ingredient for making bread in the Western world has long been refined wheat flour (Parenti *et al.*, 2020). Due to urbanization and industrialization, refined wheat bread consumption is rising quickly in developing countries (Noort *et al*., 2022), and has an associated with the prevalence of non-communicable diseases. Meanwhile, prior to the pandemic, 3 billion people, the majority of whom live in Asia and Africa, could not afford a healthy diet. This increasingly dire situation is largely caused by the dual burden of climate shocks and violent conflict in areas that are already food insecure situation (Queiroz *et al.*, 2021). More than 60% of Africa's wheat flour requirements are imported, with a significant portion reliant on wheat production in Russia and Ukraine (Araujo-Enciso & Fellmann, 2020).

Wheat bread is a popular food in the category of bakery products. Refined wheat flour is the most used raw material for this product but it has a low level of essential amino acids such as lysine and threonine, and reduced fiber content compared to whole-flour bread. In fact, many studies have been aimed at improving wheat flour's nutritional characteristics by the incorporation of new functional ingredients and at developing safe, healthy, nutritious products. An alternative could be bakery products enriched with a high amount of dietary fiber, amino acids, and bioactive compounds from whole flours or entire grains of cereal/ pseudocereal that help to prevent diseases associated with metabolic syndromes such as cardiovascular diseases, arteriosclerosis, and colon cancer (Salas-Salvadó et al., 2006).

Consumer attention in recent years has been directed toward the nutritional and health aspects of foods. The application of new ingredients in the basic product formulation could result in products with higher nutritional value and improved sensory quality (Šimurina *et al.*, 2009).

The baking industry is now recognized as one of the most important food processing segments, with baked goods becoming increasingly popular due to their availability, readiness and relatively long shelf life. They are consumed worldwide as a snack and widely in developing countries where protein and calorie deficiencies are common (Omobolanle *et al.*, 2017). The pseudocereals amaranth and wheat are currently emerging as healthy alternatives to gluten-containing grains in the gluten-free diet. Not only they are naturally gluten-free, but they are also high in a wide range of nutrients (Kupper, 2005; Pagano, 2006).

Grain-based foods containing amaranth, quinoa, and buckwheat as a compound with wheat flour (pasta, noodles, breakfast cereals, cookies, and bread) are already on the market, but few of these products are gluten-free. Amaranth flour could improve the nutritional quality of bread and baked goods because amaranth is a very high-protein, high-carbohydrate pseudocereal that meets the needs of people with celiac disease, people with wheat allergies, and vegetarians (Coțovanu & Mironeasa, 2021).

Bread made from refined wheat flour, besides being a good source of energy, is considered to be nutritionally poor bread is the major component of the diet for the majority of the human population of which more than half consumes wheat-based bread. Today, there is a tendency to consume bread and bakery products made from refined or white wheat flour (WWF) that is characterized by lower nutritional value (Bodroža-Solarov *et al*., 2008).

Various studies have been conducted to investigate the possibility of improving the nutritional value of wheat bread by supplementing it with natural ingredients (Giami *et al.*, 2003). The Amaranthus genus belongs to the Foxtail family and includes more than 60 species, of which three, namely Amaranthus hypochondriacus, A.cruentus and A.caudatus, are the most important cereal species (Stallknecht & Schulz-Schaeffer, 1993).

Amaranth is a very versatile crop that can be grown in a variety of agro-climatic conditions; it is resistant to drought, heat, and pests, and it adapts easily to new environments, including those that are inhospitable to traditional cereal crops (Kaur *et al*., 2010).

Its grains have a higher protein content (12-18%) than most other cereal grains, as well as a significantly higher lysine content and acceptable levels of tryptophan and methionine, both of which are found in low concentrations in cereals and leguminous grains (Bressani, 1988; *R . Teutonico and D . Knorr,*).

Grain products are a good source of dietary fiber, minerals like magnesium, phosphorus, copper, and manganese, as well as protein. Squalene is present in significant amounts (8%) in Amaranth grain oil (Maurya & Arya, 2018). Which has many important direct and indirect health benefits and has the potential to replace other squalene sources like whale or shark liver oil. Anti-cancer and hypocholesterolemic properties of squalene have been shown (Das *et al*., 2003; Shin *et al*., 2004).

Squalene's importance as a food component has been attributed to its ability to lower cholesterol levels by inhibiting cholesterol synthesis in the liver (Escudero *et al*., 2006). Amaranth grains are an excellent choice for a gluten- and casein-free diet. In addition to the unique properties of the main components of protein, carbohydrates, and lipids, amaranth grains have a high content of calcium, iron, and sodium compared to cereal grains (BECKER *et al*., 1981). Also, the same reported grain amaranth has several appealing characteristics such as gluten-free ingredients, high-quality protein, and an abundance of fiber and minerals such as calcium and iron (Ballabio et al., 2011; *Moreno ML, Comino I, and Sousa C.*, 2014).

Amaranth contains more protein than most other grains, including wheat, rice, and corn, which are low in lysine. It has recently been used in developing countries to combat protein malnutrition, and it is also becoming popular among celiac disease patients because it does not cause allergic reactions in the intestinal mucosa, so it is used in the preparation of gluten-free products (Gebreil *et al.*, 2020).

One possibility is to incorporate an *Amaranthus* species grain into bread or bakery products. Amaranths are non-grass plants with broad leaves that produce a lot of cereal-like grains. Amaranth (family *Amaranthaceae*) is an underutilized plant with high nutritional value. Grain amaranth is a versatile food ingredient that can diversify farming operations and is expected to prevent food depletion and feed the world (Emire & Arega, 2012).

The nutritional quality of amaranth protein has consistently outperformed that of other cereal grain and legume seeds by FAO/ WHO protein scoring or by PER (Masciarelli *et al.*, 2002).

1.2 Problem Statement

For many years, public health concerns have focused on iron deficiency because it is a health issue in the majority of the world's countries. More than 25% of the world's population has signs of anemia, and more than 50% of them, particularly women and children under the age of 7years, have iron deficiency anemia (IDA) (Pivina *et al.*, 2019). This issue is exacerbated in populations that consume monotonous plant-based diets with little meat, where the majority of the dietary Fe is in nonheme form. Nonheme Fe is primarily found in plant foods such as cereals, legumes, fruits, and vegetables, and its absorption is frequently impaired by <10% (Zimmermann & Hurrell, 2007). Furthermore, the absorption of this kind of iron can be influenced by a variety of dietary components that can act as enhancers or inhibitors (Zimmermann & Hurrell, 2007). Bread is a staple food in many countries and thus plays an important role in international nutrition. The most practical and long-term strategy for preventing Fe deficiency is food fortification, such as fortified cereal flour. However, Fe addition frequently results in undesirable sensory changes in food vehicles and has low bioavailability (Gupta *et al.*, 2020).

It is critical to diversify nutrient-deficient cereal-based foods. Wheat is a cereal grain that is low in lysine, tryptophan, and amino acids containing sulfur. Wheat has an inherently poor nutritional profile, and the commercial milling process depletes wheat's inadequate nutrients (white wheat flour). Increasingly unaffordable demand, tougher agronomic requirements for production, and some people are gluten sensitive. White bread has a poor natural flavor (sensory property). Raising consciousness about the emptiness of refined wheat flour (empty calory food). White wheat bread (WWB) is Popular because of the unique viscoelastic behaviour of dough;

- High bread loaf volume

- Fluffy (spongy) texture of the bread crumb

Amaranth its high nutritional value, such as essential amino acids, iron, fiber, and unsaturated fatty acids, amaranth is an appropriate candidate for use as composite flour. Ethiopia produces a wide range of agricultural products in order to generate foreign currency and meet domestic demand. Cereals such as wheat and maize are traditional and widely consumed as major cereal crops among the various cereal crops produced by the country. Most resource-poor people and those vulnerable to malnutrition in Ethiopia consume starchy staples like maize, which contain insufficient protein, essential amino acids (lysine and sulfur amino acids), and most micronutrients (Endris *et al*., 2017).
As a result, many children are at risk of malnutrition. Malnutrition, also known as "hidden hunger," affects nearly 31% of all children under the age of five, and has become a major public health issue in many developing countries (Andini *et al.*, 2013).
The chenopodium family, which includes amaranths, is well-known for being a good source of protein-rich leafy vegetables as well as an inexpensive source of valuable nutrients such as dietary fiber, minerals, vitamins, and antioxidants (Andini *et al.*, 2013).

Processing these grains flour into products has several limitations, and there is very little information available on their rheological and pasting properties when compared to wheat and other cereals. The quality of baked goods is primarily determined by the flour's pasting and rheological properties. Thus, detailed research into the rheological and pasting properties, as well as the nutritional values of the composite flour, could aid in the formulation and promotion of various nutritional baked foods such as bread. Furthermore, the purpose of this research is to evaluate the effect of amaranth grain flour on the overall quality of wheat flour and its bread.

1.3 Objective

1.3.1 General Objective

The general objective of this research is to evaluate the effect of wheat flour substitution with amaranth flour on bread quality

- **1.3. 2 specific Objective**

- To investigate the effect of amaranth flour on the physicochemical properties of wheat bread
- To determine the water absorption, dough development time, dough stability, and degree of softening of dough made of a mixture of wheat and amaranths grain flours.
- To determine the influence of amaranth grain flour on the organoleptic and nutritional quality of wheat bread.

1.4 Significance of the research

This study looks at the impact of locally grown Amaranth species on the potential use of nutritionally dense bread. The research involves the creation of bread from amaranth and wheat. The rheological properties of the flour will be investigated to determine their impact on the quality of baked goods (bread). As a result, amaranth and wheat may be promising sources of protein for gluten-sensitive people.

To improve baking quality, prepare a nutritionally dense product, and answer the feasibility question, will be blending to the grain amaranth and evaluating for various quality parameters. The preparation of gluten-free bread (GFB) containing from amaranth flour will solve the problem for gluten-sensitive persons or celiac disease patients (gluten-sensitive enteropathy) and the major problems with baked products from wheat, barley, and rye. People with celiac disease are very interested in gluten-free products. On the basis of gluten-free cereals, several recipes could be used to produced gluten-free products, and they already are. Cases of wheat allergy and gluten sensitivity have been identified. CD is an upper gastrointestinal disease caused by the consumption of gluten, a protein found in wheat (gliadin), rye (secalin), and barley (hordein). It is widespread throughout the world, affecting approximately one in every 100 to 300 people. People are becoming more aware of their own health, diet, and nutrition. They are looking for foods that are convenient, tasty, affordable, and nutritious. The evaluation of the nutritional and functional properties of Amaranthus species aids in the diversification of agriculture away from conventional crops such as wheat and teff, as well as the cultivation and utilization of this underutilized crop for meeting human needs for food, fodder, fiber wood, medicine, and so on. The current study's goal is to evaluate the potential use of amaranth and wheat grains grown in Ethiopia as an alternative to conventional cereal crops. This research will also contribute to national food and nutrition security by producing a document that can be used as a reference material for large-scale processing and utilization of this underutilized food crop. Amaranth grain, like some other grains and grasses, is gluten-free, which is good news for those with Celiac disease.

Chapter 2

2. Literature Review

2.1 Production and Nutrient Profile of Grain Amaranth

Amaranth is a nutritionally dense pseudocereal with excellent seed quality and the greatest potential for use as a food ingredient that grows in Africa and Asia, where it is now planted as a grain crop in widely dispersed regions such as Ethiopia's mountains, South India's hills, Nepal's Himalaya, and Mongolia's plains. When compared to other cereals, it has an excellent nutritional profile, with a high level of protein, essential amino acids, particularly lysine, minerals, dietary fiber content, and fat (Umaraw *et al*., 2020; Mekonnen *et al*., 2018). Its grain can be dried, milled, and used as whole-meal amaranth flour, crackers, gluten-free brown bread, biscuits, and cookies in human nutrition Mekonnen *et al*., (2018), the crop has a high level of genetic diversity in South America. Although only a small sample has been introduced to other continents, Northern India germplasm collections have shown a high level of genetic diversity (Akin Idowu *et al*., 2013). The incorporation of pseudocereals into the global food production system, such as bread making has been promoted to ensure food security due to their current underexploitation. In the study by Lin *et al*., (2009), Buckwheat was incorporated 15% to increase the antioxidant activity of wheat bread. By using up to 25% whole amaranth flour in baking goods, the content of protein, dietary fiber, and minerals (e.g. Fe and Zn) has been significantly increased. Miranda-Ramos *et al*., (2019), Sanz-Penella *et al*., (2013), with relatively minor changes in the physicochemical and rheological properties of the wheat dough (Guardianelli *et al.*, 2019).

2.1.1 Overview of Amaranths Grain Production

The domestication and cultivation of amaranth began around 8,000 years ago in the Maya civilization of South and Central America, according to historical evidence. According to the most significant historical records, amaranth, also known as huahtli, was a staple crop grown in Mexico during the Aztec civilization. The Aztecs believed it had magical properties that gave it strength. roughly equivalent to corn. However, in the 16th century, the Spanish conquistadors banned the cultivation of amaranth to suppress Aztec culture and religion, encouraging its adoption and production in other parts of the world (Mohil & Jain, 2014).

During the twentieth century, it was grown in China, India, Africa, and Europe, as well as North and South America. Despite being the leading producer of grain amaranth used in retail food products, China is thought to have had the largest production area in the last decade. Instead of harvesting amaranth for grain, the Chinese use it as hog forage (Rastogi & Shukla, 2013).

Amaranth is a perennial plant that can grow to be 0.5-3 m high depending on the species. Plants are densely packed with thick stalks. It is similar to pigweed in many ways. Flowers are predominantly purple, red, pink, orange, or green in color. The leaves are fairly broad, and the blossom strands can reach lengths of up to 90 cm. They can grow in either upright or prostrate positions (Amico & Schoenlechner, 2017).

The amaranth seed is a pseudocereal with over 60 species, but only three are used for edible seed production (A. cruentus, A. caudatus, and A. hypochondriacus) (Narwade & Pinto, 2018). To understand the nutritional composition of amaranth and its protein value, you must first understand the structure of amaranth seeds. Amaranth seeds are very small (1–1.5 mm in diameter) and consist of several layers like a seed coat; perisperm, rich in starch; endosperm; and the embryo, composed of two protein-rich cotyledons; Exchange; Root; and root (Montoya-Rodríguez *et al*., 2015).

Amaranth is a simple-to-grow, nutrient-dense, and underutilized pseudo-cereal that can aid in the fight against hunger and malnutrition caused by insufficient rainfall. Because of their unusual characteristics, amaranth species have piqued the interest of atypical agricultural plant researchers. The "modern" interest in this crop began in the early 1970s, when an Australian researcher highlighted A. edulis' nutritional value due to its high lysine concentration. During the same time period, the book "Unexploited Tropical Plants with Promising Economic Value" published by the National Academy of Sciences proposed amaranth exploitation, encouraging studies and research.

The Rodale Foundation and the Rodale Research Center (United States of America) supported and funded amaranth cultivation, production, and commercialization projects and research a few years later, in the mid-1970s. Later, new amaranth research centers sprang up across the globe. The most interesting countries in Europe for the amaranth crop are Austria, Czech and Slovak Republic, Germany, Hungary, Poland, Slovenia, Spain, and Italy (Valcárcel-Yamani & Lannes, 2012). There isn't any global records at the cultivation of amaranth (Amico & Schoenlechner, 2017).

South America's tropical regions produce the most amaranth, but Africa (particularly for amaranth plant leaves), Central and Southeast Asia (particularly India), and, to a lesser extent, North America's warmer regions also produce amaranth. The production area in Europe is quite small, only about 1000 ha (Amico & Schoenlechner, 2017). The high content of carbohydrates, proteins, fats, fibers, and essential amino acids in these species makes their seed quality comparable to, if not superior to, cereals (Marin *et al*., 2011).

2.1.2 Utilization of Amaranth

The importance of amaranth as an underutilized plant with high economic potential has recently been recognized. The grain can be used in a variety of ways. The whole seed can be roasted and added to other foods, wet-cooked to make a type of porridge, or ground into flour. Amaranth grain can be toasted, popped, extruded, or milled into flour and used to make bread, cakes, muffins, cookies, dumplings, crepes, noodles, and crackers. According to some studies, amaranth grain could be used in gluten-free products such as crackers, maize tortillas, and chips (Muyonga *et al*., 2014, Rai *et al.*, 2018, Gebreil *et al.*, 2020). The seeds and leaves of the amaranth are used as food and animal feed (Stallknecht & Schulz-Schaeffer, 1993). It is also used in foods to supplement nutrients typically lacking in the diet of people with celiac disease (Kupper, 2005).

In Mexico, amaranth seeds are roasted and mixed with a sugar solution to make "alegria", sweets (happiness). Ground and roasted amaranth seeds are used to make 'atole', a traditional Mexican 'alegria' drink"Amaranth seeds are consumed in Nepal in the form of porridge known as 'sattoo' or ground into flour to make chappati (Tiwari, 2018). Conventional cereals and pasture crops. The most essential nutritional features of grain are undoubtedly its protein content and quality. Amaranth seeds are a type of pseudocereal (Palombini *et al.*, 2013). Have both cereal and leguminous seed characteristics (Karuthedath, 2012).

2.1.3 Amaranth in Ethiopia

In contrast to the cream-colored grains, the tiny seeds (*Amaranthus caudatus*) are typically shiny black. There could be up to 3000 seeds per gram. The tiny, lens-shaped seeds are typically light in color.

The crop is primarily grown and consumed in Oromiya, Benashangule Gumuz, Gambella, and SNNPR regions. It is a staple food of the Meenit, Dizei, and Surma peoples in the Benchi Maji area (Mekonnen *et al*., 2018). Amaranth is mainly grown in the Benchi Maji areas of Menit Shasa, Meenit Goldya, Maji, and Yeki Woreda. Three varieties of Amaranthus caudatus are grown in Ethiopia: white, red, and black. It is grown three times a year in monoculture or in conjunction with corn or sorghum. It is drought-tolerant and can easily be grown with little water (Mekonnen *et al*., 2018). Currently, amaranth production is 2527 hectares, with the zone of Bench Maji accounting for 689 hectares of the total (ARBMZ (Annual Report of Bench Maji Zone) *(2016).*

The most common method of amaranth consumption because there are numerous species of amaranth plant grown as a grain, this study focused solely on Amaranthus caudatus (a tiny shiny black plant grown in Ethiopia).

These people use grains after popping, milling, and mixing the flour with other cereal flours such as wheat, teff, sorghum, and barley, and to make foods such as porridge (Amare *et al*., 2015). In Ethiopia, the name varies from place to place. In Amharic, it is also known as lishalisho, Aluma, and Ferenji teff; in Oromifa, it is known as Iyaso and Jolili; in Arigna, it is known as Zapina; and in Wolayita, it is known as Gegabsa. In Guraferda, Jimma, amaranth is used to make alcoholic drinks called borde, unleavened bread called 'kita,' and a thin porridge called 'atmit' for babies and new mothers (Emire & Arega, 2012).

Figure 2. 1 Amaranth grain (Sullivan, 2003).

2.1.4 Overview of Nutrient Contents of Amaranth

In several studies (Kauffman, 2016; Terrill, 2001; Pond & Lehmann, 2018; Neelesh & Pratibha, 2018; Odwongo *et al*., 1980; Stallknecht & Schulz-Schaeffer, 1993) Amaranth has been shown to have nutritional properties that outperform conventional cereals and pasture crops. Grain's most important nutritional characteristics are undoubtedly its protein content and quality. Have both cereal and leguminous seed characteristics (Karuthedath, 2012) and are gluten-free (Gebre & Emire, 2018).

In summary, the nutritional value of amaranth seeds is made up of the amaranth grain contains a lot of carbohydrates (48–69%), protein (12–18%), and fat (5–8%) (BECKER *et al*., 1981; Bressani, 1989). 62–74% of Starch (Martinez-Lopez *et al.*, 2020). 2.14–2.91% of ash (Tapia-Blácido *et al.*, 2007).

The main minerals contained in amaranth seeds are Ca, Fe, Mg, Zn, K, P, S and Na, Amaranth is also rich in B vitamins (BECKER *et al*., 1981; Montoya-Rodríguez *et al.*, 2015; Venskutonis & Kraujalis, 2013). Besides, from its nutritional benefits, amaranth is a good source of lysine and other bioactive compounds like phenolic compounds, squalene, folate, phytates, and tocopherols (Martinez-Lopez *et al*., 2020).

It has a high lysine concentration, 0.73% to 0.84% protein, which is an essential amino acid deficient in all of the world's major cereal crops (BECKER *et al*., 1981; Bressani, 1989).

It also contains high sulfur-containing amino acids, which are uncommon in pulse crops (M. W. Mburu *et al.*, 2011). Gluten-free amaranth grain is used in breakfast cereals, pancakes, soup, bread, cookies, gluten-free foods, extruded snacks, and confections (Gebre & Emire, 2018).

Amaranth flour (AMF) is especially well-suited to the production of unleavened flatbreads like tortillas and chapattis), where it can be used as the sole or major cereal ingredient. Because amaranth flours (AMF) lack gluten and cannot retain gas on their own, they must be combined with wheat flour when baking yeast-raised bread or other leavened goods. Amaranth grain contains more protein than most cereals and has been identified as a good source of protein. Furthermore, amaranth flour boosts the bread's protein content, which is especially beneficial for children. The unique nutritive composition of amaranth grain makes it more appealing for use as a blending food source to increase the biological value of processed foods (Stallknecht & Schulz-Schaeffer, 1993).

Amaranth starch has been used in the preparation of custards, pastes, and salad dressings, according to the researchers Stallknecht and Schulz-Schaeffer (Stallknecht & Schulz-Schaeffer, 1993). In addition, amaranth flour is also used in tea cookies and bakery products (Hozová *et al*., 2000). Ayo, (2001) reported Amaranth flour could be used up to 15% (using wheat: amaranth flour blend of 85:15). All factors must be considered in the production of wheat-amaranth grain composite bread without affecting physical and sensory properties, as well as consumer acceptance. In order to make wheat-amaranth cookies, use a higher percentage of amaranth flour (25-30%) (Sindhuja *et al*., 2005). Thermal treatment of popped amaranth grain gelatinizes starch, which may affect water absorption, crumb quality, taste, and overall bread acceptance, in addition to optimizing the protein efficiency ratio and minimizing the loss of available lysine.

Although amaranth is theoretically close to the ideal, combining it with another grain raises the quality to the United Nations FAO/WHO ideal amino acid reference pattern established in 1998. Amaranth grain contains iron, which is required by several enzymes involved in oxygen metabolism. Iron deficiency anemia reduces oxygencarrying capacity and interferes with aerobic functions. Extremely severe anemia is associated with a higher risk of death during childhood and pregnancy (M. W. Mburu *et al.*, 2011)**.** Nutritional benefits Amaranth is becoming a superfood due to its high-quality carbohydrates, dietary fiber, lipids like omega-3 and omega-6, essential amino acids, and other important constituents like squalene, tocopherols, phenolic compounds, flavonoids, phytates, vitamins, and minerals (Soriano-García *et al.*, 2018).

The high nutritional value of amaranth seeds (Amaranthus spp..) confirms recent worldwide interest in using species of this genus as crops (Aufhammer *et al*., 1998).

Table 2-1. A comparative account of the nutritive value of grain amaranths and other cereals

Sr. No	Crop	Protein	Fat	CHO	Iron	Calcium	Food Energy (Kcal/g)
1	Amaranth	16.0	3.1	60.0	17.5	0.49	391
2	wheat	13.0	2.0	71.0	10.5	0.41	333
3	Maize	9.2	3.9	73.7	3.5	0.2	355
4	Finger Millet	7.3	1.3	74.0	9.9	358	334
5	Rice	7.0	1.0	78.0	3.5	0.2	345
6	Sorghum	12.0	3.4	71.0	5.7	0.21	356
7	Buck wheat	11.7	2.4	72.9	15.5	0.21	335

Source: (Devi *et al.*, 2014**;** Jnawali *et al.*, 2016**;** "Lost Crops of Africa: Volume I: Grains," 1996; Pinto, 2018**;** (Sarita & Singh, 2016)**.**

2.1. 5 Nutrient Complimentary Nature of Amaranth

Wheat, corn, and rice proteins are deficient in the essential amino acid lysine as well as the sulfur-containing amino acids methionine and cysteine. Amaranth, on the other hand, is high in both lysine and iron Bressani, (2018)**,** and sulfur-containing amino acids (Chauhan & Singh, 2013)**.** Grain amaranth protein contains approximately 5% lysine and 4.4% sulfur amino acids, both of which are limiting amino acids in other grains**.** Leucine is commonly reported as the limiting amino acid for amaranth (Rodríguez *et al.*, 2020)**.** Amaranth protein's amino acid composition is compares well with the FAD/WHO protein standard. Although amaranth is theoretically close to the ideal, combining it with another grain raises the quality to levels that are very close to FAO/WHO standards (M. W. Mburu *et al.*, 2011). Amaranth protein's complementary nature has been studied by combining it with wheat, rice, and maize (Martinez-Lopez *et al.*, 2020).

The grain has a high protein content and an amino acid balance that can supplement the amino acids found in cereals such as maize, wheat, and rice (which are low in lysine) and legumes (which are deficient in the sulphur amino acids). Amaranth is thought to have up to 27% more lysine than wheat and 14% more lysine than maize.

Amaranth grain is thought to have a higher mineral content than other cereals. Calcium, magnesium, and iron levels are especially high (Garuda, 2004). Ordinary maize meal supplemented with as little as 12.7% (by weight) toasted amaranth flour provides a nutritionally superior source of protein that can satisfy a significant portion of young children's protein requirements and provide approximately 70% of diet. A 1:1 mixture of rice and amaranth has been reported to meet FAO/WHO protein specifications (Bhatiwada, 2007).

Amaranth grain contains five times more iron than wheat grain and three times more fiber than wheat. Contains twice as much calcium as milk. When combined with wheat, corn or rice, amaranth provides a complete protein with a high nutritional value comparable to fish, red meat or poultry. The starch content of amaranth is unique. The 1-3 mm-diameter polygonal starch granules have a high swelling capacity..Starch has an amazing gel quality. Waxy and non-waxy starch granules were found.

2.1.6 Health benefits of amaranth grain

2.1.6.1 High source of protein

The high-quality protein found in Amaranth seeds is important because it contains many essential amino acids, which are key molecules in the formation of new cells and tissues, allowing proper neuronal function, aiding the immune system, and promoting muscle recovery (Negro *et a*l., 2008).

2.1.6.2 Reduce inflammation

Consumption of amaranth may help prevent inflammatory diseases, as extruded amaranth protein hydrolysates have been reported to prevent inflammation by activating bioactive peptides that reduce the expression of several pro-inflammatory markers (Montoya-Rodríguez et al., 2014). Because of this, eating pseudocereals can help to lower inflammation (Laparra & Haros, 2016). In this context, it is recommended that

Amaranth seeds be included in the diet to reduce inflammation and possibly help prevent chronic diseases caused by inflammation and lowers cholesterol. According to studies done on animal models, amaranth oil increases good cholesterol while lowering total and bad cholesterol (LDL) (Berger *et al*., 2003). Also, it has been proven that Amaranth affects cholesterol metabolism (Mendonça *et al*., 2009).

2.1.6.3 Fights diabetes

Amaranth is a good option for controlling blood sugar levels, which is another important advantage of including it in the diet organism as manganese helps during gluconeogenesis; thus, when manganese is obtained in sufficient amounts by consuming Amaranth, it is possible to prevent diabetes (Lee *et al*., 2013).

2.1.6.4 Helps pregnant women

Folic acid is especially recommended for pregnant women to avoid spina bifida and heart defects. Butterworth & Tamura, (1989), reported prevent constipation it helps to avoid constipation because its starch binds water and because it contains more insoluble fiber (around 80%) than soluble fiber (Lamothe et al., 2015a).

Globally, the campaign to consume grains and vegetables to maintain good heart health has grown in popularity (Lillioja *et al*., 2013). Tagwira *et al*., *(*2006) reported that the consumption of amaranth species was discovered to have an impact on health benefits among local communities.

The communities claimed that eating grain amaranth made them healthier, and they also noticed significant improvements in the health of their children, such as an increase in appetite, faster healing of mouth sores, and a decrease in overweight. It was also discovered that eating amaranth grain increased milk production in nursing mothers; this experience was viewed as a positive contribution to food and nutrition security (Tagwira *et al*., 2006).

In some areas of Benin, amaranth has been recommended for newborns, nursing mothers and patients with constipation, fever, bleeding, anemia or kidney problems (Akubugwo *et al*., 2007). In Ghana, the water from the macerated plants is used to wash aching limbs.Cruentus is used in Ethiopia as a tapeworm killer. The ash of the stems is used as a spice in Sudan. Amaranthus tricolor and A.caudatus is used both externally and to treat inflammation and internally as an aduretic (Achigan-Dako *et al.*, 2014). Amaranthus tricolor and A. caudatus are used both externally and to treat inflammations, and internally as adiuretic (Aderibigbe *et al.*, 2020).

2.2 Development of amaranth-wheat-based bread

Amaranth grain can be used to make breakfast foods, bakery goods, gluten-free foods, and extruded products either alone or in composite flour form. To make leavened bread, it needs to be blended with wheat (Elizabeth, 2010). Amaranth flour is used to make various flatbreads in Latin America and the Himalayas (Teutonico & Knorr, 1985).

Emire & Arega, (2012) reported developed amaranth-wheat-based bread using various blend formulations and adopted commercial bread-making technology used at the Kality food share Company in Addis Ababa, Ethiopia.

2.3 Amaranth in Bread Baking

Blend of 30% amaranth with 70% creals (Almirudis-Echeverria *et al*., 2022). The addition of amaranth to cereal flour improved protein quality without affecting energy utilization (Kamotho, 2020). Amaranth flour (AMF) is particularly well-suited for unleavened (flat) bread, where it can be used as the sole or dominant cereal ingredient. The flour is used to produced a variety of flat breads in Latin America and the Himalayas (for example, tortillas and chapattis). In addition, the enriched bread contained more lipids, minerals, and myo-inositol phosphates, could provide a high percentage of Fe and Zn of the daily human requirement. Pseudocereal amaranth is a gluten-free grain, and incorporating it into composite flour during the bread-making process is a challenge. Due to the reduction of gluten content in composite flour, some authors demonstrated that less than 20% amaranth replacement positively influenced bread's physical parameters, especially loaf volume (Mlakar *et al.*, 2009; Sanz-Penella *et al.*, 2013).

Amaranth meal or flour must be blended with wheat meal or flour to create yeast-raised bread or other leavened foods because it lacks functional gluten. The high lysine content of amaranth in such blends likely improves the protein quality of foods that would typically be produced using flour from other grains, like corn, rice, or wheat. This is especially beneficial for infants, children, pregnant and lactating women, and others. Although the physical properties of amaranth flour are quite different from those of wheat flour, amaranth flour contains a high level of starch, which is important for bread quality (Morita *et al*., 2002).

Amaranth flour has been used as an ingredient for improving wheat bread. Tosi *et al*., (2002) found that up to 0.10 or 0.15 of the wheat flour in a formulation could be replaced with amaranth flour with a little detrimental effect on bread quality. In bread, the second rate qualified as fair quality and to have low volume with a 0.20 decrease with respect to the wheat flour control. A 0.10 replacement reduced loaf volume by 0.07 - 0.10 and produced a darker-colored bread; however, a taste panel preferred the 'nutty' flavor of these breads over those made using only wheat flour (Sanchez-Marroquin *et al*., 1980**;** Alvarez-Jubete *et al*., 2009)**.**

2.4 Wheat (Triticum aestivum L.)

Wheat (*Triticum spp.*) is a cereal crop that belongs to the family Poaceae (order Poales). About 40% of the world's population relies on wheat as a staple food source for nutrients. For more than one-third of the world's population, wheat is the most important stable food crop, providing more calories and proteins to the global diet than any other cereal crop (Awofadeju *et al.*, 2015; (Adams *et al*., 2002; Shewry, 2009). It can be transformed into different kinds of food, is nutritive, and is simple to store and transport. According to (Kumar et al., 2017), wheat is regarded as a good source of dietary fiber, B-group vitamins, minerals, and protein although the environmental conditions can affect the nutritional composition of wheat grains with its essential coating of bran, vitamins, and minerals and it is an excellent health-building food. The whole wheat grain consists of three parts: bran, germ, and endosperm. Only the carbohydrate-rich endosperm remains after conventional milling. This results in a significant loss of nutritionally valuable biochemical compounds such as dietary fiber, vitamins, minerals, and antioxidant compounds, all of which play an important role in CVD prevention (cardiovascular disease). Wheat's importance stems primarily from the fact that its seed can be ground into flour, semolina, and other basic ingredients of bread and other bakery products, as well as pasta, and thus it serves as the primary source of nutrients for the majority of the world's population (Sramkova & Agricultural, 2017).

Wheat's nutritional value is extremely important because it is one of the few crop species that is widely grown as a staple food source. The demand for cereals is predicted to significantly increase if the food needs for the estimated world population growth are to be met. But there is another potentially great benefit to these communities and that is the possibility to ensure such staple crops are nutritionally-balanced and help remove the millions of cases of nutritionally-related deficiency disease that afflict them. It should be emphasized that in the past there has not been a single instance where plants have been bred to improve their nutritional content. If this has occurred it is purely by accident, not design (Lindsay, 2002).

Bread wheat (*Triticum aestivum L.)*, more than 35% of the world's population relies on this food as a staple, with an annual global production of 772.6 million tons (Nigus *et al.*, 2022). The biggest producers of wheat Globally are China, India, and Russia, while South Africa and Ethiopia are the biggest producers in sub-Saharan Africa(SSA) (Nigus *et al., 2022)*. The population consumes a lot of wheat bread. This kind of bakery product is made with wheat flour, but Ethiopia's wheat harvest isn't enough to meet the nation's demands. Ethiopia produces approximately 5.8 million tons per year, with mean productivity of 3 tons per hectare (tha1) (Central Statistical Agency (CSA), 2020). that's incredibly decrease than the crop's manageable yield of attaining as much as 5 tha-1(Zegeye *et al.*, 2020). Wheat accounts for approximately 17% of total grain production in Ethiopia, ranking it third after teff [Eragrostis tef (Zucc.) maize and trotter (Zea mays L.) (Central Statistical Agency (CSA), 2020).

2.5 Nutritional quality and improvement strategies

A protein's nutritional quality can be measured using a variety of criteria, but in essence, it is the relative amounts and balance of essential amino acids in the dietary protein that determine its nutritional value. In comparison with meat, producing plant protein is much more cost-effective, but most plant proteins are nutritionally deficient when used as a source of dietary protein for people and monogastric animals because they lack a number of essential amino acids (EAAs). Lack of some amino acids decreases the availability of others that are abundant. Cereal proteins are generally low in Lys (1.5-4.5% vs. WHO recommendation of 5.5%), tryptophan (Trp, 0.8-2.0% vs. 1.0%), and threonine (Thr, 2.7-3.9% vs. 4.0%). Because of this deficiency, these EAAs become the limiting amino acids in cereals. Therefore, increasing the EAAs in plant proteins has important implications for both the economic and nutritional (Bicar *et al*., 2008).

2.6 Nutritional value of cereals and the impact of milling

Although the shape and size of the seed may vary, all cereal grains have a fairly similar structure and nutritional value. Wheat is used as a reference in this thesis research because it is the base of more foods than, discussion refers to wheat grain because it is the primary grain used to make leavened bread and can substitute for any other grain.

Provides the nutritive value of the three main different parts of wheat. Bran contains the majority of the grain fiber, essentially cellulose and pentosans, accounting for 7% of the grain. It is a source of phytochemicals and B vitamins, and this outer layer contains 40–70% of the concentrated mineral content. The endosperm, the main part of the grain (80-85%), contains mostly starch. It is deficient in vitamins and minerals and has a lower protein and lipid content than the germ and the bran. B group vitamins are abundant in the germ, the tiny inner core of the grain that accounts for about 21% of the grain, proteins, minerals such as potassium and phosphorous, healthful unsaturated fats, antioxidants, and phytochemicals. Cereals are high in glutamic acid, proline, leucine, and aspartic acid, but low in lysine. The germ has the highest concentration of amino acids

2.7 Bread quality: instrumental, sensory, and nutritional quality

Bread quality is a highly subjective term that is heavily influenced by individual consumer perception, which is influenced by social, demographic, and environmental factors. Individuals' perceptions of bread quality and how one bread compares to another differ greatly. Whereas studies focused on consumer preferences emphasize the significant relationship between sensory quality and consumer perception, scientific reports focused on the bread-making process or recipes typically refer to instrumental methods for assessing quality. Alternatively, healthy concepts related to nutritional value are emerging as fundamental quality attributes of bread products. The concept of bread quality as a whole could therefore be integrated by instrumental attributes, those that can be measured objectively; sensory perceptions, such as descriptive attributes related to consumer quality perceptions; and nutritional aspects related to the healthiness and functionality of the bread product.

Chapter 3

3 Materials and methods

3.1 Materials

The grains of *Amaranthus caudatus* with pale-black color were collected from Bahir Dar and Zenelema area. Grains were harvested by hand-cutting the heads of the plant to which the seeds are attached. The grains were sun-dried, manually threshed, and cleaned to remove dust and other non-seed material. The collected samples were transported to the laboratory in polyethylene bags and stored refrigerated until use. White wheat flour with about around 75% extraction rate (Martha Flour Factory, Bahir Dar, Ethiopia) was purchased from the Bahir Dar market. Dry instant yeast was purchased from a local market in Bahir Dar, Ethiopia.

3.2 Methods

3.2.1 Preparation of Flours

The amaranth flour was prepared from amaranth grains and was ground to fine flour using a bench-top laboratory hammer mill (Sinograin, Sichuan, China), the hammer mill is a grinding chamber containing a horizontal rotating shaft on which hammers are mounted (Figure 3.1). The rotor is spun at a high speed inside the grinding chamber while amaranth grains are fed into a feed hopper. The hammer mill with an installed sieve of 250 µm and packed in polyethylene bags. The amaranth flour (AMF) is then stored refrigerated until used for analysis and the experiment. The white wheat flour (WWF) was sifted through a 500 µm sieve, followed by packing it in a polyethylene bag before keeping it in the refrigerator until used for analysis and the experiment.

3.2.2 Experimental treatment and design

3.2.2.1 Preparation of composite flour

Wheat flour (WWF) was blended with AMF to produce WWF-AMF composite flours of different proportions. The two flours were mixed using a laboratory mixer (Shepherd

King, SM-168JGX, China). In polyethylene bags, the composite flours were stored until use in the refrigerator blending ratio of the composite flour.

WWF was blended with AMF at a 5% interval from 0% to 25%. Hence, the WWF-AMF proportions were 100-0% (control), 95-05%, 90-10%, 85-15%, 80-20%, and 75-25%.(Sanz-penella *et al.*,2013).

3.2.2.2 Experimental design

The experimental treatment was a WWF-AMF blending ratio with six levels, including the control. The experiment was conducted in a completely randomized design (CRD) of the blending ratio with three replications. A total of 18 runs were carried out randomly.

3.2.3 Bread Baking

The bread-baking process was conducted according to the recipe described in Filipčev *et al*. (2007), with little modification that instead of hearth bread, pan bread was produced. The farinograph data of each blending ratio of the composite flours were used to decide about the amount of water added to the composite flours and the mixing time to ensure that the mixing time remains between the time needed for the dough to develop and that needed to maintain its strength (stability time). Each flour mixture (and the yeast + salt) was mixed using the farinograph mixer used to collect the farinograph data (Toposun, Model: TPS-JMLD, Shanghai, China). The developed dough was moulded and allowed to ferment and proofed for 30 min, followed by baking in an oven **(**mini-oven, model DL-4) at 220°C for about 30 min(Filipčev *et al*. 2007), After baking, the bread loaves were kept at room temperature for cooling before wrapping them using a cling film.

3.2.4 Data collected

3.2.4.1 Moisture content (mc)

The moisture content (mc) of WWF and AMF was determined by the method described in AOAC, (1999) in an air convective drying oven AOAC 925.10, (AOAC, 1999). About 2-3 grams of the flour sample was weighed in a moisture dish and heated at 130 ^{o}C for 1 hour. The sample was then cooled to room temperature in a desiccator, and the residue was weighed. The moisture content (mc) was determined by comparing the weight of the sample before and after drying as follows.

$$\text{mc (\%)} = \frac{Sample\ weight\ before\ drying - Sample\ weight\ after\ drying}{Sample\ weight\ before\ drying}\ \text{x}\ 100$$

3.2.4.2 Protein content

Protein was determined using Kjeldahl method as described in Nielsen, (2010). Briefly, the organic nitrogen of the flour (about 0.5 g) was digested in concentrated sulfuric acid at 400 °C by a digestion system (SBS 5000L, Omnilab, Bremen, Germany) to converted it into ammonium sulfate in the presence of 1g catalyst (made of copper sulfate and sodium sulfate at 1:10 ratio). The ammonium sulfate in the digested sample was made alkaline with 50% (w/v) sodium hydroxide and nitrogen was distilled off as ammonia in a Kjeldahl digester (Qian Jian Instruments CO. LTD. Shanghai, China). While distilling, the ammonia gas was trapped in a boric acid solution. The amount of ammonia nitrogen in this solution is quantified by titration with a standard HCl solution. A reagent blank is carried through the analysis and the volume of HCl titrant required for this blank is subtracted from each determination.

$$\%\ \text{N} = \text{Normality HCl x} \frac{\text{corrected acid vol. (ml)}}{\text{g dry matter of sample}}\ \text{x}\ \frac{\text{14g N}}{\text{mol}}\ \text{x 100}$$

The obtained nitrogen content was then translated into protein content using 5.7 for WWF and 6.25 for AMF. Percentage protein content was reported on a dry basis.

3.2.4.3 Oil content

Fat was determined by the Soxhlet extraction method as elaborated by Carpenter, (2010). About 5 grams of the flour sample was placed in a thimble, which was placed in the extraction chamber of the Soxhlet extractor that bridges the round-bottomed flask and the condenser. After connecting all parts of the extractor units, the condenser with the cooling water system, and placing the round-bottomed flask with a heating mantle, a sufficient amount of n-hexane was carefully poured from the condenser through the extraction chamber into the round-bottomed flask. The n-hexane in the round-bottomed flask was then heated and volatilized, condensed above the sample. The condensed n-hexane dripped onto the sample and soaks it to extract the fat. At 15–20 min intervals, the solvent (with the dissolved fat) is siphoned to the round-bottomed flask to start the process again for 6 hours. Oil content was measured by weighing the oil in the round-bottomed flask after the solvent was removed by evaporation. The percentage oil content was calculated by dividing it to the dry matter weight of the sample and reported on a dry weight basis.

$$\text{Oil (\% db)} = \frac{Weight\ of\ oil}{Dry\ matter\ weight\ of\ sample} \text{ x } 100$$

3.2.4.4 Fiber content

Fibre was determined as the residue after sequential treatment with hot H_2SO_4 (1.25 %) and hot NaOH (1.25 %) by the Weende method as described in ISO 6865:2000 (using FC 221 FiberCap^{TM}, Foss Instruments, Hilleroed, Denmark).

3.2.4.5 Ash content

The amount of ash in flour was determined using method 923.03 described in the official methods of analysis of the Association of Official Analytical Chemists (*AOAC 2000).* A porcelain crucible was prepared by cleaning, drying, and igniting at 550 ^{o}C in a muffle furnace (Model: WLF-2000, Italy) for three hours, and cooled in a desiccator. The mass of the crucible was measured by analytical balance (M1). About 5g of the sample was weighed into the porcelain crucible (M2). The sample was carbonized first and was placed in the muffle furnace at 550 ^{o}C for about 8 hrs, until free from carbon and turns white. It was removed from the furnace and placed in the desiccators until it cools down to room temperature. Finally, its mass was recorded (M3).

$$\textbf{Ash}\ (\%) = \left(\frac{M2 - M3}{M2 - M1}\right) * \mathbf{100}$$

Where, (M_1) is weight of crucible, (M_2) is weight of sample with crucible, and (M_3) is weight of crucible with ash.

3.2.4.6 Total Digestible carbohydrate (TDC)

The total digestible carbohydrate (TDC) was calculated as the residual weight after subtracting the amounts of protein, fat, fiber, ash, and water from the weight of the sample, as used by USDA (Pehrsson *et al.*, 2015).

3.2.4.7 Rheological properties of dough

Water absorption, dough development time, dough stability, and degree of softening of the flour blends were carried out according to AACC, (2011) using the 300 g bowl of an electronic Farinograph (Toposun, Model: TPS-JMLD, Shanghai, China). The farinograph (Figure 3.2) is a recording measurement of the resistance to deformation of flour/water dough against the mixing action of blades over time and at 65 rpm and 30 ^{o}C. The resistance of the dough is expressed as motor torque, in dimensionless units known as Farinograph or Brabender unit (FU or BU).

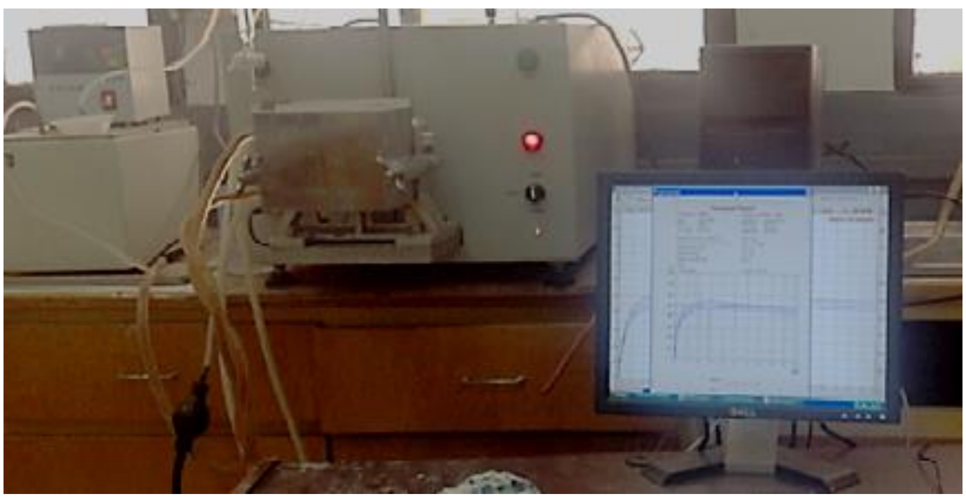

Figure 3. 1. An electronic farinograph used to measure physical dough properties

The dough is developed and further broken down during the test. The farinograph is used to determine the water absorption of the composite flours, the duration of the mixing process, the dough's resistance to over-mixing, and its rheological characteristics. Farinograph water absorption indicates the amount of water to be added to flour in order to bring the consistency of the dough to 500 ± 20 FU (Figure 3.3).

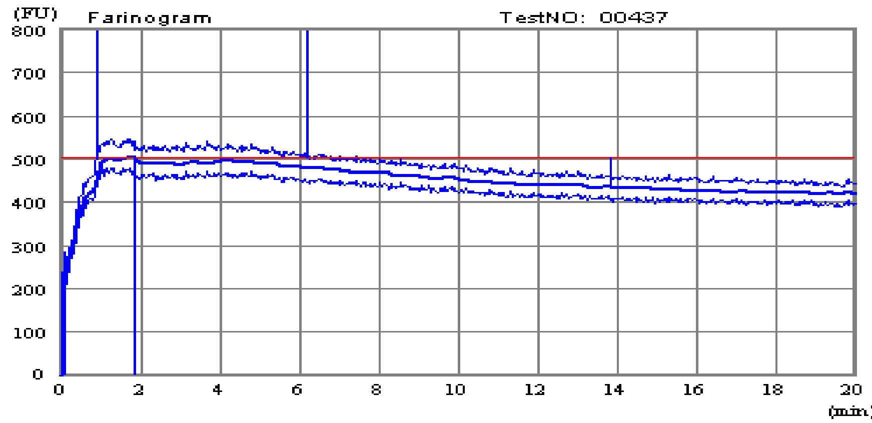

Figure 3. 2 Farinogram showing key data points of farinograph dough analysis

Dough development time gives the time (in minutes) required for the curve to reach the peak counted after the origin (time zero). Provided that the flour particle size is uniform, stronger flours with higher protein content will have a longer development time than weaker flours. Stability is the difference in minutes between the time at which the top of the curve reaches the 500-FU line and the time at which the top of the curve falls below the 500-BU line. It is a measurement of how well flour resists over-mixing. Stronger flours are more stable than weaker flours. Degree of softening measures the difference in FUs between the top of the curve at the optimum and the point on the curve 12 minutes later (Figure 3.3)

3.2.4.8 Bread loaf volume determination

The weight of the bread was determined and the Loaf volume was determined by the rapeseed displacement method as described by Feili *et al.*, (2013). The measurements were performed 1h after the baking method. The rapeseeds were poured into a rectangular box and leveled by removing the excess rapeseeds, followed by measuring its volume (ml) using a measuring cylinder (recorded as V1). Next, the bread was placed in the empty container, which was then filled with rapeseed. The rapeseed was then carefully separated from the bread loaf and its volume was measured (ml) using the measuring cylinder (V2).

$$\text{Loaf volume (ml)} = V1 - V2$$

3.2.4.9 Baking loss

Baking loss is a measure of weight loss exhibited after baking. It is caused by the loss of moisture and other volatile substances occurred during the baking process. It was measured using the formula described in Filipčev *et al*., (2007). Measurement of weight was done an hour after baking.

$$\text{Baking loss (\%)} = \frac{weight\ of\ dough - weight\ of\ bread}{weight\ of\ dough} \text{ x } 100$$

3.2.4.10 Texture profile of bread

Crumb firmness was measured by texture analyzer TA.XT Plus(Model-TA-XT plus Stable Micro Systems, UK). Bread crust cutting force was also measured using the same instrument by installing the TA-42 knife blade. Pre-test, test, and post-test speeds were 1.5, 2, and 10 mm/s, respectively. The peak force to snap the bread was reported as fracture force in (N).

3.2.4.11 Color Analysis of flour and bread crumb

Flour and bread crumb colors were measured using Spectrophotometer (Model CM-600d, Konica Minolta, INC, Japan, 2012). The color difference was represented by the color notation (M. Mburu *et al*., 2012). The spectrophotometer displayed values for L*-value represents the brightness or whiteness), a*-value represents the degree of redness (-a*) or greenness (+a*); and b* b*-value represents the degree of yellowness (-b*) or blueness (+b*).

3.2.4.12 Bread Sensory analysis

Loaves were cooled for 1-2 h at room temperature (25 ^{o}C) in a sealed plastic bag. The bread was then cut into slices using a bread knife. Sensory evaluation was performed using 10 panelists comprising graduate students and staff members of the untrained judges randomly selected (students and staff of Dept of Food Engineering and Applied human nutrition, using a seven-point Hedonic Scale (1 representing “strongly disliked” and 7 representing “like strongly”. Bread made from refined wheat flour (WWF) and those composited with amaranth flours were subjected to sensory evaluation. The sensory evaluation of the bread samples was carried out as described by See *et al*., (2007). The samples were evaluated based on the following characteristics assessed; include color, aroma, taste, texture, and overall acceptability. Commercially bottled water at room temperature was served for the panelists to rinse their mouths between evaluations.

3.2.4.13 Iron (Fe) and Zinc (Zn) determination

Iron and Zink were measured in the individual flours (WWF and AMF) by using Microwave Plasma Atomic Emission Spectrophotometric method (Model 4200 MP-AES, Agilent Technologies, Santa Clara, USA). Calibration curves for each mineral (Fe and Zn) were constructed using known concentrations versus intensities of the emitted radiations at the respective wave lengths (for Fe 371.933 nm and for Zn 213.857 nm). The Fe and Zn concentrations (mg/kg) in the WWF and AMF were then determined based on the calibration curves.

3.3 Data analysis

Triplicate data were analyzed using one-way ANOVA. When ANOVA shows a significant difference among the treatments ($P < 0.05$), treatment means were separated using Tukey's Honest Significance Difference (HSD) test at a 5% level of significance. Data were analyzed using R version 4.2.1 (R, core Team, 2018). Graphs were plotted using Sigma Plot, version 12.5 (*Anonymous, 2013).*

Chapter 4

4. Results and discussion

4.1 Properties of WWF and AMF

Whole white flour: Made from refined wheat, generally high in carbohydrates and low in fiber, protein, and fat. Contains all parts of the wheat grain, including the bran and germ. Contains B vitamins including thiamine, riboflavin, niacin, pantothenic acid. White flour is milled to a fine texture, and it has an extended storage life.

Amaranth flour: Made from ground amaranth grains that are naturally gluten-free, Higher in fiber than traditional wheat flour with a nutty flavor and slightly gritty texture when used alone in baking recipes. Rich source of minerals such as calcium, iron, magnesium, phosphorus as well as other trace minerals and A good source of dietary antioxidants and phytic acid compounds which can help reduce free radical damage associated with health conditions like heart disease and cancer.

4.2 Properties of composite flour

Composite flours are mixtures of different types of flours. They are typically made up of one or more grains, amaranth grain, nuts, seeds, and legumes that have been ground into a fine powder. These combinations can greatly vary depending on the intended use and dietary preferences of the user. Some common properties found in composite flours include: High in protein and fiber content: Composite flours tend to be high in both protein and fiber content due to their diverse ingredients. This provides greater nutritional benefits than standard white flour alone. Healthy fats: Most composite flours contain healthy fats such as those found in nuts and seeds. This can help keep you feeling fuller for longer periods of time and help with weight management. Versatile: Because it is composed of multiple grains, nuts, and legumes, composite flour have a broad range of uses from baking goods to soups and stews, as well as other savory dishes. Prebiotic effect: Many composite flour also contain prebiotic fibers which aid in digestive health by promoting the growth of beneficial bacteria in the gut microbiome.

Gluten-free: Composition flours often do not contain wheat or related grains which makes them naturally gluten free for individuals with intolerances or allergies to gluten products.

4.3 Properties of bread

Bread made from amaranth flour and white wheat flour can vary depending on the ratio of ingredients used, the types of white wheat and amaranth flour used, and the type of recipe. Generally, breads made from amaranth flour can have a sweeter flavor than those made from white wheat flour. Amaranth flour also has higher amounts of protein, fiber, and minerals than white wheat flour which gives breads made with it more nutritional value. Additionally, amaranth flour tends to create a denser crumb structure so breads can have more moist texture as compared to those made with white wheat flour.

In genral Breads vary significantly in their physical and chemical properties, depending on the type of flour used, the amount of leavening agent, the addition of other ingredients such as fats and fruits, and the length of time for which it is baked. Generally, breads are known to be high in carbohydrates, with a moderate content of fiber and protein. Some reconstituted breads may also contain added nutrients such as calcium or iron. Breads usually contain small amounts of fat, but some specialty breads contain more than others. Certain types of breads have a lower glycemic index value than others due to the added fat they contain. Bread can also have different textures depending on the ingredients used to prepare it; these include moist and chewy rustic sourdough loaves or lighter airy sandwich loaf breads. The shelf life of bread can be extended by freezing it soon after baked or purchase, while high-moisture bread should be kept refrigerated in order to prevent molding or spoilage.

4.2 Physicochemical characteristics of WWF, AMF, and composite flours

4.2.1 Proximate composition of WWF and AMF

The proximate composition of white wheat flour (WWF) and AMF are depicted in (Figure 4.1). Amaranth flour (AMF) had significantly higher protein, crude fat, crude fiber, and ash content than WWF (Figure 4.1). However, the TDC value of amaranth flour (AMF) is significantly lower (P < 0.001). than that of WWF at the expense of the other proximate constituents.

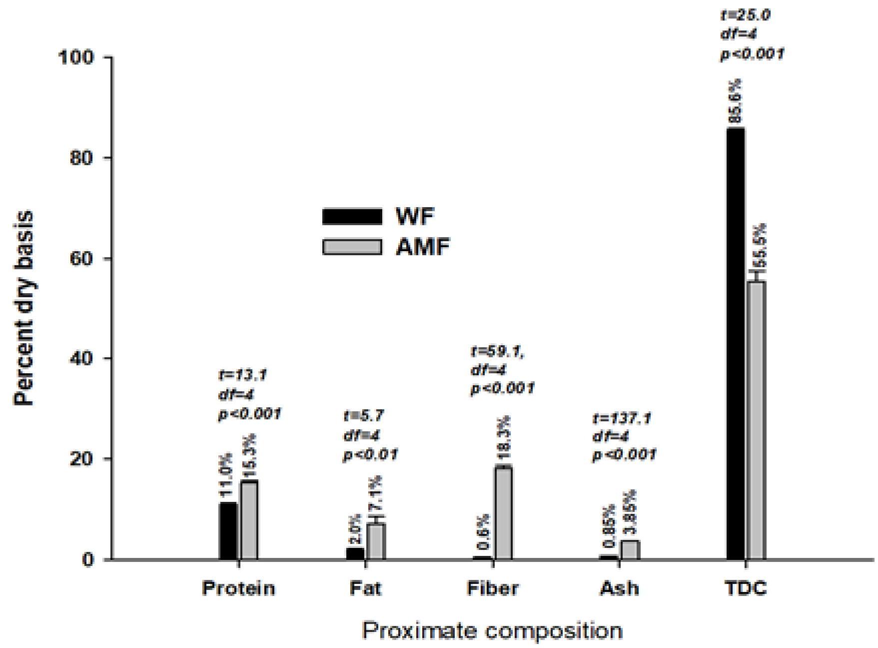

Figure 4. 1 Proximate composition of WWF and AMF

Amaranth flour (AMF) had a significantly higher protein, crude fat, crude fiber, and ash content than WWF (Figure 4.1). However, the TDC value of amaranth flour (AMF) is significantly lower (p<0.001) than that of WWF at the expense of the other proximate constituents. The findings of the proximate composition analysis of AMF in the present research are comparable with that reported by Vitali *et al.*, (2010). The results also agree with Bojórquez *et al*., (2018), except for the nearly three-fold lower fiber (18.3%) and higher carbohydrate contents(55.6%) reported by those authors. The differences in fiber and carbohydrate in the present research and that reported by Bojórquez-Velázquez *et al.*, (2018) could be due to varietal differences. Bojórquez-Velázquez *et al*., (2018) pointed out that amaranth grains with higher carbohydrate content are bigger and with higher thousand kernel weight (TSW). Those authors also concluded that wild species with the smallest size, like in the present research, had the highest protein and lowest carbohydrate contents.

(Adhikary et al., 2020) also reported that dark-colored *Amaranthus* grains have a much higher fiber content (16%). Moreover, Serna-Saldivar, (2022) indicated that the palecolored amaranthus grains contain about 8% fiber, but the darker ones have nearly twice as much fiber (18%). The moisture contents of WWF and AMF were 9.55±0.13 and 7.09±0.35, respectively.

4.2.2 Proximate composition of composite flours

The incorporations of increasing percentage of amaranth flour (AMF) in the flour formulations progressively and significantly increased protein, crude fat, crude fiber, and ash content than WWF (Table 4.1), with respect to the control sample. Proximate composition of refined wheat-amaranth blended flour presented in (Table 4.1). The protein content increased significantly with the increased levels of incorporation of amaranth flour due to the higher protein content in amaranth flour as these are rich in some essential amino acids than refined wheat flour (Venskutonis & Kraujalis, 2013). The fiber and ash content increased by increasing the amaranth flour as it contains bran which is a rich source of dietary fiber and minerals. However, during milling in refined white wheat flour, bran/germ is removed, and thus, no fat content was found in refined wheat flour. Moreover, fat content increased with the increase in different substitution levels of whole amaranth flour which is similar to some earlier reports (Masciarelli *et al*., 2002, Nasir *et al*., 2020). On the other hand, with the increase in the substitution level of amaranth flour, there was a significant decrease in moisture content as amaranth flour has less moisture content that justifies its suitability for long-term storage without deterioration (Emire & Arega, 2012).

Table 4-1 Proximate composition of composite flour (WWF:AMF)

Blending ratio (BR) (WWF: AMF)	Moisture (%)	Protein (% db)	Fat (% db)	Fiber (% db)	Ash (% db)	TDC (%db)
100:0	9.55±0.13	$11.02{\pm}0.38^{b}$	$2.03{\pm}0.20^{c}$	$0.55{\pm}0.01^{f}$	$0.85{\pm}0.17^{e}$	$85.54{\pm}0.40^{a}$
95:5	9.64±0.25	$11.23{\pm}0.38^{ab}$	$2.29{\pm}0.26^{bc}$	$1.44{\pm}0.10^{e}$	$1.00{\pm}0.23^{e}$	$84.04{\pm}0.47^{ab}$
90:10	9.55±0.18	$11.44{\pm}0.36^{ab}$	$2.54{\pm}0.28^{abc}$	$2.32{\pm}0.09^{d}$	$1.15{\pm}0.28^{d}$	$82.54{\pm}0.55^{bc}$
85:15	9.36±0.38	$11.66{\pm}0.35^{ab}$	$2.78{\pm}0.35^{abc}$	$3.21{\pm}0.09^{c}$	$1.30{\pm}0.35^{c}$	$81.04{\pm}0.62^{cd}$
80:20	9.31±0.12	$11.87{\pm}0.34^{ab}$	$3.05{\pm}0.42^{ab}$	$4.09{\pm}0.10^{b}$	$1.45{\pm}0.42^{b}$	$79.53{\pm}0.70^{de}$
75:25	9.16±0.13	$12.08{\pm}0.34^{a}$	$3.30{\pm}0.49^{a}$	$4.97{\pm}0.12^{a}$	$1.60{\pm}0.49^{a}$	$78.03{\pm}0.78^{e}$
$F_{5,12}$	**2.05**	**3.8**	**5.84**	**794.7**	**875.9**	**65.57**
***P*-value**	**0.144**	**0.028**	**<0.01**	**<0.001**	**<0.001**	**<0.001**

Values are expressed as means of triplicate samples ± standard deviation (n =3). Values with the same superscripts in a column are statistically similar while values with different superscripts are significantly different (p ≤ 0.05). The moisture content of WWF is 9.55±0.13, and that of AMF is 7.09±0.35.

4.2.3 Iron and Zinc contents of WWF and AMF

The iron and zinc (contents of AMF and WWF are shown in (Figure 4.2). Both iron (137.7mg/kg) and zinc contents (9.8mg/kg) in AMF are significantly higher p< 0.0001 than those in WWF (Fe: t-value = 77.9, df= 4, *P*<0.0001; Zn: t-value = 32.6, df = 4, *P*<0.00001). The Fe and Zn contents of AMF in the present study agree with those reported by (Marcel *et al*., 2021). The Fe and Zn contents of AMF reported by Marcel *et al.*, (2021) were 130 mg/kg and 48 mg/kg, respectively. However, the AMF reported by Castro - Alba *et al*., (2019) has a lower level of Fe (70 mg/kg) than that reported in the present study. The differences might be due to varietal or environmental origin.

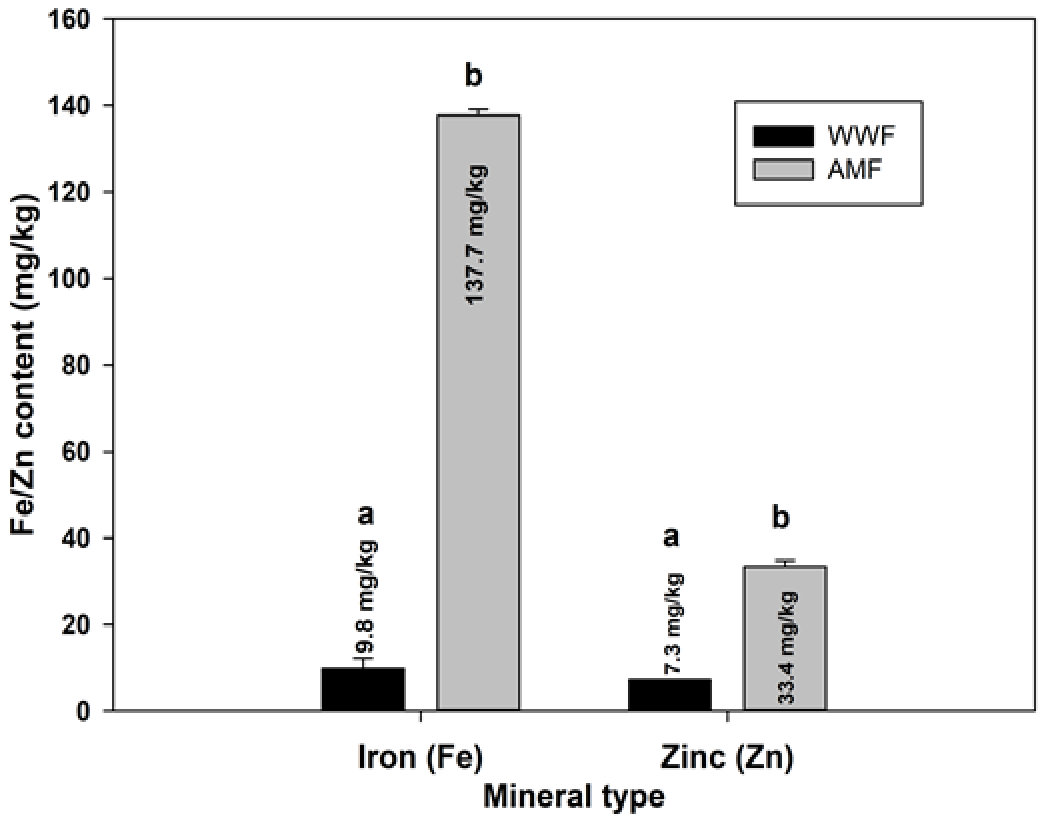

Figure 4. 2 Iron and zinc contents of WWF and AMF (mg/kg)

Bars in each mineral with different letters are significantly different.

Iron and zinc contents of wheat flour are generally lower than amaranths flour. The inherently low levels of Fe and Zn are further aggravated by the milling process, as the minerals are concentrated in the germ and bran portions of the wheat kernel. The germ and bran portions of the wheat grains are separated from the starchy endosperm, resulting in WWF with a lower level of Fe and Zn. For instance, a WWF produced from wheat grains had mean concentrations of Fe and Zn of 6.7 mg/kg and 8.4 mg/kg, respectively, compared to the mean Fe (28.2 mg/kg) and Zn (28.4 mg/kg) contents in the whole wheat flours. This substantial difference between WWF and AMF shows that AMF can be an excellent candidate to supplement WWF with Fe and Zn contents (Tang *et al.*, 2008). Due to its involvement in multiple cell energy metabolism processes, iron is essential for humans in which its presence is essential, and zinc is important for human metabolic, immunological along with many other biological functions (Lawal *et al.*, 2021). The result of the present study confirmed that amaranth grain flour is a valuable source of minerals and an excellent source of iron and zinc.

The high levels of iron and zinc in the AMF relative to WWF were carried over to the composite flours. The iron ($F_{5,12}$ = 103.9, P<0.00001) and zinc ($F_{5,12}$ = 427.8, P<0.00001) contents of the composite flours significantly increased as the level of AMF increased (Figure 4.3). Foods made of such composite flours could make a significant contribution to addressing iron and zinc deficiencies, two of the most deficient micronutrients in most of Ethiopian dishes. For instance, the daily requirement of 8.7 mg iron by adult men could be met by consuming foods made of 272 grams (db) of the WWF/ AMF composite flour of 85:15. Consumption of 463 grams (db) of the above-mentioned composite flour by adult women could meet their daily iron requirement of 14.8 mg. The adult men and women, if they depend on WWF alone, their daily consumption shall be 806 and 1000 grams (db) of WWF, respectively.

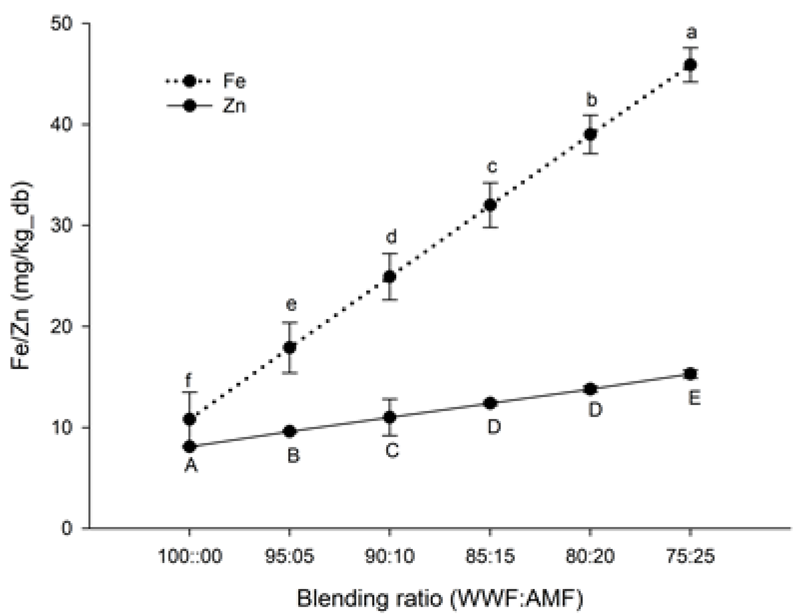

Figure 4. 3 Iron and zinc contents in composite flours

4.2.4 Color of white wheat flour (WWF) and *Amaranthus* flour (AMF)

The result of the color of the two individual flours (WWF and AMF) is depicted in (Figure 4.4). The WWF has a significantly higher L* value (t = 190, df = 4, P < 0.0001) and b* value (t = 10.8, df = 4, P < 0.001) than those of AMF. However, AMF has a significantly higher a* value than the a* value of WWF($t = 73.6$, $df = 4$, $P < 0.0001$).

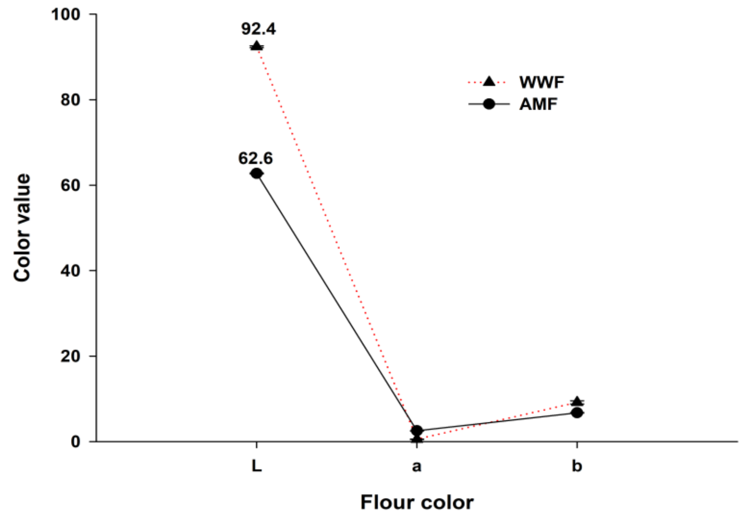

Figure 4.4 Mean ± SD of color (L, a, and b values) of WWF and AMF

The higher WWF L* value indicated the whiteness (paleness) of the flour than the AMF. This difference is due to the white color of the wheat endosperm, which is separated during the wheat milling process, from the grain's darker exterior and germ. The Amaranthus grain, on the other hand, was dark in color, and the amaranth flour (AMF) was whole-grain flour containing the darker outer part of the grain. The color measurements of the composite flour substituted with different levels of amaranth flour (WWF: AMF) are presented in (Figure 4.5). The results obtained for color analysis of WWF (refined wheat flour or (control) and different amaranth flour substitution levels showed that there was a decrease in lightness (L ∗) value and an increase in redness and yellowness (a ∗ and b ∗) values with the increase in the percentage of amaranth flour (AMF).

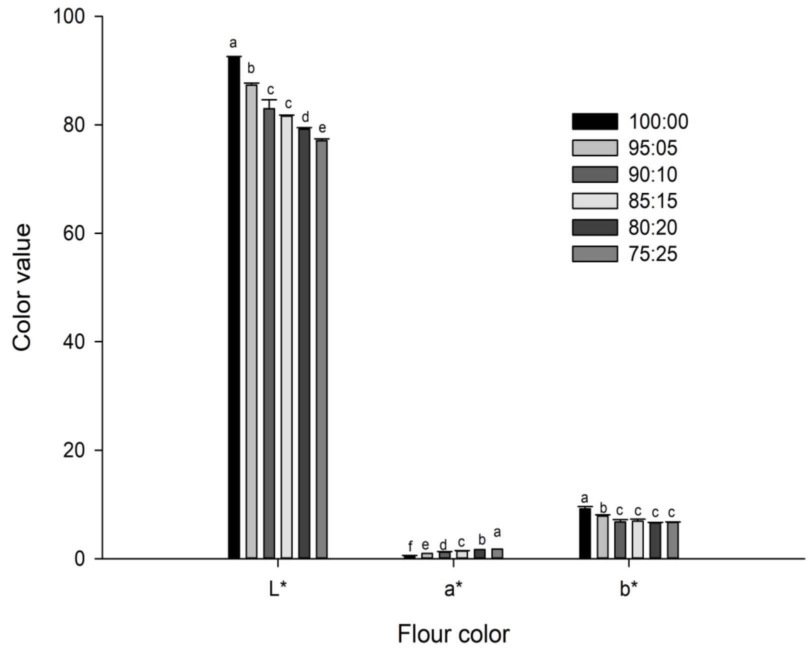

Figure 4. 5 Color analysis of refined wheat and amaranth composite flour

The L* value of the composite flour was significantly affected by the blending ratio($F_{5,\,12}$ = 189.7, P<0.00001). The paleness of the WWF: AMF composite flours, as explained by the magnitude of the L* value, depends on the level of AMF in it (Figure 4.5). Similar findings were noted by Sanz-Penella *et al*., (2013).

4.2.5 Farinograph characteristics

The farinograph properties of the WWF/AMF composite are shown in (Table 4.2). Water absorption and the degree of softening of the dough were significantly affected by the blending ratio, while development time and dough stability This is because of the compositional difference between AMF and WWF. For example fiber interfers with the gluten structure and weakens the gluten as mixing time increases. Since the fiber in the AMF is insoluble one, its water absorption is lower than the endosperm content of wheat. So water absorption reduces as the AMF increases. Remain the same regardless of the blending ratio (Table 4.2). Water absorption has shown a decreasing trend as the level of *Amaranthus* flour increases. An increased level of *Amaranthus* in the composite flour means increased fiber content, which usually contributes to higher water absorption (Ayo, 2001). According to Lamothe *et al.*, (2015), the fiber in *Amaranthus* flour is largely insoluble and does not significantly absorb water. A higher level of *Amaranthus* flour (AMF) also resulted in increased degrees of softening. An increased fiber level is known to interfere with gluten-protein interactions, weakening the dough as manifested by the increased degree of softening (Emire & Arega, 2012).

Mlakar *et al*., (2009) observed an increase in the degree of softening with an increase in amaranth flour (AMF) substitution.

Table 4.2 Farinograph values of WWW: AMF composite flour

Blending ratio	Water absorption (ml/100g)	Development time (min)	Dough stability (min)	Degree of softening (FU)
100:00	52.2±0.2[a]	1.3±0.1	7.7±0.6	56.0±2.7[bc]
95:5	51.1±0.3[b]	1.7±0.3	6.5±2.0	53.3±3.8[c]
90:10	49.8±0.5[c]	1.4±0.2	6.0±1.3	68.3±4.7[a]
85:15	48.0±0.4[c]	1.3±0.1	7.4±0.9	68.3±6.0[a]
80:20	49.5±0.2[c]	1.5±0.3	6.7±0.4	68.7±5.1[a]
75:25	49.8±0.2[c]	1.7±0.4	5.8±0.5	67.0±1.0[ab]
$F_{5,12}$	32.4	1.4	1.5	8.2
P-value	<0.001	0.298	0.269	<0.01

Mean ± SD; Mean values in the same column followed by different letters are significantly different (p≤0.05)

4.3 Bread properties

4.3.1 Proximate Composition of bread

Proximate composition of bread made of WWF: AMF composite flour is presented in (Table 4.3). The proximate composition result shows that increasing the AMF level in the composite flour resulted in a significant increase (*P*<0.05) in the proximate composition, except for total digestible carbohydrates (TDC). The increase in the proximate composition of the composite bread is obviously, due to the high level of those proximate components in the AMF compared to the WWF. These results agree with other studies on bread incorporating different types of amaranth (Roa *et al*., 2015**;** Dyner *et al*., 2007).

The proximate analysis of bread prepared with varying levels of refined wheat flour (WWF) replaced with amaranth flour (AMF) is presented in (Table 4.3). Bread that had been supplemented showed a progressive increment in moisture content, from 33.9% to 36.2% with the substitution of amaranth flour (AMF) for refined white wheat flour(WWF), which suggests that amaranth flour's starch granules have a higher capacity to absorption water than refined wheat starch flour (Sanz-Penella et al., 2013). Similar results were observed by (See *et al.*, 2007; Ho *et al*., 2014). The Ash content of bread varied from 2.28% to 2.91%, higher ash content directly affected the bread quality as amaranth flour is high in mineral content. As the level of substitution increased, data in table 4.3 reveals that crude fiber and fat content significantly increased from 0.56% to 5.04% and 2.31% to 4.15%, respectively, as a result of the substitution of amaranth flour, which has higher lipids and dietary fiber (Luis *et al*., 2007; Ayo, 2001). The protein content of bread increased from 13.5% to 16.3% with the increase in the AMF content as it is rich in essential amino acids and contains easily digestible proteins like albumins and globulins which are considered amaranth flour highly nutritious (Venskutonis & Kraujalis, 2013). The increase in moisture was primarily due to the inclusion of more insoluble dietary fiber with the amount of amaranth flour, whereas fiber remained nearly constant without a significant change.

Table 4.3 Proximate composition of bread made of WWF and AMF at different blending ratios

Blending ratio (BR) (WWF: AMF)	Moisture (%)	Protein (%db)	Fat (%db)	Fiber (%db)	Ash (% db)	Digestible carbohydrate (% db)
100:0	33.9±1.9	12.6±0.3^{d}	2.31±0.22^{d}	0.56±0.06^{e}	2.28±0.05^{c}	82.2±0.3^{a}
95:5	34.2±1.0	13.5±0.9^{cd}	3.54±0.14^{bc}	1.45±0.03^{d}	2.54±0.02^{b}	78.9±0.8^{b}
90:10	35.4±0.5	14.1±0.5^{bc}	3.13±0.21^{c}	2.44±0.17^{c}	2.60±0.01^{b}	77.7±0.6^{b}
85:15	35.9±1.8	14.6±0.1^{bc}	3.84±0.12^{ab}	3.17±0.48^{b}	2.63±0.02^{b}	75.8±0.3^{c}
80:20	35.9±1.6	15.3±0.4^{ab}	3.94±0.17^{ab}	3.45±0.24^{b}	2.84±0.04^{a}	74.5±0.5^{c}
75:25	36.2±1.6	16.3±0.4^{a}	4.15±0.34^{a}	5.04±0.16^{a}	2.91±0.06^{a}	71.6±0.2^{d}
$F_{5,12}$	**0.3**	**21.1**	**30.1**	**129.4**	**104.9**	**176.9**
P value	**0.316**	**<0.01**	**<0.01**	**<0.0001**	**<0.0001**	**<0.0001**

Means with the same letter in the same column are not significantly different at p=0.05

4.3.2 Bread Volume

Bread volume is affected by WWF: AMF blending ratio. The bread volume significantly decreased with increasing levels of AMF. The volume of bread made with composite flour showed a slight decrease from 1183.3 to 876.7 (cm^3). Amaranthus, included as a non-glutenous replacement for refined wheat flour, lowers bread volume by reducing the gluten concentration of the mixture (Sharoba *et al*., 2009). Bread volume is affected by the quantity of gluten, which, in turn, influences the amount of carbon dioxide retained in the dough being baked in the oven before the gluten sets. There is a tendency for bread volume to decrease as WWF replacement levels increase, which can be explained by the amaranth grain's lack of gluten protein (Saeid & Ahmed, 2021).

The volume of bread decreased is by a number of variables, including the viscosity of the dough, the ratio of amylose to amylopectin, and the presence of protein aggregation with an increase in temperature during heating (Abdel-Aal & Gallagher, 2009).

Table 4.4 Baking loss and textural properties of bread made of WWF and AMF at diffrenet blending ratios.

Blending ratio	Baking loss (%)*	Bread volume (cm^3)*	Crumb cutting force (N)*	Crust cutting force (N)*	Crumb sponginess(N)*
100:0	11.4 ± 0.3^{a}	1183.3 ± 115.9^{a}	17.581 ± 1.64^{ab}	23.69 ± 2.12^{ab}	3.3 ± 1.0^{d}
95:5	11.2 ± 0.9^{a}	1280.0 ± 26.5^{a}	15.31 ± 1.466^{ab}	18.37 ± 3.27^{b}	4.6 ± 0.8^{cd}
90:10	10.9 ± 1.1^{ab}	1103.3 ± 126.6^{ab}	15.113 ± 1.408^{b}	26.32 ± 3.54^{ab}	4.4 ± 0.4^{cd}
85:15	9.3 ± 0.4^{bc}	1006.7 ± 49.3^{ab}	16.41 ± 2.36^{ab}	23.92 ± 3.01^{ab}	5.4 ± 0.7^{bc}
80:20	9.3 ± 0.3^{bc}	1022.3 ± 46.1^{ab}	19.55 ± 2.71^{ab}	26.92 ± 3.43^{a}	7.8 ± 0.2^{a}
75:25	9.1 ± 0.3^{c}	876.7 ± 196.6^{b}	21.6 ± 3.63^{a}	27.91 ± 2.76^{a}	6.8 ± 0.5^{ab}
$F_{5,12}$	**8.7**	**5**	**3.6**	**3.8**	**19.9**
P value	**<0.001**	**<0.011**	**<0.033**	**<0.027**	**<0.001**

*Means with the same letter in the same column are not significantly differen

4.3.3 Baking loss

The baking loss was significantly affected by the blending ratio (Table 4.4). Baking loss decreased as the level of amaranthus flour increased in the composite bread ($p<0.001$). Baking loss is the relative change in weight before and after baking. The substance responsible for the loss of weight is the loss of moisture. The incorporation of AMF into WWF resulted in lesser water absorption of the dough and lesser loss of water after baking. The fact that AMF is rich in insoluble fiber, which absorbs less water than the control, is believed to cause a relatively lower loss of water and hence lower baking loss. If the fiber in the AMF were soluble, it is expected that more water and higher baking loss would have resulted in incorporating AMF into WWF. Baking loss Percent of baking loss significantly decreased ($p<0.001$) from 11.2% to 9.1%.

4.3.4 Bread Texture Profile

Due to the increase in the content of AMF cutting the crumb and crust of the bread prepared, the bread crumb, and crust cutting forces increased significantly affect when an increase in the level of AMF 5% to 25%, from 17.581 to21.6 and 23.69 to 27.91respectively, (but not significant) shows in Table 4.4. The firmer the bread, the greater the firmness required since the moisture content is low and there is an interaction between the gluten and the fiber material present in the AMF (Gómez-Guillén *et al*., 2002). As the AMF content increases from 5 to 25%, the sponginess (N) of bread crumbs increases (3.3 - 6.8) due to the presence of sticky starch and dough containing gluten, making the dough more elastic and hence a continuous dough spongy structure forms of the bread after baking (Bbosa *et al*., 2020).

The results agree with the report made by Alvarez-Jubete et al., (2010) and Nasir *et al*., (2020), Amaranth male flour contains a higher amount of fat that traps air bubbles and thus creates porosity that increases the elasticity of the samples.

Sensory Evaluation

The results of sensory analysis are represented in (Figure 4.7). Pan bread made from wheat-amaranth blends had a slightly dark cream color which was rated higher than wheat bread. The color and texture of the bread were found to be similar to control and no particular trend was observed with an increasing percentage of AMF. The characteristic nutty aroma and taste of amaranth were observed in the bread made from the blends. Mlakar *et al*., (2010) also reported a nutty flavor in bread incorporated with amaranth.

In this study, the (75%-25%) amaranth bread scored low in color but acceptable all the parameters and overall acceptability. Based on sensory analysis it was found that bread with good sensory acceptability can be made by replacing wheat flour with amaranth up to a level of 25%. Replacement of up to 30% of wheat with amaranth has been found to be acceptable for preparing Iranian bread (Kiskini *et al.*, 2012). In other studies, it has been reported that amaranth-wheat and popped amaranth-wheat composite bread can be made with up to 15% amaranth without affecting the physical and sensory characteristics (Ayo, 2001; Bodroža-Solarov *et al.*, 2008).

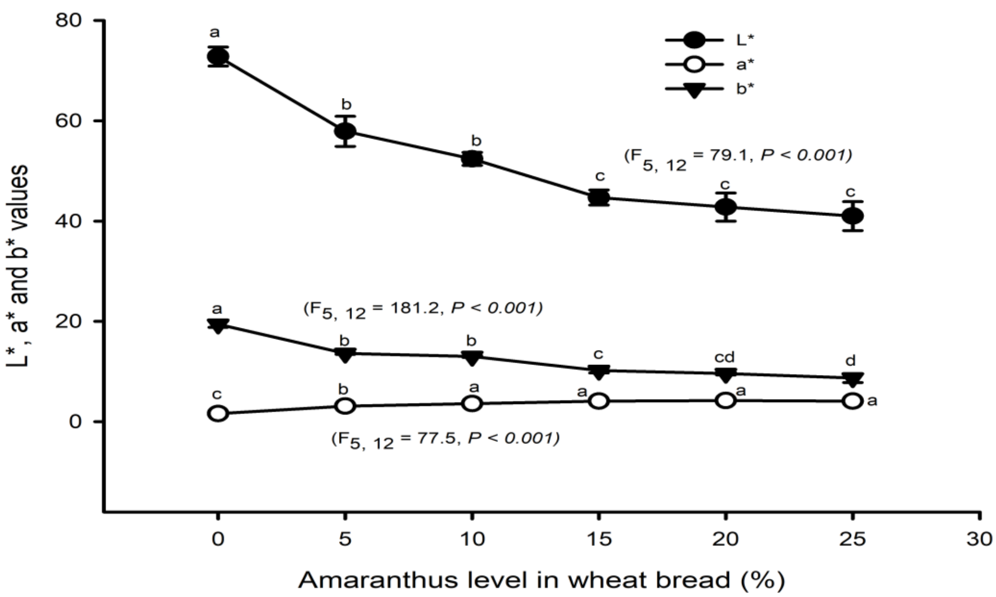

Figure 4. 6 Colour analysis of bread

4.3.5 Bread Color

AMF addition caused a significant change in the crumb color of bread (Figure 4.6). In general, when AMF concentration was raised, the tristimulus color values (L *, a *, and b *) in the crumb were affected. For the bread crumb, lightness decreased while redness and yellowness increased with the increased percentage of amaranth flour. Amaranth flour (AMF) causes significant changes in the crumb color of bread. In general, increasing the AMF concentration affected the tristimulus color values (L*, a*, and b*) in crumb. The bread crumb's lightness (L*) decreased significantly ($p < 0.001$) when the amount of amaranth flour (AMF) was increased in the composite flour bread. This was due to the amaranth flour (AMF) high protein content, which brought about the Maillard browning during baking. The crumb a^* values ranged between positive values (red), increasing significantly ($p < 0.001$) when the proportion of percentage of amaranth flour (AMF) increased. The bread darker crumb color and higher yellowness. These reactions may have been facilitated by the sugar and amino acid composition of AMF (Gibson, 2012). When the level percentage of (AMF) was increased, the bread's yellowness value (b^*) significantly increased ($p < 0.001$). Similarly, Sanz-Penella *et al*., (2013), found that as the amount of AMF in the composite flour increased, the darkness and redness of the bread increased.

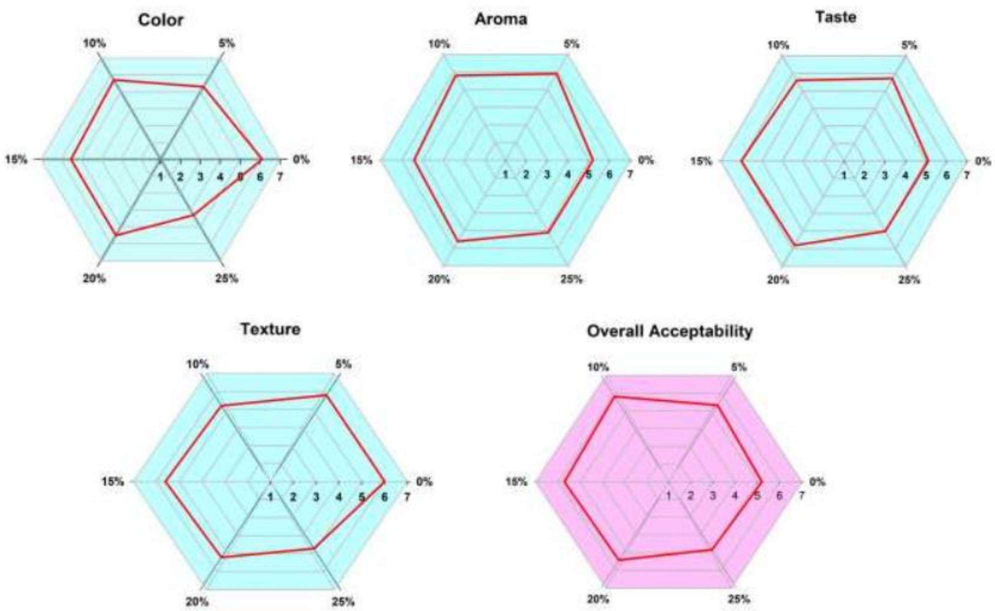

Figure 4. 7 Sensory radar chart showing color, texture, aroma, taste, and overall acceptability of bread substituted with various percentages of AMF.

Figure 4.7: A sensory radar chart showing the color, texture, aroma, flavor, and overall acceptability of bread substituting varying percentages of amaranth (AF) flour. A5: 5% amaranth flour + 95% wheat flour; A10: 10% amaranth flour + 90% wheat flour; A15: 15% amaranth flour + 85% wheat flour; A20: 20% amaranth flour + 80% wheat flour; A25: 25% amaranth flour + 75% wheat flour

Chapter 5

5. Conclusion and Recommendation

5.1. Conclusion

Considering that many citizens in developing countries suffer from food shortages and malnutrition, the application of the underutilized pseudocerial, amaranth in diversified foods is inevitable.

This study found that amaranth flour was a good source of protein, fiber, and fat compared to wheat flour. Amaranth contained more fat (7.1%), fiber (18.3%), and protein (15.3%), than wheat which had 2.0%, 0.6%, and 11.0% fat, fiber, and protein respectively. It also contained 137.7mg/kg and 33.4mg/kg iron, and zinc respectively which again were more than the contents in wheat with 9.8mg/kg, 7.3mg/kg iron, and zinc respectively. Proximate analysis of the amaranth and wheat flour blend showed a significant imporvment in nutritional value as the amount of amaranth in the blend was increased. Besides, the levels of iron and zinc, which are deficient in WWF, are improved by mixing WWF with AMF for bread making. The WWF/AMF composite bread enhanced taste, aroma, and overall acceptability, Color characteristics of the bread were significantly influenced by the addition of amaranth flour.

The physical properties of the amaranth-enriched bread were positively impacted by demonstrating a decrease in bake loss. It can be concluded that *Amaranthus* grain is an economical and nutritious part replacer of wheat, especially nowadays, when food and nutritional insecurity is a notorious issue in the developing world. Intake of products with high substitution of amaranth could cover the protein requirement in adults and could contribute substantially to intake of dietary fiber, Fe, and Zn according to daily recommendations.

5.2 Recommendations

Based on the results obtained in this study and comparison with other researchers& scientific findings, the following recommendations were made: Grain amaranth cultivation should be encouraged at both the household and community levels in Ethiopia to improve the nutritional and healthy living status of the population and contribute to food security. Also the results can be used to determine the amount of water required to make a dough, the amount of flour needed for blending, and to predict processing effects.

It is advised to include grain amaranth in your diet and to use it to make high-protein, high-energy weaning foods for both adults and infants. Although *Amaranthus* is a cultivated superfood in many parts of the world, it still is a weed in Ethiopia. Hence cultivation and variety trials in the country's agricultural research system shall be encouraged. A follow-up study about the antinutritional factors, amino acid profile, fatty acid profile, and digestibility shall be conducted.

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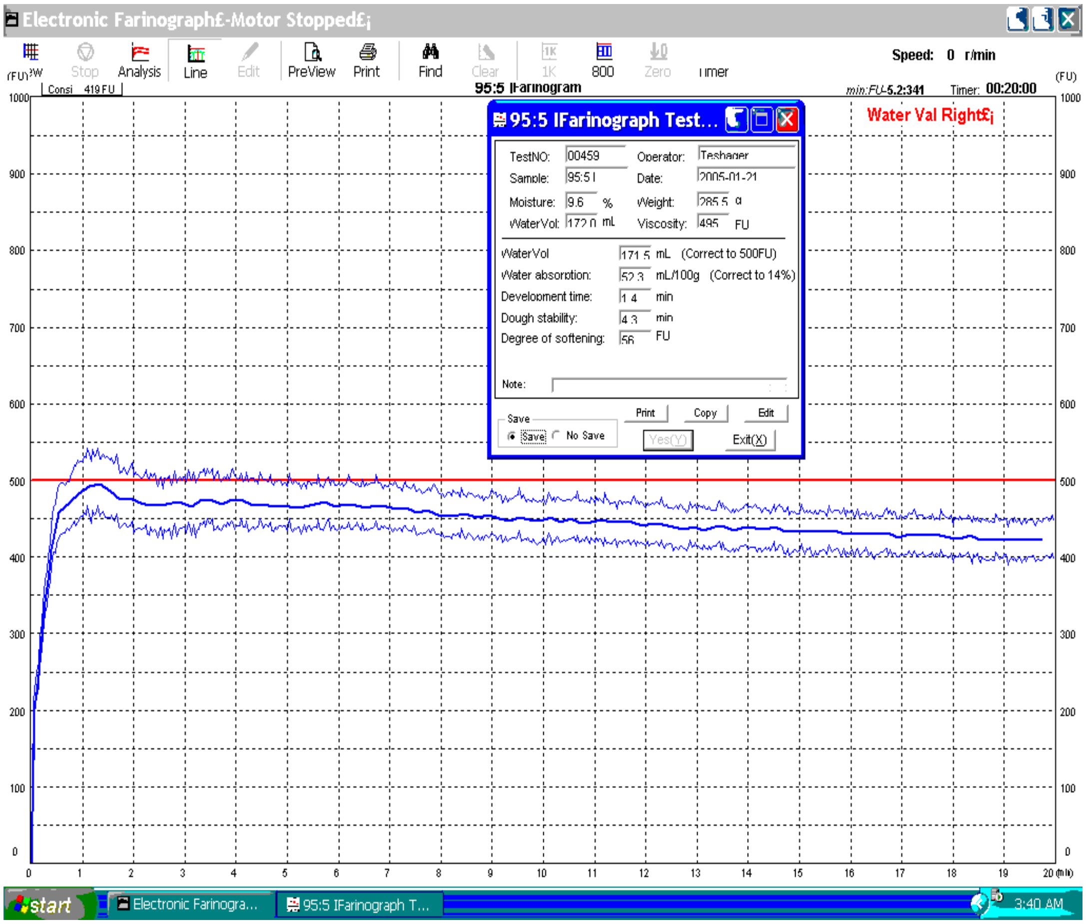
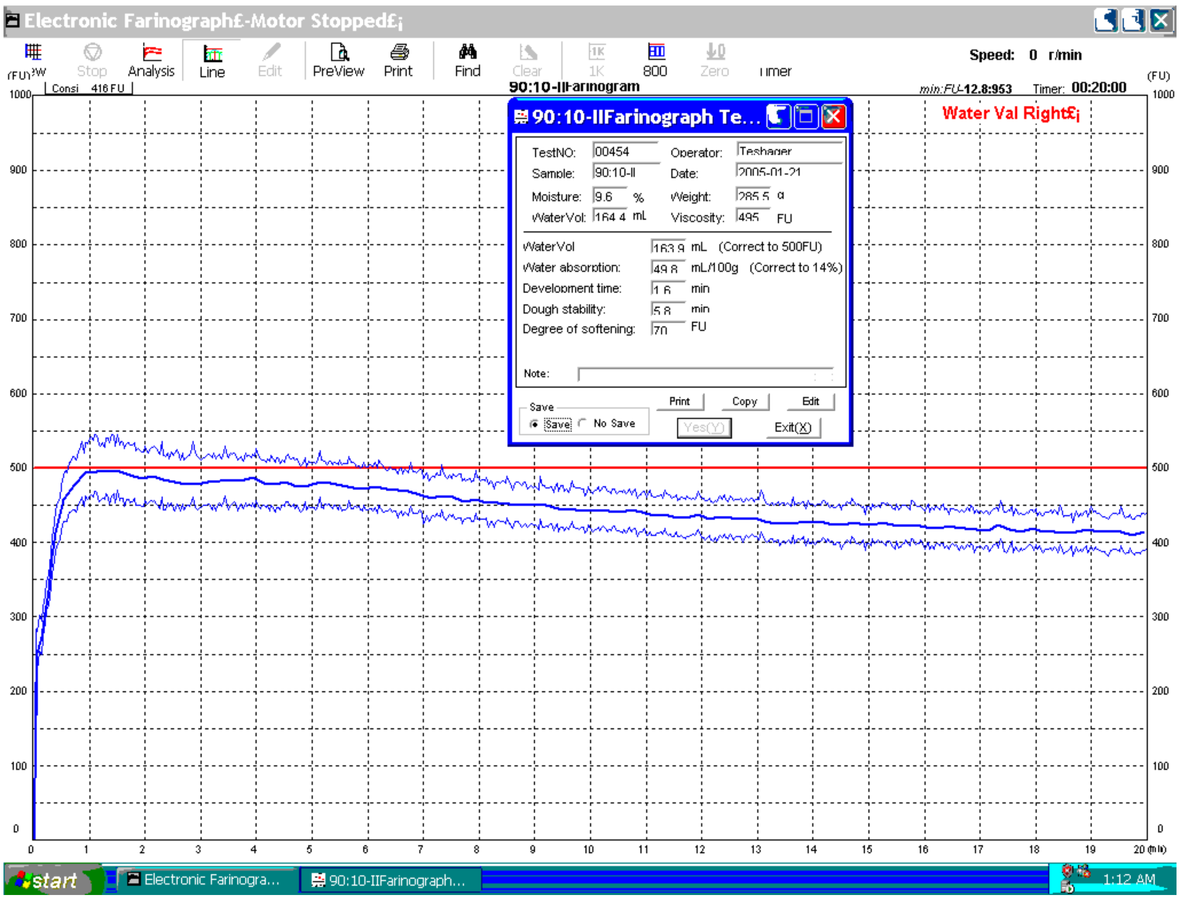
Appendix

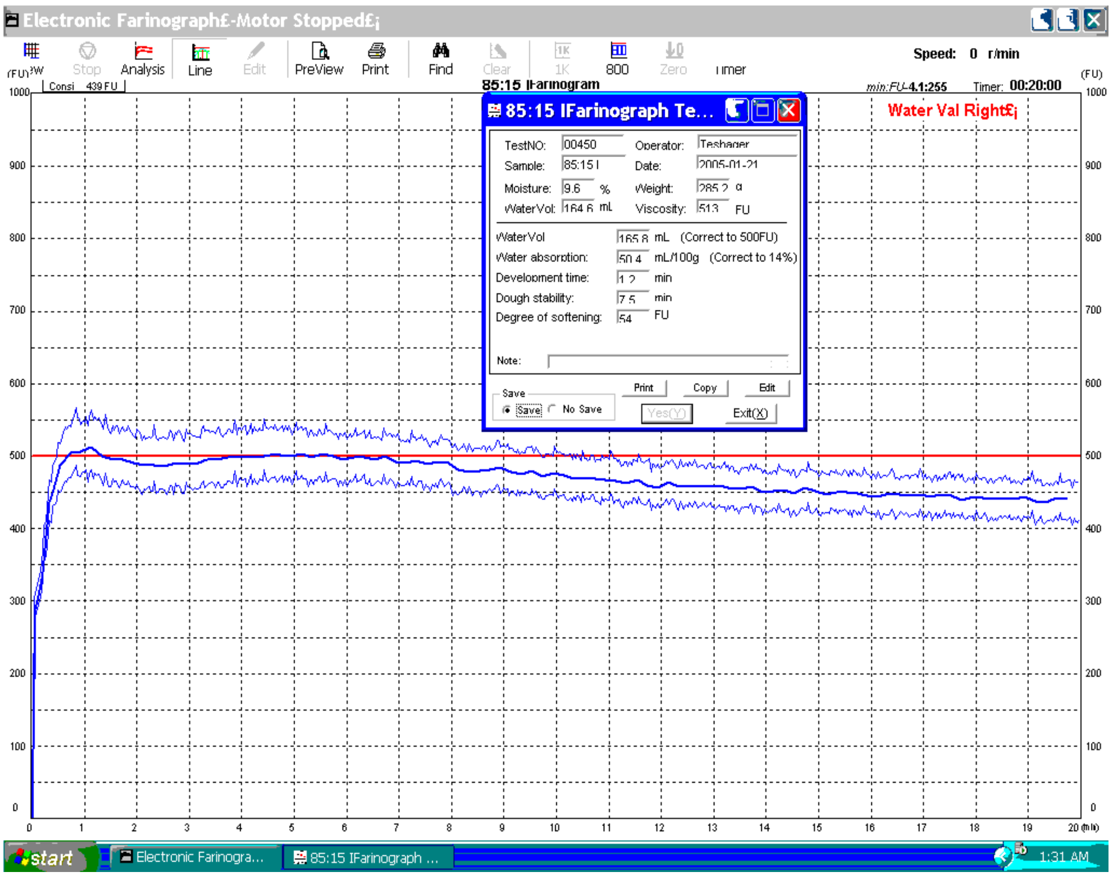
Appendix A. Farinographic characteristics of control and composite flour



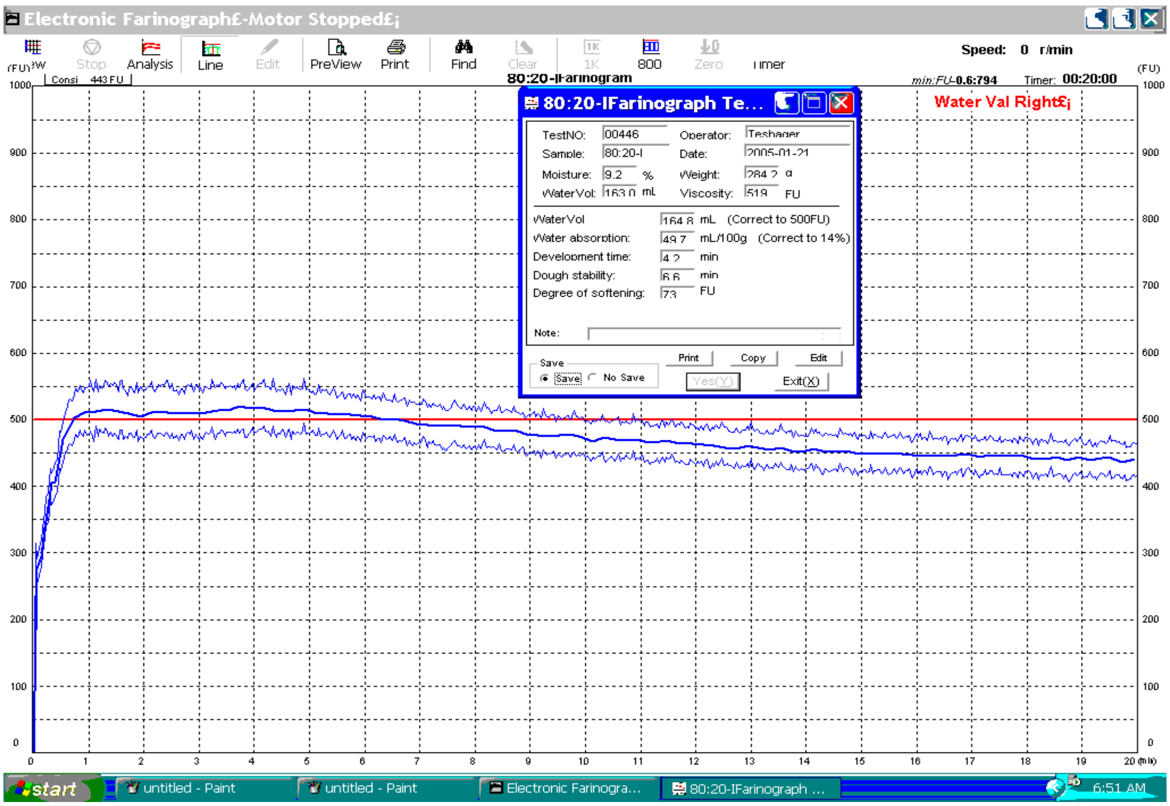
Farinograph of 5% amaranthus flour and 95% refined wheat flour



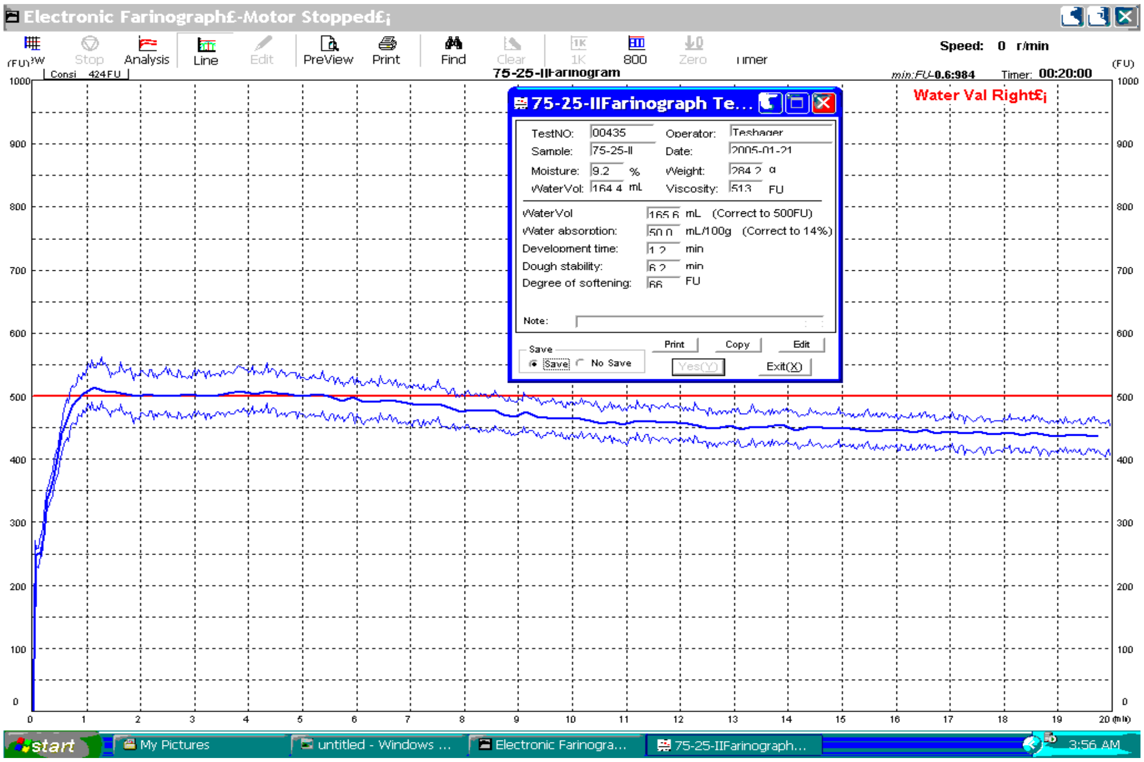
Farinograph of 10% *amaranthus* flour and 90% of refined wheat flour



Farinograph of 15% amaranthus flour and 85% refined wheat flour



Farinograph of 20% amaranthus flour and 80% of refined wheat flour



Frinograph of 25% amaranthus flour and 75% of refined wheat flour

Appendix B. Sensory quality attributes evaluation form

Sensory scorecard for composite flour made of refined wheat and whole Amaranthus flour

1='strong disliked'; 2= 'moderatly disliked' ;3= 'slightly disliked'; 4='Indiffrent'; 5= 'slightly liked '; 6= ' Moderatly liked' and 7= 'Strongly liked'

Put 'X' mark in the cell you preferred

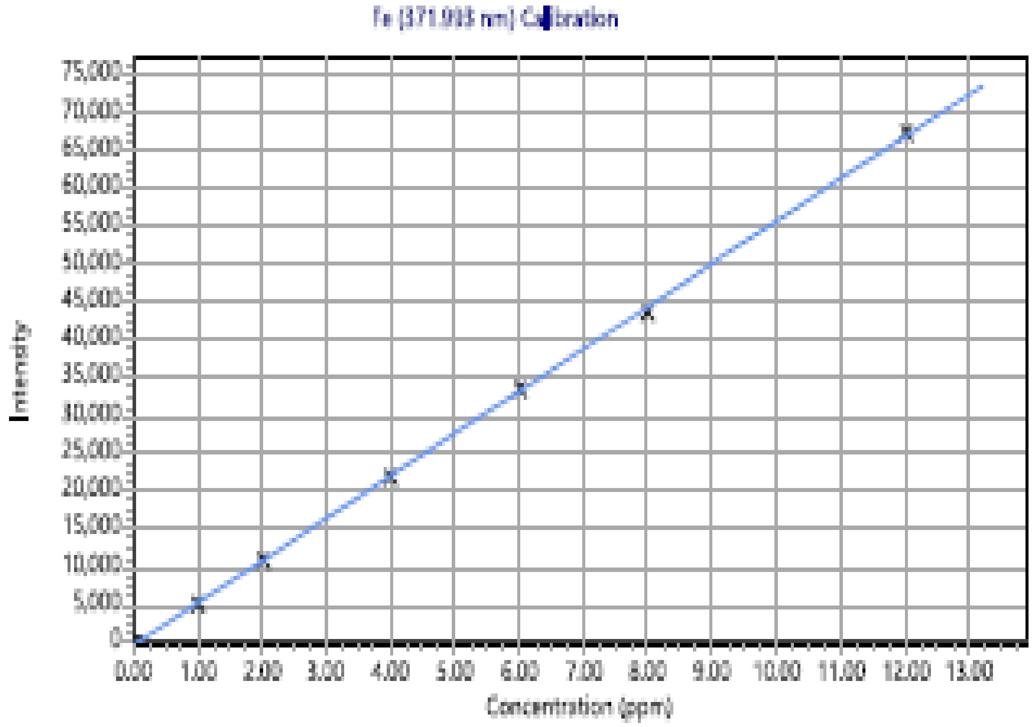
	color							Aroma							Taste							Texure							Overall acceptability						
code	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
962																																			
258																																			
614																																			
168																																			
825																																			
715																																			
976																																			
515																																			
389																																			
199																																			
227																																			
855																																			
493																																			
958																																			
300																																			
786																																			
672																																			

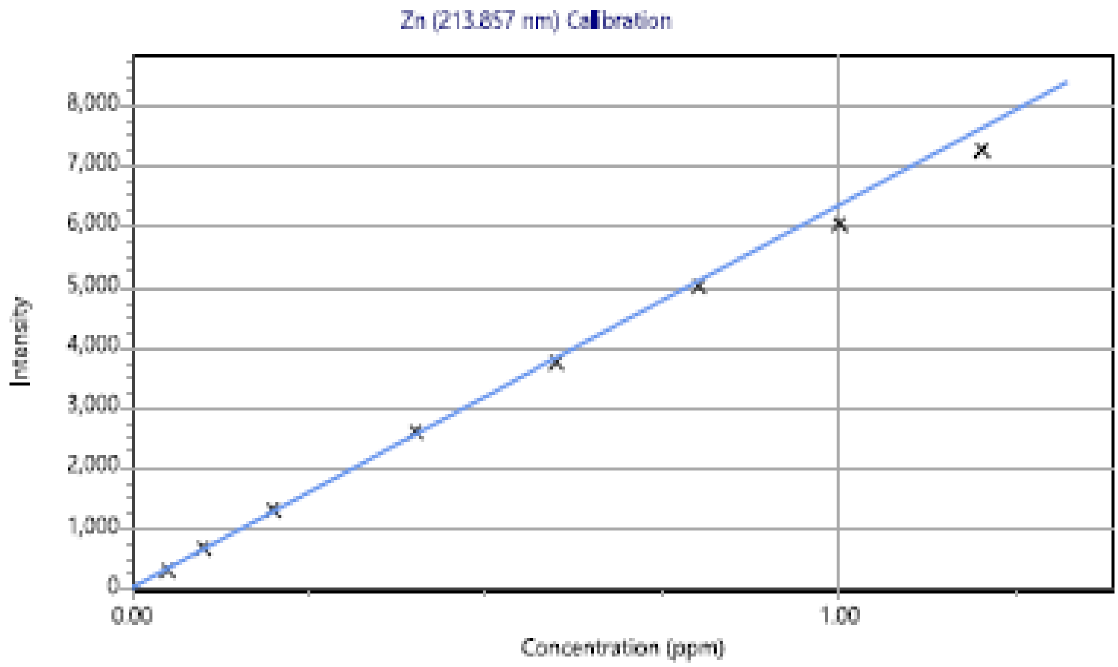
If you get access to amaranthus grain supplemented bread, would you consume the bread with the level of amaranthus you chose?

Yes ☐ **No** ☐

Appendix B. Sensory Evaluation form

Appendix C. Calibration and standard curve for mineral analysis





Appendix C. Linearity standard solution demonstrating

Appendix D. Laboratory pictures were taken during the research work

Harvesting amaranth grain drying and collecting seed.

Working on laboratory miller for preparation of flour and Brabender Farinograph for evaluation of Rheological properties of composite flour (food research lab)

Loaf volume determination and bread crumb and crust color determination

Baking of biscuit samples using Baking Oven (at food processing lab)

Sensory evaluation of prepared bread samples (Food Research Lab)