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Department of Food Science and Technology

Improving Sorghum Injera Quality Using Germinated Cereal as Fermentation Starters

By:

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A Thesis Submitted to the Department of Food Science and Technology in Partial Fulfillment of the Requirements for the Master of Science Degree in Food Processing Technology

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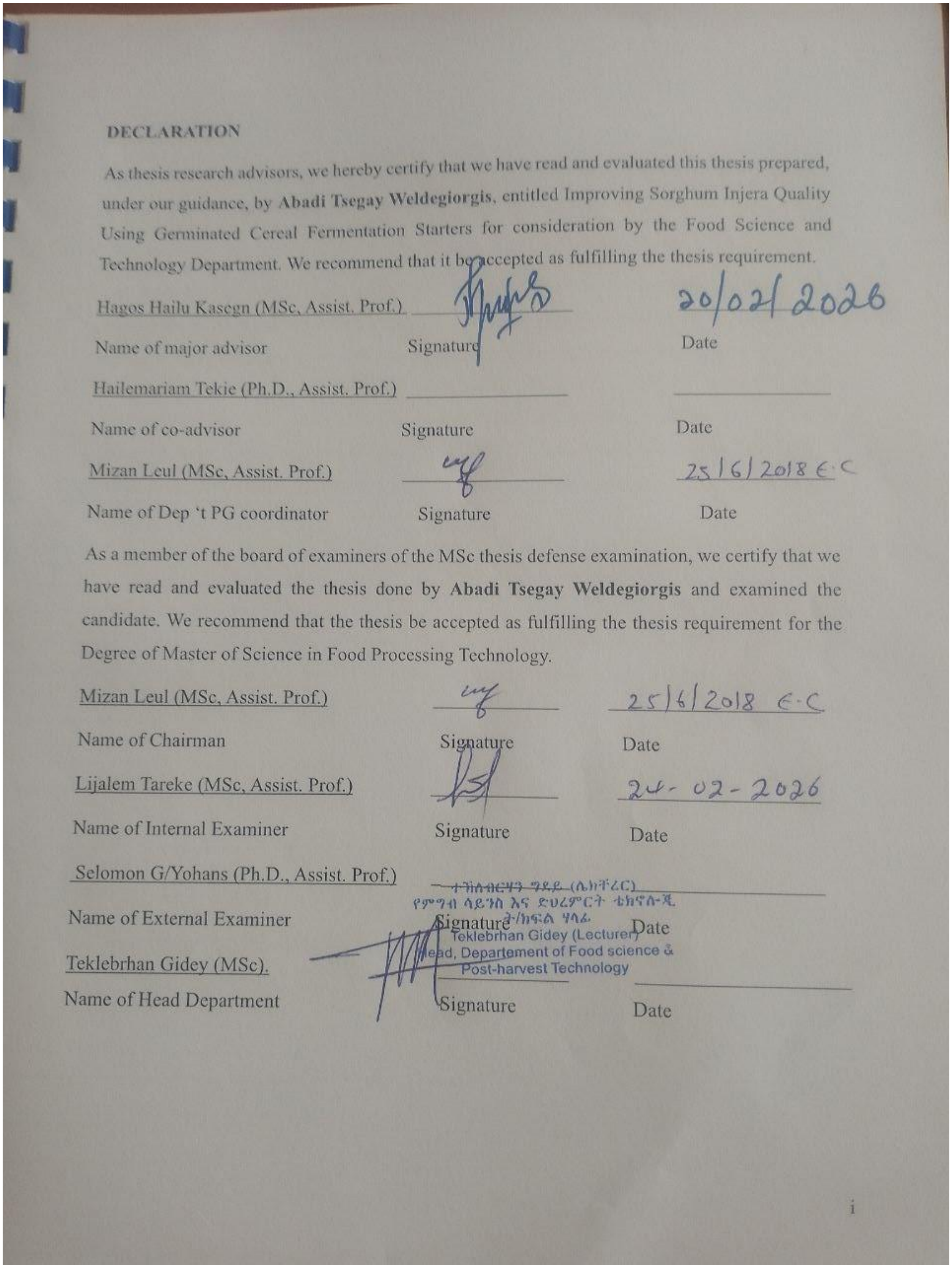

DECLARATION

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by **Abadi Tsegay Weldegiorgis**, entitled Improving Sorghum Injera Quality Using Germinated Cereal Fermentation Starters for consideration by the Food Science and Technology Department. We recommend that it be accepted as fulfilling the thesis requirement.

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As a member of the board of examiners of the MSc thesis defense examination, we certify that we have read and evaluated the thesis done by **Abadi Tsegay Weldegiorgis** and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Food Processing Technology.

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DEDICATION

This work is dedicated to my beloved family for their unwavering affection and support, which have contributed significantly to the success of my study.

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STATEMENT OF THE AUTHOR

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LIST OF ABBRIVATIONS

AAS	Atomic Absorption spectrophotometer
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
ANF	Antinutritional Factor
AOAC	Association of Official Analytical Chemists
BD	Bulk Density
BR	Blending Ratio
CHO	Carbohydrate
CRD	Completely Randomized Design
CT	Cereal type
CV	Coefficient of Variance
EC	Emulsion Capacity
EU	Eye-uniformity
FC	Foaming Capacity
LAB	Lactic acid bacteria
LSD	List Significant Difference
MC	Moisture Content
NIR	Near-infrared transmission spectroscopy
OA	Overall acceptability
SD	Standard Deviation
T&B/S	Top and Bottom Surfaces
WAC	Water Absorption Capacity
Kg	Kilo gram

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ABSTRACT

Injera is a staple fermented food in Ethiopia. It can be prepared from mono or mixed cereals such as teff, sorghum, barley, millets, and others. Sorghum is actually, a common fair cost cereal for injera preparation. But the maximum consumption of sorghum-injera is mostly limited to the local producers and low-income individuals, due to less quality comparing to teff-injera. So, the main objective of this study was improving of sorghum injera quality using germinated cereal flours as starters. Sixteen percent of ungerminated barley, sorghum, and teff starters by weight of dough flour were used as control units; to formulate 8%, 12%, and 20% germinated mix starters from the three cereals each. The study was conducted by using a survey and practical experiment. Functional attributes of the flours and proximal contents of the prepared injeras were investigate using the AOAC official method, and NIR grain analyzer. Mineral-analysis, and sensory acceptability of the injeras were also evaluated using AAS assay, and seven-point hedonic scale methods, respectively. ANOVA and Tukey were applied to analyze all the obtained data. Result of (1.44 and 1.54 g/g); (1.53 and 1.62 g/g); and (1.53 and 1.61 g/g) were scored for OAC of the barley, sorghum, and teff flours at the ungerminated, and 20% germinated mix blending ratios. Values of (54.42 and 56.88%); (54.84 and 55.92%); and (56.96 and 58.28%) were also scored for the EC of the cereal-flours at ungerminated, and 20% germinated mix proportions. But (9.22 and 9.97%); (9.92 and 9.97%) and (9.81 and 10.01%) were scored for the BD of the cereal-flours for the respective proportions. Results of (9.22 and 9.97%); (9.92 and 9.97%); and (9.81 and 10.01%) for protein, and (1.57 and 1.84%); (1.48 and1.78%); and (1.66 and 1.79%) for fiber contents of the prepared injeras were also scored for the ungerminated, and 20% germinated mixes of barley, sorghum, and teff starter-injeras, respectively. Ca, Fe and Zn contents were increased in the teff starter-injeras; Ca and Zn contents also increased in the barley starter-injeras. But Zn content was increased in all the prepared injeras. All the prepared injeras were sensory accepted. Injeras prepared using; ungerminated teff, 12% germinated mix barley, and 20% germinated mix teff starters had the highest sensory acceptance of all the prepared injeras. Therefore, using of such germinated cereal starters could have a great probability to improve sorghum-injera qualities.

***Keywords**: Sorghum-Injera, Quality-improvement, Germinated cereal fermentation starters, physicochemical attributes.*

CHAPTER ONE: INTRODUCTION

Background of the Study

Worldwide, sorghum grain is one of the available food crops under high-stress situations, where other food crops fail (Sirany et al., 2022). This cereal grain is used to make wide range of foods in Africa. Injera, which is a known food in Ethiopia, Eritrea, and Somalia (Godebo et al., 2019), can be prepared from this cereal grain. Injera is a thin, pancake-like, spongy, circular flatbread with a small honeycomb-like structure on the top surface, and gluten-free food (Neela and Fanta, 2020).

It is mainly prepared using cereal flours, fermentation starter, and pure water with no need of extra ingredients or food enhancers. Cereal grains such as: teff, sorghum, barley, maize, wheat, and millets are commonly used for injera preparation in Ethiopia. Although teff-based injera is the most preferrable injera product, the current economic-instability highly influences its consumption for the lower-class economy consumers. Almost, it becomes limited for the upper economy consumers. Hence, improving of the other less costly injera sources, like sorghum-based injera, is mandatory to assure the food security issue of many Ethiopians. In Ethiopia, all age groups of the whole family consume injera at least once a day for the three daily meals (Dasa and Binh, 2020).

Sorghum-injera is consumed next to the teff-injera but, sometimes as a third, next to maize-injera (Bogale, 2022). So, it has a great contribution for the sustainable food system of the nation, by reducing the lack teff-injera demands. Fast-staling during storage time is a common problem of sorghum-injera product, that limit its maximum consumption level. In this study, fermentation starters were developed from germinated and ungerminated cereals (barley, sorghum, and teff) to improve the sorghum-injera quality problem.

The quality improvement concept is projected due to the potential advantages of the starter cereals germination. Germination of cereals can improve certain physical attributes, such as the softness (spongy characteristics) of food products (Wang et al., 2013), bioavailability of minerals (Liu et al., 2022), improve enzymatic activities (protease, amylase, and lipase) and release desirable compounds such as phenols (Guzmán-Ortiz et al., 2019), and reduce Antinutritional factors such tannins and phytates, which combine nutrients (Pająk et al., 2014). Limited research has been conducted or explored on the use of germinated cereal flour starters to improve sorghum injera quality. Therefore, the present study aims to improve sorghum injera quality perceptions, so as to assure food security system of many Ethiopians.

1.2 Statement of the Problem

Although, sorghum-injera is widely consumed in many parts of Ethiopia, its quality is still censured compared to the quality of teff-injera product. As a result, its consumption is limited almost to the poor rural societies. Currently, consumption of teff-injera becomes difficult to consume due to the economic inflation and rapid population growth in Ethiopia. Hence, the supply of teff-injera is not satisfactory for the demand of many Ethiopians. This scarcity of teff-injera consumption may cause a food security problem in the nation. This issue is more complex and a serious problem in the study area (Tigray, northern Ethiopia), due to the recent continued political instabilities and genocidal war. Sorghum-injera has a great potential to minimize the scarcity of teff-injera demands; however, the problem of rapid-friability/fast-staling during storage time (Yetneberk, 2006; Onesmo, 2011) limits its maximum consumption level. This quality problem could be minimized by using of germinated cereal starters. So, the need of sorghum injera quality improvement using germinated cereal starter in Tigray, Ethiopia is timely, unquestionable. In the best knowledge of the author, no study and has been done regarding to this topic.

1.3 Significance of the Study

Comparing, identifying, and recommending the best preparation way of sorghum-injera quality, may assure life of many consumers. So, the study is mainly expected to cope with the current food insecurity problems in the nation, especially in the study area-Tigray. The study could also be a source of scientific information for further investigations in sorghum injera preparations.

1.4 Objectives

1.4.1 General Objective

The main objective of the study was to improve sorghum injera quality using germinated cereal flours as a fermentation starter.

1.4.2. Specific objectives

- ✓ To assess indigenous practices in sorghum-injera preparations.
- ✓ To investigate germination effect of germinated cereal flour as fermentation starters on, functional and chemical profiles of the sorghum-injera.
- ✓ To evaluate the effect of germinated cereal fermentation starters on sensory acceptability of the sorghum-injera product.

CHAPTER TWO: LITERATURE REVIEW

2.1 Definition of Grains

According to Godswill and Nwankwere (2018), grains are hard, small dry seeds with or without attached hull or fruit layers. In a sense, they are durable than other staple foods, like starchy fruits and tubers, harvested for animal or human consumption. Grains are categorized as cereal grains and legume grains. They can grow in different seasons, agro-ecologies, have different production systems, and are distributed across the world to save the lives of millions at different consumption levels. Sorghum [*Sorghum bicolor (L.) Moench],* Barley (Saesa-two raw variety/*Hordeum vulgare*), and Teff (*Eragrostis tef*) are among the common cereal grains used for human consumption. They are also sometimes income (cash) generating crops for the poor producers (farmers) (Abraham, 2015). Those cereals are naturally nutrient-abundant and used in different forms of foods, like injera, the most stable food item in Ethiopia.

2.2 Properties of Cereal-Based Foods

Cereal-based foods are vital stable foods characterized by a diverse range of physical and chemical properties. Their properties largely depend on their compositions and processing methods. Nutritional and chemical properties of cereal-based foods may depend on the macro and micronutrients, dietary fibers, phytochemicals, and their antinutrients. Physical and functional properties of those foods may also be characterized by their texture/mouthfeel, water absorption/solubility, bulk density, porosity and gluten content. Processing methods like: the germination, fermentation, milling, refining and cooking could influence all those properties. The physicochemical, thermal, and rheological properties, such the gelatinization, retrogradation, paste, viscosity, gelation, and α-amylase digestibility properties of cereal-based foods, may be determined by their amylose and amylopectin starch structures (Sang et al., 2008). Especially the branched chains of amylopectin are highly affected by those properties (Ai, 2013).

2.3 Functional Properties of Cereal -Based foods

Grains have different biochemical compositions, component conformations, and molecular structures; hence, they show a diverse functional properties and characteristics (Alleoni, 2006). Functional properties of foodstuffs like hydration properties (protein solubility, protein dispersibility, water- and fat-holding capacities), surface properties (emulsion and foam), structural and textural properties (viscosity, gelation, and viscoelasticity) enable us to predict the industrial usage and applications of cereal flours, how the finished food looks, tastes, and feels (Awuchi et al., 2019). Thus, these properties modify the general structural quality, nutritional value, and acceptability of food materials.

Variations in functional properties in different flours are attributed to their relative proportions of carbohydrates, protein, and lipids. These expressions may not only depend on the relative proportions of these macronutrients but mainly depend on the relative strength of the hydrophilic and the hydrophobic groups of the starch and protein molecules. The more polar amino acid-proteins have a stronger hydrophilic strength, while the non-polar amino acid-proteins show the reverse (Alleoni, 2006). Processing conditions and environmental factors may also affect the functional properties of final food products. During processing, favorable transformations in form, texture, and taste of foods due to proximal changes may occur. Therefore, functional properties of foods enable the prediction of how the interactions of food components and their structures will affect properties of foods. This indicates how ingredients behave during processing (preparation and cooking), storage, and transport.

2.4 Germination in Cereal Grains

Germination is an environmentally friendly, convenient, and inexpensive biochemical process. It terminates (breaks down) seed dormancy and activates endogenous enzymes such as protease, amylase, and lipase (Guzman-ortiz et al., 2019; Oliveira et al., 2022). Activated enzymes break down complex molecules of proteins, carbohydrates, and lipids into their simpler forms (amino acids, basic sugars, and unsaturated fatty acids) (Benincasa et al., 2019).

2.4.1 Advantage of Germination in Cereal Grains

Germination causes a change in the nutritional profile and morphological structure of grain seeds, ultimately improving the bio-accessibility of nutrients, digestibility, and functional properties of the grains (Allai et al., 2022). Germination of cereals also generates new bioactive compounds such as the polyphenols and flavonoids (Donkor et al., 2012). These compounds are beneficial for their antioxidant activity, lowering the risk of many non-communicable diseases such as cardiac diseases, cancers, have cholesterol-lowering properties, reduce gastrointestinal disorders, diabetes, neuro-related health problems, and obesity (Chu et al., 2020). Laetitia et al. (2005) also reported that germination of sorghum improves the level of water-soluble proteins like lysine, methionine, soluble sugar, diastase activity, and the availability of iron, calcium, and phosphorus.

2.4.1.1 Physical Characteristics of Germinated Cereal-Based Foods

a. Effect of Germination on Foods-Texture

Softness of food may be dependent on the network interaction bonds of food molecules, like the starch–protein network bonds. These networks could be modified due to the germination process. The weaker interaction bonds of starch granules and protein network molecules cause an improvement in the swelling capacity of starch and the water absorption capacity of protein molecules (Ding et al., 2019). These capacities also increase foaming and emulsifying activities in germinated foods (Millar et al., 2019). Activated enzymes also increase fermentable sugars in foods of germinated cereals. These sugars can also increase the gas (CO_2) formation during dough fermentation (Motahar et al., 2021) and increase their spongy character (Wang et al., 2013). This character is an indicator of quality to consumers. α-amylase activity of grains is well-increased through the germination process (Amigo et al., 2021). Effective α-amylase activity is good for starch degradation and gelatinization of food items. The sum-up of these effects could improve the texture profile of germinated cereal-based foods.

b. Germination Effect on Foods-Taste

The Maillard reaction between the released reduced sugars and amino acids during the baking process of the food sources may influence the taste and color of the foods (Perri et al., 2021). The modified starch molecules during germination may promote sweetness and develop caramel-smelling compounds (Heiniö et al., 2003). Paiva et al. (2022) also indicated that germination promotes the sensory acceptability (appearance, flavor, and taste) of germinated food products. Therefore, germination boosts grain consumption by enhancing their eating quality (nutrients and physicochemical attributes) and overall health.

2.5 Sorghum Grain

Sorghum [*Sorghum bicolor (L.) Moench*] is one of the most promising crops to ensure food security of societies under high-stress situations, where the availability of other food crops has failed (Sirany et al., 2022). Worldwide, sorghum is the fifth most significant cereal after maize, rice, wheat, and barley (Faostat, 2023). This crop is also evidenced as it saves the lives of millions in semi-arid tropical regions of Africa, Asia, and Latin America (Malabadi et al., 2022). Mostly, the survival of many food-insecure societies of the world depends on such starchy staple cereals, like sorghum, wheat, rice, maize, and millet-based foods (Mayer et al., 2008). In Ethiopia, sorghum is also one of the most important staple food crops for a large number of people (Motuma et al., 2016). So, expanding notions of sorghum food systems, their dynamic use, and functionality in food security discourses, such as in Tigray, Ethiopia, is necessary to assure food security concerns.

2.5.1 Properties of Sorghum Grain

Sorghum starches display higher gelatinization temperatures, a higher degree of retrogradation, but slower enzymatic hydrolysis rates than starches from other cereals, such as maize, wheat, and barley (Duodu et al., 2003). These properties may influence the quality of the finished products of these cereals. Sorghum-based foods have a significant level of protein but are lower in digestibility than other cereal proteins. Albumins, globulins, glutelins, prolamins (protease resistant), and kafirins (hydrophobic) are the protein proportions of sorghum grain. The condensed kafirin and prolamin in sorghum storage proteins cause lower protein digestibility and solubility in sorghum-based products than in other cereal-based products (Taylor and Duodu, 2018).

2.5.2 Nutritional Values and Health Benefits of Sorghum-Based foods

2.5.2.1 Nutritional composition

Comparable chemical compositions, like those of the other common cereals, are presented in sorghum grain. It is reported to be a significant source of many macro nutrients such as protein, starch, fat, and dietary fiber; micro nutrients such the vitamins like the riboflavin, thiamine, and vitamin B6 of the B vitamins, and vitamin E; minerals such as calcium, iron, manganese, magnesium, potassium; and many phytochemicals (Musa et al., 2012). According to Khan et al. (2013), sorghum's starch was reported to be in the range of 55.6–70.0% of dry grain weight depending on the cultivar. Phytochemicals such as the condensed tannin and phytate (phytic acid) in sorghum may affect the human digestive system as well as the availability of some nutrients (Adhikari, 2024). In general, some common compositions of sorghum-based foods are presented in the following table (Table 2.1).

Table 2. 1 Nutritional compositions of sorghum-Based products

Constituents	Range (%)
Protein	6.2 - 14.9
Starch (CHO)	54.6–85.2
Crude fiber	1.4–26.1
Fat	1.3–10.5
Ash	0.9–4.2
Calcium (Ca)	44.57–477.04 (mg/100 g)
Iron (Fe)	13.50–55.13 (mg/100 g)
Zinc (Zn)	0.90 – 20.00 (mg/100 g)
B_1	0.69–0.73(mg/100 g)
B_2	0.12–0.14(mg/100 g)
B_3	2.99–3.01(mg/100 g)

Source: Adebo (2020)

2.5.2.2 Health Benefits of Sorghum-Based Foods

Sorghum-based foods are reported to be good for human health. Researchers, become interested in study of sorghum-based foods in different angle of perspectives. These investigations are mostly concerned with the nutritional compositions, health potentials, and processing ways of sorghum-based foods. Sorghum-based foods are good health promoters as the other common cereal-based foods. These foods are safe for the celiac disease, diabetes, and obesity patients, and are nutritious for healthy individuals (Palavecino et al., 2019). Tannins in sorghum-based foods reduce starch and protein digestibility of the foods (Hargrove et al., 2011). But, this effect may result in low glycemic body's response/good to control the risk of metabolic diseases, such the diabetes (Anunciação et al., 2018). Cancer, cardiovascular diseases, chronic inflammation, and oxidative stress can be suppressed or prevented by consuming of sorghum-based foods. This advantage is due to the array of phenolic compounds in sorghum, which are not common for other cereal grains (Taylor and Duodu, 2023). About 0.5 to 2.0 percents of the total worldwide population is estimated to be affected by celiac disease or wheat allergies (Umwungerimwiza, 2014). So, consumption of sorghum-based foods could be a solution to alleviate this worldwide celiac disease problem.

As mentioned in the previous topic, sorghum is also a good source of dietary fibers, as the other cereals. Dietary fibers have different health benefits for humans. The insoluble dietary fibers, like cellulose, hemicellulose, and lignans have hygroscopic properties, so they may increase the water up taking property and regulate to have a healthy bowel movement, as it may clear the buildup of junk in the intestine (Jha et al., 2017). The soluble dietary fibers (beta-glucans and AX) may also form viscous solutions to slow intestinal transit, delay gastric emptying, and reduce glucose and sterol absorptions by the intestine (Rawat et al., 2023). Therefore, health problems such as constipation and fecal removal system problems, diabetes, obesity, and the development of tumors in the large intestine can be reduced or prevented through the consumption of dietary fibers.

2.6. Sorghum Production and Consumption Trends in Ethiopia

2.6.1 Sorghum Production

Ethiopia produces one million metric tons of sorghum every year (Neela and Fanta, 2020). According to Teshome et al.(2018), approximately one-third of the Ethiopian cereal diet is accounted for the cereal-based foods. Smallholder farmers produce approximately 2.5 tons/ha of sorghum per year in the nation (Getnet et al., 2017). Sorghum is well produced by subsistence farmers, and consumed in most parts of Ethiopia, mainly in Tigray, Harari, Dire Dawa, Amhara, Oromia, Benishangul Gumuz, Somali, and Gambella regional states (Adem and Abera, 2024; Adem et al., 2025).

Table 2. 2 Regional annual consumption of some common cereal foods (average of kg per adult).

Cereals	Tigray	Afar	Amhara	Oromia	Somali	Benishangul	SNNP	Gambella	Harari	Addis Ababa	Dire Daw
Teff	59	39	64	74	7	41	58	51	40	118	36
Wheat	49	92	36	44	101	22	34	48	70	47	99
Maize	16	57	20	50	23	43	75	151	32	1	31
Barley	14	1	8	12	1	1	5	1	1	2	1
Sorghum	52	4	30	19	22	56	5	14	77	0	49

Source: Adem et al. (2025).

2.6.2 Sorghum-Based Foods in Ethiopia

In Ethiopia, most of the produced sorghum is used for human consumption. According to Neela and Fanta (2020), about eighty percent (80%) of the produced sorghum is used to make injera product in the nation. The rest is used for home-brewed beverages (Tella and Kattikala), porridge (Genfo), Bread, Quitta (unleavened bread), Hanza, boiled whole grain (Nifro), roasted grain (Kolo), and animal feed. All these kinds of foods, except injera, are consumed as snacks or as special meals. But injera is normally used every day by the whole family for the three daily meals. Adults can normally serve it as injera and wot, while children are normally fed injera in the form of fitfit (a mixture of broken pieces of injera and wot). So, sorghum-based foods have a great contribution to assure food security of millions in Ethiopia. According to Biswas et al. (2012), food security exists when all people always have physical and economic access to sufficient and nutritious foods that suit their dietary needs and food preferences. Food and nutritional security can vary based on the economic status and resource availability of countries. Therefore, the matter of food insecurity status is mainly solved by checking the availability (productivity), accessibility of nutritious and healthy food distributions.

2.7 Blending of Injera Ingredients and Role of Fermentation Starter

Incorporation of different food materials for the purpose of nutritional and sensory quality improvement is a common practice in a food processing. Combining of different cereals is also likewise a common practice in injera preparation process. Thus, blending of injera starters could improve the nutritional and sensory quality of sorghum-injera product. Blending of cereal flours also significantly affect functional properties of the blended materials (Okafor et al., 2021).

Fermentation starter is an essential element during injera making process. This element initiates and catalyzes the fermentation progress of batter during the process. Consistent quality of fermentation starter could be formulated by "identifying and understanding the right property and proportion of the raw materials from which it is made up of'' (local mothers-survey). This implies as quality of fermentation starter has a considerable effect on the consistent quality determination of any injera product.

Fermentation is one of the most significant techniques in food processing, in which raw materials of foods are intentionally transformed into new, desirable products/forms through microbial action. Numerous changes (modifications of food substrates) could occur during fermentation. Such alterations influence taste, appearance, texture, color, flavor, shelf life, and nutritional properties of derived products. It changes the inherent constituents and metabolites, increase microbial actions, activation of enzymes, decrease in pH, ANF levels, and contaminants in food products (Adebo, 2020).

According to Adebo et al. (2018), lactic acid bacteria (most dominant microorganisms) during injera fermentation, are exploitative competitors, inhibit other spoilage and poison microorganisms through rapid utilization of abundant carbohydrates and accumulation of acetic and lactic acids.

Those bacteria also favor proteins, sugars, vitamins, nucleotides, and fat-rich environments. So, the most common form of sorghum-based fermentation type is lactic acid fermentation, which is mainly carried out by the safe and beneficial LABs. This type of fermentation reduces the risk of fermentation failure and fermentation period, while improve values of end products. So, actions of yeasts and LAB in fermented foods enable to synthesize organic acids, inhibit food poisoning and spoilage bacteria through their antimicrobial, and bactericidal effects (Adebo et al., 2018). Fermented foods are also well in shelf life, and safe for consumption (Tadesse et al., 2019). Pająk et al. (2019) also draw out that fermented foods are nutrient-dense, noted for their flavor, aroma, visual appearance, texture, shelf life, taste, superior in cooking and processing abilities. So, fermented foods are attractive/appealing, nutritious, well digestible and healthy foods.

2.8 Quality Attributes and Nutritional Compositions of Injera

Injera is mostly described in terms of sensory characteristics. But, its physicochemical, microbial, and nutritional qualities require instrumental investigations. A typical injera has a round shape, honeycomb-like "eyes" evenly distributed on the upper surface, soft (supple or rolls easily), pliable, not fluff up and non-sticky upper and bottom surfaces to fingers, smooth, slightly sour taste, and not rusted or brown. While poor quality injera had been reported to crumble easily, have a powdery, dry, or sticky appearance, and have large, unevenly spaced eyes or tiny eyes (Abraha et al., 2013; Mengesha et al., 2022). Nutritional compositions of the major cereals are presented in the below table (table 2.3).

Table 2. 3 Nutritional Composition of major cereals per 100 g.

Nutritional Composition	Sorghum	Teff	Maize	Finger-Millet	Barley	Wheat
Food energy (cal)	193	162	185	172	167	172
Moisture (%)	52	59.8	54.0	56.1	58.0	57.4
Protein (g)	7.1	4.2	5.00	3.8	3.5	5.4
Fat (g)	0.6	0.6	0.7	0.3	0.3	0.9
Fiber (g)	0.9	1.7	0.7	4.0	0.9	0.9
Ash (g)	0.5	1.5	0.7	1.4	0.7	0.7
Calcium (mg)	10	64	27	169	16	28
Iron (mg)	3.5	30.5	2.1	17.3	4.2	3.3

Source: Taylor and Duodu (2023)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experimental Site

The study was conducted in Tigray regional state, northern Ethiopia, at Mekelle University main campus (Food Science and Technology and Hydro-Geochemistry) research LABs.

3.2 Experimental Material Collection

All the raw materials (Sorghum, Teff, and Barley) for injera preparation were purposively collected from farmers of a local market in Esra Adi-Wejerat district (southeast of Tigray). Approximately 12kg of cereal samples (10 kg of sorghum,1kg of teff, and 1kg of barley) were collected. All the collected cereals were famous and well-adapted food grains in the collecting area. Materials were carefully collected to avoid adventitious contamination.

3.3 METHODS

3.3.1 Baseline Data Collection

A baseline survey was conducted purposively at two kebeles (Adikeyh and Tsehafti) and eight kushets/villages (Beri, Ezgadele, Afogedem, Mamet, Ago, Hele, Atsemba, and Bor-emni) in Esra-Addi Wejerat district, southeast of Tigray. These local areas were selected because the three named cereals were grown well in the agrology of the societies. These areas cover both the lowland and midland climate categories. (Ago, Boremni, and Ezgadele) were selected from lowlands, but (Afogedem, Atsemba, Beri, Hele, and Mameat) were selected from the midland agroecological zones of the district. Those communities were selected to gather valid information on the sorghum-based injera preparation and to estimate the ecological effects of the local communities on the properties of the product. Three respondents were selected from each of the communities where the sorghum grain was dominantly grown, while two respondents were selected from the communities where less sorghum was grown compared to the dominant communities.

A total of twenty household respondent mothers (35-72 years old) were selected. The high range of age groups is intentionally selected to gather valid information's from the oldest and youngest generations regarding the sorghum-injera preparations. The respondents were estimated to have basic local knowledge and information regarding sorghum injera preparations. The survey assessment was mainly focused on the traditional methods of sorghum-injera preparation. Especially, in the estimation of the amount of fermentation starter to the weight of the total dough flour ratio. Primary data was collected using structured questionnaires developed to record the basic information. What main ingredients, amounts, qualities, properties and acceptability of materials are used in local sorghum-injera preparation were the main questions involved in the questionnaire of the study.

The investigator focused especially on the amount of fermentation starter to the main dough flour ratio, and what happened to the quality of the final injera? If, the fermentation starter was germinated cereal flour-based. Five (5) kilograms of sorghum flour was assumed to be taken as the main dough flour to estimate the amount of the fermentation starter flour, and a digital balance was used to measure each estimated flour from the respondents. The statement was summarized in the figure below (figure 3.1).

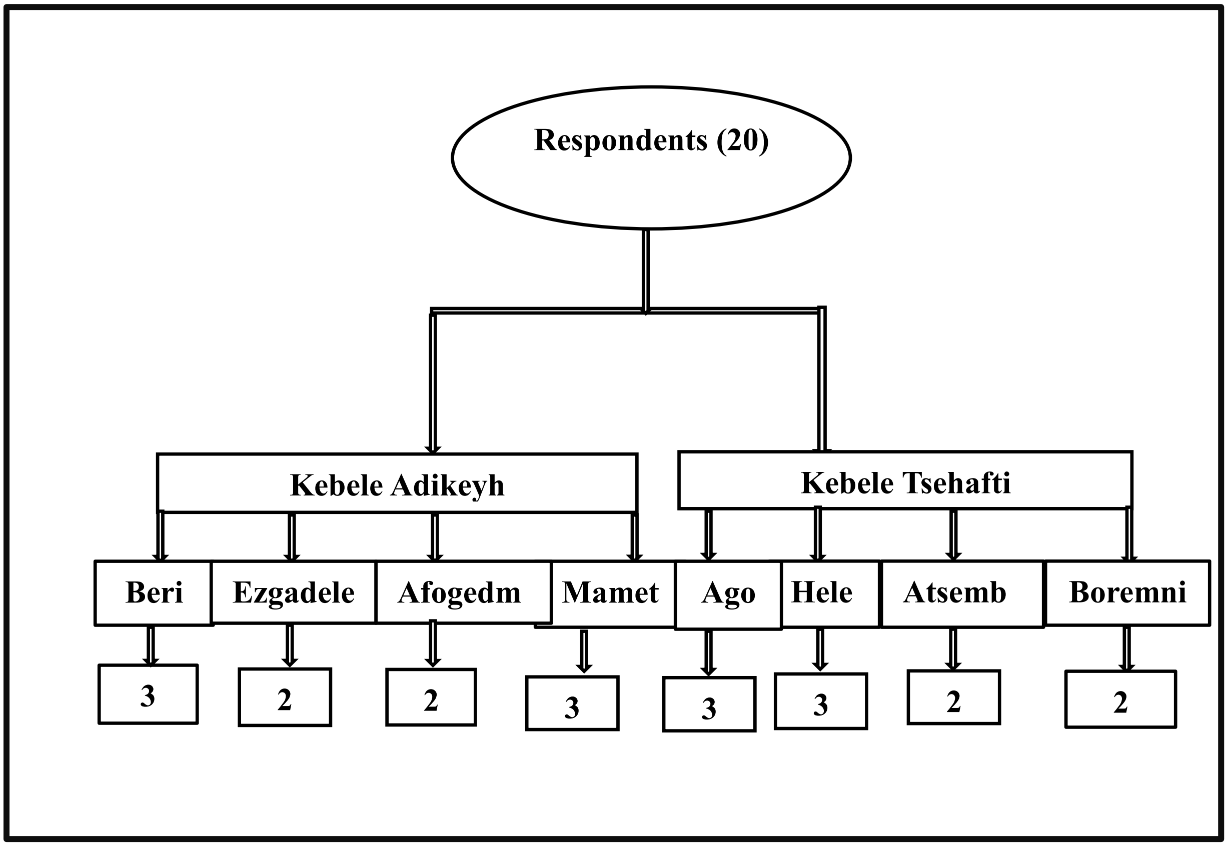


Figure 3.1 Diagrammatic presentation of the survey representative respondents from each kushets/villages of the two kebeles.

3.3.2 Preparation of Sample Cereals

The collected cereals were cleaned, and categorized into germinated and ungerminated cereals. Sorghum grain was served as the main dough flour and as part of the fermentation starter flour. Approximately nine (9) kg of the sorghum (kodom variety) was decorticated manuallly by the traditional method; where, the grain was made slightly moist, and pounded with a timber in a wooden vessel. Sorghum kernel was separated from the bran by winnowing. Finally, the grain for the main dough flour was air-dried in the sun. But, one (1) kg of each cereal type (barley, sorghum, and teff) was transported to the experimental LAB research rooms at Mekelle University.

3.3.3 Germination Potential of the Cereal-Starters

A preliminary test was conducted to determine the germination potentials of the cereals in percent (%) for 72 hours. This test was conducted following the principle of Kaur and Gill (2021); one hundred (100) seed grains were counted from each of the cereal types (barley, sorghum, and teff). Then the counted samples were washed, soaked for 12 hours using potable water, rewashed with the water, rinsed, and drained, and stored until sprouting for the 72 hours. At the end of the 72 germination hours, the germination potentials for the sorghum, barley, and teff grains were found to be 96, 97, and 98 percents, respectively. After the preliminary test, about 250g from each of the cereal types were cleaned, washed, and steeped in a volume of water 3 times the weight of grains (3:1) for a soaking process at room temperature in a steeping vessel. This was also performed according to the method used to the preliminary test of the cereals. The germinated grains were washed twice using distilled water to protect the growth of microorganisms during the germination process, then water was drained off. The washed grains were soaked, drained off, then allowed to germinate at room temperature for 72 hours. Finally, the germinated sample grains were placed in cleaned plates and left to dry in a drying oven (DHG-9055A: Model) for 36 hours, adjusted at 50 °C, which was the appropriate drying temperature and time as checked by pre-trial.

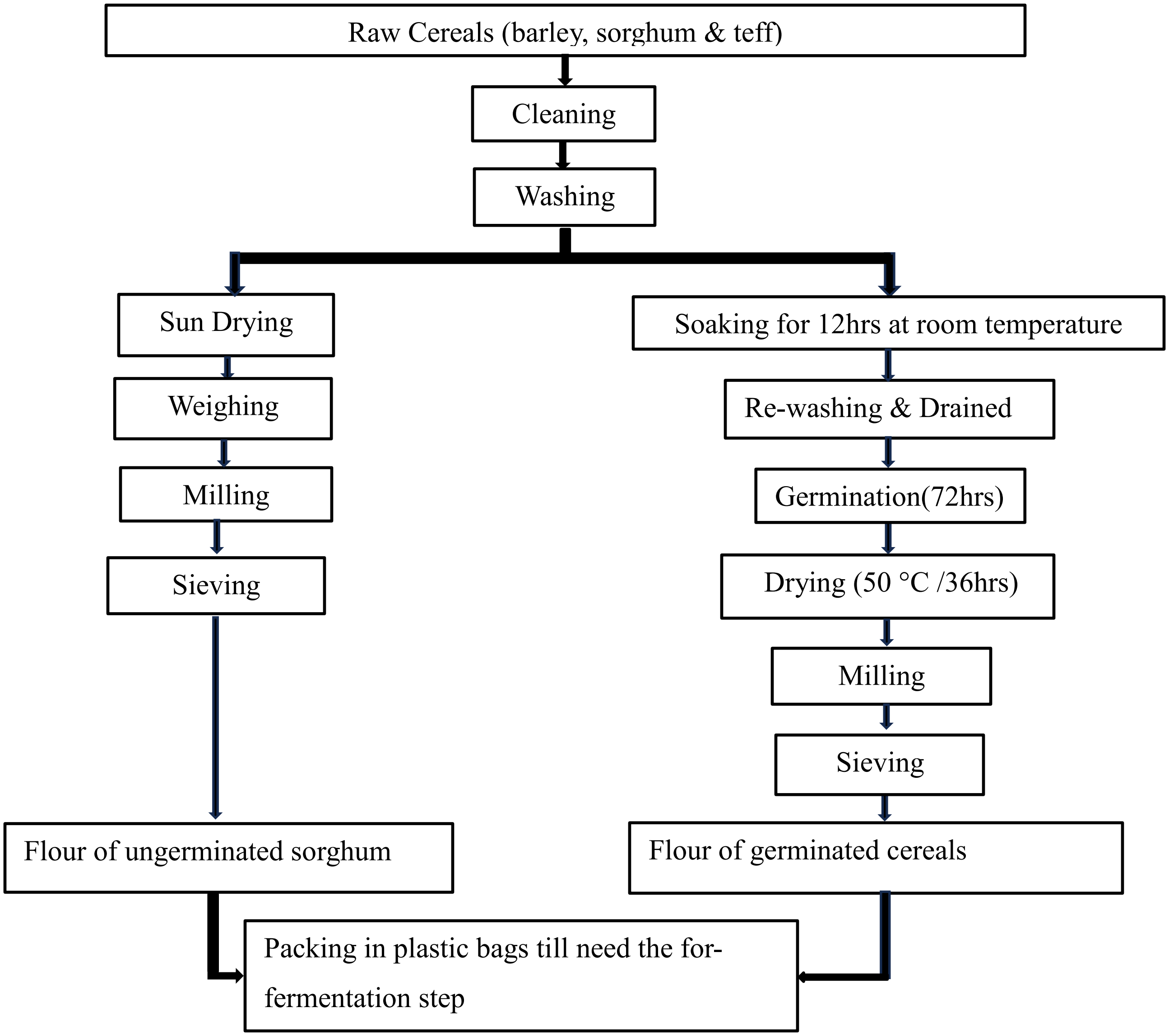
$$\text{Germination percentage (\%)} = \frac{\text{No of germinated grains}}{\text{Total grains}} \times 100$$

A. Germinated Barley **B.** Germinated Sorghum **C.** Germinated Teff

Figure3. 2 Pre-testing of the germination potential of the cereals

3.3.4 Germinated and Ungerminated Flour Preparations

The dried germinated cereals and ungerminated sorghum were ground into normal injera flours independently. Grains were milled using a grinder machine (RRH-200g, Hi-speed multifunctional grinder, China: Model) and sieved with a 0.5 mm aperture size of a laboratory test sieve. The flour was then stored in airtight plastic bags in a safe (dry, clean, and cold) manner until the dough preparation. The packed sample flours were labeled with the type and attribute of the cereal flour.



Source: Siddiqua et al. (2019)

Figure 3. 3 Flow chart for the flour preparations.

3.3.5 Procedures of Injera Preparation

3.3.5.1 Experimental Set up and Design for Fermentation Starter Preparations

Fermentation starter was prepared following to the principles of Ashagrie and Abate (2012), where 16 percent(%) of starter by weight of teff-dough flour was used. The weight of the fermentation starter to main dough flour was also checked during the distribution of the survey questionnaires, using a digital balance. The starter was prepared by combining water and flour of the cereals to the right consistency, and leaving the mixture to ferment for 72 hours based on the response of the (local mothers). Sixteen percent (16%) from each ungerminated (barley, sorghum, and teff) flour was taken as control units to compare all the other formulated starter-based injeras.

The experiment was conducted in a completely randomized design (CRD) method using two (3×4) factorial design for the cereal type and blending ratio; at fixed germination time, fermentation time, and temperature. Experiments were tested at four blending ratios ($R_{0,}$ R1, R2, and R3), for the three cereal types (CT: Barley, Sorghum, and Teff). R_0 stands for the control ones (100% of ungerminated flour starters from each of the three cereal types). While, the R1, R2, and R3 presented for the 8%,12%, and 20% from each of the germinated mix cereal-flour starters. The blending ratios (R1, R2, and R3) indicated that, fermentation starts were formulated by mixing of 8%, germinated flour from each cereal with ungerminated sorghum flour; 12%, germinated flour from each cereal with ungerminated sorghum flour; and 20% germinated flour from each cereal with ungerminated sorghum flour. So, nine (9) blended type, and three (3) control of fermentation starters were prepared to produce a total of twelve (12) sorghum-injeras. The control starter- injeras were used as reference units for all dependent variables of the injera treatments. Analytical runs were assessed using Analysis of variance (ANOVA). The experiment was also triplicated for all the analytical runs. Cereal starters were formulated based on the proportions (R_s) indicated in the experimental table plan (Table 3.1).

Table 3. 2 Experimental layout for the fermentation starter preparation.

Cereal Type	**Blending Ratio (R)%**			
Cereal's Name	$R_{0,}$16%	R1,8%	R2,12%	R3,20%)
S	SR0	SR1	SR2	SR3
B	BR0	BR1	BR2	BR3
T	TR0	TR1	TR2	TR3

Where: S= Sorghum, B= Barley, T= Teff, R= Blending Ratio, R_0 (16%) = 100% Ungerminated cereals starter flour, R1= Blending Ratio of 8% geminated cereal starter mixed flour, R2=Blending Ratio of 12% geminated cereal starter mixed flour, and R3=Blending Ratio of 20% geminated cereal starter mixed flour

3.3.6 Dough Preparation (Fermentation)

Dough was prepared from three common components:(flour, water, and fermentation starter) following to the principles of Mihrete and Bultosa (2017) and local practices with some modifications. Specifically, the dough was prepared from mixtures of ungerminated sorghum flour, pure water, and the prepared fermentation starters in 300g:600ml:48g ratios respectively. The mixture was mixed and kneaded well for about 6 minutes by hand and allowed to ferment for three days at room temperature (25°C). Then, batter was made by adding water to the right consistency and made it ready for the injera preparation.

3.3.7 Injera Baking Process

Sorghum-based injera was baked based on the procedures of Mihrete and Bultosa (2017) injera-studies, as well as the guidance of local mothers. Baking pan (Metad) surface was greased with rapeseed flour using a piece of clean cloth. Injera was then backed around (220°C**)** by pouring about 450ml of batter using a circular motion from the outer perimeter toward the center onto the hot "Metad" surface. Finally, "Metad" was covered with its lid ("Akimbalo") to hold the steam and baked for three minutes (Anberbir et al., 2023). Injeras were finally stored for 2 hours.

3.4 Dried Injera-Flour Preparation

Fresh injera was partially dried in a hot air oven dryer (DHG-9055A, China: Model) by spreading on aluminum foil at 70°C for 24 hours. Dried injera was then powdered using the miller machine, used for milling of the cereals and sieved through a 0.5mm sieve. Finally, nutritional contents were analyzed.

Figure 3. 4 Drying and Milling of the prepared Injeras, using Oven dryer and Maltifunctional Miller Machines.

3.5 Functional property investigations

3.5.1 Water absorption capacity (WAC)

Water absorption capacity expresses the weight of water bound by 1g of dry flour. WAC of the flour samples was determined according to the methods of Beuchat (1977). One (1)g of each flour sample was measured, then mixed with 10ml of distilled water and shaken using a (GEMMY INDUSTRIAL CROP, TIWAN R.O.C) vortex shaker for 1 minute. The content was then centrifuged at 3000rpm for 45min. Finally, water absorption capacity was determined from a 10ml graduated cylinder. The volume of supernatant was calculated and recorded from the graduated cylinder as: **WAC (g/g) =**

$$\frac{\text{Weight of water bound} * 100}{\text{Weight of sample (dry basis)}}$$

Where, Weight of water bound weight of centrifuge tube plus the sediments- weight of centrifuge tube plus sample (dry basis)

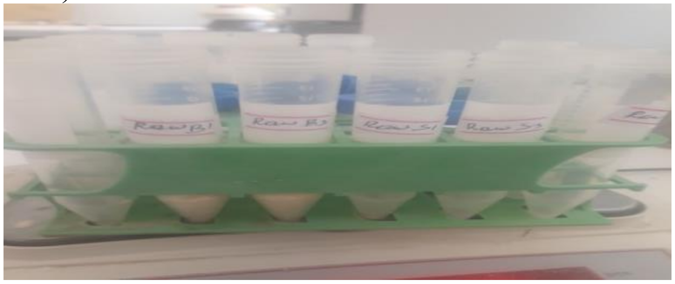


Figure 3. 5 Testing of the Water absorption capacity of the flours

3.5.2 Oil Absorption Capacity (OAC)

OAC of the flours was determined according to the method of Chau and Huang (2003). One (1)g of each sample flour was measured and mixed with 10ml of oil. The mixture was stirred for 30 min at room temperature. After sample centrifugation at 2500rpm for 30min, the supernatant was transferred to a graduated cylinder of 10ml, where the volume was measured. OAC was expressed as the weight of oil bound by 1g of dry flour.

$$\textbf{OAC (g/g)} = \frac{\text{Weight of oil bound}}{\text{Weight of sample (dry basis)}} * 100$$

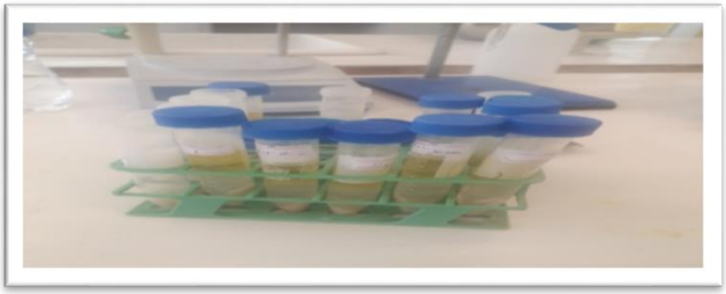


Figure 3. 6 Testing of oil absorption Capacity of the flours

3.5.3 Emulsifying Activity

Emulsifying activity of the flour samples was determined based on the method of Yasumatsu et al. (1972), where two grams(2g) of each sample, 20 ml of distilled water, and 20 ml of olive oil were vortexed (mixed) for 1 minute in a 50 ml test tube. Samples were then centrifuged at 4000rpm for 10 min. Finally, the emulsion activity of each sample was determined, considering the height of the emulsified layer as a percentage of the total height of the test tube.

$$\textbf{EA (\%)} = \frac{\text{Height of emulsion layer}}{\text{Height of the whole layer}} * 100$$

3.5.4 Foaming Capacity

Foam capacity of the flours was determined according to Klunklin and Savage (2018). Two(g) sample flour, and 50ml of distilled water were mixed in 250 mL graduated cylinder at 30 °C; then Shaked vigorously for 5 min using an electrical homogenizer for foam formation. The foam volume after 30 seconds of shaking was expressed as foam capacity of the flours.

$$\textbf{FC (\%)} = \left[\frac{V_O - V_1}{V_O}\right] * 100$$

Figure 3. 7 Shaking of the cereal flours using an electrical homogenizer

3.5.5 Bulk Density

Bulk density of the flour samples was also determined following the method of Asoegwu et al. (2006). Ten (10) grams of the samples were placed in a 25 mL measuring cylinder. The cylinder was tapped until there was no further change in volume. Finally, weight of the measuring cylinder and its contents was taken and recorded. Bulk density was computed as weight per unit volume (g/mL) of the sample.

$$\textbf{BD(g/ml)} = \frac{\text{Weight of flour(g)}}{\text{Volume of flour (mL)}}$$

3.6 Proximate analysis

Moisture, protein, lipid, ash, and crude fiber contents of the prepared sample injeras were determined by scanning of the samples using a near-infrared transmission spectroscopy (NIR) Grain Analyzer and the AOAC (2005) official methods modified by Ben-Gigirey et al. (2012).

3.6.1 Moisture Content (MC)

Moisture content of the samples was determined based on the AOAC (2005) official method. Five (5) g of sample was dried at 50 °C for about 6 hours in (10-D1390/10, D1390/25, D1390/40: model) drying Oven and cooled at room temperature. Moisture content of the samples in percent is expressed and computed as loss in weight per weight of sample times hundred.

$$\text{MC (\%)} = \frac{\text{W2-W1}}{\text{W3}} \times 100$$

Where, W1 Empty test dish mass(g), W2=Initial sample weight(g), W3=mass of content after drying (test dish and dried sample) (g)

3.6.2 Crude Protein

Protein content was determined using the AOAC (2005) method. Half (0.5) g of sample flour was added to the digestion bomb tubes, then four (4) mL (5% of concentrated Nitric acid and 95% of sulfuric acid) acid mixture was added into each test tube and mixed well. Then 3.5 mL of 30% hydrogen peroxide(H_2O_2) was added for a digestion case. Digestion was continued until it was completed. Nitrogen value of the crude protein was then read using (UV-VIS Spectrophotometer: Mode) UV Spectrophotometer.

Protein (%) = 6.25*% Nitrogen; where: 6.25 is the conversion factor for protein from %nitrogen.

3.6.3 Crude Fat

Crude fat was determined by the AOAC (2005) official method using a Soxhlet apparatus. Samples were boiled for 15 min using hexane as a solvent in a boiling flask. Two (2) g of sample flour content in an extraction cylinder and 40 mL of ether were moved into the heating plank. The extraction was running at 50 °C for about 6 hours. The extraction cylinder was placed in an oven dried at 85 °C for about an hour and cooled for 30 minutes. Finally, the extraction cylinder was weighed.

Crude Fat (%) =

$$\frac{\text{Weight of dried ether} \times 100}{\text{Soluble material}}$$

3.6.4 Ash Content

The ash content of the prepared injera samples was determined based on the AOAC (2005) official method. Samples were igniting in a (LEICESTAR LE675T, ECF3, ENGLAND: Model) muffle furnace, at 500°C for 24 hours.

Total Ash (%) =

$$\frac{W_A \times 100}{W_I}$$

Where, W_A = Weight of Ash and W_I = Initial weight of dry matter

3.6.5 Crude Fiber

Crude fiber content of the prepared injeras was analyzed using the official method of AOAC (2005). About 3 g of sample and 200 mL of 1.25% H2SO4 were added to a beaker and boiled for about 30 minutes. Again, about 20 mL of 20% KOH was added and boiled for 30 minutes. The residue was washed and filtered twice using hot distilled water in a crucible. Residue was again washed with 1% of H2SO4, filtered, re-washed with 1% of KOH, and filtered. Finally, the crucible content was dried in (10-D1390/10, D1390/25, D1390/40, ITALY: Model) Laboratory oven dryer for about 2 hours at 130 °C and cooled for about 30 minutes in a desiccator and weighed (W2). The crucible was placed into a muffle furnace for about 12hours at 500 °C, and after cooling, the content was finally weighed (W3).

Fiber content (%) was computed as:

$$\frac{(W3-W1) * 100}{W2}$$

Where, W1=Weight of fresh sample; W2=Weight of crucible with sample after oven drying, and W3=Weight of crucible with the sample after ashing.

3.6.6 Utilizable Carbohydrate (CHO)

Total carbohydrate content was computed as: **CHO (%)** = 100 - % (moisture + protein+ fat +fiber + ash**).**

3.6.7 Total Food Energy

The gross food energy was computed using Atwater's conversion factors modified by Al Hasan et al. (2020). **Energy** (Kcal/100g) = **(**9×Fat%+4×Protein%+4×Carbohydrate%).

3.7 Mineral Analysis

Two grams of sample flours were weighed and digested in a digestion bomb using 20 ml of concentrated HNO3 and 4 ml of 70HClO4 reagents. Then, samples were filtered in to 100ml volumetric flasks using distilled water and filled up to (50ml) of the volumetric flasks. Finally, samples were analyzed for zinc, iron, and calcium contents using the official methods of AOAC (2000). JUN-AIRBENELUX BV (Model: Beckeringhstroat37, NL-3762EV Soest, Holland) Atomic Absorption Spectrophotometer was used to determine these minerals. Mineral concentrations were determined from the absorbance of the samples in the flame atomic absorption spectrometer against the standard readings. Absorbance was determined at 248.3 nm, 422.7 nm, and 213.9 nm for iron, calcium, and zinc, respectively.

3.8 Sensory Evaluation of Sorghum-Injera

Sensory acceptability of the prepared injeras were determined based on the methods of Lim (2011). Seven-point hedonic scale was used for color, taste, top and bottom surfaces, texture, eye uniformity, rollability and overall acceptability evaluations of the prepared injeras. Those parameters were evaluated, by students who regularly consume sorghum-injera in their local society, and food science and postharvest technology department staff members after two hours of 0wbaking. Participants were oriented just before the test how to evaluate; then the injeras were arranged in random order on plates, and served to the panelists with a glass of water for rinsing between the samples.

3.9 Statistical Data Analysis

A replicate data was used and analyzed using Minitab 19 statistical software package and Tukey's multiple comparison tests was used to determine the significance of variation between treatments at 95% confidence level. Results were given as mean ± standard deviation. General liner model analysis of variance (ANOVA) was used to investigate factors effect on the response variables.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Traditional Sorghum-Injera Preparations

As usual, sorghum-injera was prepared using the three main ingredients (sorghum flour, fermentation starter, and water). Mixing of the common ingredients need a special art of wisdom. Blending of the main ingredients and primary fermentation were the first phase activities; while thinning of thick dough to the right consistency, secondary fermentation, and baking processes were the second phase activities for the sorghum-injera making process (Local mothers). All (100%) of the respondents were consented in the mentioned procedures of sorghum injera preparation.

Germinated cereal flours for "fermentation starters" were still not commonly practiced in the local injera preparations (Local mothers). But as concept, using of germinated fermentation starter in sorghum-injera preparation was soundly supported by 98 percent of the respondents. Quantity and quality of fermentation starter play a critical role in any injera preparation. The benchmark weight of starter flour (16 percent) by weight of dough-flour in this study was also cross-checked by the baseline survey, using the household mother's response. Five (5) kg of dough flour was taken to measure the weight of starter flour needed for this assumed dough flour(5kgs**)**. Each respondent was asked to share their experiences on how much weight of starter flour they used for 5 kgs as dough flour for the time.

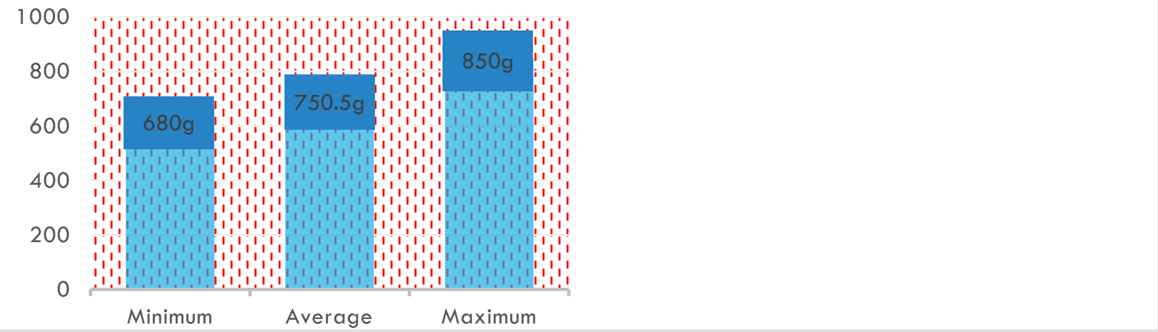


Figure 4. 1 Minimum, average, and maximum survey responses for the starter flour ratio by weight of the main dough flour.

As presented in the (figure 4.1) above the mean weight of the starter flours for the taken **(**5kg) main dough flour were recorded as 680,750.5, and 850 grams as minimum, average, and maximum values, respectively. On average, the 16 percent (benchmark) of 5kg flour became about 800g; while the average value recorded for the survey was 750.5g, for the 5kg main dough flour. These estimations indicated that, as it was possible to use this ranges (680-850g/13.5-17%) as starter ratio, rather than the bench mark (16%).

Effectiveness of germinated cereal starter, during fermentation process was also supported as assumption, 57% of the respondents as compared to the ungerminated ones. This could be related to the facts of natural enzymes in germinated cereals promote fermentation process than in ungerminated ones (Veluppillai et al., 2009). Thus, starters germination could promote the latter fermentation process in a short period of time, in addition to the quality improvements.

4.2 Assessments of Cereal type and Blending Ratio (Germinated) Interaction Effects on Functional Properties of the Sorghum, barley and Teff flours.

Some functional properties of the germinated and ungerminated mixed cereal starter fours were presented in (Table 4.1). Water absorption capacity, oil absorption capacity, emulsion capacity, foaming capacities, and Bulk density attributes of the flours were assessed.

4.2.1 Water Absorption Capacity (WAC)

Though increments were insignificant ($P > 0.05$), water absorption capacity (WAC) of the flours was slightly increased, due to germination of the cereals. Initially, the pure ungerminated teff flour had the highest (1.47 g/g) WAC of the two cereal (barley and sorghum) flours. In contrast, the pure ungerminated barley had the lowest (1.38 g/g) WAC value. After germination of the cereals, WAC of their flours were slightly increased from: (1.38 to 1.46 g/g); (1.43 to 1.49 g/g); (1.47 to 1.51 g/g) for the ungerminated, and 20% germinated mixing ratio of barley, sorghum, and teff flours, respectively (Table 4.1). Structural, and compositional differences of could be the probable causes for such differences in WAC of the cereal flours.

The current WAC values of the sorghum flour were slightly higher compared to the previous values of 1.2 and 1.38 g/g, respectively for WAC of ungerminated, and germinated sorghum flours found by Ocheme et al. (2015). WAC values for the barley flour were slightly lower to the values of 2.19 g/g for barley flour which found by Adem and Abera (2024); and that of the current teff 's WAC was also in line to the ranges of (1.39 -2.04 g/g) for WAC of teff-flours found by Boka et al. (2023).

4.2.2 Oil Absorption Capacity (OAC)

Oil absorption capacity of the flours was significantly affected by the interaction effect of the germination ratio, and cereal-type (barley, sorghum, and teff) ($P<0.05$). OAC was significantly

increased from: (1.44 to 1.54 g/g); (1.53 to 1.62 g/g); and (1.53 to1.61 g/g) for the ungerminated, and 20% germinated mixed flours of the barley, sorghum, and teff cereals, respectively.
Overall, 20% germinated added sorghum-flour showed the highest (1.62g/g) increment in OAC; while the ungerminated barley-flour had the lowest (1.44 g/g) (Table 4.1). According to Wang et al. (2020), OAC is well developed under good rate of protein association with fat contents under the conditions of oil limitations. Gemination by itself may also cause to increases the OAC of the cereal flours. Oil absorption capacity of food materials can be enhanced through the germination process (Adedeji et al., 2014). Large surface area of hydrophobic amino acids in germinated food materials could bind more oil than ungerminated ones (Amanipour et al., 2024). Hence, variations in protein and fiber contents, the strength of non-polar interaction sites to the oil hydrocarbon chain, enhancement in protein to fat rate associations may the probable causes for the OAC increments of the germinated cereal flours. The current OAC values (1.44-1.62g/g) of the flours were less than the value (2.35g/g) observed by Atuna et al. (2022) for OAC of malted sorghum, maize, and millet flours. Incorporation of germinated cereal flours could be better eating qualities (good flavor retainers and increase the mouth feel of consumers).

4.2.3 Emulsion Capacity (EC)

Emulsion capacity of the flours was significantly influenced by the interaction effect of the germination ratio, and cereal-type (barley, sorghum, and teff) ($P<0.05$). The ungerminated teff, sorghum, and barley cereal flours were ordered in descending order based on their EC values. But, after germination those values were raised from:(54.42 to 56.88); (54.84 to 55.92); and (56.96 to 58.28) percents for the ungerminated and the 20% germinated mix barley, sorghum, and teff flours, respectively (Table 4.1). In general, the ungerminated barley-flour showed the least (54.42 percent) value; whereas the 20% germinated-mix teff flour showed the highest (58.28 percent) values in emulsion capacity.

Germination of cereals was reported to improve EC of the respective flours (Sofi et al., 2020). Abd Elmoneim and Bernhardt (2010) had also observed a 33% increment in the emulsion capacity of three-day germinated sorghum flour. Increment stabilized oil droplet at the interface, and reduction of surface tension in germinated foods increase emulsion capacity of flours (Awuchi et al., 2019). According to Iwe et al. (2016), the higher the protein content of the cereal food, the higher the emulsion capacity of the flour. Variations in shapes, dipole neutrality, and hydration power of polar groups of protein molecules may cause variation in emulsion capacity of foods (Albano et al., 2019). Therefore, increment in the area of stabilized oil droplet at the interface, and hydration power of polar groups, reduction of surface tension, protein content and structural shape variations probably cause to increase the emulsion capacity of the germinated cereal flours.

4.2.4 Foaming Capacities (FC)

Foaming capacity of the germinated mix-flours had shown an increment to some extent; however, the overall increment was statistically not significant ($P>0.05$) for all of the subjected cereal flours. The cereals were ordered as barley, sorghum, and teff flours in ascending order for their foaming capacities. Values of (12.97 and 13.41); (13.16 and 13.81); and (13.85 and 14.35) in percents were scored at the ungerminated, and 20% germinated mix of barley, sorghum, and teff flours, respectively.

4.2.5 Bulk Density (BD)

Results of the interaction effect was significant on BD of the flours ($P<0.05$). BD of the flours was significantly reduced by germination ratio, and the cereal type (Table 4.1). Germination process was reported to reduce the bulk density of germinated food flours (Sibian et al., 2017). Average reductions by 4.1, 6.4, and 12.6% in BD of germinated sorghum, maize, and millet flours were observed by Atuna et al. (2022) comparing to their ungerminated samples. Bioactive enzymes might be consumed some energetic parts of the grain’s/cereal’s carbohydrate and protein contents as energy during germination. Hence, reduce BD of the flours. Reduce BD flours would be convenient for easily digestible food formations with small amount of water (Fonmboh et al., 2024). So, they could be important for infant foods formulations with desired energy and nutrients. All result values in the whole study were in means of three replicates ± Standard Deviation.

Table 4. 1 Cereal type and blending ratio interaction effects on functional properties of barley, sorghum and teff flours.

CT*R	WAC(g/g)	OAC(g/g)	EC (%)	FC (%)	BD g/ml
B*R_0	*1.38*±0.01^a	1.44 ±0.01^e	54.42±0.39^f	12.97±0.02^a	0.51±0.01^d
B*R1	*1.41*±0.01^a	1.46 ±0.01^e	56.90±0.03^c	13.22±0.01^a	0.41±0.01^g
B*R2	*1.42*±0.02^a	1.47 ±0.01^e	56.91±0.02^c	13.24±0.01^a	0.41±0.01^g
B*R3	*1.46*±0.01^a	1.54 ±0.02^d	56.88±0.01^c	13.41±0.01^a	0.42±0.01^g
S*R_0	*1.43*±0.01^a	1.53 ±0.01^c	54.84±0.01^e	13.16±0.01^a	0.55 ±0.01^b
S*R1	*1.46±0.01*^{a}	1.6 ±0.02^{ab}	55.91±0.01^d	13.52±0.01^a	0.46±0.01^f
S*R2	*1.48*±0.01^a	1.6 ±0.03^{ab}	55.91±0.01^d	13.58±0.01^a	0.48±0.01^{ef}
S*R3	*1.49*±0.01^a	1.62±0.01^{bc}	55.92±0.01^d	13.81±0.02^a	0.49±0.01^{de}
T*R_0	*1.47*±0.01^a	1.53 ±0.06^d	56.96±0.01^c	13.85±0.01^a	0.62±0.01^a
T*R1	*1.48*±0.01^a	1.56±0.06^{cd}	57.92±0.01^b	14.2±0.01^a	0.52±0.01^{cd}
T*R2	*1.5*±0.01^a	1.58±0.06^{bc}	58.17±0.01^{ab}	14.26±0.02^a	0.54±0.01^{bc}
T*R3	1.51±0.01^a	1.61±0.06^{ab}	58.28±0.01^a	14.35±0.01^a	0.51±0.01^d
CV (%)	3.64	4.01	2.12	3.34	12.37
P- value	0.052	0.001	0.000	0.938	0.000

Values in the same column followed by the same letter are not significantly different at the 0.05. **Where:** CV=Coefficient of variance, SD=Standard Deviation, WAC = Water Absorption Capacity, OAC=Oil Absorption Capacity, EC=Emulsion Capacity, FC=Foaming Capacity, BD=Bulk Density; B*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix barley flour; S*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix sorghum flour; and T*(R_0, R1, R2,and R3)=ungerminated (16%),8%,12%, and 20% germinated mix teff flour.

4.3 Assessments of the Cereal Type and Blending Ratio Interaction Effect on Proximate Content of the Formulated Injeras.

Results of proximate content of the prepared injeras are presented in (Table 4.2) below. Proximate contents were assessed based on the procedural standard of AOAC (2005) official methods and the NIR grain analyzer.

4.3.1 Protein content (%)

The interaction effect of the starters cereal type and their respective blending ratio had shown a significant influence on, protein content of the prepared injeras($P<0.05$). The barley, sorghum, and teff starter-injeras were ordered based on their protein contents, at the ungerminated, 8%, 12%, and 20% germinated mixing ratios, respectively. Protein content for each of the ungerminated, and 20% germinated mix barley, teff, and sorghum starters-injeras were: (9.22 and 9.97); (9.81 and

9.97); and (9.92 and 10.01 %), respectively (Table 4.2). Maximum values for the protein content were obtained from all the germinated mix teff starter- injeras. A study by Melaku, revealed that sorghum contains less protein content (9.597%) than teff grain (10.752%) (Melaku, 2022). In contrast, Yetneberk (2006) reported that sorghum contains higher protein content (7-14 %) than teff protein (6-10 %). According to (Amtataw et al. (2025); Cherie et al. (2018)), protein content of injeras is mostly found in the range of 7 to 15 percents.

In average, as mixing ratio of the germinated cereals were increased, protein content of the prepared injeras were also increased. Thus, germination of all the cereal starters influenced protein content of the prepared injeras. A study by Kayisoglu et al. (2024) similarly revealed that as crude protein content was increased by 23 and 19 % in germination of red and white sorghum varieties foods. Synthesis of new proteins, free amino acids (potential advantages of the enzymatic activity); intrinsic factors of the cereals, and agricultural practices may be some incidences to increase protein content of the germinated cereal-based starter injeras. Reduction of antinutritional factor**s** due to the germination process may also another probable cause for promotion of the injera protein content.

4.3.2 Fat Content (%)

Interaction of the cereal type, and blending ratio (germination) effect was significant on fat content of the formulated injeras ($P<0.05$). Fat content of the injeras was significantly reduced by the germination process for all the cereal type starters. The highest (2.75 percent), and lowest (2.5 percent) values were obtained from the ungerminated sorghum, and the 20% germinated mix barley starter-injeras (Table 4.2). Thus, germination of the cereal starters had a reduction impact on fat content of the sorghum-injera product. According to Xu et al. (2019), vital energy of foods might be generated (consumed) to support the emerging seed development during germination process, by β-oxidation of free fatty-acids in the cytosol and mitochondria.

Reversely, Kayisoglu et al. (2024) reported that as germination of sorghum had increased fat content of the final germinated sorghum-food; due to lipid biosynthesis pathways. Complex contents might also be hydrolyzed via Enzymes. So, dilution and consumption of those stored nutrients may the probable causes for the fat content reduction of the injeras. The current fat content values were almost inline to the previous ranges of (2.5-10.85%) for injera fat content reported by Awulachew et al. (2023).

4.3.3 Moisture Content (MC%)

Interaction effect of the cereal type, and blending ratio (germination) of the starters had, a significant influence on moisture content of the formulated injeras($P<0.05$). Moisture content was significantly increased for both the barley, and sorghum starter-injeras; but reduced for the teff starter-injera (Table 4.2). Germination process in grain/cereals sometimes increase moisture content of the final germinated food products (Rodríguez-España et al., 2022). Differences in physiological and compositional characteristics, enzymatic activation, cell expansion, osmotic pressure, and environmental adaption of the cereals might also be, the probable causes for such moisture content variations in the three cereal-starter injeras. Moisture content of the current injeras was comparable to the values of (4.88- 8.36%) for moisture content of maize-sorghum-wheat-injera reported by Nibret et al. (2024).

4.3.4 Dietary Fibers (%)

Crude fiber was significantly influenced by the type of fermentation starter; injera made from the barley, followed by the teff-starters showed higher crude fiber content, compared to the sorghum starter-injera (Table 4.2). The crude fiber content was generally increased due to the germination process of the cereal starters. All the three cereals starter-based injeras had shown increments in crude fiber, due to germination of the cereal starters. Hence, interaction effect of both the cereal type and blending ratio of the cereal- starters germination had considerable effect on the crude fiber content of the formulated injeras. The highest (20%) germinated mix barley starter-injera had the highest (1.84%) fiber content, compared to the other starter-injeras (Table 4.2). This could be attributed due to higher presence of fiber in the barley grain than the sorghum and teff grains, besides to the germination process of the cereals. According to Sibian et al. (2017), cereals germination could increase the dietary fiber of their final foods.

Formation of new cell walls, and some fiber-compounds like: cellulose and lignin (Thakur et al., 2021) in germinated foods, probably increased the total fiber content of the prepared injeras. Ohtsubo et al. (2005),and Loikaeo (2024) also noted that incorporation of pregerminated rice, and beans-flours, to ordinary wheat flour in bread preparation increase fiber contents of the final bread. Dietary fiber contents of the current formulated injeras were in line to the ranges of (1.23- 2.79%) for dietary fiber of sorghum-faba bean injera reported by Mihrete and Bultosa (2017).

4.3.5 Ash Content (%)

Ash content of the prepared injeras was significantly influenced by the type of the cereal starter (P<0.05). Ash content values were better recorded for the teff followed by barley starter-injeras, respectively. Ash content in all of the three-cereal starter-injeras had significantly reduced, as germinated cereal starters were added (Table 4.2). A similar observation was reported for the ash content of regular**,** malted, fermented, and malted-fermented sorghum-based flours, by Gawande et al. (2018).

In general, injeras produced using 20% germinated mix, and ungerminated teff starters had higher (1.67-1.74%) ash content, whereas injeras made using of 20% germinated mix, and ungerminated sorghum-starters had the lowest (1.53-1.55%) contents (Table 4.2). The inherent variations and agroecological factors of the cereal types may some of the factors for such variations in the injeras ash content. The current injera's ash content was similar to the respective values of (1.57 and 1.75%) for sorghum flour and injera products found by Mohammed et al. (2011).

4.3.6 Carbohydrate (CHO%) and Energy (kcal/100 g) Contents

Significant effect due to the cereal type and blending ratio interaction effects of the cereal starters had been shown, on carbohydrate content (CHO) of the prepared injeras (P<0.05). CHO of all the baked injeras were significantly reduced, due to germination of the starters. The pure ungerminated barley starter-injera had the highest value (77.27%), for the CHO content; while the 20% germinated mix teff starter- injera had the lowest in CHO value (75.87%) (Table 4.2).

According to Thakur et al. (2021), germination of food materials may reduce their CHO contents. Hydrolysis of the cereal's carbohydrate contents, may probably cause to the reduction of the final injera's carbohydrate contents. Cereal type and variety, growing and processing conditions could also be another probable cause for the CHO variations. The current CHO interval is comparable to the intervals of (59.54-77.74%) and (73.89-86.8%) reported by Awulachew et al. (2023), and Woldemariam et al. (2019) for the CHO contents of injera products. This interval also agrees to the daily need amount of food's CHO content to be (>57 %), as reported by Boza et al. (2017).

Energy content had also been reduced, due to germination of all the cereal-starters. Fat content of germinated foods could be utilized as energy (Rodríguez-España et al., 2022).This fat utilization, bio and physicochemical changes during the starter's germination probably cause, for energy reduction of the final injeras; as fat provides about twice of food energy values of protein and carbohydrate (Munarko et al., 2025).

Table 4. 2 Interaction of the starters cereal type and blending ratio effect on proximate contents of the prepared sorghum-injeras.

Injera product	Protein %	Fat %	MC %	Fiber %	Ash %	CHO %	Energy Kcal/100g
B*R0	9.22 ± 0.02^{d}	2.72 ± 0.01^{a}	7.57 ± 0.02^{d}	1.57 ± 0.02^{g}	1.65 ± 0.01^{d}	77.27 ± 0.06^{a}	370.44 ± 0.16^{b}
B*R1	9.81 ± 0.01^{c}	2.61 ± 0.03^{de}	7.9 ± 0.02^{b}	1.83 ± 0.01^{ab}	1.61 ± 0.01^{e}	76.24 ± 0.03^{cde}	367.69 ± 0.16^{ef}
B*R2	9.84 ± 0.02^{c}	2.57 ± 0.01^{e}	7.97 ± 0.01^{a}	1.84 ± 0.01^{a}	1.59 ± 0.01^{ef}	76.2 ± 0.02^{def}	367.29 ± 0.11^{fg}
B*R3	9.97 ± 0.02^{ab}	2.5 ± 0.01^{f}	7.99 ± 0.01^{b}	1.84 ± 0.01^{a}	1.58 ± 0.017^{f}	76.1 ± 0.02^{f}	366.78 ± 0.06^{g}
S*R0	9.92 ± 0.06^{b}	2.75 ± 0.02^{a}	7.98 ± 0.01^{a}	1.48 ± 0.01^{i}	1.55 ± 0.01^{g}	76.3 ± 0.05^{c}	369.63 ± 0.15^{c}
S*R1	9.94 ± 0.01^{b}	2.64 ± 0.01^{cd}	7.97 ± 0.01^{a}	1.66 ± 0.01^{g}	1.53 ± 0.01^{g}	76.3 ± 0.02^{cde}	368.72 ± 0.01^{d}
S*R2	9.98 ± 0.02^{ab}	2.63 ± 0.02^{cd}	7.98 ± 0.01^{a}	1.73 ± 0.01^{ef}	1.53 ± 0.01^{g}	76.14 ± 0.02^{ef}	368.15 ± 0.06^{e}
S*R3	9.97 ± 0.01^{ab}	2.61 ± 0.01^{d}	7.83 ± 0.01^{c}	1.78 ± 0.02^{cd}	1.53 ± 0.01^{g}	76.28 ± 0.03^{cd}	368.49 ± 0.7^{de}
T*R0	9.81 ± 0.02^{c}	2.73 ± 0.01^{a}	7.97 ± 0.02^{a}	1.66 ± 0.01^{g}	1.74 ± 0.01^{a}	76.1 ± 0.04^{f}	368.21 ± 0.63^{f}
T*R1	10.01 ± 0.02^{a}	2.68 ± 0.01^{b}	7.98 ± 0.01^{a}	1.70 ± 0.02^{f}	1.7 ± 0.01^{b}	75.89 ± 0.06^{g}	367.72 ± 0.14^{ef}
T*R2	10.01 ± 0.01^{a}	2.65 ± 0.02^{bc}	7.00 ± 0.03^{e}	1.75 ± 0.01^{de}	1.67 ± 0.01^{cd}	76.91 ± 0.04^{b}	371.53 ± 0.14^{a}
T*R3	10.01 ± 0.01^{a}	2.65 ± 0.01^{bc}	7.99 ± 0.01^{a}	1.79 ± 0.03^{bc}	1.69 ± 0.01^{abc}	75.87 ± 0.03^{g}	367.37 ± 0.15^{fg}
CV (%)	2.14	2.62	3.61	6.38	4.46	5.1	3.7
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values in the same column followed by the same letter are not significantly different at the 0.05. Where: CV=Coefficient of variance, SD=Standard Deviation, MC=Moisture Content, and CHO=Carbohydrate Content; B*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix barley flour; S*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix sorghum flour; and T*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix teff flour.

4.4 Assessments of the Cereal Type and Blending Ratio Interaction Effect on, Mineral Content of the Formulated Injeras.

Statistically, the interaction effect of the starters cereal type and blending ratio showed, a significant effect on: Calcium/Ca, Iron/Fe, and Zinc/Zn contents of the prepared injeras ($P<0.05$). From the recorded values (Table 4.3), calcium contents were increased due to germination of the cereal starters, for the barley, and teff starter-injeras, whereas the sorghum starter-injeras were reduced in their calcium contents. So, calcium content of sorghum-injera could effectively improve by using such germinated barley, and teff as fermentation starter sources; but not sorghum as starter source. Agricultural practices and natural characteristics of the cereal types might be caused differences in calcium content of the injeras. Sorghum, for instance might have condensed antinutritional factors than barley and teff grains. Naturally, teff has also best mineral contents among cereal grains (Baye, 2014).Thus, the germinated teff followed by barley starter-injeras showed highest level of calcium concentrations in this study.

Iron concentration showed a significant reduction in the germinated barley, and sorghum starter-injeras; compared to their ungerminated starter-injeras. According to Karki et al. (2024), minerals could also be utilized by the developing seedling during germination of foods. Leaching of the mineral during the germination might also another probable cause for this reduction.

In contrast, the teff starter-injera showed an increment in the iron content, due germination of the cereal as fermentation starter source; the 20% germinated mix starter-injera particularly, had the highest (7.7 mg/100g) iron content (Table 4.3). Mineral content of foods depend on, the species and variety of the food source, agricultural factors, especially the soil where they are grown (Miller, 2017). Therefore, interaction of the cereal type and blending ratio (germinated) was effective, on Iron content of the teff starter-injera, but not to the barley, and sorghum-starter injeras.

The interaction effect of the starters cereal type and germination ratio, showed a significant increment in Zink content of all the prepared injeras ($P<0.05$). Over all, teff followed by the barley-starter injeras had higher Zink content; whereas the sorghum starter injera had the lower Zink content. Values of (1.53 mg/100g); and (1.44 mg/100g) were the higher values for the 20% germinated mix teff, and barley starter-injeras, respectively. Enzymes like phytase boost mineral contents of germinated foods (Bassey et al., 2023).This motivation could be due to releasing of bound minerals for absorptions. The current mineral values were almost similar to the values of Ca, Fe, and Zn contents of injera product reported by Anberbir et al. (2023).

Table 4.3 Assessments of the cereal type and blending ratio interaction effect on mineral content of the formulated injeras (means of three replicates ± SD).

Injera product	Ca (mg/100g)	Fe(mg/100g)	Zn(mg/100g)
B*R0	49.5±0.01^{g}	*7.42*±0.01^{d}	*1.41*±0.01^{fg}
B*R1	50.24±0.01^{e}	*7.36*±0.03^{de}	*1.42*±0.01^{ef}
B*R2	50.26±0.01^{de}	*7.37*±0.01^{e}	*1.45*±0.01^{de}
B*R3	50.28±0.01^{d}	*7.39*±0.01^{e}	*1.44*±0.01^{de}
S*R0	49.79±0.01^{f}	*7.24*±0.01^{f}	*1.34*±0.01^{i}
S*R1	49.22±0.01^{j}	*7.21*±0.01^{g}	*1.36*±0.01^{hi}
S*R2	49.32±0.01^{i}	*7.17*±0.02^{h}	*1.38*±0.01^{gh}
S*R3	49.37±0.02^{h}	*7.22*±0.01^{fg}	*1.39*±0.01^{h}
T*R0	51.12±0.01^{c}	*7.59*±0.01^{c}	1.46±0.01^{cd}
T*R1	51.17±0.02^{b}	*7.64*±0.01^{b}	1.49 ±0.01^{bc}
T*R2	51.19±0.01^{ab}	*7.66*±0.01^{b}	1.51 ±0.02^{ab}
T*R3	51.22±0.01^{a}	*7.7*±0.02^{a}	1.53 ±0.01^{a}
CV (%)	1.53	2.51	4.00
P-Value	0.000	0.000	0.000

Values in the same column followed by the same letter are not significantly different at the 0.05. **Where:** CV=Coefficient of variance, SD=Standard Deviation; B*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix barley flour; S*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix sorghum flour; and T*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix teff flour.

4.5 Interaction-Effect of the Cereal-Type, and Germinated Blending Ratios on Sensory Acceptably of the Formulated Injeras.

Assessments of sensory acceptability of the prepared sorghum injeras were presented in Table 4.4. Color assessments of the prepared injera had shown significant effect (P<0.05), due to the interaction effect of the cereal type, and blending ratio of the starters. Injeras made using of ungerminated teff, 8% germinated mix sorghum, 20% germinated mix teff, 12% germinated mix barley, and ungerminated sorghum starters, respectively had the highest mean values for the color assessments (Table 4.4). While, the ungerminated barley starter-injera had the lowest (4.33) value in the color assessment. Over all, the color values were rated in the range of above neither like nor dislike (4), to the like moderately (6) on the scale of seven (7) hedonic points. The color acceptability results showed as all the prepared injeras were color accepted, and germination of the cereal starters had no negative impact on the color of the injeras. Color acceptance of the current prepared injeras is almost matched to the previous color acceptance values of teff-sorghum-fenugreek injera reported by Awulachew et al. (2023).

Response of the panelists to taste of the injera products exhibited significant influence was observed, due to the interaction of the cereal type and blending ratio effects of the germinated starters (P<0.05). The recorded data for the injeras taste was varied from 3.91 to 5.75 on the scale of seven points. The highest (5.2, 5.25, 5.42, 5.42, and 5.75) taste values were recorded for the injeras prepared using the ungerminated-sorghum, 20% germinated mix-barley, 8 % germinated mix-sorghum, 20% germinated mix-sorghum, and ungerminated-teff starters, respectively. While, the lowest values (3.91, 4.58, and 4.58) were scored for the injeras made from the ungerminated barley, 8% germinated mix-barley, and the 12% germinated mix-sorghum starter-injeras. Generally, response of the panelists for the injeras taste highlighted that all the prepared injeras were acceptable in taste. Variation of the cereal's behavior, agrological factors and germination effects of the cereals may some probable cause for this taste variations.

Significant effect was exhibited on the top and upper surfaces assessments of the prepared injeras (P<0.05). Most of the prepared injeras had received scores above the slightly like (5) range. But, injeras of the ungerminated barley, 12% germinated mix-teff, 8% germinated mix-teff, and the 12% germinated mix-sorghum starter-injeras had received scores under the like slightly (5) points in the seven liking scores. The lowest (4) value was scored for the pure ungerminated barley starter-injera; as the highest (*5.75*) value was scored for the 12% germinated mix barley starter-injera (Table 4.4).

Scores of texture-acceptability had also exhibited a significant interaction effect (P<0.05), of the cereal-type and blending ratio of the germinated flour starters.

The scored data for texture of the prepared injeras were generally ranged from 3.3 to 6 based on the starter type. The highest values (5,5.67,5.25,6,5 and 5.58) were scored for the 8% germinated mix barley, 12% germinated mix barley, 20% germinated mix sorghum, ungerminated teff, 8% germinated mix teff, and 20% germinated mix teff starter-injeras, respectively. Hence, the prepared injeras had received perceptions of like slightly (5) to like moderately (6.00) on the scale of (7) points. The pure-ungerminated sorghum starter-injera had received the lowest (3.3) perception in the texture test. From judgment of the evaluators, texture of the prepared sorghum injera was certainly improved, due to germination of the cereal starters. Cereals germination was reported, to improve texture of their final products (Dahiya et al., 2020; Sarabhai et al., 2021).

Evolutions, for eye-uniformity of the different starter-based injeras also exhibited significant variations (P<0.05). Minimum (4.42) value was scored for the eye-uniformity of the pure ungerminated barley starter- injera. While the highest (5.75) acceptance was recorded for the 20% germinated mix barley starter-injera. Those values were almost in the range of neither like nor dislike (4), near to the like-moderately (6) on the scale of seven points. Regarding the eye uniformity, injeras of the germinated barley, followed by the teff, starters received better acceptances compared to the sorghum starter-injeras (Table 4.4).

Rollability evolutions for the different cereal starter-injeras, showed significant differences (P<0.05). Values for rollability evaluations were scored in the range of (4.25-6.25). Except the pure ungerminated teff starter-injera, both the pure ungerminated barley, and sorghum starter-injeras showed lower rollability acceptances compared to their germinated mix starter-injeras. But, the ungerminated teff starter-injera had shown the maximum (6.25) value for the rollability acceptance of all the prepared injeras; while, the lowest (4.25) value for the injeras rollability was scored, for the pure ungerminated sorghum starter-injeras, respectively (Table 4.4).

The interaction effect of the cereal type and blending ratio of the germinated cereal starters result also reflected a significant influence (P<0.05), on overall acceptability of the prepared injeras. Except the in the teff starter, the overall acceptability was mostly increased in the barley, and sorghum-starters, as germinated cereal flour in the starters was added. But the overall result showed that the pure ungerminated teff starter-injera had maximum (6.67) value for the overall acceptability judgment. Whereas, the lowest value (4.33) was scored for the overall acceptability of the pure ungerminated barley starter-injera (Table 4.4).

On average, the sensory quality evaluations demonstrated that as the germinated cereals particularly the (barley and sorghum) fermentation starters had positive influences on sensory qualities, compared to their ungerminated starter forms in sorghum-injera preparation. This all might be due to the potential advantages of the cereal starters' germination. A study by Boukid et al. (2019), implies that incorporation of 15% germinated legume flours improved sensory profiles of a bread product. According to (Perri et al. (2021)), germination of food materials improve the appearance, taste, color, texture and smell of their final foods.

Table 4. 4 Assessments of the cereal type and blending ratio interaction effect on sensory acceptability of the formulated injeras (means of three replicates ± SD).

Injera product	Color	Taste	T&B/S	Texture	EU	Rollability	OA
B*R0	4.33± 0.65	3.91± 0.67^{c}	4± 0.74^{c}	4.42± 0.51^{c}	4.42± 0.9^{c}	4.75± 0.75^{cde}	4.33± 0.49^{e}
B*R1	4.75± 0.62^{bcd}	4.58± 0.51^{bc}	5.33± 0.65^{ab}	5± 0.84^{bc}	5.67± 0.99^{ab}	5.67± 0.78^{abc}	5.41± 0.58^{abcd}
B*R2	5.66± 0.89^{ab}	4.92± 0.67^{abc}	5.75± 0.75^{a}	5.67± 0.65^{ab}	5.67± 0.98^{ab}	5.83± 0.58^{ab}	6± 0.74^{ab}
B*R3	4.92± 0.79^{bcd}	5.25± 0.45^{ab}	5.41± 0.67^{ab}	4.91± 0.57^{bc}	5.75± 0.87^{a}	5.41± 0.51^{abcd}	5.17± 0.72^{abcde}
S*R0	6.08± 0.79^{a}	5.2± 0.94^{ab}	5.41± 0.79^{ab}	3.3± 0.65^{d}	5.41± 0.99^{abc}	4.25± 0.75^{e}	4.58± 0.67^{de}
S*R1	5.25± 0.62^{abcd}	5.42± 0.67^{ab}	5.33± 0.78^{ab}	4.58± 0.51^{c}	5.58± 0.99^{abc}	5.33± 0.65^{abcd}	5.41± 0.51^{abcd}
S*R2	4.92± 0.79^{bcd}	4.58± 0.67^{bc}	4.75± 0.62^{bc}	4.58± 0.51^{c}	4.5± 0.67^{bc}	5.17± 0.72^{bcde}	5± 0.74^{bcde}
S*R3	4.92± 0.79^{bcd}	5.42± 0.67^{ab}	5.08± 0.67^{ab}	5.25± 0.62^{abc}	5.5± 0.91^{abc}	4.67± 0.65^{de}	5.17± 0.72^{abcde}
T*R0	5.08± 0.67^{bcd}	5.75± 0.75^{a}	5.41± 0.90^{ab}	6± 0.85^{a}	5.67± 0.65^{ab}	6.25± 0.75^{a}	6.67± 0.65^{a}
T*R1	4.58± 0.51^{cd}	4.66± 0.65^{bc}	4.66± 0.65^{bc}	5± 0.74^{bc}	4.67± 0.89^{abc}	5.25± 0.45^{bcd}	4.83± 0.72^{cde}
T*R2	4.83± 0.72^{bcd}	4.83± 0.72^{abc}	4.58± 0.67^{bc}	4.83± 0.72^{bc}	4.91± 0.79^{abc}	5.17± 0.72^{bcde}	4.92± 0.79^{bcde}
T*R3	5.5± 0.52^{abc}	4.66± 0.65^{bc}	5± 0.6^{ab}	5.58± 0.9^{ab}	5.58± $0.9a^{bc}$	5.58± 0.79^{abcd}	5.75± 0.62^{abc}
CV (%)	16.27	16.38	16.39	19.1	18.94	15.91	15.35

Means that do not share a letter are significantly different at 0.05. **Where**: CV = Coefficient of variance, SD= Standard Deviation, T&B/S = Top and Bottom Surfaces, EU =Eye uniformity, OA=Overall acceptability; B*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix barley flour; S*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix sorghum flour; and T*(R_0, R1, R2, and R3) = ungerminated (16%),8%,12%, and 20% germinated mix teff flour.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study was held on sorghum-injera quality improvement using germinated barley, sorghum, and teff starters at (8, 12, and 20 percents) blending ratios. From the result, it can be concluded that mixing of the germinated cereal flours at the mentioned proportions were positively influence some functional attributes (OAC, EC, and BD) of the final flours; proximate (protein and dietary fibers); and mineral (Zn-in all the three cereal starter-injeras, Ca-in both the barley, and teff starter-injeras, and Fe-in the teff starter-injera) contents were significantly improved, due to mixing of the germinated cereal flour-starters. Sensory acceptability of the prepared-injeras was effectively improved; as germinated cereal starters were added to the fermentation starter flours. This was particularly effective for the barley, and sorghum-based starter injeras. The ungerminated teff, 12% germinated mix barley, and 20% germinated mix teff-starter injeras, respectively had notable positive influences on sensory acceptability of the formulated injeras. Therefore, sorghum-injera quality can be improved by using of such germinated mix cereal starters. This intern can increase the consumption mode and level of sorghum-injera; so, ensures food security of many Ethiopians.

5.2 RECOMMENDATIONS

Actual results of this study, provide valuable insights to use germinated cereals as fermentation starters to improve sorghum-injera quality. So, in sorghum-injera preparation using of germinated cereal flours as fermentation starter is recommended to have nutritional, sensory accepted, and healthy injera product. From the actual and particular result, the ungerminated teff,12% germinated mix barley, and 20% germinated mix teff starter-injeras, respectively were better in their sensory acceptability. Nutritional contents such, the minerals (Zn, and Ca); macronutrients (protein, and dietary fiber) contents of prepared injeras also improved due to the germinated mixed cereal starters. So, using of such cereal type and blending ratio for the future sorghum injera preparation will have great contributions, in sorghum-injera sensory and nutritional quality improvements. It is also better to invite and persuade food processers/makers to adopt germinated cereals, as food adjuncts/starters in their food preparations/production; such in sorghum-injera preparations. Microbial-safety, shelf life, using of combined, and multiple germinated cereal starters effect on, sensory and nutritional qualities are some of the not addressed issues by this study, so need further investigations.

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Appendixes

Appendix I: Sensory Evaluation Sheet **Date of test**…………

First, thank you for coming here. I am **Abadi Tsegay** MSc. Student in Food Processing Technology, FSPT-Department at Mekelle University. I am researching **"Improving sorghum Injera quality using germinated cereal fermentation starters"**. Scores of preferences you put here regarding the sensory attributes of the injera in front of you may contribute to improving sorghum injera quality scientifically, in Tigray, Ethiopia. Please put a number that represents your preference about the sensory attribute of the product, ranging from1to7.

Panelist's Name __________________Age____ Sex _____ Occupation_______

Type of sensory test: Seven-point hedonic scale

Instruction: Please observe the injera samples exactly, which are coded by their representatives on the plates in front of you. After you observe, score a value that is assumed to represent the color, taste, top & bottom surfaces (degree of being powdery& sticky), texture (degree of softness), eye-uniformity, rollability, and overall acceptability based on your perceptions.

Sensory Attributes							
Sample-code	Color	Taste	Top& bottom-surfaces	Texture	Eye-uniformity	Rollability	Overall-acceptability
R0B							
R1B							
R2B							
R3B							
R0S							
R1S							
R2S							
R3S							
R0T							
R1T							
R2T							
R3T							

Acceptability level for the presentative sample using a seven-point hedonic scale (1-7) ranges.

Like extremely	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

Comment---

Thank you very much, for your evaluation!!

Appendix II: Questionnaire format sheet for the survey of sorghum injera preparation at the traditional level.

Title: “**Improving Sorghum Injera Quality Using Germinated Cereal Fermentation Starters**”

Responses are marked with (X).
1. Name of household mother (Respondent)______________________________
2. Age_____________________________
3. Marital status: A. married☐ B. unmarried☐ C. widowed☐
4. Education level: A. Literate☐ B. Illiterate☐ C. Semi-literate☐
5. Religious: A. orthodox☐ B. Muslim☐ C. protestant ☐
6. Do you know Injera? Yes☐ No ☐
7. How is it? Sorghum-injera produced at the home level for own consumption or selling?

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8. What main ingredients are used to produce Sorghum-injera at the home level?

---.

9. Put your best assumptions for the germination time of the given cereal crops that can be better for fermentation starter preparation for future sorghum injera preparation.

Cereal type	Germination time			
Barley (Saesa)	24hours	48hours	72hours	96hours
Sorghum (Kodem)				
Teff (Boni)				

Conditions to be considered during the germination time---

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10. Identify which cereal grain is best? For fermentation starter preparation, to prepare sorghum injera, produce and rank them.

Cereal type	1st	2nd	3rd
Barley			
Sorghum			
Teff			

11. What changes happen if germinated (barley, teff, and sorghum) cereals are used for fermentation starter in sorghum injera preparation? Especially in the quality of the sensory attributes of the injera produced?

12.**A**. How many grams of flour will be required to prepare a fermentation starter (Ersho), if 5Kg of sorghum flour is assumed to make a fermented dough?
B.------------grams of flour is required.

Grams of flour	Average (Mean)	Minimum	Maximum

General suggestions--

1. Were you familiar with the practice of germinated fermentation starter in injera preparation?
Yes ☐ No ☐ If your response is no, why? And if yes, how?

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2. Please draw up your suggestions about the preparation of sorghum injera using germinated cereal fermentation starter flours briefly.

Thank you for your willingness!!!

Appendix III: ANOVA Tables

General Linear Model: Analysis of Variance (ANOVA)

Table 1: Analysis of Variance for WAC of the flours

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.059172	0.029586	304.31	0.000
blending ratio	3	0.036942	0.012314	126.66	0.000
cereal type*blending ratio	6	0.001450	0.000242	2.49	0.052
Error	24	0.002333	0.000097		
Total	35	0.099897			

Table 2: Analysis of Variance for OAC of the flours

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.087339	0.043669	285.84	0.000
blending ratio	3	0.037922	0.012641	82.74	0.000
cereal type*blending ratio	6	0.005528	0.000921	6.03	0.001
Error	24	0.003667	0.000153		
Total	35	0.134456			

Table 3: Analysis of Variance for EC of the flours

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	30.3757	15.1879	1178.88	0.000
blending ratio	3	16.7031	5.5677	432.16	0.000
cereal type*blending ratio	6	2.8789	0.4798	37.24	0.000
Error	24	0.3092	0.0129		
Total	35	50.2669			

Table 4: Analysis of Variance for FC of the flours

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	5.71020	2.85510	102.31	0.000
blending ratio	3	1.33771	0.44590	15.98	0.000
cereal type*blending ratio	6	0.04776	0.00796	0.29	0.938
Error	24	0.66973	0.02791		
Total	35	7.76540			

2. Table 5: Analysis of Variance for BD of the flours

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.072800	0.036400	504.00	0.000
blending ratio	3	0.053211	0.017737	245.59	0.000
cereal type*blending ratio	6	0.003556	0.000593	8.21	0.000
Error	24	0.001733	0.000072		
Total	35	0.131300			

Table 6: Analysis of Variance for protein

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.47257	0.236286	509.36	0.000
blending ratio	3	0.61027	0.203425	438.52	0.000
cereal type*blending ratio	6	0.47512	0.079186	170.70	0.000
Error	24	0.01113	0.000464		
Total	35	1.56910			

Table 7: Analysis of Variance for fat

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.039506	0.019753	109.40	0.000
blending ratio	3	0.108764	0.036255	200.79	0.000
cereal type*blending ratio	6	0.015694	0.002616	14.49	0.000
Error	24	0.004333	0.000181		
Total	35	0.168297			

Table 8: Analysis of Variance for moisture content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.25841	0.129203	637.16	0.000
blending ratio	3	0.52393	0.174644	861.26	0.000
cereal type*blending ratio	6	2.01848	0.336414	1659.03	0.000
Error	24	0.00487	0.000203		
Total	35	2.80569			

Table 9: Analysis of Variance for fiber content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.070350	0.035175	197.86	0.000
blending ratio	3	0.294697	0.098232	552.56	0.000
cereal type*blending ratio	6	0.052161	0.008694	48.90	0.000
Error	24	0.004267	0.000178		
Total	35	0.421475			

Table 10: Analysis of Variance for ash content

Source	DF	Adj SS	Adj MS	Table 11	P-Value
cereal type	2	0.162872	0.081436	1047.04	0.000
blending ratio	3	0.013786	0.004595	59.08	0.000
cereal type*blending ratio	6	0.003172	0.000529	6.80	0.000
Error	24	0.001867	0.000078		
Total	35	0.181697			

Table 11: Analysis of Variance for CHO content

Source	DF	Adj SS	Adj MS	Table 12	P-Value
cereal type	2	0.44457	0.222286	148.74	0.000
blending ratio	3	1.39628	0.465425	311.44	0.000
cereal type*blending ratio	6	3.50412	0.584019	390.79	0.000
Error	24	0.03587	0.001494		
Total	35	5.38083			

Table 12: Analysis of Variance for Energy Content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	4.072	2.03586	41.95	0.000
blending ratio	3	22.358	7.45271	153.55	0.000
cereal type*blending ratio	6	38.577	6.42942	132.47	0.000
Error	24	1.165	0.04854		
Total	35	66.171			

Table 13: Analysis of Variance for Ca content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	18.7792	9.38959	68984.71	0.000
blending ratio	3	0.1195	0.03983	292.59	0.000
cereal type*blending ratio	6	1.7664	0.29441	2163.00	0.000
Error	24	0.0033	0.00014		
Total	35	20.6684			

Table 14: Analysis of Variance for Fe content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	1.17721	0.588603	5045.17	0.000
blending ratio	3	0.00793	0.002644	22.67	0.000
cereal type*blending ratio	6	0.02595	0.004325	37.07	0.000
Error	24	0.00280	0.000117		
Total	35	1.21389			

Table 15: Analysis of Variance for Zn content

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
cereal type	2	0.098839	0.049419	555.97	0.000
blending ratio	3	0.012542	0.004181	47.03	0.000
cereal type*blending ratio	6	0.001050	0.000175	1.97	0.001
Error	24	0.002133	0.000089		
Total	35	0.114564			

Table 16: Analysis of Variance for color

Source	DF	Table 17	Adj MS	F-Value	P-Value
cereal type	2	3.722	1.8611	3.72	0.027
blending-ratio	3	2.139	0.7130	1.43	0.238
cereal type*blending-ratio	6	25.444	4.2407	8.48	0.000
Error	132	66.000	0.5000		
Total	143	97.306			

Table 17: Analysis of Variance for taste

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	5.681	2.8403	6.18	0.003
blending-ratio	3	2.083	0.6944	1.51	0.215
cereal type*blending-ratio	6	24.875	4.1458	9.02	0.000
Error	132	60.667	0.4596		
Total	143	93.306			

Table 18: Analysis of Variance for top and bottom surfaces

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	1.542	0.7708	1.52	0.223
blending-ratio	3	1.021	0.3403	0.67	0.572
Cereal type*blending-ratio	6	28.792	4.7986	9.44	0.000
Error	132	67.083	0.5082		
Total	143	98.437			

Table 19: Analysis of Variance for texture

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	20.847	10.4236	21.67	0.000
blending-ratio	3	8.333	2.7778	5.77	0.001
Cereal type*blending-ratio	6	34.875	5.8125	12.08	0.000
Error	132	63.500	0.4811		
Total	143	127.556			

Table 20: Analysis of Variance for eye uniformity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	0.722	0.3611	0.44	0.648
blending-ratio	3	6.722	2.2407	2.70	0.048
Cereal type*blending-ratio	6	25.944	4.3241	5.21	0.000
Error	132	109.500	0.8295		
Total	143	142.889			

Table 21: Analysis of Variance for Rollability

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	13.431	6.7153	14.34	0.000
blending-ratio	3	2.611	0.8704	1.86	0.140
Cereal type*blending-ratio	6	23.014	3.8356	8.19	0.000
Error	132	61.833	0.4684		
Total	143	100.889			

Table 22: Analysis of Variance for total acceptability

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cereal type	2	1.500	0.7500	1.68	0.191
blending-ratio	3	5.444	1.8148	4.06	0.009
Cereal type*blending-ratio	6	24.056	4.0093	8.97	0.000
Error	132	59.000	0.4470		
Total	143	90.000			

Appendix IV. Some Photos of the Laboratory and the survey activities

Appendix V:Photos of the prepared Injeras

Photos of Sorghum injera, prepared using **barley-based** fermentation starters.

Photos of Sorghum Injera, prepared using **sorghum-based** fermentation starters.

Photos of Sorghum Injera, prepared using **teff-based** fermentation starters.