# Regional Review Workshop on Completed Research Activities

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## Preface

Oromia Agricultural Research Institute (IQQO) is undertaking various applied research programs that is fundamental for food security and poverty reduction in the region since its establishment. This is undertaken across its 17 research centers which address various agro-ecologies of the region working on Crop, Livestock, Natural Resources, Agricultural Engineering and others. Furthermore, IQQO established Food Science Research Directorate and currently started the operation of Food Science Laboratory to undertake applied research on the agricultural products (food crops and livestock products) and other food related issues. This is planned to integrate quantitative research to the qualitative approach with the generated technologies from various disciplines of agricultural research at different agro-ecology-based research centers of IQQO.

To undertake this research, IQQO has built a Food Science Research Laboratory with the necessary facilities and cutting edge Laboratory equipment in its head quarters' compound. The Food Science Laboratory and its Research Directorate is established at a time when the country is urgently demanding competent laboratories to support the implementation of activities set under the Growth and Agricultural Transformation Plan to ensure food safety, improve quality and nutritional content.

Food Science is a field of integrated study of basic sciences and the application of science and engineering to the production, processing, distribution, preparation, and evaluation of food. Furthermore, it is the study of the transformation of biological materials into food products acceptable for human consumption. This requires studying diverse scientific disciplines related to food, including Chemistry, Microbiology, Biochemistry, Toxicology, Biotechnology, Engineering technology for the development and management of food resources ; and effectively applying the industrial and practical aspects to product development, food processing, preservation, and marketing.

In the past, the areas of food technology and nutritional research in agriculture has not been given due attention in the research institute. Consequently, agricultural researchers of the country have focused most of their attention on improving the production and productivities of the crops and livestock. However, in any major crop/livestock improvement program, attention must not only be given to the quantitative aspect of production but also nutritional considerations , processing quality and consumer acceptance be considered in order to bridge the gaps between nutritional needs and food supply. Hence, the Food Science Research Directorate with its Laboratory is established for the purpose of working to improve the safety and quality of the food supply from the farm to the table by providing high quality research and services to public and the food industry.

Accordingly, it is realized that the following three disciplinary research teams are indispensable in the establishment of IQQO's Food Science Research Directorate: Food Chemistry and Nutrition, Food Microbiology and Food Technology and Process Engineering Research Teams. Each team has at least three research components under which numbers of qualified researchers and Laboratory technicians are being assigned to undertake pertinent research activities.

Components under each research teams are:

1. Food Chemistry and Nutrition Research Team (Food Analysis, Food Assessment and Nutrition Analysis and Food Chemical Safety and Toxicology)
2. Food Microbiology Research Team (Food Pathology, Food Probiotics and Food Biological Safety and Toxicology)
3. Food Technology and Process Engineering Research Team (Food Biotechnology, Food Processing and Product Development and Food Marketing and Value Chain and Food Processing and Environmental safety)

These Research components under respective teams are organized to undertake Food Science research activities accordingly and a couple of researchers having pertinent professions are transferred from IQQO research centers, a few junior researchers are recruited and we still critically anticipate more researchers to be recruited yearly to fill the human power gap.

Since the establishment of Food Science Research Directorate, a number of research activities have been proposed and carried out by these researchers supported by IQQO and AGP-II budgets. Some of the completed activities have been reviewed during the IQQO annual regional completed research review Workshop and published in the proceedings of AGP-II, Crop and Livestock research directorates as well as Food Science research directorate completed research proceeding , volume-I in the past three years. Now our Food Science Research Directorate has published its second volume of completed research proceedings following the Regional Annual Review Workshop 2022.

Food Technology and Process Engineering Research

# Formulation and fortification of Soybean and Yam to Wheat flour on nutritional and sensory quality of bread.

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Abstract

*The research was carried out to evaluate the effect of blending ratio of soya bean and yam flours on the quality of wheat bread. They were processed into flour, mixed with wheat and bread was baked. The nutritional value and sensory attributes of bread was computed using design expert software. The mixed flour have the mean value for (M.C.), Fiber, crude fat, Ash, Zn, Fe, Mg, Ca , Na, K,P, protein, Carbohydrate and energy with the following ranges: 6.93–9.4%,2–2.99%,2.14–11.39,1.42–2.77,15.29.69,17.3–37.79 and 647–1239.19,670.14–1187.22,132.1–260.6,4395.69–6633.43,2116.7–660.93,7.58 19.63 %,57.32–76.66% and 356.75–408.63kcal respectively. The baked bread samples have the mean value with the ranges of 3.51–4.27%,1.92–3.04%,2.41–97,2.45–3.84,20.97–41.96ppm,26.98–38.98,697.82–1252.3,691.4–199.4,2169.99–2260,4501.87–612.5ppm,3954.75–5277.34ppm,5.99–13.71%,68.81–83.36%and374.08–406.59kcal for (M.C.),Fiber, crude fat, Ash, Zn, Fe, Mg ,Ca, Na, K, P, protein, Carbohydrate and energy , respectively. The result of the proximate composition showed that the blending ratio of soya bean and yam had effect on nutritional and sensory evaluation of bread with significant difference in the protein content of bread (p<0.05). There was an increase in the values of overall acceptability, texture and flavor of the bread samples with increasing levels of soya bean and yam flours but the decrease was not significantly different. It was concluded that a substitution of optimum soy bean and yam flour into wheat flour gave the bread with the best overall quality acceptability.*

**Keywords:** *Fortification, Wheat flour, Soybean flour, Yam flour, Nutritional value and Sensory attributes*

## Introduction

Ethiopia has a high prevalence of Acute and Chronic Malnutrition, with almost half of Ethiopian children chronically malnourished and one-in-ten children wasted. About 47% of children under-five are stunted, 11% are wasted and 38% are underweight. It is estimated that 53% of deaths among pre-school children in the developing world including Ethiopia are due to the underlying effects of malnutrition on diseases such as measles, pneumonia, and diarrhea (Mercedes, 2004). Ethiopia had a very high level of undernourishment in 2006-08, the latest period available; 41% of the total population was undernourished. In Oromia region, prevalence of child malnutrition indicated that 34.4% are underweight with 11% severe underweight, 9.6% of the children are wasted (2.4 % severe wasting) and 41 % of the children are stunted with 21.8% sever stunting (EDHS, 2010).

Protein Malnutrition is widely recognized as a major health problem in worldwide due to cereal-based dietary pattern. In countries where malnutrition poses a serious problem especially among children, composite flours which have better nutritional quality would be highly desirable (Okpala and Okoli, 2013). According to Ethiopian Public Health Institute; Ethiopian national food consumption report in 2013, the energy distribution of carbohydrate in the diet of children, women and men from Oromia were 65.6 %, 74.6 % and 65.8 % respectively. While, the energy distribution of protein and fat in the diet of children, women and men from Oromia were 10.4 %, 9.8% and 9.7 % and 24.2%, 15.8 % and 24.8 % respectively. Thus, the diet of an average Ethiopian consists of foods that are mostly carbohydrate based, there is a need for strategic use of inexpensive high protein resources that complement the amino acid profile of the staple diet in order to enhance their nutritive value.

The protein quality of the cereal-based diet can be improved by fortifications. Fortified cereal with soy protein, especially when mixed with proper ratio, is one of the best sources of protein (Wadud &Shah, 2004). The fortification of bread and other cereal based confections with legume flours particularly in regions where protein utilization is inadequate has long been recognized. According to FAO standards (FAO, 1995) suggestion, to meet the recommended dietary allowances of infants, preschool children, adolescent girls, pregnant and lactating women, low-cost supplementary foods could be processed domestically by simple, inexpensive processing technology. Bread is a dietary staple in human nutrition (Dewettinck et al., 2008).

Bread is one of the most important staple foods and it is consumed by people in every socioeconomic class and is acceptable to both adults and children and also it is good source of carbohydrate and micronutrient such as vitamin and minerals. There is increasing demand for bread substitute's wheat in bread. The successes have been achieved with the use of flours from cereal, legumes, roots and tubers as wheat substitutes in bread (Amber et al., 2009). To increased trend towards health eating which has result in development of many novel functional food including use of other 14 locally available crops for bread production, portion or total substitution wheat flour with other cereals (Oyewol ,1996).

Research on bread is globally conducted to improve its nutritional value (macronutrients: carbohydrates, proteins, fat and dietary fibers; micronutrients: minerals and vitamins), health supporting bioactive compounds, sensory acceptability, shelf life and to match with the affordability

In many countries, particularly in sub- Saharan Africa, bread wheat production and supply is inadequate to meet the bread eating habit of consumers, which is increasing with an increase in urbanization. One method to alleviate the shortage of wheat flour, increase the nutritional quality and bioactive contents of the bread is to use composite flours prepared from different crops like protein rich legumes, tubers rich in starches and/ or other cereal grain flours (Nwanekezi, 2013).

Wheat flour for bread has starches and functional protein glutens that favor the processing of leavened aerated bread, but is limited in fat and balanced amino acids (Goesaert et al., 2005). Partial substitution of wheat flour by flour products from Sorghum, Millet, Maize, yam and cassava is therefore being explored and evaluated in bread quality parameters such as specific volume, structure, texture and sensory qualities. Although it is shown that substitution of wheat flour up to a level of 20% results in acceptable composite loaves of bread, an increase substitution level may adversely affect bread and sensory qualities (Khalil et al., 2000).

Studies on human nutrition have shown that worldwide nutrition transition is taking place, in which people shift towards more affluent food consumption patterns (FAO, 2003; Grigg, 1995; Popkin, 2002). Levels and composition of food consumption are major determinants of the nutritional wellbeing of individuals, which in turn, have important implications for health, productivity, and income.

Soybean (Glycine max L) has been the primary source of proteins for use as a functional ingredient in food system (Gernah and Chima, 2006). It enhances the protein quality of wheat bread because of its lysine content which is deficient in wheat. Soybean is an essential source of protein, oil and micronutrients in human and animal diets and become an increasingly important agricultural commodity, with a steady increase in worldwide annual production due to its excellent nutritional value and health benefit (Liu, 1997).

Soybean is now the world's fourth most important crop, only surpassed by wheat, maize and rice. The bulk of the harvest is solvent-extracted, and the defatted soymeal (50% protein) makes the raising of farm animals possible on an industrial scale never before seen in human history. A relatively small proportion of the crop is consumed directly by humans. In East Asia Soybean has been consumed for centuries in many forms. In China, Japan, and Korea, soybean products made are popular parts of the diet. The Chinese invented tofu and also made use of several varieties of soybean paste as seasonings. The beans can be processed in a variety of ways. Common forms of soybean include soy meal, soy flour, soy milk, tofu, textured soybean protein (TSP), which is made into a wide variety of vegetarian foods, (some of them intended to imitate meat), tempeh, soy lecithin and soybean oil. Soybeans are also the primary ingredient involved in the production of soybean sauce.

Yams (*Dioscorea* spp.) are the Dioscoreaceae vine plants grown and staple food in tropical and sub-tropical regions that produce underground tubers (IITA, 2010). Yams are edible energy-rich tuber crops developed from modified and thickened underground stems storage organs which they are bulky, perishable, and vegetative propagated by the tuber (Tamiru, M. 2006, Bradshaw, J. E, editor. 2010). Among different type of root and tuber crops, yarms (*Dioscorea* spp.) are the common usable staple food, livestock feed, or as raw materials for the production of different industrial products (Tamiru, and Maass, 2007). Yams are grown widely throughout tropical regions around the world and are a staple food for millions people (Bourke, 2004).

Over 58.8 million tons of yams were produced in the world in 2012, out of which 92.2% were from West Africa. Nigeria is the largest producer of yams in the world, followed by Ghana, Cote D'Ivoire, Benin, Togo, and Cameroon (FAO, 2013). The total annual production of yam in

Ethiopia was estimated at about 277,000 metric tons from an area of about 68,000 ha, corresponding to a yield of about 4 tons per hectare (FAO, 2005).

Yam is assumed to be the fourth most important tuber crop in the world next to potato, cassava and sweet potato (Edgerton, 2009.). These crops can be grown at most any time of the year so long as temperature does not freeze. And be very careful about spearing them or breaking them when digging the longer tubers. Store tuber in low light and cool temperatures where they can last up to several months. Yam (*Dioscorea* spp.) is widely grown in many parts of Ethiopia particularly in southern and southwest parts of the country and plays a vital role in local subsistence in the region. It serve as a 'life saving' plant group for the marginal farming and forest dwelling communities, during periods of food scarcity (Agbaje, 2003). True yams are ubiquitous lowland tropical food plants (Ikeorgu, 2000.); and are a staple foodstuff and also important as a secondary (famine) food. Yam is an attractive crop in poor farms with limited resources. Yam is also available all year round making it preferable to other unreliable seasonal crops. These characteristics make yam a preferred food and a culturally important food security crop in some sub-Saharan African countries (Izekor and Olumese, 2010). Yam is a staple food for millions of people in the world, providing an important source of carbohydrate and more protein on dry weight basis than is commonly assumed (IITA, 1992). It is considered to be the most nutritious of the tropical root crops (Wanasundera and Ravindran 1994). It contains approximately four times as much protein as cassava, and is the only major root crop that exceeds rice in protein content in proportion to digestible energy and Yam is also a good source of vitamins A and C, and of fiber and minerals. Its relatively low calcium content is related to low concentrations of calcium oxalate, an ant nutritional factor (Bradbury and Holloway 1988).

Food consumption patterns in Ethiopia are diverse, and unlike in many other countries, no single crop dominates the national food basket (e.g., rice in most of East Asia, maize in Latin America, or cassava in Central Africa). The Ethiopian food basket consists of a wide variety of grains and other staples. However, consumption levels and mixes of these grains vary widely according to differences in agro-ecology, socioeconomic levels, and livelihood strategies.

Wheat is one of the important grain crops produced worldwide. Ethiopia is the second largest wheat producer in sub Saharan Africa, next to South Africa, area under wheat cultivation expanded from 1.4 million hectare 2004/05 to 1.6 million hectare by 2010/11 and from these the production yield was 2.9 million tones(Wheat import trade in Ethiopia /2005-13/). Wheat accounts for more than 10 percent of the food budget in many regions, including Tigray, Amhara, Oromia, Somale, and Afar. Again, it is important to note that these regions are among the largest wheat food aid recipients in the country. In general, calorie consumption across Ethiopia is low, but a high percentage of this consumption is coming from cereals.

Wheat is used for the manufacturing of flour for different purposes. Bread, biscuits and pasta products such as macaroni, spaghetti and noodles are some of the industrial products. Wheat is known to be a major source of energy and protein. Traditionally, wheat is used for making "dabo", "dabokolo","ganfo", "kinche" and other types of food. In terms of quality, wheat provides an optimum amount of energy, protein, calcium and iron. Chemically, wheat contains 339 kcal of energy, 10.3 g of protein, and 49 mg of calcium and 1.5 mg of iron/100 g of whole grain (Aberra Bekele, 1991). The study output going to optimize bread nutrient quality and sensory acceptability by fortification of the three crops which is different from the traditional one.

The aim of this study was to use soybean and yam in wheat bread in order to maximize the potential of soybean protein and yam carbohydrate and protein in an attempt to address Protein Energy Malnutrition.

## Materials and Method

### Experimental Site

The activity was conducted at Bako Agricultural Engineering Research Center and Food Science Research Directorate Laboratory of Oromia Agricultural Research Institute.

### Samples Collection

The raw materials of wheat (Limu variety), Soybean (Boshe variety) and yam (Bulcha variety) were collected from Bako Agricultural Research Center. Other ingredients and raw materials were Purchased and stored in the freezer in laboratory. Other facilities and utensils were sourced from Food science research directorate laboratory of Oromia Agricultural Research Institute.

## Preparation of Raw Materials

### Preparation of Soybean Flour

The soybean was processed before converted to flour to remove all anti-nutritional factors. The flour from soy bean was produced by cleaning, sorting, soaking, DE hulling and drying at 70°C for 12hours and milled into coarse particles, winnowed later milled into powder and the obtained flour was sieved in a standard sieve of 400µm particle size.

### Preparation of Yam flour

The method of (Binta et al, 2010) was adopted in the preparation of yam flour. 10kg of yam was washed, peeled, thinly sliced, washed and blanched in boiling water for 4 min and then sun dried until the moisture content was between 10% and 13%. The dried chip was milled to pass through 400 µm mesh sieve to obtain the flour. The Yam flour was packaged in Polyethylene bags and stored until ready for further use.

(A) *yam Slicing* (B) *yam Drying* (C) *dried yam Milling*

(D) Sieving (E) filling and packing (F) packed samples (G) Blended flours

***Figure 1. Preparation of Yam flour***

**Preparation of wheat flour**

The wheat was sorted, cleaned, milled into coarse particles and milled again into fine powder after which it is sieved using a standard sieve of 400µm particle size. The obtained flour was packaged in Polyethylene bags and stored in the freezer.

**Experimental design and formulation of composite flours**

As the first step, the food quality characteristics of the targeted food product/*Bread* were identified and selected, then the quality indices that are specific and important for the food product were selected as responses and must be determined and presented numerically to make modeling possible. Thus, iron, zinc, potassium, phosphorus, magnesium, phytic acid, crude fat, crude protein and overall sensory acceptability were identified as bread quality characteristics. As important component variables, the raw materials in the recipe that significantly affect the desired quality of the food products were identified considering cost and availability. Therefore, wheat, Soybean and yam were identified as a raw material.

Table 1. Experimental design for ratio of mixture components

| Std | Run | Block | Component 1 A,Wheat Flour (%) | Component 2 B,Soya Bean Flour(%) | Component 3 C,Yam Flour (%) |
|---|---|---|---|---|---|
| 12 | 1 | Block 1 | 0.600 | 0.000 | 0.400 |
| 10 | 2 | Block 1 | 0.596 | 0.297 | 0.107 |
| 3 | 3 | Block 1 | 0.800 | 0.000 | 0.200 |
| 7 | 4 | Block 1 | 0.688 | 0.182 | 0.130 |
| 11 | 5 | Block 1 | 0.697 | 0.280 | 0.023 |
| 8 | 6 | Block 1 | 0.652 | 0.084 | 0.264 |
| 14 | 7 | Block 1 | 0.600 | 0.400 | 0.000 |
| 4 | 8 | Block 1 | 0.550 | 0.223 | 0.227 |
| 13 | 9 | Block 1 | 1.000 | 0.000 | 0.000 |
| 16 | 10 | Block 1 | 0.550 | 0.223 | 0.227 |
| 1 | 11 | Block 1 | 0.600 | 0.400 | 0.000 |
| 9 | 12 | Block 1 | 0.865 | 0.067 | 0.068 |
| 5 | 13 | Block 1 | 0.800 | 0.200 | 0.000 |
| 15 | 14 | Block 1 | 0.800 | 0.000 | 0.200 |
| 6 | 15 | Block 1 | 0.600 | 0.000 | 0.400 |
| 2 | 16 | Block 1 | 1.000 | 0.000 | 0.000 |

The maximum and minimum ranges of ingredients were determined based on the existing knowledge. Thus, Wheat maximum 100% and minimum 55%, soybean: maximum 40% and minimum 0%, Yam: maximum 40% and minimum 0%

***Baking of Bread***

Bread baking was carried out by straight dough methods (mixing and kneading, fermentation, molding, proofing, baking, cooling and packaging), All ingredients which included the 200 gram of flour, 3 gram of yeast, 4 gram of bread improver, 2 gram of salt ,6 gram of sugar and 140-

200ml of water were mixed for 15 minutes in a dough mixer. The different dough samples were placed in baking pans smeared with vegetable oil and was covered for the dough to ferment resulting in gas production and gluten development for about 1 hour. The dough was then baked in the oven at recommended temperature for recommended time ($220^{0C}$ for 30 minutes). The baked bread was carefully removed from the pans and allowed to cool and packaged in polyethylene bags for further analysis.

**Proximate composition analysis**

Proximate composition of the resulting wheat, soybean flour, Yam flour and their blends was determined by the methods of AOAC, 2005 on dry matter basis. Ash, crude protein (N x 6.25), fat (ether extract) and fiber are evaluated. All measurements are made in triplicate. Total carbohydrate was calculated by difference.

**Moisture Content**

Moisture Content of raw materials and the product was determined by hot air oven method as described by AOAC (2005). An empty crucible was weighed and 2g of the sample was transferred into the crucible. This was taken into the hot air oven and dried for 24 hours at 100°C. The crucible and its contents were cooled in the Incubator and their weights taken. The loss in weight was regarded as moisture content and expressed as

$$\text{Moisture Content} = \frac{Weight\ loss}{weight\ of\ samples} \text{x}100$$

**Ash Content**

The ash dish was washed and placed into a muffle furnace for 30 min at 5500C. The dish was then removed and cooled in desiccators for about 30 minutes to room temperature after which each dish was weighed 3 g of flour and bread sample were added into each dish. Then dishes were place on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the sample become thoroughly charred. The dishes was placed inside the muffle furnace at 550 0C for 6 hrs, and removed from the muffle and then placed in desiccators for 1hr to cool. Finally the clear white ashes were obtained. Weight of total ash were calculated by difference and expressed as percentage of sample.

Ash Content (%) =$\frac{Weight\ of\ ash}{weight\ of\ samples}$x100

**Protein Content**

Crude protein content was determined by the micro-Kjeldahl procedure by taking about 0.5g flour samples using a K2SO4 - CuSO4 catalyst in according to AACC (2000) method 46-12.

**Mineral Content**

The mineral content of bread sample was determined by using the method described by AOAC (1998). The ash obtained from the ash analysis earlier was used in the determination of the minerals content. The ash was placed in porcelain crucibles, and dissolved with few drops of distilled water, followed by 5ml of 2N hydrochloric acid and filtered through Whiteman filter paper into 100 ml volumetric flask. The minerals such as calcium (Ca), magnesium (mg), Zinc (Zn), Sodium (Na), Iron (Fe) and Potassium (K) was then determined by using Flame photometer while phosphorous (P) content was determined using spectrophotometer finally crude fat, Carbohydrate and energy was calculated.

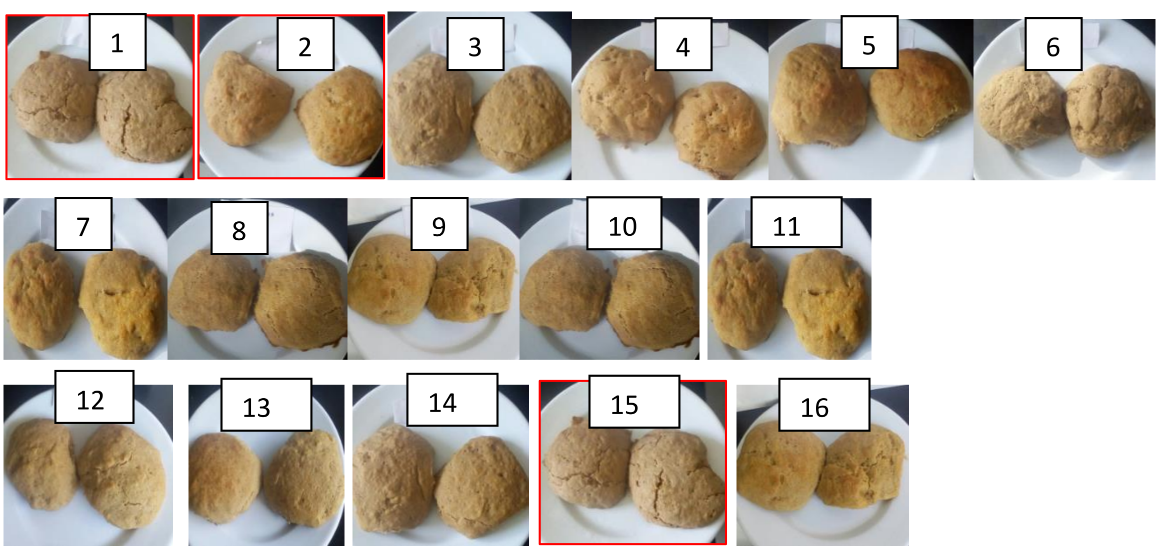


***Figure 2 :Baked bread samples***

**Sensory Acceptability Test of Bread**

The physical attributes of products are key measures of quality, including the sensory parameters such as color, odor, flavor, texture and overall acceptability using standard methods. Sensory evaluation of bread samples by nine consumer panelists were carried out. The samples were served in random order, identified by the coded (Br1 to Br16) on disposable plates. This analysis was conducted at Oromia Agricultural Research Institute, in the Food science Laboratory, where most of the judges were employees in the Institution, who are aware of sensory evaluation of food. Just before each test session, panelists were given orientation about the procedure of sensory evaluation. They were asked to fill questionnaire prepared for the evaluated sensory attributes of the bread samples, i.e., color, odor, flavor, texture and overall acceptability using a 9- point hedonic scale where 9 indicates extremely like and 1 extremely dislike AOAC (2005). Drinking water was provided for rinsing their mouth between samples.

*Figure 3. Panelists during sensory evaluation*

**Statistical Analysis**

The study was conducted to determine wheat, Soybean flour and Yam flour blending ratio for improved nutritional and processing quality of bread production by varying the blending ratio. Data were subjected to analysis of variance (ANOVA). The analysis of variance (ANOVA) of the different data was carried out using appropriate software (design expert Version 7.0.). Mean values were considered at 95% significance level ($p < 0.05$). The hedonic scores for the sensory evaluation were analyzed by ANOVA. Means were compared for the significant factors by least significant difference (LSD) test and significance was accepted at 5% level.

## Result and Discussion

### Proximate composition of Raw Materials

Table 2A.Chemical Composition of Mixture Component Flours (on dry weight basis)

| Flour | MC % ± *SD* | Fiber% ± *SD* | Crude Fat%± *SD* | Ash % ± *SD* | Zinc(ppm) ± *SD* | Iron(ppm) ± *SD* | Magnesium(ppm) ± *SD* |
|---|---|---|---|---|---|---|---|
| Wheat | *8.88 ± 0.07* | *2.7 ±0.21* | *0.3 ± 0.07* | *4.6 ± 0.1* | *38.5 ± 0.64* | *44.82 ± 4.3* | *2066.01 ± 191.20* |
| Soybean | *5.8 ± 0.08* | *3.6 ± 0.5* | *21.6 ± 0.11* | *2.6 ±0.20* | *8.22 ± 1.64* | *6.69 ± 0.70* | *367.06 ± 12.37* |
| Yam | *9.57 ± 0.02* | *1.7 ± 0.19* | *3.8 ± 0.02* | *1.6 ±0.14* | *26.92 ± 5.52* | *34.03 ± 1.83* | *937.52 ± 25.95* |
| Mean ± *Sd* | 8.05 ± 0.06 | 2.67 ± 0.3 | 8.6 ± 0.07 | 2.6 ± 0.16 | 24.64 ± 2.6 | 28.51 ± 2.28 | 1123.53 ± 76.51 |

Raw materials flour used for preparation of blended bread wheat, Yam flour and Soybean flour contains 8.88, 5.8, 9.57 % moisture content and 4.6, 2.6 and 1.6 % ash content, respectively and also contains mean value: 8.05%, 2.67, 8.6, 2.6, 24.64, 28.51, 1123.53, MC, Fiber, crude fat, Ash, Zinc, Iron, Magnesium,

Table 2B.Chemical Composition of Mixture Component Flours (on dry weight basis)

| *Flour* | Calcium(ppm) ± SD | *Sodium(ppm) ± SD* | *potassium(ppm) ± SD* | *Phosphorous(ppm) ± SD* | *Protein % ± SD* | *Carbohydra %± SD* | *energy kcal ± SD* |
|---|---|---|---|---|---|---|---|
| *Wheat* | *1232.2 ±12.08* | *101.8 ±5.31* | *9122.4± 54.1* | *5255 ± 38.3* | *4.5 ± 0.15* | *79.02 ± 0.14* | *336.9 ± 0.49* |
| *Soybean* | *544.71± 31.86* | *154.81 ± 10* | *7156.24 ± 22.1* | *1159.8±17.2* | *38.70±1.2* | *27.68±1.77* | *459.67±3.6* |
| *Yam* | *819.03± 56.18* | *84.51 ± 5.17* | *4468.1 ± 15.15* | *2538.53± 43.16* | *14.7 ± 0.6* | *68.64 ± 0.53* | *367.3 ± 0.79* |
| Mean ± *Sd* | *865.31±33.37* | *113.71±6.83* | *6915.57±30.4* | *2984.44±32.89* | *19.3±0.65* | *58.45±0.81* | *387.93±1.62* |

Raw materials flour used for preparation of blended bread wheat, Yam flour and Soybean flour contains 865.31ppm, 113.71, 6915.57ppm, 2984.44, 19.3, 58.45 and 387.93 Calcium, Sodium, potassium, Phosphorous, protein, carbohydrate and energy, respectively.

**Nutritional compositions of Blended flours**

Table 3A. Proximate composition of blended flour samples before bread baking

| F.N | M.C.% ± *SD* | Fiber% ± *SD* | Crude Fat% ± *SD* | Ash% ± *SD* | Zinc(ppm) ± *SD* | Iron(ppm) ± *SD* | Mag(ppm) ± *SD* |
|---|---|---|---|---|---|---|---|
| F1 | *9.4±0.1* | *2.04±0.17* | *2.14±0.18* | *2.05±0.04* | *15.6±1.15* | *17.3±0.41* | *647 ±11.67* |
| F2 | *8.15±0.1* | *2.60±0.09* | *10.02±0.17* | *2.28±0.26* | *26.05±0.98* | *36.07±0.41* | *1209.36±31.70* |
| F3 | *8.26±0.01* | *2.24±0.17* | *2.82±0.18* | *1.71±0.11* | *18.90±0.55* | *27.44±0.63* | *730.17±37.04* |
| F4 | *7.74±0.27* | *2.60±0.11* | *6.39±0.12* | *2.25±0.19* | *23.40±2.23* | *31.04±0.80* | *967.84±6.53* |
| F5 | *7.22±0.22* | *2.99±0.14* | *8.71±0.33* | *2.13±0.08* | *26.38±1.75* | *32.59±6.12* | *1115.14±23.13* |
| F6 | *7.87±0.08* | *2.41±0.07* | *4.21±0.12* | *2.11±0.10* | *20.37±3.09* | *26.05±0.71* | *799.07±23.47* |
| F7 | *6.93±0.12* | *2.37±0.19* | *11.39±0.46* | *2.77±0.31* | *29.69±5.57* | *37.79±0.68* | *1239.19±30.12* |
| F8 | *8.43±0.16* | *2.05±0.07* | *6.90±0.29* | *2.45±0.10* | *24.87±4.30* | *30.92±0.56* | *985.50±12.35* |
| F9 | *8.99±0.12* | *2.00±0.19* | *3.34±0.14* | *1.42±0.05* | *20.59±0.50* | *35.34±0.47* | *832.07±13.50* |
| F10 | *8.43±0.16* | *2.05±0.07* | *6.90±0.29* | *2.45±0.10* | *24.87±4.30* | *30.92±0.56* | *985.50±12.35* |
| F11 | *6.93±0.12* | *2.37±0.19* | *11.39±0.46* | *2.77±0.31* | *29.69±5.57* | *37.79±0.68* | *1239.19±30.12* |
| F12 | *8.30±0.13* | *2.85±0.13* | *4.01±0.04* | *1.67±0.07* | *25.01±2.87* | *34.95±0.56* | *860.09±19.94* |
| F13 | *7.67±0.09* | *2.92±0.14* | *7.24±0.29* | *2.12±0.11* | *25.78±0.98* | *34.91±5.90* | *1146.54±44.65* |
| F14 | *8.26±0.01* | *2.24±0.17* | *2.82±0.18* | *1.71±0.11* | *18.90±0.55* | *27.44±0.63* | *730.17±37.04* |
| F15 | *9.4±0.1* | *2.04±0.17* | *2.14±0.18* | *2.05±0.04* | *15.6±1.15* | *17.3±0.41* | *647 ±11.67* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| F16 | *8.99±0.12* | *2.00±0.19* | *3.34±0.14* | *1.42±0.05* | *20.59±0.50* | *35.34±0.47* | *832.07±13.50* |
| Mean ± *Sd* | 8.19±0.012 | 2.36±0.13 | 5.86±0.098 | 2.09±0.099 | 22.89±0.37 | 30.82±0.30 | 935.37±2.32 |
| Cv(%) | 0.15 | 5.33 | 1.68 | 4.76 | 1.60 | 0.96 | 0.25 |
| Max | 9.4 | 2.99 | 11.39 | 2.77 | 29.69 | 37.79 | 1239.19 |
| Min | 6.93 | 2 | 2.14 | 1.42 | 15.6 | 17.3 | 647 |

*Where Fl_16 were mixed flours of samples ratio according to table 1 which was produced mixer design software CV=coefficient of variation, SD=standard Deviation, max=maximum and min=minimum value*

According to table 3A the maximum value proximate composition of bread sample like (M.C.), Fiber, crude fat, Ash, Zinc, Iron, and Magnesium were obtained 9.4%,2.99%,11.39,2.77, 29.69ppm,37.79ppm and1239.19ppm on F1,F5,F11,F11,F11,F1,F11, and the minimum value 6.93%,2%,2.14,1.42,15.6ppm,17.3ppm and 6479ppm was obtained on F1,F16,F15,F16,F15,F15 and F15 respectively .The mean value of Moisture content (M.C.), Fiber, crude fat, Ash, Zinc, Iron, and Magnesium were in ranges 6.93_9.4%, 2_2.99%, 2.14_11.39, 1.42_2.77, 15.29.69ppm, 17.3 – 37.79ppm and 647–1239.19ppm, respectively. From tables 3A determined minerals in bread samples magnesium consists of highest ratio when compared to other minerals. Therefore, the difference observed from physicochemical among bread samples might be due to composition of soya bean and yam to wheat.

Table 3 B. Mineral composition flours mixed

| F.N | Calcium<br>± *SD* | Sodium<br>± *SD* | potassium<br>± *SD* | Phosphorous<br>± *SD* | Protein%<br>± *SD* | CHO%<br>± *SD* | Energy kcal<br>± *SD* |
|---|---|---|---|---|---|---|---|
| F1 | *703.4 ± 18.7* | *132.1 ± 31.24* | *6134.5 ± 200.5* | *2116.7 ± 41.4* | *8.7 ± 1.3* | *75.7 ± 1.5* | *356.75±0.25* |
| F2 | *1111.8 ± 27.6* | *141 ± 4.1* | *6124.5 ± 178.3* | *3441.3 ± 26.9* | *19.63 ± 1.35* | *57.32 ± 1.1* | *397.97±1.04* |
| F3 | *673.7 ± 43.8* | *260.6 ± 76.15* | *5016.94 ± 18.28* | *2696.3 ± 5255* | *10.43 ± 1.6* | *74.53 ± 1.5* | *365.27±1.72* |
| F4 | *967.84 ± 46.95* | *226.5 ± 76.09* | *5940.98 ± 63.6* | *2844.54 ± 20.9* | *9.97 ± 0.86* | *71.04 ± 0.8* | *381.58±1.49* |
| F5 | *1128.13 ± 42.67* | *239.56 ±71.08* | *6162.58 ± 36.12* | *3146.03 ± 140.9* | *12.94 ± 0.13* | *66 ±0.7* | *394.14±1.17* |
| F6 | *810.39 ± 20.52* | *216.11 ±40.71* | *5890.85 ± 28.32* | *2423.51 ± 21.36* | *7.82 ± 0.26* | *75.59 ±0.2* | *371.51±0.75* |
| F7 | *1187.22 ±55.2* | *203.58 ±60.9* | *6633.43 ± 51.1* | *3660.93 ± 76.60* | *13.95 ± 0.01* | *62.57 ±0.79* | *408.63±1.22* |
| F8 | *968.14 ± 30.59* | *195.62 ±53.77* | *6451.41 ± 22.97* | *2978.05 ± 70.29* | *10.17 ± 0.36* | *70 ± 0.21* | *382.81±1.41* |
| F9 | *670.14 ±42.28* | *168.09 ±13.8* | *4395.69 ± 29.89* | *2517.85 ± 33.41* | *7.58 ± 0.4* | *76.66 ± 0.64* | *367.00±0.59* |
| F10 | *968.14 ± 30.59* | *195.62 ±53.77* | *6451.41 ± 22.97* | *2978.05 ± 70.29* | *10.17 ± 0.36* | *70 ± 0.21* | *382.81±1.41* |
| F11 | *1187.22 ± 55.2* | *203.58 ±60.9* | *6633.43 ± 51.1* | *3660.93 ± 76.60* | *13.95 ± 0.01* | *62.57 ± 0.79* | *408.63±1.22* |
| F12 | *813.84± 26.09* | *191.45 ±87.3* | *5028.63 ± 42.74* | *2566.45 ± 27.01* | *7.99 ± 0.32* | *75.18 ± 0.24* | *368.78±0.34* |
| F13 | *1006.22 ± 29.27* | *193.79 ±48.03* | *5670.40 ± 48.03* | *3017.76 ± 59.55* | *10.54 ± 0.5* | *69.51 ± 0.58* | *385.34±1.95* |
| F14 | *673.7 ± 43.8* | *260.6 ±76.15* | *5016.94 ± 18.28* | *2696.73 ± 5255* | *10.43 ± 1.6* | *74.53 ±1.5* | *365.27±1.72* |
| F15 | *703.4 ± 18.7* | *132.1 ±31.24* | *6134.5 ± 200.5* | *2116.7 ± 41.4* | *8.7 ± 1.3* | *75.7 ± 1.5* | *356.75±0.25* |
| F16 | *670.14 ± 42.28* | *168.09 ±13.8* | *4395.69 ± 29.89* | *2517.85 ± 33.41* | *7.58 ± 0.4* | *76.66 ± 0.64* | *367.00±0.59* |
| Mean ± *SD* | 890.2±9.14 | 195.52±7.74 | 5755.11±36.93 | 2836.26±25.49 | 10.66±0.74 | 70.84±0.86 | 378.77±0.4 |
| Cv (%) | 1.03 | 3.96 | 0.64 | 0.90 | 6.98 | 1.21 | 0.11 |
| Max | 1187.22 | 260.6 | 6633.43 | 3660.93 | 19.63 | 76.66 | 408.63 |
| Min | 670.14 | 132.1 | 4395.69 | 2116.7 | 7.58 | 57.32 | 356.75 |

*Where Fl_16 were mixed flours of samples ratio according to table 1 which was produced mixer design software CV=coefficient of variation, SD=standard Deviation, max=maximum and min=minimum value*

According to table 3B the maximum value proximate composition of Mixed flours sample like Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy were obtained1187.22ppm,260.6ppm,6633.43ppm,3660.93ppm,19.63%,76.66% and 408.63kcal respectively on F7,F3,F7,F7,F7,F9 and F7, and the minimum value 670.14ppm, 132.1ppm, 4395.69ppm,2116.7ppm,7.58%,57.32% and 356.75kcal respectively obtained on F9,F1,F9,F1,F9,F2 and F1 respectively .The mean concentration of Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy value ranges were 670.14–1187.22ppm,132.1_260.6ppm,4395.69_6633.43ppm,2116.7_3660.93ppm,7.58_19.63%, 57.32_76.66% and 356.75-408.63kcal, respectively.

Among determined minerals in bread samples potassium consists of the highest ratio when compared to other minerals. Therefore, the difference observed from physicochemical among bread samples might be due to composition of soya bean and yam to wheat. Therefore, chemical compounds in the food such as the amount of protein or carbohydrates a food contains may affect a consumer’s acceptance of the product (Shepherd, R. 1988).The mean values for proximate and minerals composition of wheat flour, Soybean flour and Yam flour were calculated in 100g of flours and the obtained results are presented in Table **2**. Yam flour compared to wheat flour contains lower level of crude protein and higher level of moisture content and ash contents. Considering to minerals and proximate composition, Yam flour has higher level of sodium, potassium, and Iron than wheat flour. It also contains lower level of Phosphorus, Zinc , Magnesium and calcium than wheat flour .Therefore, blending of Yam flour to wheat flour and Soybean flour would contribute to increase nutrients which were lower in one of the component of composite flours of breads.

**Nutritional compositions of Baked Bread**

Table 4 A. Proximate composition of blended Bread samples

| S.N | Moisture ± *SD* | Fiber ± *SD* | Crude Fat% ± SD | Ash% ± *SD* | Zinc(Ppm) ± *SD* | Iron(Ppm) ± *SD* | Magnesium(ppm) ± *SD* |
|---|---|---|---|---|---|---|---|
| 1 | *4.01 ± 0.01* | *1.92 ± 0.14* | *1.97 ± 0.43* | *2.76 ± 0.1* | *20.97 ± 1.35* | *27.57 ± 0.79* | *697.82 ± 10.41* |
| 2 | *4.26 ± 0.05* | *2.40 ± 0.04* | *8.52± 0.28* | *3.75 ± 0.03* | *41.96 ± 5.88* | *37.49 ± 0.98* | *1165.77 ± 38.51* |
| 3 | *4.27 ± 0.14* | *2.50 ± 0.07* | *2.51 ± 0.06* | *2.84 ± 0.01* | *25.80 ± 1.26* | *29.72 ± 0.88* | *757.12 ± 8.95* |
| 4 | *3.81 ± 0.15* | *2.39 ± 0.02* | *5.52 ± 0.04* | *3.40 ± 0.06* | *31.70 ± 0.10* | *33.93 ± 0.40* | *1082.5 ± 11.79* |
| 5 | *3.83 ± 0.17* | *2.25 ± 0.10* | *7.59± 0.12* | *3.53 ± 0.14* | *26.83 ± 0.33* | *26.98 ± 0.42* | *795.01 ± 25.58* |
| 6 | *4.06 ± 0.17* | *2.17 ± 0.08* | *3.72 ± 0.53* | *3.24 ± 0.07* | *28.10 ± 0.99* | *31.11 ± 1.16* | *965.10 ± 27.85* |
| 7 | *3.51 ± 0.08* | *3.04 ± 0.44* | *9.63 ±0.28* | *3.84 ± 0.07* | *29.72 ± 1.08* | *35.89 ± 1.64* | *1099.7 ± 39.47* |
| 8 | *4.03 ± 0.21* | *2.19 ± 0.23* | *6.20 ±0.19* | *3.53 ± 0.04* | *26.65 ± 1.15* | *29.66 ± 0.57* | *823.27 ± 3.29* |
| 9 | *3.79 ± 0.05* | *2.36 ± 0.03* | *2.41 ±0.03* | *2.69 ± 0.08* | *33.21 ± 0.14* | *38.98 ± 0.55* | *1252.3 ± 18.10* |
| 10 | *4.03 ± 0.21* | *2.19 ± 0.23* | *6.20 ±0.19* | *3.53 ± 0.04* | *26.65 ± 1.15* | *29.66 ± 0.57* | *823.27 ± 3.29* |
| 11 | *3.51 ± 0.08* | *3.04 ± 0.44* | *9.63 ±0.28* | *3.84 ± 0.07* | *29.72 ± 1.08* | *35.89 ± 1.64* | *1099.7 ± 39.47* |
| 12 | *4.23 ± 0.08* | *2.68 ± 0.08* | *3.84 ±0.03* | *3.07 ± 0.06* | *30.75 ± 0.42* | *33.24 ± 0.33* | *976.01 ± 22.35* |
| 13 | *3.83 ± 0.05* | *3.01 ± 0.23* | *6.34 ±0.04* | *2.45 ± 0.10* | *40.46 ± 0.66* | *34.88 ± 0.45* | *826.23 ± 13.35* |
| 14 | *4.27 ± 0.14* | *2.50 ± 0.07* | *2.51 ±0.06* | *2.84 ± 0.01* | *25.80 ± 1.26* | *29.72 ± 0.88* | *757.12 ± 8.95* |
| 15 | *4.01 ± 0.01* | *1.92 ± 0.14* | *1.97± 0.43* | *2.76 ± 0.1* | *20.97 ± 1.35* | *27.57 ± 0.79* | *697.82 ± 10.41* |
| 16 | *3.79 ± 0.05* | *2.36 ±0.03* | *2.41 ± 0.03* | *2.69 ± 0.08* | *33.21 ± 0.14* | *38.98± 0 .55* | *1252.3 ± 18.10* |
| Mean ± *sd* | 3.95±0.16 | 2.43±0.089 | 16.94 ± 2.08 | 3.17 ± 0.14 | 29.53 ± 0.022 | 32.58 ± 0.83 | 941.94 ± 7.73 |
| C.v (%) | 4.02 | 3.67 | 12.28 | 4.41 | 0.075 | 2.56 | 0.82 |
| Max | 4.27 | 3.04 | 9.63 | 3.84 | 41.96 | 38.98 | 1252.3 |
| Min | 3.51 | 1.92 | 2.41 | 2.45 | 20.97 | 26.98 | 697.82 |

*Where S.N 1_16 was bread of samples which was produced mixer design, CV=coefficient of variation, SD=standard Deviation, max=maximum and min=minimum value*

According to table 4A the maximum value proximate composition of bread sample like (M.C.), Fiber, crude fat, Ash, Zinc, Iron, and Magnesium obtained value of 4.27%,3.04%, 9.63,3.84, 41.96ppm,38.98ppm and 1252.3ppm on F2, F7, F11, F7, F2, F9, F16, and the minimum value 3.51%, 1.92%, 2.41, 2.45, 20.97ppm, 26.98 ppm and 697.82ppm obtained on F7, F15, F9 , F13, F15, F5 and F15, respectively .The mean value of Moisture content (M.C.), Fiber, crude fat, Ash, Zinc, Iron, and Magnesium have got the ranges of 3.51_4.27%, 1.92_3.04%, 2.41_97, 2.45_3.84, 20.97–41.96ppm, 26.98 – 38.98ppm and 697.82–1252.3ppm, respectively.

Table 4 B. Mineral Composition of bread from different flour mixtures

| S.N | Calcium | Sodium | potassium | Phosphorous | Protein | CHO | Energy kcal |
|---|---|---|---|---|---|---|---|
| 1 | *720.7 ± 32.3* | *2248.1 ± 29.5* | *5836.1 ± 18.1* | *4100.2±49.3* | *5.99±0.3* | *83.36 ±0.76* | *375.16±1.30* |
| 2 | *1199.4 ±19.2* | *2253.73±14.7* | *6439.35 ± 65* | *5028.3 ± 60.4* | *12.25±0.42* | *68.81±0.44* | *400.99±1.14* |
| 3 | *746.15±48.14* | *2199.63±27.69* | *5266.59±38.05* | *4113.12±50.25* | *7.34±0.02* | *80.53±0.23* | *374.08±0.47* |
| 4 | *1030.65±9.5* | *2245.01±8.45* | *6454.82±16.84* | *4944.35±96.42* | *10.36±0.26* | *74.53±0.10* | *389.20±0.49* |
| 5 | *691.40±28.29* | *2216.92±16.87* | *5255.96±43.61* | *4020.10±33.15* | *12.67±0.39* | *70.13±0.55* | *399.52±1.33* |
| 6 | *805.33±119.56* | *2180.52±31.31* | *5894.32±83.69* | *4404.67±146.07* | *13.71±0.82* | *73.10±1.37* | *380.72±1.84* |
| 7 | *1017.19±24.77* | *2207.43±17.53* | *6010.02±113.8* | *4877.49±159.48* | *7.94±0.62* | *72.04±0.72* | *406.59±1.82* |
| 8 | *791.65±32.41* | *2260.00±0.69* | *5883.70±62.56* | *4528.21±157.37* | *7.33±0.36* | *76.72±0.67* | *391.99±1.74* |
| 9 | *1172.42±21.43* | *2185.78±14.30* | *6612.50±32.38* | *5277.34±53.35* | *7.99±0.05* | *80.78±0.17* | *376.70±0.32* |
| 10 | *791.65±32.41* | *2260.00±0.69* | *5883.70±62.56* | *4528.21±157.37* | *7.33±0.36* | *76.72±0.67* | *391.99±1.74* |
| 11 | *1017.19±24.77* | *2207.43±17.53* | *6010.02±113.8* | *4877.49±159.48* | *7.94±0.62* | *72.04±0.72* | *406.59±1.82* |
| 12 | *893.54±64.48* | *2204.76±10.02* | *6406.67±34.72* | *5027.47±204.23* | *8.07±0.56* | *78.11±0.64* | *379.31±0.19* |
| 13 | *725.38±25.68* | *2169.99±26.63* | *4501.87±69.28* | *3954.75±48.44* | *9.98±0.12* | *74.40±0.13* | *394.55±0.42* |
| 14 | *746.15±48.14* | *2199.63±27.69* | *5266.59±38.05* | *4113.12±50.25* | *7.34±0.02* | *80.53±0.23* | *374.08±0.47* |
| 15 | *720.7 ± 32.3* | *2248.1 ± 29.5* | *5836.1 ± 18.1* | *4100.2±49.3* | *5.99±0.3* | *83.36 ±0.76* | *375.16±1.30* |
| 16 | *1172.42±21.43* | *2185.78±14.30* | *6612.50±32.38* | *5277.34±53.35* | *7.99±0.05* | *80.78±0.17* | *376.70±0.32* |
| Mean±*sd* | 890.11±25.92 | 2217.05±7.67 | 5885.68±20.24 | 4573.27±34.76 | 8.75±1.85 | 76.62±2.51 | 387.08±0.15 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CV | 0.35 | 0.35 | 0.34 | 0.76 | 21.19 | 3.27 | 0.04 |
| Max | 1199.4 | 2260 | 6612.5 | 5277.34 | 13.71 | 83.36 | 406.59 |
| Min | 691.4 | 2169.99 | 4501.87 | 3954.75 | 5.99 | 68.81 | 374.08 |

*Where S.N 1_16 was bread of samples which was produced mixer design, CV=coefficient of variation, SD=standard Deviation, max=maximum and min=minimum value*

According to table 4B, the maximum value proximate composition of bread sample like Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy were obtained 1199.4ppm, 2260ppm, 6612.5ppm, 5277.34ppm,13.71%, 83.36% and 406.59kcal on F2,F10,F16,F9,F6,F1 and F7, and the minimum value 691.4ppm,2169.99ppm,4501.87ppm, 3954.75ppm,5.99%,68.81%and374.08kcal obtained on F5,F13,F13,F13,F1,F2 and F13, respectively .The mean concentration of Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy ranges were 691.4–1199.4ppm, 2169.99_2260ppm, 4501.87_6612.5ppm,3954.75_5277.34ppm, 5.99_13.71%, 68.81_83.36 %and 374.08-406.59kcal respectively. Among determined minerals in yam varieties, potassium consists of the highest ratio when compared to other minerals. Therefore, the difference observed from physicochemical among bread samples might be due to variation in the ratio of the mixture of raw materials.

**Sensory Evaluation of the Bread**

***Table 5 Result of sensory evaluation of Bread samples***

| *S.N* | *Bread Sensory Attributes* | | | | |
|---|---|---|---|---|---|
| | *Color ±SD* | *Odor± SD* | *Texture± SD* | *Flavor± SD* | *Overall acceptance ± SD* |
| *1* | *4 ± 1.49* | *5 ± 1.37* | *4 ± 1.56* | *4 ± 2.23* | *5 ± 1.73* |
| *2* | *7 ± 1.75* | *5 ± 2.49* | *6 ± 1.73* | *5 ± 2.28* | *6 ± 1.52* |
| *3* | *6 ± 2.62* | *5 ± 2.17* | *5 ± 1.69* | *5 ± 1.83* | *6 ± 1.41* |
| *4* | *6 ± 2.15* | *6 ± 2.02* | *6 ± 1.57* | *5 ± 2.2* | *6 ± 1.56* |
| *5* | *7 ± 1.69* | *6 ± 1.93* | *6 ± 1.91* | *6 ± 1.97* | *7 ± 1.25* |
| *6* | *5 ± 1.94* | *5 ± 1.57* | *5 ± 1.83* | *4 ± 1.73* | *5 ± 1.49* |
| *7* | *7 ± 2.0* | *6 ± 1.94* | *6 ± 1.89* | *5 ± 2.4* | *7 ± 1.50* |
| *8* | *6 ± 1.94* | *5 ± 2.02* | *6 ± 2.10* | *6 ± 1.89* | *6 ± 2.18* |
| *9* | *7 ± 1.99* | *5 ± 1.56* | *6 ± 1.94* | *6 ± 2.2* | *6 ± 1.31* |
| *10* | *6 ± 1.94* | *5 ± 2.02* | *6 ± 2.10* | *6 ± 1.89* | *6 ± 2.18* |
| *11* | *7 ± 1.99* | *6 ± 1.85* | *6 ± 1.89* | *5 ± 2.3* | *7 ± 1.50* |
| *12* | *5 ± 2.25* | *5 ± 2.47* | *5 ± 1.76* | *5 ± 1.15* | *5 ± 1.52* |
| *13* | *8 ± 1.07* | *6 ± 2.0* | *6 ± 2.18* | *6 ± 2.16* | *6 ± 1.17* |
| *14* | *6 ± 2.62* | *5 ± 2.17* | *5 ± 1.69* | *5 ± 1.83* | *6 ± 1.41* |
| *15* | *4 ± 1.49* | *5 ± 1.37* | *4 ± 1.56* | *4 ± 2.23* | *5 ± 1.71* |
| *16* | *7 ± 1.99* | *5 ± 1.56* | *6 ± 1.94* | *6 ± 2.2* | *6 ± 1.31* |
| *Mean* | *6.13±0.58* | *5.31±0.25* | *5.5±0.23* | *5.19±0.15* | *5.94 ± 0.18* |
| *CV (%)* | *9.55* | *4.67* | *4.21* | *2.82* | *3.09* |
| *Max* | *8* | *6* | *6* | *6* | *7* |
| *Min* | *4* | *5* | *4* | *4* | *5* |

*Note: In each are significantly different. Where, 1=Dislike extremely, 2= Dislike very much, 3= Dislike moderately, =Dislike slightly, 5=neither like nor dislike, 6=Like slightly, 7=Like moderately. 8= Like very much, 9=like extremely*

All accessed sensory quality attributes strongly significant among bread samples. The perceptions of sensory attributes may be defined as the evaluated adequacy of the product in terms of its set of desirable eating quality characteristics like, color, texture, odor, Flavor and overall acceptance (Nord test T. 2002).

The mean values of color, odor, texture, flavor and overall acceptances of sensory attributes that were given by panelist for bread samples range 4_8, 5_6, 4_6, 4_6 and 5_7 or dislike slightly – like very much, neither like nor dislike- Like slightly, Dislike slightly- Like slightly, Dislike slightly- Like slightly, and neither like nor dislike- Like moderately for color, odor, texture flavor and overall acceptance, respectively.

**Conclusions and Recommendations**

This study determined Moisture content (M.C.), Fiber, crude fat, Ash, Zinc, Iron, Magnesium, Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy) in raw materials flour (wheat, soya bean and yam), mixed flour before bread baking and after baking bread .The study has shown that blending ratio on wheat, Soybean and Yam could be used to produce bread that would be preferred by the consumers. The bread produced from wheat, Soybean and yam had increased nutrients and sensory quality of produced bread and had significantly increased levels of nutrition and mineral content of bread.

Generally the produced flours from soya bean and yam flour fortification to wheat flour have the mean value of Moisture content (M.C.), Fiber, crude fat, Ash, Zinc, Iron, Magnesium , Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy in the following ranges 6.93–9.4%, 2–2.99%, 2.14–11.39, 1.42–2.77, 15.6–29.69ppm, 17.3–37.79ppm, 647–1239.19ppm,670.14–1187.22ppm,132.1–260.6ppm,4395.69–6633.43ppm,2116.7–3660.93ppm, 7.58–19.63%, 57.32–76.66% and 356.75–408.63kcal , respectively.

The produced bread from soya bean and yam Flours fortification to wheat have the mean value of Moisture content (M.C.), Fiber, crude fat, Ash, Zinc, Iron, Magnesium , Calcium (Ca), sodium (Na), potassium (K), phosphorus (P), protein, Carbohydrate and energy had ranges of 3.51–4.27%, 1.92–3.04%,2.41–97,2.45–3.84, 20.97–41.96ppm, 26.98 – 38.98ppm ,697.82–1252.3ppm, 691.4–1199.4ppm,2169.99–2260ppm,4501.87–6612.5ppm, 3954.75–5277.34ppm, 5.99–13.71%, 68.81–83.36 %and 374.08–406.59kcal, respectively .

Based on the current study, it is recommended that:

- ✓ Bread should be prepared by blending yam with other crops rather than preparing from single crops in order to improve nutritional value and sensory attributes.
- ✓ Bread prepared from soya bean and yam flour fortification have more nutritional and sensory attributes than the bread which produced from only wheat flour without any fortification or formulation
- ✓ However, further research is still needed for better improvement of the wheat bread quality from the blending ratio of soybean and yam.

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Food Chemistry and Nutrition Research

# Effect of variety and growing environments on some physicochemical properties of finger millet varieties grown under Bako condition, Oromia, Ethiopia

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## Abstract

*Finger millet is important nutrient and food security cereal crop that can grow at diverse agro ecologies. This research was conducted to study the effect of varieties and growing conditions on some physicochemical properties of different finger millet varieties. The research experiments were performed at Bako and Gute study sites. R statistical software was used to analyze the data and ANOVA was used to determine significant difference and mean separation performed at LSD 5 % significance level. The study revealed that growing environment and cropping seasons had effect on some physicochemical properties of finger millet varieties. The crude protein, crude fat and moisture content of finger millet varied from 8.75 % (Wama) to 10.85 % (Gudatu); 1.27 % (Gudatu & Wama) to 1.70 % (Bareda); and 9.06 % (Meba) to 10.01 % (Diga I) respectively. The mineral contents of finger millets varieties such as Ca, Mg, P and K varied from 277.1 mg/100g (Bako 09) to 416.2 mg/100g (Diga-2); 158.0 mg/100g (Diga-1) to 200 mg/100g (Paddet); 222.5 mg/100g (Addis 01) to 281.0 mg/100g (Paddet); and 335.3 mg/100g (Addis 01) to 496.5 mg/100g (Paddet) respectively. Finger millet varieties that were grown at Bako location contained higher amount of Crude Fat, Crude Fiber, Phosphorus, Ash, Potassium, Magnesium whereas finger millet grown at Gute site contained higher crude protein, Iron, Zinc, Calcium and Manganese. The physicochemical properties of finger millet were also affected by the variety. Black seeded finger millet contains higher moisture, calcium, crude fiber content than white and brown seeded finger millet. Due to its high calcium content food product processed from finger millet can be good source of calcium for children, pregnant and lactating mothers and elderly population. Further study on anti-nutritional content of the Finger millet varieties need to be assessed. Consumers/farmers perception on the preferences of finger millet varieties (**Brown, white and black seedes**) for commercial/food including beverages need to be assessed.*

*Key words*

*Finger Millet, physicochemical properties, growing conditions, Variety*

**Introduction**

Finger millet (Eleusine coracana) is an annual tetraploid cereal widely grown under different agro ecologies (Hulse et al, 1980) and can be grown on poor sandy soil (Purseglove, 1972). It is a climate change resilient crop compared with other cereal crops (Kumar et al., 2018). Finger millet is staple food in parts of Eastern and Central Africa, and India (FAO, 2005). It is indigenous to Ethiopia and occupies 4 % of total area devoted to cereal production and common in Wollega, Iluababor, Eastern Haraghe, Central rift valley (Arsi Negele, Siraro), Gamo Gofa, Tigray, Gojjam and Gonder (Chimdo *et al*.,2006). Finger millet is used for the preparation of different food products such as Injera, Porridge, Bread, Soup and Local beverages (Tela and Areki) (Assefa et al.,2009)

Often finger millet is considered as "Super cereal" due to its high nutrient content and gluten-free grain (Kumer et al., 2016) and its grain can also be stored from 5-10 years. Its quality increases with storage time (Adugna A. 2007). There is a perception that Finger millet is poor man's crop and it is grown by rural poor farmers (Assefa *et al.*, 2009, Ayalew, 2015). Its production and yield is increasing which is attributed to release of improved finger millet varieties (Ayalew, 2015). Consumption of finger millet prevents cancer, cardiovascular diseases; reduce tumor incidence and lower blood pressure-risk of heart disease. It also lower rate of fat absorption and supply gastrointestinal bulk (Saleh et al., 2013).

Breeders are interested most of the time on the agronomic traits but not on physicochemical properties of the grain. There is no sufficient data on physicochemical properties of different finger millet varieties grown in West Oromia, therefore this study was conducted to study the effect of growing environment and variety on some physicochemical properties of different finger millet varieties.

## Materials and Methods

### The study area

The study was conducted at Bako and Gute research stations in Ethiopia for two cropping seasons (2014-2016). Bako Agricultural Research Center (BARC) is located at 9º6'N latitude and 37º09'E longitude with altitude of 1650 m.a.s.l. The soil is deeply weathered and slightly acidic in reaction (Wakene, 2000). Gute sub-station is also found at west and lies at $09^0$ 01.06'N and $036^0$ 38.196'E with altitude of 1915 m.a.s.l. The average rain fall of 1431mm per annum and clay loom soil with slightly acidic property. The two research stations have unimodal pattern of rain distribution, with the rainy period running from April to October (Kebede et al, 2019)

### Experimental design

The experiment was laid out in RCBD on 15 finger millet varieties released by agriculture research centres with three replications for two consecutive growing seasons in 2018/19 and 2019/20 at Bako and Gute research sites. All recommended agronomic practices were applied in the study. Grain samples of each finger millet varieties were collected each year and taken to IQQO Food Science Laboratory for the physicochemical analysis. During the second year experimental year, soil samples were taken for physicochemical analysis.

Two-three kg sample per treatment were collected in plastic bags and transported to the Food Science Research Laboratory. All of the test samples were kept clean and broken grains, dust and other foreign materials were removed before the commencement of test. The finger millet samples were ground in analytical mill to fine flour and passed through 0.2 mm sieve size. All chemicals and reagents used were either analytical or reagent grade.

Table 1, Finger millet varieties studied and year of release

| Variety | Year | Breeder |
|---|---|---|
| Addis 01 | 2015 | Addis Ababa University, Bako Agriculture Research Center |
| Axum | 2016 | Melkassa Agriculture Research Center |
| Bako 09 | 2017 | Bako Agriculture Research Center |
| Bareda | 2009 | >> |
| Diga-2 | 2018 | >> |
| Boneya | 2002 | >> |
| Diga-1 | 2016 | >> |
| Gudatu | 2014 | >> |
| Gute | 2009 | >> |
| Meba | 2016 | Melkassa Agriculture Research Center |
| Paddet | 1998/99 | >> |
| Tadesse | 1998/99 | >> |
| Tesema | 2014 | >> |
| Urji | 2016 | Bako Agriculture Research Center |
| Wama | 2007 | >> |

The color of the finger millet varieties studied could be classified in to three groups. These are brown, white (Urji) and black (Diga-1 and Diga-2) Figure 1.

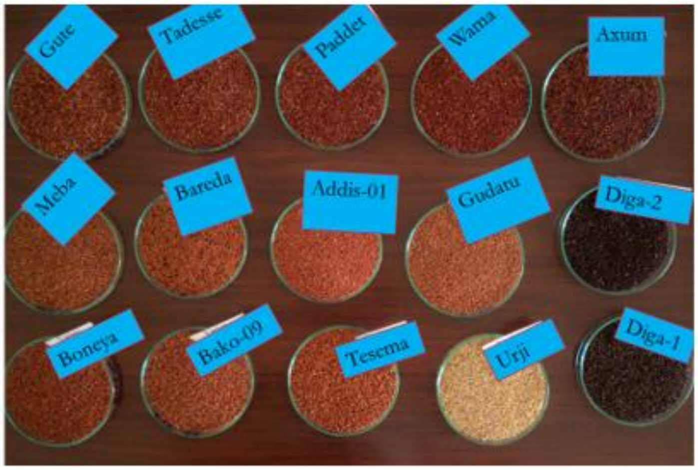


*Figure 1. Color of Finger millet varieties studied*

**Physicochemical analysis**

The moisture content was analysed using AACC 2000 Method 44-15A. Thousand seed weight (TSW) were counted automatically by seed counter from a bulk of threshed seeds of each experimental plot. Crude protein, crude fat, minerals and phosphorus content of finger millet flour were analysed by using AOAC methods 2003.05, 978.10, 975.03 and 986.24 respectively. Composite soil samples were analysed for exchangeable cations and cation exchange capacity according to Pansu and Gautheyroy (2006).

**Data Analysis**

The data generated was subjected to ANOVA using R-statistical software (R-4.1.1 version). Descriptive statistics were used to describe the soil parameters analy used for the study areas. Mean separation was conducted for significant parameters using LSD at 5%.

## Results and discussion

### Crude protein, phosphorus and moisture content of finger millet verities

The crude protein, phosphorus, ash and moisture content of finger millet verities are presented in Table 1. The crude protein content of finger millet grown in the study areas were affected by variety, environmental condition and were significant ($P<0.05$). It ranged from 8.75 % (Wama Variety) to 10.85 % (Gudatu) variety. The crude protein content of finger millet that was grown at Gute site is significantly higher. The moisture content of finger millet varieties ranged from 9.06 % (Meba) to 10.01 % (Diga I). The moisture content of black seeded finger millet was higher than other coloured finger millet which is in line with the study by Shimelis, et al. (2009) but Ramashia et al. (2018) found higher moisture content which could be attributed to collection of samples harvested in different cropping season. Low moisture content enables good storage ability of finger millet.

Phosphorus content of finger millet varieties ranged from 222.5 mg/100g for Addis 01 to 281.0 mg/100g for Paddet finger millet varieties. Finger millet varieties had higher amount of phosphorus content than reported by Shimelis et al. (2009). Bugum and others reported phosphorus content of finger millet to be 283 mg/ 100 g which is comparable to paddet variety (Bugum et al. 2017).

Table 2. Combined Mean for Crude protein, Phosphorus, Ash and Moisture content of finger millet varieties (Whole seed grain).

| Variety | Protein (%) | P (mg/100 g) | Ash (%) | Moisture (%) |
|---|---|---|---|---|
| Addis 01 | $9.46^{cde}$ | $222.5^{f}$ | $2.22^{ef}$ | $9.51^{bcde}$ |
| Axum | $9.21^{cdef}$ | $247.7^{bcd}$ | $2.23^{ef}$ | $9.73^{abc}$ |
| Bako 09 | $8.90^{def}$ | $224.6^{f}$ | $2.13^{f}$ | $9.79^{abc}$ |
| Bareda | $8.82^{ef}$ | $236.9^{de}$ | $2.36^{bc}$ | $9.33^{def}$ |
| Diga-2 | $8.89^{def}$ | $242.0^{d}$ | $2.40^{ab}$ | $10.00^{a}$ |
| Boneya | $9.02^{cdef}$ | $244.5^{cd}$ | $2.19^{ef}$ | $9.53^{bcde}$ |
| Diga- 1 | $9.03^{cdef}$ | $227.3^{ef}$ | $2.39^{abc}$ | $10.01^{a}$ |
| Gudatu | $10.85^{a}$ | $247.3^{bcd}$ | $2.25^{de}$ | $9.87^{ab}$ |
| Gute | $8.90^{def}$ | $242.8^{d}$ | $2.28^{cde}$ | $9.58^{bcd}$ |
| Meba | $9.53^{bcd}$ | $245.8^{bcd}$ | $2.36^{bcd}$ | $9.06^{f}$ |
| Paddet | $10.16^{b}$ | $281.0^{a}$ | $2.45^{ab}$ | $9.61^{bcd}$ |
| Tadesse | $9.57^{bc}$ | $272.4^{a}$ | $2.47^{a}$ | $9.41^{cdef}$ |
| Tesema | $9.26^{cdef}$ | $256.4^{b}$ | $2.36^{bc}$ | $9.47^{cde}$ |
| Urji | $9.53^{bcd}$ | $240.8^{d}$ | $2.21^{ef}$ | $9.18^{ef}$ |
| Wama | $8.75^{f}$ | $255.0^{bc}$ | $2.24^{e}$ | $9.46^{cde}$ |
| Location | | | | |
| Bako | $9.17^{b}$ | $279.8^{a}$ | $2.43^{a}$ | $9.54^{a}$ |
| Gute | $9.48^{a}$ | $211.8^{b}$ | $2.12^{b}$ | $9.59^{a}$ |
| Year | | | | |
| 2018 | $8.82^{b}$ | $233.1^{b}$ | $2.18^{b}$ | $8.33^{b}$ |
| 2019 | $9.82^{a}$ | $258.7^{a}$ | $2.42^{a}$ | $10.81^{a}$ |

Mean of Variety, Location and Year followed by the same letter within same column are not significantly different ($P < 0.05$)

**Potassium, sodium, Iron, Zinc, Calcium, Magnesium content of Finger Millet Varieties**

The Potassium (K), Sodium (Na), Iron (Fe), Zinc (Zn), Calcium (Ca) and Magnesium content of finger millet varieties showed significant difference (Table 3). Paddet finger millet varieties contained the highest potassium whereas Addis contained the lowest potassium content among the varieties studied. The current study on finger millet varieties showed that the calcium contents ranged from 277.1 mg/100g for Bako 09 to 416.2 mg/100g for Diga-2. Study by Bachar and others reported for different finger millet accessions that ranged from 162 mg/100g to 487 mg/100g (Bachar et al, 2013). The study revealed that black seeded finger millet varieties contained higher amount of calcium than others. Diga 2 finger millet variety contained the highest calcium content but Bako 09 variety contained minimum among the varieties studied. High calcium content of black seeded grain finger millet is in line with other study (Shimelis et al., 2009). The calcium content of finger millet variety was higher than other cereal crops (Figure 2).

There were significant differences between study locations for Potassium, sodium, Iron, Zinc, Calcium and Magnesium contents. This could be attributed to difference in mineral content of soil (Table 5). Na and Ca contents were not affected by growing years which could imply variation in temperature and rainfall distribution in the study areas. The iron contents of finger millet varieties ranged from 28.1 ppm for Wama to 43.7 ppm for Diga-1 variety. Other study reported the iron content of finger millet to be 39 ppm (Bugum et al. 2017).

Table 3. Combined mean for some chemical properties of finger millet varieties (Whole seed grain)

| Variety | K (mg/100 g) | Na (ppm) | Fe (ppm) | Zn (ppm) | Ca (mg/100 g) | Mg (mg/100 g) |
|---|---|---|---|---|---|---|
| Addis 01 | $335.3^{j}$ | $36.2^{abcd}$ | $34.0^{d}$ | $20.6^{efgh}$ | $345.0^{cde}$ | $183.5^{bc}$ |
| Axum | $453.5^{bcd}$ | $33.6^{cde}$ | $30.0^{e}$ | $25.8^{abcde}$ | $278.9^{i}$ | $158.5^{e}$ |
| Bako 09 | $413.6^{gh}$ | $47.2^{a}$ | $36.6^{bcd}$ | $18.8^{gh}$ | $277.1^{i}$ | $169.8^{de}$ |
| Bareda | $352.7^{j}$ | $26.1^{def}$ | $35.3^{cd}$ | $22.1^{efgh}$ | $356.5^{bc}$ | $172.4^{cd}$ |
| Diga-2 | $304.7^{k}$ | $22.7^{ef}$ | $37.0^{bcd}$ | $28.1^{abcd}$ | $416.2^{a}$ | $165.5^{de}$ |
| Boneya | $438.4^{cde}$ | $36.7^{abcd}$ | $34.3^{d}$ | $19.9^{fgh}$ | $284.2^{hi}$ | $162.9^{de}$ |
| Diga-1 | $382.0^{i}$ | $35.0^{bcd}$ | $43.7^{a}$ | $31.0^{a}$ | $364.8^{b}$ | $158.0^{e}$ |
| Gudatu | $394.3^{hi}$ | $39.7^{abc}$ | $36.0^{cd}$ | $28.0^{abcd}$ | $298.2^{gh}$ | $191.1^{ab}$ |
| Gute | $434.4^{def}$ | $46.9^{a}$ | $29.8^{e}$ | $25.4^{bcdef}$ | $331.9^{def}$ | $160.8^{de}$ |
| Meba | $423.5^{efg}$ | $38.4^{abc}$ | $35.9^{cd}$ | $23.6^{defg}$ | $347.3^{cd}$ | $169.2^{de}$ |
| Paddet | $496.5^{a}$ | $41.3^{abc}$ | $37.9^{bc}$ | $29.3^{abc}$ | $326.0^{f}$ | $200.0^{a}$ |
| Tadesse | $453.7^{bcd}$ | $36.1^{abcd}$ | $36.9^{bcd}$ | $30.6^{ab}$ | $328.1^{ef}$ | $185.2^{b}$ |
| Tesema | $459.2^{b}$ | $46.4^{ab}$ | $36.9^{bcd}$ | $24.0^{cdefg}$ | $308.8^{g}$ | $168.4^{de}$ |
| Urji | $414.9^{fg}$ | $21.6^{f}$ | $39.3^{b}$ | $28.1^{abcd}$ | $334.2^{def}$ | $171.8^{cd}$ |
| Wama | $454.3^{bc}$ | $30.0^{cdef}$ | $28.1^{e}$ | $18.0^{h}$ | $282.7^{hi}$ | $169.8^{de}$ |
| Location | | | | | | |
| Bako | $497.9^{a}$ | $44.7^{a}$ | $32.9^{b}$ | $23.3^{b}$ | $312.7^{b}$ | $181.1^{a}$ |
| Gute | $330.2^{b}$ | $26.9^{b}$ | $37.9^{a}$ | $26.5^{a}$ | $337.9^{a}$ | $163.8^{b}$ |
| Year | | | | | | |
| 2018 | $373.6^{b}$ | $34.8^{a}$ | $38.0^{a}$ | $28.3^{a}$ | $326.6^{a}$ | $174.7^{a}$ |
| 2019 | $454.5^{a}$ | $36.9^{a}$ | $32.9^{b}$ | $21.5^{b}$ | $324.0^{a}$ | $168.5^{b}$ |

Mean of Variety, Location and Year followed by the same letter within same column are not significantly different ($P < 0.05$)

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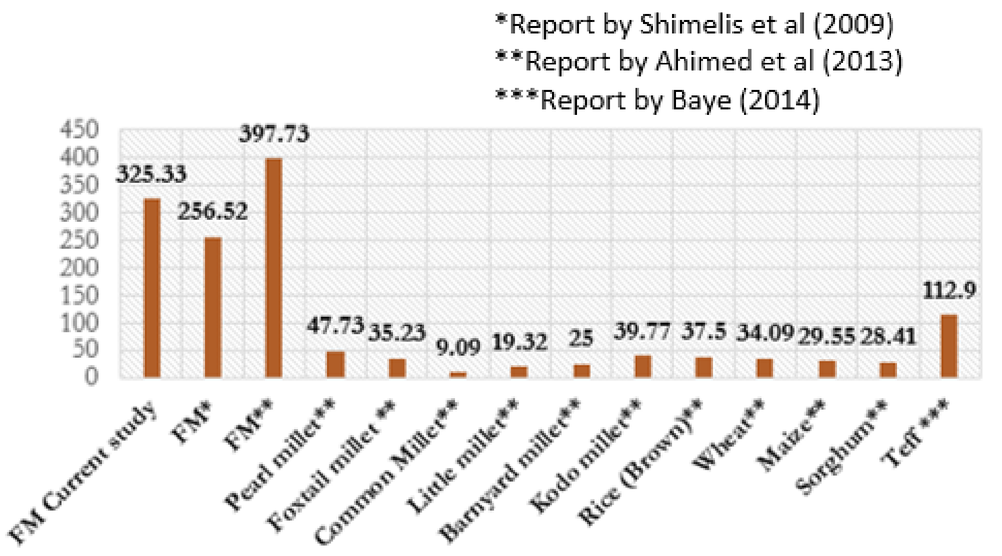


*Figure 2.Calcium content of various cereals (mg/100 g), FM=Finger millet*

**Crude fat, crude fiber, TSW and Manganese content of finger millet varieties**

The crude fat, crude fiber, TSW (thousand seed weight) and manganese contents of finger millet varieties grown at Bako and Gute study sites are presented in Table 4. These parameters were analyzed for one cropping season finger millet varieties. The crude fat content of finger millet varieties ranged from 1.27 % for Gudatu and Wama; and 1.70 % for Bareda. The crude fiber contents for the finger millet varieties ranged from 2.91 % for Bako 09 to 5.38 % for Diga-2 variety. Bugum and others reported the crude fiber of finger millet to be 3.6 % (Bugum et al. 2017). Higher crude fiber content for different genotypes was reported (Shibairo et al. 2014). A study on crude fiber contents of different finger millet accessions showed large variations that ranged from 0.93 % to 10.01 % (Bachar et al. 2013). TSW contents of finger millet varieties ranged from 1.69 g for Addis 01 variety to 2.82 g for Paddet variety. The lower seed size the higher surface area which could affect the concentration of some parameters expected to be directly related to the surface area of grain seed. 2.88 g TSW was reported for finger millet

(Bugum et al. 2017). On the study of performance and participatory variety evaluation of finger millet Tarekegne and others reported that the TSW for Bareda was about the same but the TSW for Gute, Tadesse, Paddet and Wama was reported to be higher than current study (Tarekegne et al, 2019). This difference could be attributed to adaptation of a stated variety to a given location or suitability of the agro ecology to the varieties.

*Table 4.* Combined mean for Crude fat, crude fiber, TSW and Manganese content of finger millet varieties at two different locations during 2019 cropping season (whole seed grain)

| Variety | Crude Fat (%) | Crude Fiber (%) | TSW(g) | Mn mg/100 g |
|---|---|---|---|---|
| Addis 01 | $1.50^{bc}$ | $3.77^{def}$ | $1.69^{g}$ | $33.1^{a}$ |
| Axum | $1.51^{bc}$ | $4.71^{c}$ | $2.34^{cde}$ | $22.4^{cde}$ |
| Bako 09 | 1.46c | $2.91^{k}$ | $2.60^{ab}$ | $20.5^{efg}$ |
| Bareda | $1.70^{a}$ | $3.66^{fgh}$ | $1.99^{f}$ | $34.8^{a}$ |
| Diga-2 | $1.48^{c}$ | $5.38^{a}$ | $2.25^{de}$ | $35.2^{a}$ |
| Boneya | $1.34^{ef}$ | $3.56^{hi}$ | $2.29^{cde}$ | $23.5^{cd}$ |
| Diga-1 | $1.32^{ef}$ | $4.93^{b}$ | $2.30^{cde}$ | $28.4^{b}$ |
| Gudatu | $1.27^{f}$ | $3.00^{k}$ | $2.50^{bc}$ | $24.4^{c}$ |
| Gute | $1.49^{bc}$ | $3.60^{gh}$ | $2.43^{bcd}$ | $30.3^{b}$ |
| Meba | $1.46^{c}$ | $3.28^{j}$ | $2.16^{ef}$ | $18.5^{gh}$ |
| Paddet | $1.68^{a}$ | $3.55^{hi}$ | $2.82^{a}$ | $19.4^{fgh}$ |
| Tadesse | $1.37^{de}$ | $3.83^{de}$ | $2.38^{bcde}$ | $17.8^{h}$ |
| Tesema | $1.56^{b}$ | $3.72^{efg}$ | $2.78^{a}$ | $21.4^{def}$ |
| Urji | $1.45^{cd}$ | $3.42^{ij}$ | $1.97^{f}$ | $20.5^{efg}$ |
| Wama | $1.27^{f}$ | $3.91^{d}$ | $2.38^{bcde}$ | $15.3^{i}$ |
| Location | | | | |
| Bako | $1.49^{a}$ | $3.86^{a}$ | $2.32^{a}$ | $182.2^{b}$ |
| Gute | $1.43^{b}$ | $3.77^{b}$ | $2.33^{a}$ | $306.1^{a}$ |

Mean of Variety and Location followed by the same letter within same column are not significantly different ($P < 0.05$)

On average, the crude fat content for finger millet at Bako site was high. It was only non-significant for TSW of finger millets for the study sites (Table 4). The manganese contents of finger millet varieties were affected by locations and it was higher for Gute study sites which could be attributed to higher manganese content of the soil (Table 5).

Table 5. Some chemical properties of soils from the experimental sites

| Location | Ex Na (ppm) | Ex K (ppm) | Ex Mg (ppm) | Ex Ca (ppm) | Ex Mn (ppm) | CEC (cmol/kg soil) | TN (%) |
|---|---|---|---|---|---|---|---|
| Bako | 35.4±4.9 | 363.4±14.6 | 239.2±21.3 | 1110.1±79.5 | 23.1±0.7 | 9.69±0.87 | 0.10±0.01 |
| Gute | 34.9±5.6 | 206.5±10.3 | 132.6±13.0 | 783.1±114.1 | 67.3±3.4 | 17.61±0.29 | 0.19±0.01 |

## Conclusions Recommendation

The growing environments and cropping seasons had effect on some chemical composition of finger millet varieties. Finger millet varieties that were grown at Bako location contained higher amount of Crude Fat, Crude Fiber, Phosphorus, Ash, Potassium, Magnesium whereas finger millet grown at Gute site contained higher crude protein, Iron, Zinc, Calcium and Manganese. The physicochemical properties of finger millet were also affected by the variety. Black seeded finger millet contains higher moisture, calcium, crude fiber content than white and brown seeded finger millet.

Food product processed from finger millet can be good source of calcium for children, pregnant and lactating mothers and elderly population. Further study on anti-nutritional content of the Finger millet varieties need to be assessed. Consumers/farmers perception on the preferences of finger millet varieties (**Brown, white and black seeded**) for commercial/food including beverages need to be assessed. What is special to finger millet for its very high calcium content when compared with other cereals?

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# Evaluation of Nutritional Content of Teff Varieties Grown in Oromia, Ethiopia

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**Abstract**

*Teff is among the staple cereal crops mostly produced and the daily consumption of Ethiopian's is majorly dependent on Injera. Teff has a lion's share of injera preparation might be due to nutritional qualities, shelf life merit and consumers' preference of the product. This study aimed to evaluate proximate and minerals content of fifteen teff varieties which were new and currently used in production in Oromia Region, Ethiopia. The proximate and minerals content of these varieties were analyzed by using AOAC Official methods. The result showed that mean content of moisture, ash, crude protein, crude fat, carbohydrate and energy were 9.28%, 2.92%, 9.34%, 3.06%, 75.39% and 366.56kCal respectively. While, iron, calcium, sodium, potassium, manganese and zinc mean contents were determined 548.79ppm, 1552.64ppm, 539.45ppm, 4614.08ppm, 122.79ppm and 31.67ppm respectively. The study revealed that there were significant differences ($p \leq 0.05$) among proximate and minerals content in fifteen teff varieties which could be attributed to difference in varieties. Felagot teff variety had superior protein, fat, calcium, and iron and zinc contents. Teff could be good source of protein, calcium, iron and zinc which are limited in other cereals. Proximate and mineral contents could be affected by variety and environment. Therefore, further research on the effect of environment on proximate and mineral composition need to be conducted.*

***Keywords**: Teff, variety, proximate, minerals*

**Introduction**

Teff (Eragrostis tef) is among the staple cereal crops mostly produced and consumed in Ethiopia. It is also used as animals feed in other countries. During 2019/2020 cropping season, cereals were produced on about 10,478,218.0 hectares of land and 296,726,476.9 quintals of yield were obtained in the country. From these, tef had 30% and 19% share for production area and yield, respectively (CSA, 2020). The daily consumption of Ethiopian's is solely dependent on Injera and teff has lion share for injera preparation  might be due to nutritional qualities, shelf life merit and consumers custom of the product.

There have been many finding reports that teff has good source of protein, energy, fiber and minerals. Teff has an attractive nutritional profile, being high in dietary fiber, iron, calcium and carbohydrate and also has high levels of phosphorus, copper, aluminium, barium, thiamine and excellent content of amino acids essential for humans (Abebe et al., 2007 &Hager et al., 2012). Teff is a valuable source of minerals; in particular, Ca, Fe, Mn and Zn are present in larger amounts (Eva et. al., 2018). It is free of gluten (Miller, 2010) and can provide an alternative food source for people with celiac disease. The global use of teff for human consumption has been restrained partly due to limited knowledge about its nutritional values and the processing challenges faced in making teff-based food products (Haci et al., 2018)

The overall quality may be defined as the sum (or product) of individual properties that enable a plant or plant product to meet the requirements of a user or consumer. The overall quality depends on both physical and chemical plant properties. Plant quality is predominately controlled by genetic and physiological factors. This becomes obvious in a comparison of species, cultivars, plant organs and tissues. In Ethiopia; Regional and National Agricultural Research Institute are adopting/adapting and verifying nationally and internationally varieties as to their significance to agro-ecology basis. Accordingly, more than 40 teff varieties were in Ethiopia of which 4 varieties such as Dursi, Guduru, Jitu and Kena were by Oromia Agricultural Research Institute (Bako Agricultural Research Center). However, physico-chemical food quality characteristics of these teff varieties are not well studied yet.

**Objective**

- To evaluate nutritional content of teff varieties grown in Oromia, Ethiopia

**Materials and Methods**

**Samples Collection and Study Sites**

Fifteen (15) teff varieties (figure 1) were collected from Bako Agricultural Research Center and Debra Zeit Agricultural Research Center during 2019/2020 cropping season. All proximate and mineral analysis were conducted at Food Science Laboratory of Oromia Agricultural Research Institute


*Figure 1. List of teff varieties used in this study*

### Sample Preparations for Analysis

All samples were sorted, cleaned, milled and stored at room temperature until analysis.

### Proximate and Minerals Analysis

Moisture, crude ash and crude protein were determined by Using AOAC Official Methods 2000 while, fat and minerals contents were analyzed by using AOAC Official Method 2003.05 and 975.03 respectively. Carbohydrate was determined by difference and Energy was calculated using Artwater factor. All determinations were done in triplicate.

### Data Analysis

Means and standard deviations were calculated for all proximate and minerals data. ANOVA subjected to SAS software version 9.00.

## Results and Discussions

### Proximate content

The results of proximate (moisture, ash, protein, fat and carbohydrate) and energy content determined for fifteen teff varieties were listed in terms of mean value and standard deviation on the dry weight as shown Table 1. The grand mean of moisture, crude ash, crude protein, crude fat, carbohydrate and energy quantified were 9.28±0.14 %, 2.92±0.05%, 9.34±0.41%, 3.06±0.05%, 75.39±0.46% and 366.56±0.71kcal, respectively and the result revealed that there were a significant difference ($P<0.05$) among the teff varieties. The obtained value of carbohydrate, fat, ash, protein and moisture were acceptable with Ethiopia standard requirement as teff quality which were 63%, 2% - 6%, 3% - 4%, 8% and max 12.5% respectively (Ethiopian standard, 2015). Proximate and energy value of this study compared with some cereals generated by United States Department of Agriculture (USDA, 2019) as illustrated on figure 2 to 4.

### Crude Protein Content

The mean crude protein content of teff varieties ranged from 6.48% to 11.35%. The least and highest crude protein obtained from Guduru and Tseday varieties, respectively. There was no significant difference among Boset, Felagot, Jiru, Simada and Tseday teff varieties.

Kamila(2018), Bekabil (2011) and Haci (2018) reported that teff protein content ranges from 8.9% – 10.5%, 8 – 11%, and 10.5 -11.1%, respectively. USDA reported up to 13.3 % with typical value of 11 % protein content. Bultosa (2007) also reported protein content of 13 teff varieties that ranged 8.7% - 11.1% with mean 10.4%. Even though maximum protein value was in agreement with these scholars; lower value of protein obtained in this study (6.48 %).

**Moisture Content**

The moisture content of teff varieties ranged from 7.63±0.12% to 12.17±0.16% with mean value of 9.28±0.14%, which is in the normal range for field dried teff grain.

**Carbohydrate Content**

Teff varieties had mean value of 75.39% carbohydrate and it ranged from 70.90% to 79.32%. The smallest and highest values were obtained from Felagot and Dagim varieties respectively. There was no significant difference among Dagim and Tesfa varieties. The total carbohydrate content of teff ranges from 57 to 86g/100g (Bultosa, 2007). Various Studies have reported that the content of teff carbohydrates produced in different ecologies can change the values. In this study the teff had higher carbohydrate content than maize, wheat and rice as shown on figure 2 (USDA 2019).

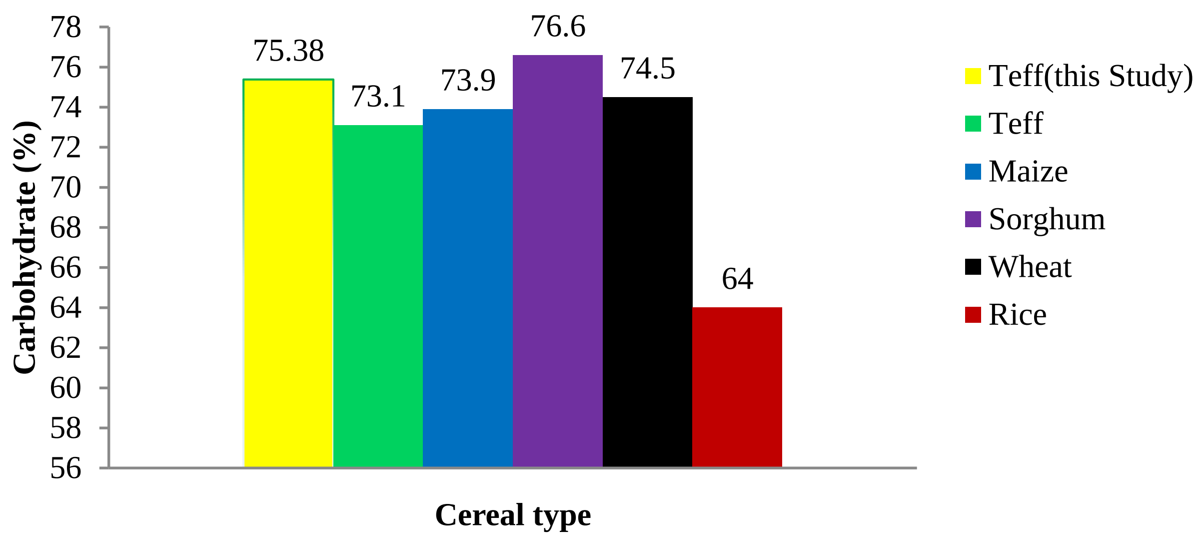



*Figure 2. Comparison of carbohydrate content of some cereal with teff*

Table 1. Proximate and Energy Content of Teff Variety

| SN | Teff Varieties | Proximate and Energy Content of Teff Varieties on the dry weight | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture (%) | Ash (%) | Crude Protein (%) | Crude Fat (%) | Carbohydrate (%) | Energy (Cal) |
| 1 | Bora | 10.58±0.05c | 1.66±0.81g | 9.62±0.23b | 2.85±0.04dc | 75.79±0.34d | 369.58±0.88d |
| 2 | Boset | 10.30±0.17d | 2.84±0.01e | 11.07±0.73a | 2.94±0.06c | 72.85±0.52h | 362.11±0.45h |
| 3 | Dagim | 7.30±0.16k | 2.95±0.01ed | 7.31±0.75d | 3.12±0.02b | 79.32±0.81a | 374.43±0.76a |
| 4 | Dursi | 9.48±0.07f | 3.67±0.01b | 7.27±0.46d | 3.35±0.22a | 76.15±0.66c | 364.14±1.42g |
| 5 | Eba | 8.05±0.31i | 2.49±0.03f | 8.92±1.20cb | 2.77±0.03de | 77.76±0.91bc | 371.50±1.33c |
| 6 | Felagot | 9.89±0.21e | 4.57±0.02a | 11.22±0.66a | 3.42±0.02a | 70.90±0.88i | 359.23±0.74i |
| 7 | Guduru | 12.17±0.16a | 3.59±0.01b | 6.48±0.57d | 3.42±0.09a | 74.29±0.74gf | 354.05±0.97j |
| 8 | Jitu | 8.53±0.04h | 3.24±0.07c | 11.17±0.04a | 3.32±0.01bc | 73.74±0.08g | 369.48±0.41d |
| 9 | Kena | 11.27±0.19b | 3.13±0.02c | 6.81±0.16d | 3.43±0.01a | 75.37±0.29dc | 359.56±0.74i |
| 10 | Kora | 8.03±0.20i | 2.46±0.02f | 8.74±0.21c | 3.13±0.01e | 77.65±0.35c | 372.84±0.68bc |
| 11 | Kuncho | 9.13±0.17g | 3.22±0.02c | 11.12±0.28a | 3.08±0.03b | 73.45±0.29gh | 366.01±0.61f |
| 12 | Nigus | 7.63±0.12j | 2.49±0.12f | 10.85±0.08a | 2.83±0.06dc | 76.20±0.22d | 373.70±0.76ba |
| 13 | Simada | 8.34±0.10h | 3.00±0.02d | 11.19±0.31a | 2.66±0.00e | 74.81±0.33de | 367.92±0.45e |
| 14 | Tesfa | 8.98±0.06g | 2.49±0.11f | 6.97±0.36d | 2.94±0.07c | 78.62±0.35ba | 368.83±0.17ed |
| 15 | Tseday | 9.51±0.02f | 2.57±0.03f | 11.35±0.15a | 2.67±0.03e | 73.90±0.17g | 365.06±0.28gf |
| Grand Mean | | 9.28±0.14 | 2.92±0.05 | 9.34±0.41 | 3.06±0.05 | 75.39±0.46 | 366.56±0.71 |
| CV | | 1.70 | 2.44 | 5.49 | 2.42 | 0.69 | 0.22 |
| LSD(α=0.05) | | 0.26*** | 0.12*** | 0.86*** | 0.12*** | 0.87*** | 1.35*** |

Values within the same column with different letters are significantly different ($p < 0.05$)

Where, CV= Coefficient of variation, LSD: List significant difference, ***: Highly significant and Cal= Calorie

**Crude Fat Content**

Tested teff varieties had crude fat content ranged from 2.66±0.00 % to 3.43±0.01% with the minimum and maximum value obtained from Simada and Guduru varieties, respectively. There was no significant difference among Dursi, Felagot, Jitu and Guduru varieties. Thirteen teff varieties had crude fat ranged 2.0-3.0% with mean of 2.3% and the value is similar with the review report of 2.00 -3.09% of previous works (Bultosa, 2004, 2007). Teff lipid content is higher than wheat and rice, but lower than maize and sorghum as shown on figure 3(USDA 2019).

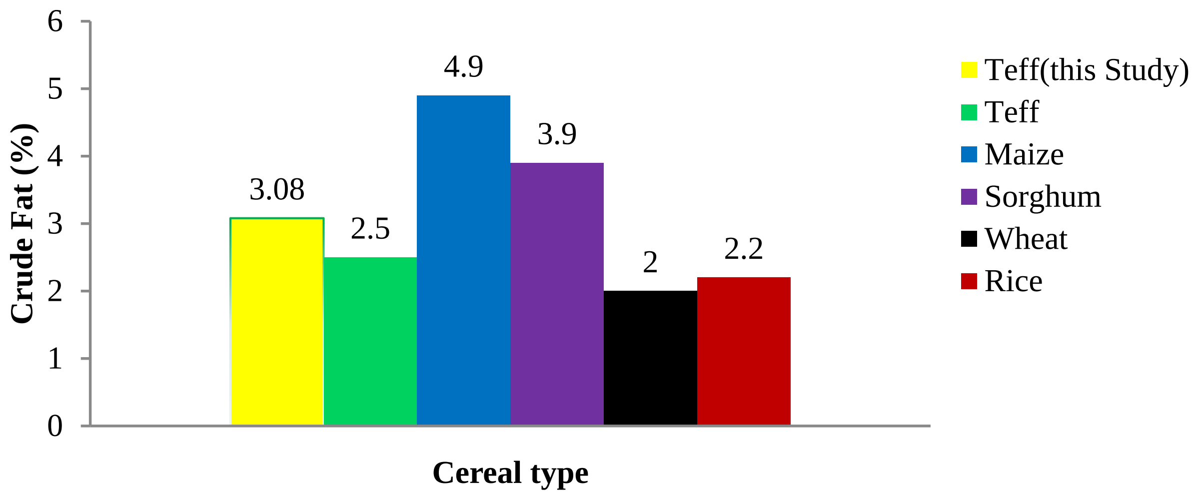



*Figure 3. Comparison of crude fat content of some cereal with teff*

**Mineral Content**

The concentration of mineral in all studied teff varieties is presented in table 2 and results are in terms of mean value and standard deviation on dry weight. The result revealed that there were a significant difference ($P<0.05$) among the teff varieties. The difference in mineral content among teff varieties was wide-ranging from the highest and least order of mineral were K > Ca > Fe > Na > Mn >Zn with grand mean value of 4614.08 mg/kg, 1552.64mg/kg, 548.79mg/kg, 539.45mg/kg, 122.79 mg/kg and 31.67mg/kg respectively. The concentration of K was the highest of all the analyzed minerals and ranges from 4190.45- 5064.97mg/kg and the least was obtained for Zn that ranges from 17.43 - 48.93 mg/kg.

Felagot variety had the highest Ca composition (1861.80±30.80ppm) while the least value was obtained from Jitu (1328.48±81.57ppm). The concentration of Ca (1206.9 - 1769.5 mg/kg) in this study agrees with the range of the value 124 - 155 mg/100 g, 168.64 ± 11.03 to 180.7 ± 14.65 mg/100 g and 1800 mg/kg reported by Alemtsehay et al., 2007, Ma et al., 2001  lower USDA 2019, respectively.

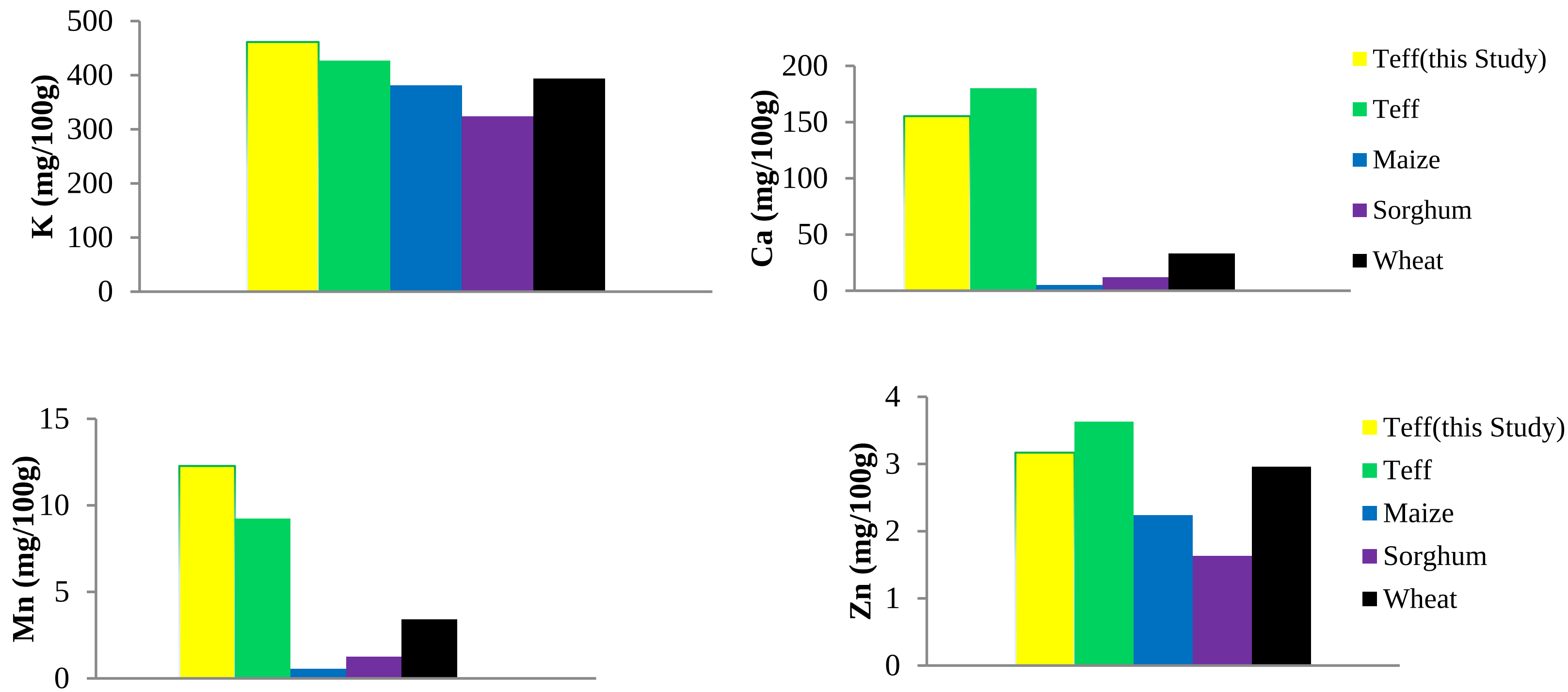
The mean mineral content of teff varieties were compared with some cereals that was generated by United States Department of Agriculture (USDA, 2019) as illustrated in figure 5. As a result of this stuy; teff has a higher  iron, calcium, manganese, zinc, potassium and sodium content than maize, wheat, sorghum and rice.

Table 2. Minerals Content of Teff Varieties

| SN | Teff variety | Teff minerals content on the dry weight(mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Fe | Ca | Na | K | Mn | Zn |
| 1 | Bora | 174.50±2.27j | 1551.15±42.17c | 355.84±30.43g | 5064.97±8.36a | 92.53±2.94e | 31.95±0.11cbd |
| 2 | Boset | 448.36±21.55g | 1824.01±57.08a | 469.19±19.28f | 4344.53±44.94hi | 40.22±6.30i | 31.02±5.75cd |
| 3 | Dagim | 498.64±27.95f | 1346.25±5.39hg | 539.84±11.28ed | 4836.46±19.57c | 62.80±2.80h | 28.40±1.38cd |
| 4 | Dursi | 793.85±21.97b | 1432.46±49.99fge | 613.88±39.44b | 4886.67±17.86c | 257.86±9.02b | 30.58±0.97cd |
| 5 | Eba | 407.57±22.49h | 1466.55±42.39dfce | 676.58±30.61a | 4759.60±23.56d | 86.93±4.26fe | 27.98±3.26cd |
| 6 | Felagot | 1128.98±20.91a | 1861.80±30.80a | 586.13±3.66bcd | 4308.79±40.59i | 91.02±4.21e | 48.93±2.01a |
| 7 | Guduru | 745.42±34.05c | 1381.35±0.34hfg | 632.30±39.88a | 4574.77±47.56e | 307.93±5.57a | 28.79±0.86cd |
| 8 | Jitu | 1140.49±14.18a | 1328.48±81.57h | 593.19±41.69bc | 4190.45±54.37j | 207.55±6.50c | 34.59±2.30cb |
| 9 | Kena | 625.19±39.82e | 1460.63±4786def | 359.11±61.63g | 4713.68±45.82d | 263.02±4.06b | 26.32±0.42d |
| 10 | Kora | 182.63±17.80j | 1510.31±38.93dce | 585.25±8.38bcd | 4981.92±28.47b | 74.16±3.18g | 29.90±6.56cd |
| 11 | Kuncho | 644.05±8.92d | 1672.41±7142b | 561.26±4.83ecd | 4510.96±19.53f | 82.83±4.56f | 32.86±0.95cbd |
| 12 | Nigus | 432.13±23.98hg | 1528.61±65.56dc | 633.72±47.47a | 4389.34±59.43hg | 89.82±2.72fe | 39.26±13.84b |
| 13 | Simada | 516.19±14.94f | 1826.37±37.97a | 534.99±37.75e | 4404.02±13.86g | 15.98±0.75j | 31.42±1.83cd |
| 14 | Tesfa | 157.99±25.55j | 1682.43±86.05b | 523.88±16.92e | 4880.92±11.61c | 157.55±1.38d | 17.43±2.77e |
| 15 | Tseday | 294.74±26.74i | 1416.82±4.01fg | 426.51±11.38f | 4364.22±18.53hgi | 4.19±4.70k | 35.66±1.41cb |
| Grand Mean | | 548.79±21.54 | 1552.64±44.13 | 539.45±24.98 | 4614.08±30.27 | 122.79±4.20 | 31.67±2.96 |
| CV | | 4.32 | 3.34 | 5.45 | 0.76 | 3.73 | 14.56 |
| LSD | | 39.67*** | 86.73*** | 49.13*** | 58.65*** | 7.65*** | 7.71*** |

Values within the same column with different letters are significantly different ($p < 0.05$)

Where, CV= Coefficient of variation, LSD: List significant difference, ***: Highly significant and Cal= Calorie



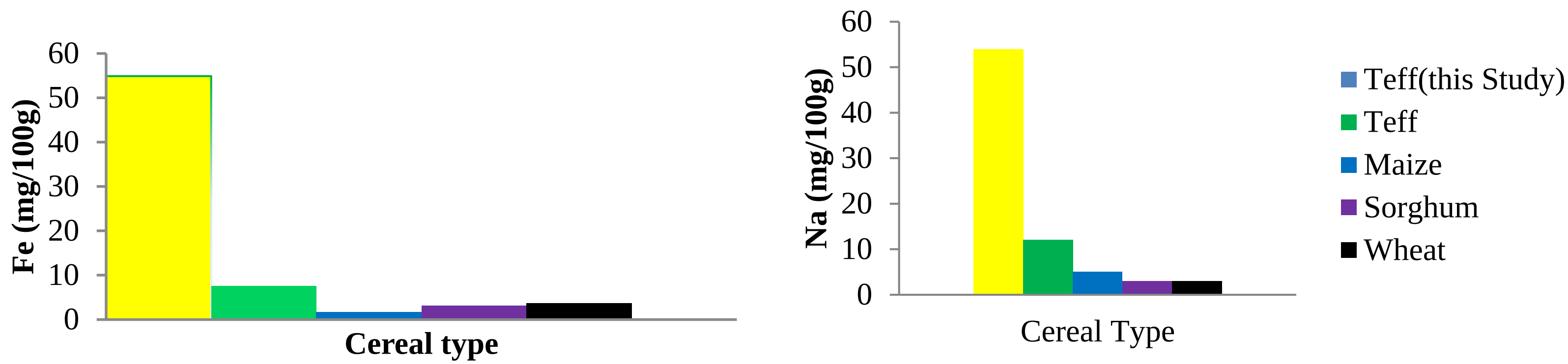



*Figure 5. Comparison of K, Ca, Mn, Zn, Fe and Na content of some cereal with teff*

## Conclusion and Recommendations

In this study, 15 teff varieties were collected and their nutrients contents were evaluated. There were significant differences observed in proximate and mineral content among the teff varieties. From the evaluated teff varieties; Felagot teff variety had superior protein, fat, calcium, and iron and zinc contents`. Teff could be good source of protein, calcium, iron and zinc which are limited in other cereals. Proximate and mineral contents could be affected by variety and environment. Therefore, further research on the effect of environment on proximate and mineral composition need to be conducted.

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# Some Physicochemical Properties of Butter Collected from Local Markets of North Shewa Zone, Oromia, Ethiopia

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Abstract

*Dairy products are very important foods that supply nutrients for human beings. Butter is one of the popular dairy products in Oromia. This study aimed to assess some of the physicochemical properties of butter samples collected from local markets of North Shewa Zone of Oromia Regional State. Butter samples were collected on the market day of the peasant associations. A total of 118 samples were collected from three districts. Standard analytical procedures were used to analyse the physicochemical properties of the butter samples. The fat, moisture, free fatty acid and ash contents of butter samples ranged between 70.35 % - 92.34 %c; 5.95 % - 27.95 %, 0.27 % - 9.69 %; and 0.04 % - 0.31 % respectively. Calcium, magnesium, potassium and phosphorus content of the butter ranged between 13.59 ppm- 537.49 pp, 2.57 ppm – 42.12 pp, 7.98 ppm – 224.17 ppm; and 12.20 ppm – 434.76 ppm, respectively. The average free fat acidity and moisture content of butter samples failed to comply with codex international standards whereas the average fat content of butter was in agreement the standards. Butter from Kuyu district contained higher amount of fat content when compared with Kimbibit and Wachale districts. The low fat content of butter at Kimbibit and Wachale can be attributed to less removal of butter milk during the dairy processing. The free fatty acid of butter samples showed large variation among butter samples which can be attributed to storage of butter for market size; and unhygienic dairy processing technique and storage materials. The concentration of mineral elements in butter samples followed the order Ca>P>K>Na>Mg>Fe>Zn. Further study on fatty acid profile, and effect of butter storage materials and mineral rich feed on the storability of butter need to be investigated.*

*Keywords*

*Butter, North Shewa, physicochemical properties, Local markets*

## Introduction

Dairy products play crucial role in the improvement of livelihoods of the community if they are properly processed, managed and with available market chain. Dairy products contain high nutrients and are rich in milk fat globule membrane, conjugated linoleic acid and fatty acids beneficial for health (Hae-Soo Kwak, 2013). Butter is one of the dairy products used for cooking and cosmetic purposes in Ethiopia (Alganesh and Yetenayet, 2017). It is essential part of nutritional value of milk that can be made from milk of different animal species. Cow milk is the dominant source of butter production (John W. Fuquay, 2011). Butter that is used for cooking is processed to ghee by adding different spices. This increases the stability of butter removing moisture which result in decrease in oxidation of lipids. Besides its use in household consumption, butter generate income for different community (Zelalem Yilma, 2011; Neijenhuis, 2014). Mettiello et al. stated that the current dairy production techniques in Africa may not guarantee safe hygienic practices which can limit their possibilities of export of dairy products (Mattiello et al, 2018).

According to the community, the quality of butter is characterized by its origin, color, smell, consistency and degree of adulteration with foreign materials (Gebremedhin et al, 2014). These are traditional butter quality indicators/features which the community uses to rate butter quality. According to the review conducted smoking of milk storage container and churning container by different plant species is believed to impart distinct flavour (Alganesh and Yetenayet, 2017). The literature supports that the quality of butter is characterized by feed, breed type and milk handlings (Gebremedhin et al, 2014). In some areas of Ethiopia feed is probably the main factor that affects the butter quality (Geographical locations). Study shows the price of Butter from Debre Birhan is high which could be attributed to consumers' preference for Butter of particular area (Ghilu *et al.,* 2012). As a result, Sheno (Kimbibit District), Wollega and Dire Inchini butter are with repute market according to the consumer perception. In Ethiopia, the geographical origin of butter affects its reputability (Diriba Idahe, 2013; Debela et al., 2016).

The cattle feed practices in Ethiopia is changing from time to time. There is a general decrease in green fodder and increase crop residue as animal feed. The breed composition of cattle according to CSA 2020/21 report is 2.29 %, 2.6 % and 15.4 % hybrid cattle for Ethiopia, Oromia and North Shewa Zone respectively (CSA, 2020) indicating large proportion of hybrid cattle composition in the study area.

Although the price of a commodity is affected by different factors, quality of a product affects its reputability. Therefore, if there is a difference in butter quality among different geographical locations the community should get market/price advantage. As far as literature is concerned there are no data that support the difference in butter quality with regard to physical and chemical properties except consumer perceptions at these locations. Therefore, this study was initiated to determine some physicochemical properties of butter collected from local markets of North Shewa Zone of Oromia Regional state.

## Materials and Methods

### Site Selection

Wachale, Kimbibit and Kuyu districts were selected from North Shewa Zone for the study by consulting with Zonal Livestock Agency. Based on the information obtained from Zonal Livestock Agency each district was consulted. The selection of peasant associations (PAs) was done purposefully with dairy products expert from each district. Three PAs were selected per district. Accordingly, Adadi Falee, Gara Chatu and Sike from Kimbibit District; Dawicha Kerenso, Wuye Gose and Biriti from Kuyu district; and Bole Bacho, Bidaru and Gimbichu from Wachale disricts were selected for the study.

### Sampling

Butter samples were collected from the market on the market day of the PAs. Development agents (DAs) helped in identifying the dwellers of the PA to take samples. Butter samples were collected separately. A total of 118 butter samples were collected (Kuyu and Wachale districts - 36 samples each; Kimbibit- 46 samples). Samples were taken in capped glass jar and sealed, and labeled appropriately. The samples were kept in ice box and brought to Food Science Research Directorate Laboratory and kept in deep freezer until analysis.

### Analysis of some physicochemical properties of Butter

Standard procedures were used to analyze the physicochemical properties of butter samples. Samples were prepared according to AOAC official method 938.05. Moisture content was analyzed using AOAC official method 920.116. Fat content (AOAC official method 938.06), ash content (AOAC official method 920.117) and free fatty acid (AOAC official method 940.28) were analyzed using the stated methods. Minerals (Fe, Zn, Ca and Mg) were analyzed using AAS, and K and Na- using Flame photometer.

## Statistical Analysis

The data obtained were analyzed using R statistical software package. Descriptive statistics were used to describe the physicochemical properties of butter samples collected from the study sites. ANOVA at 5 % significance level and LSD mean separation method were used to analyze the parameters. Correlation between some butter physicochemical compositions was analyzed using Pearson correlation coefficient model.

## Results and Discussion

### Fat, moisture, free fatty acid and ash content of butter

The fat content of butter samples collected from the study sites ranged from 70.35 % - 92.34% (Table 1). The average fat content of butter samples collected from market was 81.34±3.87 % which is higher than fat content reported by Celik and Bakirci (2000) and the standard (Codex Alimentarius International Food Standards – CXS 279-1971). Fat content of butter ranged from 68.00- 90.00 % according to study conducted on Iranian traditional butter (Sarab et al, 2019). The variation in fat content of butter samples could be attributed to the removal of butter milk by farmers at different amount (Padure, 2021). The higher the butter milk removed during the dairy processing, the higher the fat content it will be. Non washing practices of butter after its production could yield lower fat content butter (White et al, 1956). A study showed that feed had no significant difference on the fat content of butter (Middaugh et al, 1988). The study revealed that 32.20 % of butter samples collected did not comply with fat content requirement set by International food standards (Fig 1).

Butter samples contained higher amount of moisture content (17.04±3.83%) not within the standard set by Codex Alimentarius International Food Standards for butter i.e. max 16 % but similar with research reviewed by Mogessie (2006) i.e. 17.2 %.  Wider moisture content ranges obtained by other scholars ranging from 2-42 % (O'Mahony F and Bekele E, 1985*)*. A research conducted in Türkiye showed higher moisture content of butter than results obtained by this study i.e. minimum of 8.72 % and maximum 31.63 % moisture content (Akgii *et al*., 2021). A study conducted on traditional butter prepared in a laboratory contained 17.5-26.2 % moisture (Idoui et al, 2010) and 0.78-33.5 % was also reported (Sarab et al, 2019). Up to 18.86±1.02% moisture content of butter was reported by Mekdes (2008) collected from open market. 17.2 % moisture content was also reported by EMPHI 1995 report. Although analysis was for small number of butter samples, the moisture content of butter decreased along the value chain and was in accordance with the standard set (Lina et al, 2018). The study revealed that 69.49 % of butter samples collected did not comply with moisture content requirement set by International food standards (Fig 1).

The free fatty acid content showed higher variation among the samples collected from the local markets (0.27 % min and max. 9.69 %, Table 1). The average free fatty acid was 1.86 ±1.86%. This result was not in agreement with the study conducted by Goncalves MFD and Baggio SR (2012) that ranged from 0.16-0.46 %. Lina et al (2018) reported 2.09 % free fatty acid higher than this study. Other scholars reported the free fatty acid of butter samples from local markets of Dire Enchini and Ejere districts of West Shewa, Oromia, to be 0.82±0.05% and 0.62±0.1 % respectively even though the information was generated from three replications (Shitaye et al, 2018). The free fatty acid of Iranian traditional butter ranged from 0.1- 0.99 % (Sarab et al, 2019) which could be attributed to the type and storage of butter. A maximum free fatty acid of about 23 % was obtained for older butter samples in Addis Ababa (O'Mahony F and Bekele E, 1985). The large range of free fatty acid can be attributed to accumulation of butter by farmers for market size (Ghilu et al, 2012) and non-hygienic practices during the dairy processing (Lina et al, 2018; Shekhara et al, 2020). Butter storage methods can also lead to deterioration of butter (Kosikowsky et al, 1947). Producers bring butter to local market by packing it with different packaging materials which could deteriorate the butter to different degrees.

Table 1. Fat, moisture, non-fat solid, free fatty acid and ash contents of butter collected from North Shewa (n = 118)

| Parameter | Mean ±SD | Median | Min. | Max. |
|---|---|---|---|---|
| Fat content (%) | 81.34 ±3.87 | 81.24 | 70.35 | 92.34 |
| Moisture content (%) | 17.04 ±3.83 | 17.14 | 5.95 | 27.95 |
| Non-fat solid (%) | 1.62 ±0.32 | 1.62 | 0.24 | 2.59 |
| Free fatty Acid (%) | 1.86 ±1.86 | 1.04 | 0.27 | 9.69 |
| Ash content (%) | 0.13 ±0.05 | 0.13 | 0.04 | 0.31 |

The non-fat solid content of the butter samples ranged from 0.24 %-2.59% (Table 1). The average of non-fat solid was 1.62 ±0.32 %. This result is in agreement with the maximum standard set as 2.0 % according to Codex Alimentarius CXS 279-1971 for butter. Queiros et al (2016) reported that 1.05 ± 0.14% for non-fat solid content of butter. Padure (2021) also reported 0.72-1.42 % non-fat solid in his study. Other scholars reported the lowest non-fat solids (0.001% - 0.052%) (Nunes et al, 2019). In another study the non-fat solid content of traditional butter ranged from 0.44-21.7 % (Sarab et al, 2019).

The ash contents of butter samples collected from the study area ranged between 0.04-0.31 %. The average ash content 0.13 ±0.05 % higher than the average ash content reported by Shitaye et al (2018) 0.114 % but Mogessie (2006) and Enb et. al (2009) reported 0.2 % for ash content of butter. Sila et. al (2021) obtained 0.02-0.55% ash for unsalted butters.

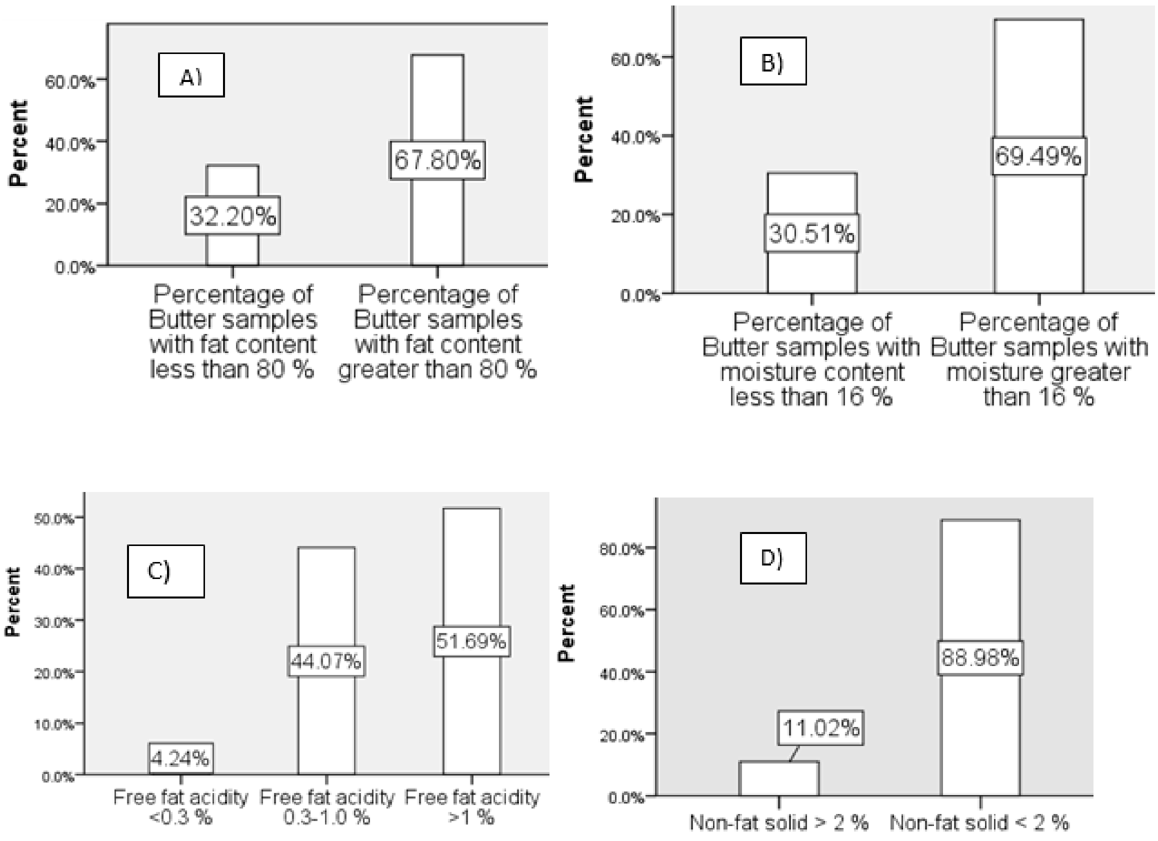


*Figure 1. Percentage of butter samples with different fat content (A), moisture content (B), free fatty acidy<0.3, 0.3-1.0 and >1%(C) and Non-fat solid content <2 and >2 % (D)*

**Calcium, Magnesium, Iron, Zinc, potassium, sodium and phosphorus content of butter**

Some mineral elements in butter samples from North Shewa were analyzed. Results showed that calcium concentration was the highest of all on average and Zinc was the one with the lowest concentration (Table 2). Although there were large range of variation among the samples, Calcium (Ca), Potassium (K), Phosphorus (P), Zinc (Zn) and Iron (Fe) contents of butter samples were higher than reported by Holland et al (1995) and Shitaye et al (2018) but lower sodium and potassium content. The average calcium content was similar with that reviewed by Mogessie (2006). The iron content of butter collected from local market in Tehran was reported to be 1.274±0.419 ppm (Vahedi et al, 2015), lower than Iron content reported by this study. The minimum - maximum and average iron and zinc contents of butter samples reported by Meshref et al (2014) were 5.0693- 13.14 ppm and 6.69±0.437 ppm 2.815 - 8.893 ppm, and 5.98±0.407 ppm respectively. Enb et al (2009) reported the iron and zinc content of butter to be 4.407 ppm and 19.086 ppm respectively. The higher content of iron may be attributed to feed, churning process or unhygienic practice that might lead to higher iron concentration in butter samples. The concentration of mineral elements in butter samples followed the order Ca>P>K>Na>Mg>Fe>Zn but according to Shitaye et al (2018) the order of magnitude of mineral elements in the butter samples increased in the order of K>Ca>Na>Mg >Fe>Zn.

Washing water and utensils used for milking and dairy processing can affect the quality of butter. Washing water of 0.4 ppm Fe could affect dairy products. Quantities of Iron higher than 1.5 ppm in milk could also affect the shelf life of butter due to their catalytic effect (Lante et al, 2006).

Table 2. Calcium, Magnesium, Iron, Zinc, Potassium, Sodium and phosphorus content of butter collected from North Shewa (n = 118)

| Parameter (on dry basis) | Mean ±SD | Median | Min. | Max. |
|---|---|---|---|---|
| Calcium (ppm) | 245.64 ±70.43 | 243.71 | 13.59 | 537.49 |
| Magnesium (ppm) | 21.36 ±6.17 | 20.94 | 2.57 | 42.12 |
| Iron (ppm) | 3.04 ±2.28 | 2.44 | 0.54 | 15.86 |
| Zinc (ppm) | 2.63 ±1.25 | 2.54 | 0.50 | 7.01 |
| Potassium (ppm) | 130.77 ±31.27 | 133.32 | 7.98 | 224.17 |
| Sodium (ppm) | 61.16 ±31.98 | 53.52 | 6.65 | 158.72 |
| Phosphorus (ppm) | 207.35 ±64.32 | 204.50 | 12.20 | 434.76 |

**Physicochemical properties of butter among districts**

The Fat contents of butter samples collected were significantly higher for Kuyu district (Table 3). The moisture content of butter samples was in a good agreement with standard for Kuyu district and it was significant.

Table 3. Comparison of fat, moisture and potassium contents of butter in North Shewa Zone, Oromia.

| District | Fat Content (%) | Moisture Content (%) | Potassium (ppm) |
|---|---|---|---|
| Kimbibit | 80.10[b] | 18.28[a] | 136.29 |
| Kuyu | 83.07[a] | 15.30[b] | 126.68 |
| Wachale | 81.19[b] | 17.21[a] | 127.79 |
| CV | 4.56 | 21.91 | 24.25 |
| P-value | 0.00157 | 0.00123 | 0.3746 |

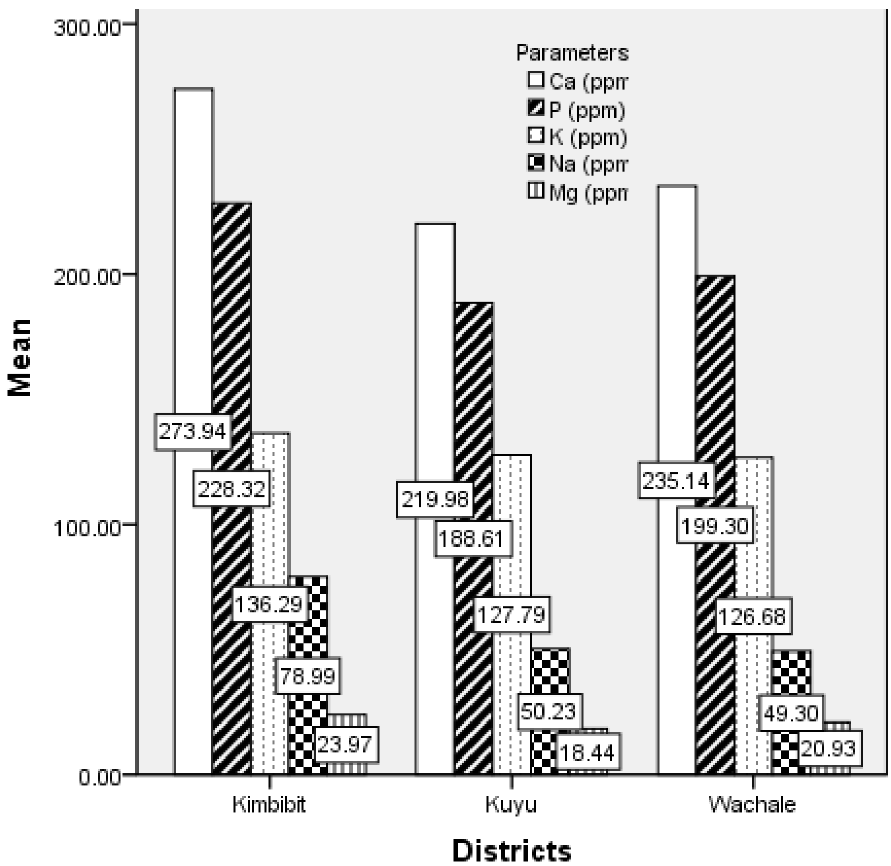


*Figure 2. Calcium (Ca), phosphorus (P), Potassium (P), Sodium (Na) and Magnesium (Mg) content of Butter collected from Kimbibit (n=46),Kuyu (n=36) and Wachale (n=36) districts of North Shewa, Oromia*

The mineral content analyzed showed similar trend along the districts (Figure 2). Calcium content of butter was the highest when compared with other minerals. Mineral content follows the order Ca>P>K>Na>Mg>Fe>Zn.

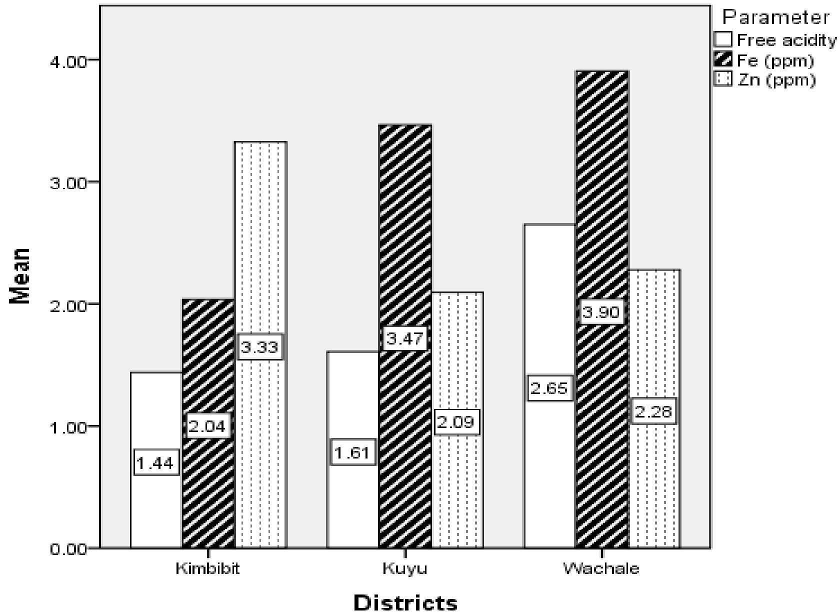


*Figure 3. Free fatty acid, Iron (Fe) and Zinc (Zn) content of Butter collected from Kimbibit (n=46),Kuyu (n=36) and Wachale (n=36) districts of North Shewa, Oromia*

The free fatty acid, Iron and Zinc content of butter samples did not show similar trends with Ca, P, K, Na and Mg (Figure 3). Iron content and free fatty acid of butter samples shown increase along Kimbibit, Kuyu, Wachale districts.

Butter handling process, proper sanitation and use of fumigated container for its storage affect the shelf life of butter according to butter producers (Gebremedhin et al, 2014). The community used to keep butter in different storage materials such as clay pot, plastic material and metal containers (Debela et al, 2016).

**Correlation coefficients between some butter physicochemical parameters**

The correlation coefficient between some physicochemical properties of butter samples collected from North Shewa Zone is shown in Table 4. Ash content moderately correlated with the phosphorus, calcium and magnesium. Phosphorus was very strongly correlated with calcium and magnesium respectively according to Patrick et al (2018) correlation coefficients interpretation.

Table 4. Correlation coefficients between some butter physic-chemical parameters

| | Ash | Moisture | Fat | Non-fat solid | P | Na | K | Fe | Zn | Ca | Mg | Free fatty acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ash | 1 | | | | | | | | | | | |
| Moisture | .279** | 1 | | | | | | | | | | |
| Fat | -.304** | -.997** | 1 | | | | | | | | | |
| Non-fat solid | .328** | .057 | -.140 | 1 | | | | | | | | |
| P | .624** | .286** | -.315** | .379** | 1 | | | | | | | |
| Na | .104 | .206* | -.225* | .245** | .458** | 1 | | | | | | |
| K | .262** | .180 | -.212* | .410** | .659** | .322** | 1 | | | | | |
| Fe | -.146 | .035 | -.037 | .030 | .029 | .038 | .084 | 1 | | | | |
| Zn | .128 | .241** | -.254** | .186* | .216* | .258** | .218* | -.079 | 1 | | | |
| Ca | .618** | .379** | -.418** | .505** | .899** | .539** | .655** | .059 | .342** | 1 | | |
| Mg | .589** | .342** | -.373** | .407** | .888** | .504** | .629** | .005 | .322** | .885** | 1 | |
| Free fatty acid | .242** | -.096 | .089 | .076 | .158 | -.109 | .040 | .095 | .119 | .074 | .234* | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

## Conclusions

Dairy products are important source of nutrients throughout the World. This study was conducted to analyze some physicochemical properties of butter collected from local markets of North Shewa Zone of Oromia Regional State. The study revealed that the average fat content of butter collected from local markets of North Shewa Zone comply to International food standard whereas the moisture content and free fatty acids failed to comply. These non-conformities make butter unfit for export to countries that are abide by the Codex International food standards even though the moisture content of the butter may comply as butter moves to higher value chain. The study shows the necessity to control the quality of butter and provide training on the hygienic practices to be followed by the butter producers. Butter from Kuyu district contained higher fat content than Kimbibit and Wachale districts which implies that more ghee yield can be obtained from butter collected from Kuyu district. The concentration of mineral elements in butter samples followed the order Ca>P>K>Na>Mg>Fe>Zn. Further study on fatty acid profile of butter in the study area is required. The effect of butter storage materials and mineral rich feed on the storability of butter need to be investigated.

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## Food Microbiology Research

# Microbial profile of some waste water irrigated vegetable types in Akaki District of Oromia Special Zone Surrounding Finfinne, Ethiopia

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***Abstract***

*Vegetables of different types are being produced following the river bank of Akaki River which is the most polluted river in Ethiopia. Thus the microbial contamination level of those vegetables need to be quantified. Samples of different vegetable types were collected using random sampling technique following Akaki river bank from three kebeles, namely Echu, Gemeda and Dawara Tino. Magnitude of microbiological profile of each sample were estimated following standard procedures. Lab analysis showed that maximum log cfu/g of SPC of 9.737 log10 cfu/g was recorded on vegetable samples from Dawara Tino Kebele whereas the minimum level of was 8.782 log10 cfu/g from Gammada Kebele obtained from lettuce samples. Most of the samples from Gammada and Dawara Tono kebeles had shown a maximum level of total coliform contamination with log10 cfu/g of 5.042. Dawara Tino showed higher TC contamination of the samples. A relatively higher faecal coliform contamination was still recorded in Dawara Tino Kebele. A maximum level of Staphyloccocus aureus was obtained from cabbage samples in Echu kebele. Samples from Echu kebele exhibited a comparatively higher mold and yeast contamination level with 4.797 log10 cfu/g on onion. The use of polluted water for irrigation greatly contributed to the microbial contamination of the samples. Exposure of all the vegetable types to the microbial contamination has a serious health hazard implication for the consumers and requires effective sanitary actions to be taken before preparation for consumption.*

*Key words: Microbial profile, waste water, vegetables, Akaki River*

## Introduction

Vegetables take a considerable share in the daily diet of Ethiopians. They are utilized by all classes of the society alike. The World Health Organization recommended that one has to get 5-9 servings of fruits and vegetables daily (Johnston *et al*., 2006). Their Significance as an important dietary component is related to their high composition of carbohydrates, proteins, vitamins, minerals, and fiber which are crucial from health point of view. According to the Central Statistical Agency (2015), farmers with holdings near big cities mostly practice vegetable production. In Akaki District, during the dry seasons, vegetable production is facilitated by irrigation water from Akaki river. Akaki river, which flows through Finfinne City is the most polluted river in the country (Yohannes and Elias, 2017). Reports show that industrial (Leta *et al*., 2004), municipal (latrine and home wastes) (CSA, 2014) and medical wastes like laboratory cultures, wound dressings, blood and other body fluids, and needles (FEPA, 2005; Mafuta *et al*., 2011) are readily disposed and discharged into the river without any treatment. As a result, the river's water has become extremely polluted by heavy metals, different ions and faecal coliforms rendering it a very bad status showing that it doesn't meet the quality river water standard and hence unsuitable for different purposes such as drinking, swimming, irrigation, aquatic ecosystem preservation, etc (Mersha, 2012). Even though farmers living in the District close to the river are seriously complaining of the deterioration of the quality of the water (Personal observation), due to absence of other alternatives, they are using the river for preparation of food, homemade beverages, irrigation and even drinking (Yohannes and Elias, 2017). Little Akaki river has been studied and some important biological and physicochemical parameters have been reported. The magnitude of all the physical and chemical parameters analyzed have extremely exceeded the standard limits set by WHO, EU, MoWR AND AAWSA. Likewise the faecal coliforms and the total coliforms count was much higher than the critical level of the microorganisms established by the above mentioned Organizations (Mersha, 2008; Gebremedehin, 2011; Mulu *et al*., 2013; Aschale *et al*. 2015). Among the major sources of contamination of vegetables by fungal, bacterial and parasitic entities is the irrigation water used. There is a scarce documentation of the level of biological contamination of the vegetables produced by Akaki District farmers from Akaki river water irrigation. Therefore, the current investigation is initiated to study the degree of microbial contamination of some vegetables produced by Akaki farmers.

## Materials and Methods

### Sample collection and preparation

Samples were collected by employing random sampling technique from the river bank irrigated vegetables, viz, lettuce, Ethiopian kale, collard greens, cabbage, beet root and onion at time of harvesting. Samples were collected from each type of vegetable at the representative sites and aseptically put into a sterile polyethylene zip bags and transported to IQQO Food Science Lab for analysis. The samples were stored at 4°C for later analysis (Chaturvedi *et al*., 2013; Pinky and Nishant, 2015). About twenty five grams of each vegetable type sample was soaked for 15 mins and washed by shaking thoroughly with 225 ml of physiological saline water. Serial dilutions of each vegetable washing was made in sterile physiological saline water at dilutions $10^{-1}$ to $10^{-8}$.

### Microbial analysis

*Standard plate count (SPC)*

Laboratory analyses to perform standard plate count was performed in accordance with the ISO 4833-1:2003 standard (Anonymous, 2003) and ISO 17410:2001 (Anonymous, 2001).

*Enumeration of total and faecal coliforms*

Samples were prepared as described above. Homogenate or the rinse fluid was prepared using PSW. For each selected dilution, 0.1 ml of sample was spread-plated onto violet red bile agar (HiMedia, India). The plates were incubated at 37 °C for 24 h. The number of pink (coliform) and purple colonies was counted (Frampton *et al*., 1988). Identification of coliforms was carried out with IMVIC tests (Andrews, 1992). Enumeration of faecal coliform was conducted by following MPN technique.

*Detection and enumeration of Staphyloccocus aureus*

A volume of 0.1mL aliquot of appropriate dilution was spread-plated in duplicate on presolidified plates of Manitol salt agar. Inoculated plates were incubated at 35°C for 24 hrs. Yellow colonies on Mannitol Salt Agar plates were picked aseptically for further identification procedures and confirmed employing cell morphology, gram staining, motility, and catalase tests.

*Enumeration of mold and yeast*

Laboratory analyses to enumerate of yeasts and molds were performed in accordance with the ISO 7954:1987 standard (Anonymous, 1987).

## Results and discussion

Sixteen samples of different vegetable types, namely, cabbage, collard greens, Ethiopian cabbage, Lettuce, beet root and onion were collected at harvesting time from three kebeles (Echu, Gammada and Dawara Tinno) of Akaki District following Akaki river bank. Each of the samples were tested for their microbial quality. Total bacteria, total coliform, faecal coliform, *Staphylococcus aureus* and yeast and mold were counted for each of the samples.

**Standard plate count (SPC)**

At Echu kebele among the vegetable types collected, the maximum mean log10 CFU/g of 9.643 (range: 9.347 to 9.913) of standard plate count was recorded from cabbage samples. Whereas the minimum mean value was obtained from onion samples (9.161 log10 CFU/g). The rest of the samples of the vegetable types tested, *viz*. collard greens, Ethiopian cabbage, beet root and Lettuce had a standard plate count level of 9.527, 9.446, 9.230 and 9.208 log10 CFU/g, respectively (Table 1).

At Gammada kebele, the maximum mean SPC of 9.737 log10 CFU/g was read from cabbage samples. The least value was from Lettuce samples with 8.782 log10 CFR/g (range: 8.737 to 8.828 log10 CFU/g). Samples of beet root and Ethiopian cabbage took the second and third places in terms of SPC with 9.470 and 9.334 log10 CFU/g (Table 1).

In Dawara Tino Kebele elevated magnitude of SPC was recorded with log10 CFU/g of 9.737. In general, Echu and Gammada were statistically similar in SPC magnitude whereas Dawara Tino was significantly higher than the two kebeles (Figure 1).

A study conducted at the upper part of the river in Finfinne municipality by Sisay *et al*., (2021) Ethiopian kale and celery reported a far lower level of SPC with a range of 3.3 to 6.8 log10 CFU/g. However, a comparable level of SPC range of 6.8 to 8.5 log10 CFU/g was reported from waste water irrigated vegetable types produced in Harar town (Getachew, 2020). The mean SPC of waste water irrigated vegetables produced around Kombolcha town, Northeastern Ethiopia showed a maximum of 4.6 log10 CFU/g which a considerably lower as compared to the current finding (Leykun *et al*., 2022). Similarly Delesa (2017) (as cited in Dejen, 2020) reported a relatively lower mean total bacteria count of 4.2 log10 CFU/g in different localities of Nekemte town.

Table 1. Standard plate count (SPC) (log10 cfu/g) values of vegetable samples (mean±SE)

| | Vegetable type | Mean | Min | Max |
|---|---|---|---|---|
| | **Beet root** | 9.230±0.233 | 8.98 | 9.441 |
| | **Onion** | 9.161±0.169 | 8.992 | 9.329 |
| **Echu** | **Green collards** | 9.527±0.099 | 9.458 | 9.597 |
| | **Ethiopian kale** | 9.446±0.471 | 8.903 | 9.737 |
| | **Cabbage** | 9.643±0.381 | 9.374 | 9.913 |
| | **Lettuce** | 9.208±0.184 | 9.043 | 9.407 |
| | **Beet root** | 9.470±0.462 | 8.936 | 9.737 |
| | **Onion** | 9.318±0.140 | 9.216 | 9.477 |
| **Gamadda** | **Green collards** | 9.334±0.326 | 9.008 | 9.659 |
| | **Ethiopian kale** | 9.136±0.414 | 8.722 | 9.551 |
| | **Cabbage** | 9.737±0.000 | 9.737 | 9.737 |
| | **Lettuce** | 8.782±0.046 | 8.737 | 8.828 |
| | **Beet root** | 9.737±0.000 | 9.737 | 9.737 |
| **Dawara Tino** | **Onion** | 9.737±0.000 | 9.737 | 9.737 |
| | **Green collards** | 9.737±0.000 | 9.737 | 9.737 |
| | **Ethiopian kale** | 9.737±0.000 | 9.737 | 9.737 |
| | **Cabbage** | 9.737±0.000 | 9.737 | 9.737 |
| | **Lettuce** | 9.737±0.000 | 9.737 | 9.737 |

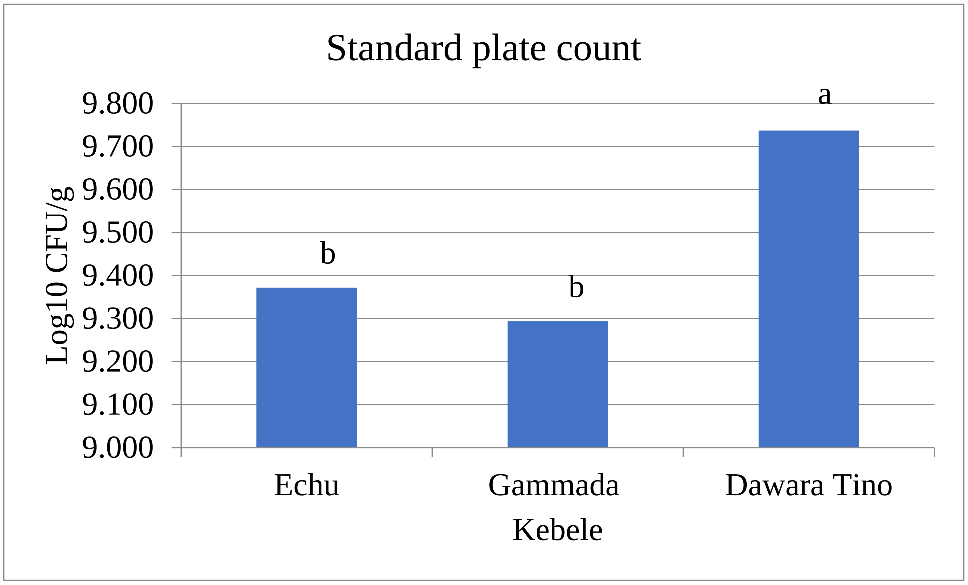


*Figure 3. Magnitude of SPC from vegetable samples from the three kebeles*

**Total coliform count (TCC)**

All the vegetable samples from each type were analyzed for contamination with coliform bacteria. Total coliform value ranged from 4.482±0.970 to 5.042±0.000 log10 CFU/g at Echu Kebele. Onion, collard greens, Ethiopian kale and cabbage were equally contaminated with total coliforms with the highest value of 5.042±0.000. On the contrary, beet root and lettuce were relatively low in coliform infestation with 4.554±0.844 and 4.482±0.970 log10 CFU/g, respectively (Table 2).

Analysis of samples showed a similar level of coliform contamination in samples from Gammada Kebele. Onion, collard greens, Ethiopian kale, cabbage and lettuce had shown an equivalent value of 5.042±0.000 log10 CFU/g. Beet root gave lower value of 4.849±0.335 log10 CFU/g. A comparable magnitude of total coliform was observed in samples from Dawara Tino kebele with an exception that lettuce gave the least value of 4.849±0.335 log10 CFU/g. Over all, there was no significant statistical variability among the Kebeles with respect to total coliform count (fig. 2). On the basis of HACCP-TQM technical guideline, all the samples have shown an "average" contamination level with respect to TCC (Table 4).

Berhanu *et al*., (2022) reported a relatively lower value of coliform contamination ranging from 3.7 to 3.9 log10 CFU/g in lettuce, cabbage, carrot and tomato in North Western Ethiopia. Similarly, a value range of 3.05 to 4.54 log10 CFU/g was reported in cabbage, lettuce and carrot samples in the different sub cities of

Nekemte town (Delesa, 2017). In contrary, Getachew (2020) reported a comparatively higher total coliform value ranging from 6.792 to 5.708 log10 CFU/g from waste water produced Lettuce, Spinach, Kale and Cabbage samples in Harar town. Similarly, Mamdouh *et al*., (2019) reported that a total coliform count value range of 5.0± 0.29 to 5.7± 0.41 in waste water irrigated vegetable samples in Egypt.

Table 2. Total coliform count (TCC) (log10 cfu/g) values of vegetable samples (mean±SE)

| Kebele | Vegetable type | Mean | Min | Max | Standard category (based on mean) |
|---|---|---|---|---|---|
| **Echu** | Beet R. | 4.554±0.844 | 3.58 | 5.042 | Average |
| | Onion | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Ethiopian kale | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Collard greens | 5.042±0.000 | 5.041 | 5.042 | Average |
| | Cabbage | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Lettuce | 4.482±0.970 | 3.362 | 5.042 | Average |
| **Gammada** | Beet R. | 4.849±0.335 | 4.462 | 5.042 | Average |
| | Onion | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Ethiopian kale | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Collard greens | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Cabbage | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Lettuce | 5.042±0.000 | 5.042 | 5.042 | Average |
| **Dawara Tino** | Beet R. | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Onion | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Ethiopian kale | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Collard greens | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Cabbage | 5.042±0.000 | 5.042 | 5.042 | Average |
| | Lettuce | 4.849±0.335 | 4.462 | 5.042 | Average |

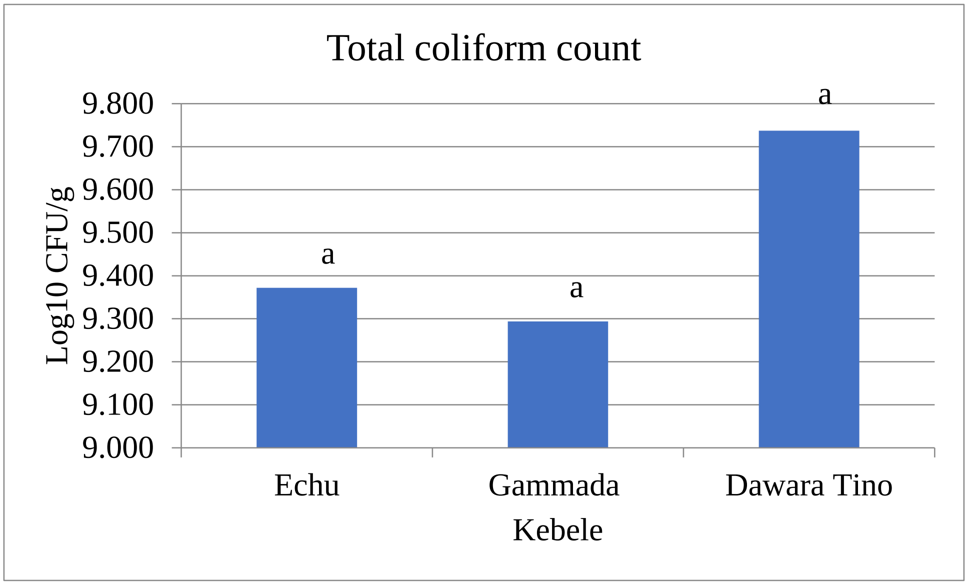


*Figure 4. Magnitude of TCC from vegetable samples in the three kebeles*

**Faecal coliform count**

Analysis of faecal coliform for the vegetable samples showed that the highest contamination level of 5.042±0.000 (minimum: 5.042; maximum: 5.042) was recorded on cabbage and the least value of 3.702±0.333 (minimum: 3.447; maximum: 4.079) was obtained from beet root samples.

A relatively higher degree of faecal coliform contamination was obtained from samples collected from Gammada kebele with a range of 3.759 log10 CFU/g in lettuce to 5.041 in beet root and collard greens (Table 1). Onion, Ethiopian kale and cabbage gave a faecal coliform value range of 4.623 to 4.695 log10 CFU/g. According to HACCP-TQM technical guideline, most of the samples have attained an "average" contamination status with samples of beet root, collard greens and lettuce taking a "good" status in FCC population (Table 4).

This finding is in agreement with the report of Desta *et al*., (2017). Lettuce samples collected from different farm sites in Addis Ababa city had shown a mean faecal coliform contamination range of 3.46±0.44 to 5.03±1.38 log10 MPN/g. However, a comparatively lower FCC (3.3 to 3.5 log10 CFU/g) was reported by Leykun *et al*., (2022) from lettuce (3.5±0.4), cabbage (3.3±0.2), carrot (3.5±0.2) and tomato (3.5±0.2) samples in Northeastern Ethiopia. Samples of Ethiopian kale, lettuce and swiss chard collected from Akaki river exhibited a FC contamination range of 10 to 2800 CFU/g in parts of Addis Ababa city and Oromia Special Zone Surrounding Finfinne (Sisay *et al*., 2021). In the current study, each of the samples collected from further downstream along the bank of Akaki river, in Dawara Tino kebele, has shown a relative increase in the faecal coliform infestation as compared to the rest of the sampling kebeles (fig 3). On average there was an increment of 2.444 log10 CFU/g down along the river.

Table 3. Faecal coliform count (FCC) (log10 CFU/g) of vegetable samples in the three Kebeles

| Kebele | Vegetable type | Mean | Min | Max | Standard category (based on mean) |
|---|---|---|---|---|---|
| **Echu** | Beet root | 3.702±0.333 | 3.447 | 4.079 | good |
| | Onion | 4.499±0.177 | 4.322 | 4.676 | average |
| | Ethiopian kale | 4.071±0.146 | 3.968 | 4.174 | average |
| | Collard greens | 3.968±0.930 | 3.431 | 5.042 | good |
| | Cabbage | 5.042±0.000 | 5.042 | 5.042 | average |
| | Lettuce | 3.769±1.115 | 2.964 | 5.042 | good |
| **Gammada** | Beet root | 5.042±0.000 | 5.041 | 5.042 | average |
| | Onion | 4.695±0.332 | 4.38 | 5.041 | average |
| | Ethiopian kale | 4.623±0.419 | 4.204 | 5.042 | average |
| | Collard greens | 5.042±0.000 | 5.042 | 5.042 | average |
| | Cabbage | 4.852±0.268 | 4.663 | 5.042 | average |
| | Lettuce | 3.759±1.283 | 2.476 | 5.041 | good |
| **Dawara Tino** | Beet root | 5.042±0.000 | 5.042 | 5.042 | average |
| | Onion | 4.922±0.120 | 4.802 | 5.042 | average |
| | Ethiopian kale | 5.042±0.000 | 5.042 | 5.042 | average |
| | Collard greens | 5.042±0.000 | 5.042 | 5.042 | average |
| | Cabbage | 5.042±0.000 | 5.042 | 5.042 | average |
| | Lettuce | 4.849±0.335 | 4.462 | 5.042 | average |

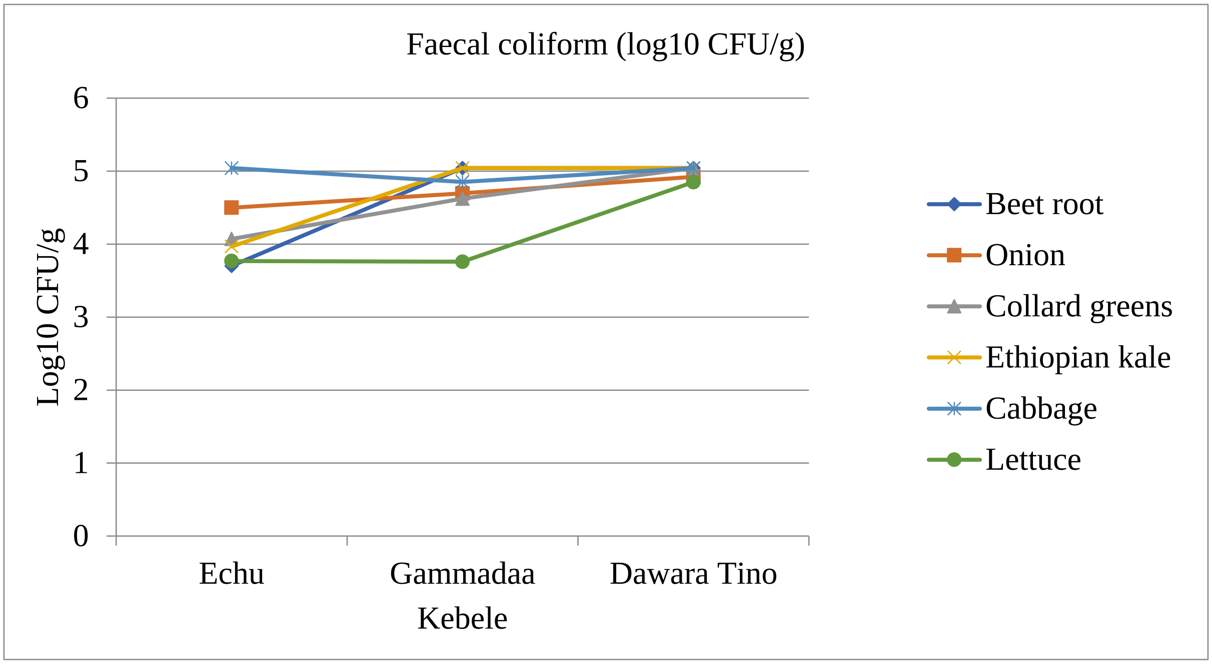


*Figure 5. Trend of faecal coliform in the three kebeles along the river*

***Staphyloccocus aureus* count (SC)**

All the samples were tested for contamination by *S. aureus.* In Echu kebele, cabbage (6.135±0.000 log10 CFU/g), collard greens (5.046±3.161 log10 CFU/g) and beet root (4.922±3.059 log10 CFU/g) took first, second and third places, respectively, in terms of *S. aureus*. Similarly, 4.644±2.815 log10 CFU/g was obtained in lettuce samples. Ethiopian kale samples have been found least contaminated with the value of 1.396±0.000 log10 CFU/g**.** Onion and Ethiopian kale attained a “good” status in *S. aureus* contamination, whereas, the rest of the vegetable types were “average” as described by HACCP-TQM technical guideline (Table 4).

In Gammada kebele, most of the vegetable types had been found to show lower SC levels. Beet root, Ethiopian kale, cabbage and lettuce contamination was reduced by 3.526, 3.65, 2.356 and 3.248, respectively. Whereas increments have also been reported in onion and collard greens. Onion and Ethiopian kale and lettuce became the highest and the least contaminated. Most of the vegetable types exhibited a “good” status in SC except onion which showed an “average” level of contamination (Table 4).

In Dawara Tino kebele however, the level of SC have been shown to be lifted as compared to Gammada with some of the samples still being lowered. The highest and the lowest levels were taken by lettuce (7.093±0.307 log10 CFU/g) and cabbage (1.396±0.000 log10 CFU/g). Ethiopian kale, collard greens and Onion, too, had a significant level of contamination ranging from 4.200±2.804 to 5.596±0.715 log10 CFU/g (Table 4). There was a significant statistical difference among the kebeles in SC (fig. 4). With most of the vegetable types being “average” in SC infestation level, cabbage had shown a “good” status, whereas, lettuce had shown a “poor” level of contamination (Table 4).

Even though most of the works in Ethiopia failed to consider *S. aureus* count in waste water irrigated vegetable samples, Desta and Diriba (2016) reported it in the vegetables produced by irrigation from Awetu river in Jimma town. A maximum *S. aureus* contamination of 2.97±0.3 was obtained in lettuce samples which is lower as compared to the finding of the current result. However, it seems to be comparable with the report of Mamdoh *et al*., (2019) in Gana. *S. aureus* ranged from 4.2 – 5.2 log10 CFU/g in samples of cucumber, lettuce and Arugula. Comparison of the three kebeles showed that there is a significant difference among them in terms of *S. aureus* showing a general increasing trend downstream along the river (fig. 4). In general, Schelin *et al*., (2011) reported that production of enterotoxin reaches when *S. aureus* count reaches 6 log cfu/g.

Table 4. *Staphyloccocus aureus* (SC) (log10 CFU/g) of vegetable samples in the three Kebeles

| **Kebele** | **Vegetable type** | **Mean** | **Min** | **Max** | **Standard category (based on mean)** |
|---|---|---|---|---|---|
| **Echu** | Beet root | 4.922±3.059 | 1.396 | 6.867 | average |
| | Onion | 2.269±0.872 | 1.396 | 3.141 | good |
| | Ethiopian kale | 1.396±0.000 | 1.396 | 1.396 | good |
| | Collard greens | 5.046±3.161 | 1.396 | 6.913 | average |
| | Cabbage | 6.135±0.000 | 6.135 | 6.135 | average |
| | Lettuce | 4.644±2.815 | 1.396 | 6.374 | average |
| **Gammada** | Beet root | 1.396±0.000 | 1.396 | 1.396 | good |
| | Onion | 4.988±3.114 | 1.396 | 6.936 | average |
| | Ethiopian kale | 1.396±0.000 | 1.396 | 1.396 | good |
| | Collard greens | 3.765±2.369 | 1.396 | 6.135 | good |
| | Cabbage | 3.779±3.370 | 1.396 | 6.163 | good |
| | Lettuce | 1.396±0.000 | 1.396 | 1.396 | good |
| **Dawara Tino** | Beet root | 4.174±3.929 | 1.396 | 6.952 | average |
| | Onion | 5.596±0.715 | 4.881 | 6.311 | average |
| | Ethiopian kale | 5.309±3.413 | 1.396 | 7.668 | average |
| | Collard greens | 4.200±2.804 | 1.396 | 7.004 | average |
| | Cabbage | 1.396±0.000 | 1.396 | 1.396 | good |
| | Lettuce | 7.093±0.307 | 6.842 | 7.436 | poor |

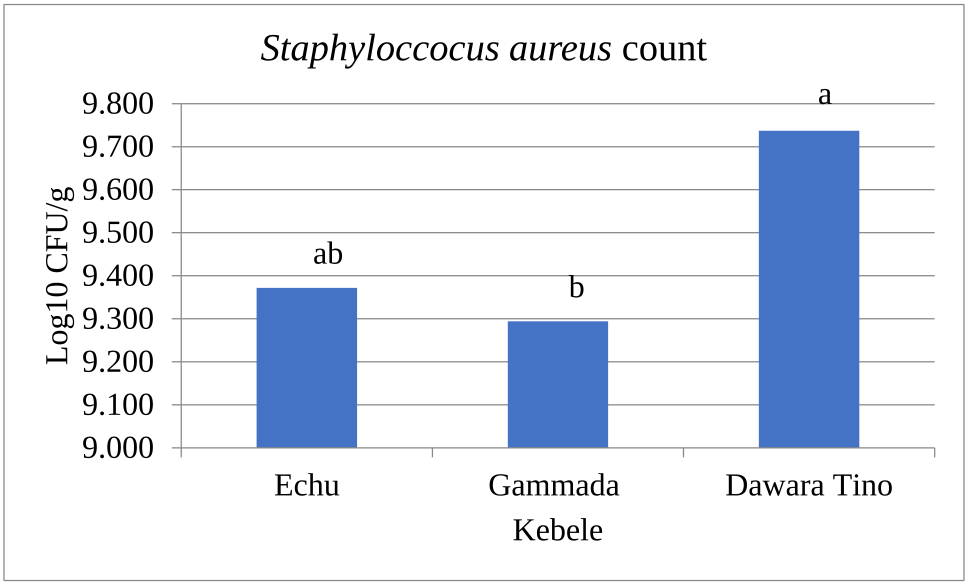


*Figure 6. Magnitude of SC from vegetable samples in the three kebeles*

**Mold and yeast count**

A maximum and minimum mold and yeast count of 4.797±0.394 log10 CFU/g and 3.931±0.618 log10 CFU/g have been obtained in onion and lettuce samples, respectively from Echu kebele. An equivalent level of contamination was observed on samples of beet root, collard greens, Ethiopian kale and cabbage with the range of 4.064±0.249 log10 CFU/g to 4.321±0.086.

Mold and yeast contamination has become relatively lower at Gammada kebele. The maximum level was observed on collard greens with 4.755 log10 CFU/g. A minimum of 2.135±1.279 log10 CFU/g was recorded on beet root samples. Whereas, the result from Dawara Tino samples has become comparable to that of Echu kebele with the maximum and minimum range value of 4.525±0.363 log10 CFU/g to 2.976±0.501 log10 CFU/g on Ethiopian kale and cabbage samples, respectively (Table 5). The three kebeles varied significantly in terms of MYC with relatively lower value observed in Gammada (fig. 5).

The current result is in line with report of Delesa (2017). A maximum and minimum mold and yeast contamination values of 4.35 and 3.33 log10 CFU/g was obtained in waste water irrigated vegetable samples in Nekemte town.

Table 5. Mold and yeast count (log10 CFU/g) in vegetable samples in the three Kebeles

| Kebele | Vegetable type | Mean | Min | Max |
|---|---|---|---|---|
| **Echu** | Beet root | 4.164±0.230 | 3.913 | 4.365 |
| | Onion | 4.797±0.394 | 4.403 | 5.192 |
| | Ethiopian kale | 4.064±0.249 | 3.888 | 4.240 |
| | Collard greens | 4.194±0.169 | 4.000 | 4.311 |
| | Cabbage | 4.321±0.086 | 4.260 | 4.382 |
| | Lettuce | 3.931±0.618 | 3.260 | 4.477 |
| **Gammada** | Beet root | 2.135±1.279 | 1.396 | 3.612 |
| | Onion | 2.611±2.105 | 1.396 | 5.041 |
| | Ethiopian kale | 3.112±1.716 | 1.396 | 4.828 |
| | Collard greens | 4.755±0.125 | 4.631 | 4.880 |
| | Cabbage | 2.962±2.214 | 1.396 | 4.527 |
| | Lettuce | 2.849±1.452 | 1.396 | 4.301 |
| **Dawara Tino** | Beet root | 4.037±0.330 | 3.804 | 4.270 |
| | Onion | 3.589±0.148 | 3.442 | 3.737 |
| | Ethiopian kale | 4.358±0.172 | 4.237 | 4.555 |
| | Collard greens | 4.525±0.363 | 4.163 | 4.888 |
| | Cabbage | 2.976±1.501 | 1.476 | 4.477 |
| | Lettuce | 3.677±0.487 | 3.357 | 4.237 |

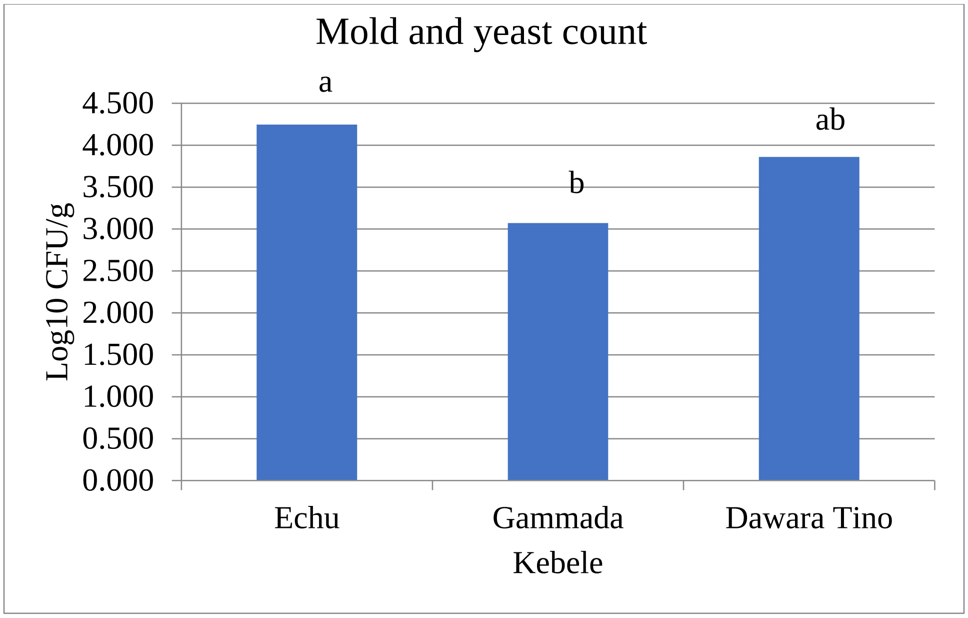


*Figure 7. Magnitude of MYC from vegetable samples in the three kebeles*

## Conclusion and recommendation

From the study, it could be understood that the microbial contamination of almost all the samples of the different types of vegetables have exceeded the standard limit set by WHO (2006).This indicates that there is potential health hazard associated with the consumption of the inadequately treated vegetables. Analysis of SPC showed that all the samples had a substandard level of hygienic quality for which the consumers should be well aware of it. Most of the samples from the different types of vegetable were also not entirely free of indicator, pathogenic and spoilage microorganisms to the level safe for human consumption. It is also critical that further research efforts made that provide safe and effective technologies for the disinfection of the produce for healthy diet. This requires that effective sanitary and disinfection actions should be taken before preparation for consumption. Moreover, since the major source of microbial contamination of the vegetables being produced along the Akaki river bank is the highly polluted irrigation water, the government should formulate and indicate a corrective policy direction that helps cease the pollution of Akaki river.

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# Assessment of microbial quality of drinking water from different sources in selected districts of Bale Zone, Oromia Regional State

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## Abstract

*Safe drinking water is a vital component of human diet which ensures the sustainability of life. Thus periodic surveillance of the quality of drinking water is critically important to guarantee the health of the society. The current study is initiated to assess the microbial quality of drinking water from different sources in selected districts of Bale Zone. Laboratory analysis was done following standard procedures. The analysis showed that a maximum of 6.396+0.010 log10 CFU $ml^{-1}$ of SPC have been recorded in samples from Tegona river in Goba district. Hand pump samples have scored a maximum total coliform value of 1023.54±77.378 MPN 100 $ml^{-1}$ in Goba district with the minimum being 32.663±21.730 MPN 100 $ml^{-1}$ value being obtained from spring in Goro district. The minimum faecal coliform population has been recorded in samples of bore hole in Goba, whereas the highest contamination was 1101 MPN 100 $ml^{-1}$ in a range of sources such as rivers Bamo and Tegona in Goba and Haro Wanji of Dello Mana. The least E. coli value of 2.990 MPN 100 $ml^{-1}$ was obtained from bore hole samples in Goba district with the highest being $972.800^{a}$±128.200 MPN 100 $ml^{-1}$ from Bamo river. Faecal coliform and E. coli followed similar trend across the three agro ecologies highland, lowland and mid altitude from top to down. Rivers and ponds being used as source of drinking water were found to be highly contaminated and categorized under high to very high risk classifications. Thus, the society, as an emergency action, should take actions like filtration, boiling and treatment with some commercially available antimicrobial agents following manufacturers' instructions. The government is expected to establish facilities for the supply of safe drinking water to avoid the likely health burden to be posed on the society from the highly contaminated water sources.*

*Key words: microbial quality, drinking water, sources, Bale*

## Introduction

Sustainability of life is ensured by the accessibility of safe drinking water (Alhassan, 2014) and satisfactory supply should be available to all (WHO, 2011). The same organization defined safe drinking water as that which does not represent any significant risk to health over a life time of consumption, including different sensitivities that may occur between life stages. Those at greatest risk of waterborne disease are infants and young children, people who are debilitated and the elderly, especially when living under unsanitary conditions. World Health Organization (2003a) reported that, more than 80% of human diseases in the World is attributed to the use of unsafe drinking water or inadequate sanitary practices. Cholera, shigellosis and Campylobacteriosis are among the important water borne diseases easily contracted due to contaminated water sources serving. Recent WHO report briefed that about 1.1 billion people, out of which 42% (WHO & UNICEF, 2010), globally drink unsafe water and the vast majority of diarrhoeal diseases in the World, estimated to be nearly 88%, are attributed to unsafe drinking water, sanitation and hygiene. The consumption of unsafe water is responsible for the 3.1% (1.7 million), of the annual deaths and 3.7% (54.2 million) of the annual health burden (Burgess and Pletschke, ). In general, most water borne outbreaks involve source contamination, breakdown of the treatment system, contamination of the distribution system and the use of untreated water (WHO, 2004).

It is estimated that about 75% of the health problems in Ethiopia is communicable arising from inaccessibility to safe and adequate water supply ((UN-WATER/WWAP, 2004). In the country, the number of companies engaged on production and trading of packaged/bottled water is increasing from time to time (Ensermu, 2014). The Ethiopian water quality standard requires that no *E. coli* or thermoduric bacteria and Coliform bacteria are detected in the treated water entering the distribution system and water in the distribution system (FDRE-MOWR, 2002). Bacteriological analysis of drinking water (tap, spring and well) from around Dire Dawa City showed that all the samples (100%) from spring and tap were positive for indicator microorganisms (total coliforms, thermotolerant (faecal) coliforms. Whereas 50% of the tap water samples were found to be contaminated with the same organisms (Desalegn *et al.*, 2013). Assessment of the microbiological quality of drinking water in four districts of Addis Ababa City reported that 34% of the samples were contaminated with faecal bacteria (Crampton, 2005). Likewise, microbial quality study of drinking water in Debre Zeyit Town found that 100% of the drinking water samples from underground sources were contaminated with total coliforms and 20% were found to be positive for faecal coliforms and faecal streprococci. Thus, regular examination of the drinking water resource with regard to microbiological and physicochemical quality is of crucial importance so that appropriate remedial actions can be forwarded to safe guard the community.

So far, quality of drinking water from different sources have not been investigated in Bale Zone in terms of microbiology. Therefore, this study was initiated to assess the microbiological quality of drinking water from different sources across the different agro-ecologies Bale zone of Oromia Regional State.

## Materials and methods

### *Sample collection*

Drinking water samples were collected from purposively selected Districts of Bale Zone, namely, Goro, Goba and Dallo Manna, Oromia Regional State by consultation of Regional Bureau, Zonal and District Offices of Water Resources. The assessments were carried out on samples from three selected Districts of the Zones. A total of 135 samples of drinking water were collected randomly from different sources in the Zone. Analyses were done in plant pathology laboratory of Sinana Agricultural Research Center during late October 2021 which is the last month of the heavy rainy season in the Zone.

### *Microbial analysis*

Microbiological analyses of water samples were done following standard methods as described in Standard Methods for the Examination of drinking water (WHO, 1996; APHA, 2005). Determination of Total and fecal coliforms were performed by the most probable number (MPN) per 100 ml sample using multiple tube fermentation technique including presumptive, confirmed and completed phases (APHA, 2005). Further identification of coliforms was done by carrying out the appropriate biochemical tests like, indole production, methyl red, voges/proskeur, citrate utilization, motility, gram staining and gas production from lactose (EHNRI, 2003).

## Results and discussion

*Total bacterial count*

All drinking water samples collected from the 9 kebeles of the three districts of Bale Zone were tested for their hygienic status in terms of total bacterial count. In Goro District, a total of 45 samples were collected out of which 42 were from tap and only 3 were from unprotected spring. There was statistically none significant variability between the kebeles of the district, with the samples from spring in Waltai Gobu scoring the highest log10 CFU $ml^{-1}$ of 6.132 and the minimum value of 6.009 log10 CFU $ml^{-1}$ was obtained from tapped drinking water from the same district (Table 1). Water samples from Garre and Chaffe Mana kebeles were non significantly different from those of Waltai Gobu. The current finding is a little higher than that reported ($5.4 \times 10^3$) by Amira *et al*., (2015).

A relatively higher log10 CFU $ml^{-1}$ was recorded from samples collected from Goba district. The maximum count (6.396 log10 CFU $ml^{-1}$) was from samples of Tegona river in Waltai Sura kebele, whereas the least record (6.159) was from samples collected from households in Waltai Tosha kebele who fetched the water from Bamo river.

Dallo Manna samples were collected from 4 sources (river (53%, tap 18%, pond 24% and spring 4%). Samples from tap collected from Barraq kebele exhibited the highest level of bacterial contamination with log10 CFU $ml^{-1}$ of 8.330. Samples from spring, Gongoma river and Haro Sora pond took the $2^{nd}$, $3^{rd}$ and $4^{th}$ places in bacterial contamination with values of 8.305, 8.204 and 8.080 log10 CFU $ml^{-1}$, respectively (Table 1).

Table 1. Total bacterial count (log10 CFU $ml^{-1}$)

| District | Kebele | Drinking water source | N | Minimum | Maximum | Mean | P (<0.05) |
|---|---|---|---|---|---|---|---|
| **Goro** | Waltai Gobu | Tapped | 12 | 5.538 | 6.244 | $6.009^{cd} \pm 0.068$ | <0.0001 |
| | | Spring | 3 | 5.925 | 6.239 | $6.132^{c} \pm 0.104$ | 0.0003 |
| | Garre | Tapped | 15 | 5.621 | 6.244 | $6.014^{cd} \pm 0.050$ | <0.0001 |
| | Chafe Mana | Tapped | 15 | 5.834 | 6.436 | $6.026^{cd} \pm 0.044$ | <0.0001 |
| **Goba** | Waltai Sura | River (Tegona) | 4 | 6.368 | 6.410 | $6.396^{c} \pm 0.010$ | <0.0001 |
| | | River (Tegona-Household) | 4 | 6.301 | 6.417 | $6.349^{c} \pm 0.024$ | <0.0001 |
| | | River (Micha-Household) | 5 | 6.073 | 6.394 | $6.245^{c} \pm 0.064$ | <0.0001 |
| | | Bore hole (Burgullo) | 2 | 6.354 | 6.382 | $6.368^{c} \pm 0.014$ | 0.001 |
| | Waltai Tosha | River (Bamo) | 8 | 5.837 | 6.428 | $6.307^{cde} \pm 0.057$ | <0.0001 |
| | | River (Bamo-Household) | 7 | 6.135 | 6.436 | $6.159^{c} \pm 0.080$ | <0.0001 |
| | Aloshe | Hand pump | 13 | 6.089 | 6.401 | $6.238^{c} \pm 0.037$ | <0.0001 |
| | | Bore hole | 2 | 6.089 | 6.401 | $6.231^{c} \pm 0.097$ | 0.218 |
| **Dallo Manna** | Haya Oda | River (Yadot-Household) | 6 | 5.842 | 5.851 | $5.847^{cde} \pm 0.004$ | <0.0005 |
| | | River (Erba) | 4 | 5.374 | 5.718 | $5.516^{de} \pm 0.104$ | <0.0005 |
| | | River (Erba-Household) | 5 | 5.189 | 5.640 | $5.384^{e} \pm 0.095$ | <0.0004 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Barraq | Tapped | 4 | 8.307 | 8.362 | $8.330^{a}\pm0.013$ | <0.0001 |
| | | Pond (Haro Sora) | 8 | 7.675 | 8.360 | $8.080^{ab}\pm0.077$ | <0.0001 |
| | | Pond (Haro Wanji) | 3 | 7.658 | 8.293 | $8.012^{ab}\pm0.187$ | 0.0005 |
| | Gongoma | Tapped | 4 | 6.403 | 8.348 | $7.810^{b}\pm0.469$ | 0.0005 |
| | | Spring | 2 | 8.260 | 8.350 | $8.305^{ab}\pm0.045$ | 0.0034 |
| | | River (Gongoma) | 9 | 7.882 | 8.393 | $8.204^{ab}\pm0.081$ | 0.0001 |

*Total coliform count*

A highly significant difference was observed in total coliform count among the drinking water samples of Goro district. Samples from spring at Waltai Gobu and tap water from Chafe Mana kebeles had the least coliform contamination of 32.663 and 60.661 MPN/100 ml, respectively, of water with non significant statistical variation. On the contrary, a higher total coliform count of 95 CFU/100 ml was recorded from tap water samples in Nekemte town (Gonfa *et al*., 2019). However, tapped water from Garre kebele had a coliform contamination of 549.933 MPN/100 ml (Table 2). A lower value ranging from 1.50±0.71CFU/100ml to 133.67±21.25 CFU/100ml was reported by Desalegn *et al.,* (2013) from unprotected well and tap water samples in Dire Dawa Administrative council. A similarly lower level of contamination was reported in drinking water samples from spring (2 - 70 MPN/100ml) and hand pipe (2 – 9 MPN/100ml) by Negera *et al*., (2017) in a study conducted in Shashemene rural districts. In Fiche town still lower total coliform count range of 3.93 to 9.29 CFU/ml was recorded in dry and wet seasons, respectively from samples of piped drinking water (Israel *et al*., 2021).

Total coliform counts have been shown to radically increase in samples from Goba district. The test showed that samples from Tegona river (Waltai Sura), Bamo river (Waltai Tosha), hand pump and unprotected bore hole (Aloshe) showed coliform count range of 1101.0 to 1023.54 MPN/100 ml of sample. This finding agrees with the result of Edessa *et al*., (2017) reporting a total coliform range of 270 to 1600 MPN/100 ml of drinking water samples from river. Similarly, a total coliform range of 67 to 1366 MPN/100 ml of sample drinking water from wells.  Household drinking water samples (rivers and unprotected bore hole) from Waltai Sura kebele had a cell number range of 670.000 to 756.000 MPN/100 ml (Table 2).

In Dallo Manna district, out of the 9 sources used for sampling, drinking water samples from the 7 sources had a total coliform cell count of 885 MPN/100 ml. Only samples of tap from Barraq kebele scored a relatively lower count of 593.75 MPN/100 ml of sample (Table 2). The analysis result showed that none of the samples comply with the WHO guideline or the National standards of faecal coliform count per 100 ml for drinking water.

Table 2. Total coliform count 100 $ml^{-1}$ of water sample

| District | Kebele | Drinking water source | N | Minimum | Maximum | Mean | p (<0.05) | %detection |
|---|---|---|---|---|---|---|---|---|
| **Goro** | Waltai Gobu | Tapped | 12 | 2.990 | 1101.000 | $368.999^{cd}\pm 128.661$ | 0.015 | 91.7 |
| | | Spring | 3 | 2.99 | 75.000 | $32.663^{d}\pm21.730$ | 0.272 | 66.7 |
| | Garre | Tapped | 15 | 5.621 | 6.244 | $549.933^{bc}\pm124.432$ | 0.001 | 100 |
| | Chafe Mana | Tapped | 15 | 2.990 | 150.000 | $60.661^{d}\pm17.978$ | 0.005 | 46.7 |
| **Goba** | Waltai Sura | River (Tegona) | 4 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | | River (Tegona-Household) | 4 | 240.000 | 1101.00 | $670.500^{abc}\pm248.549$ | 0.074 | 100 |
| | | River (Micha-Household) | 5 | 240.000 | 1101.00 | $756.600^{abc}\pm210.901$ | 0.0230 | 100 |
| | | Bore hole (Burgullo) | 2 | 240.000 | 1100.00 | $670.000^{abc}\pm0.014$ | 0.3632 | 100 |
| | Waltai Tosha | River (Bamo) | 8 | 240.000 | 1101.00 | $1014.90^{ab}\pm86.100$ | <.0001 | 100 |
| | | River (Bamo-Household) | 7 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | Aloshe | Hand pump | 13 | 95.000 | 1101.00 | $1023.54^{ab}\pm77.378$ | <.0001 | 100 |
| | | Bore hole | 2 | 1100.00 | 1101.00 | $1100.50^{a}\pm0.500$ | 0.0003 | 100 |
| **Dallo Manna** | Haya Oda | River (Yadot-Household) | 6 | 5.988 | 5.988 | $1100.67^{a}\pm0.333$ | 0.000 | 100 |
| | | River (Erba) | 4 | 240.000 | 1101.00 | $885.750^{ab}\pm215.250$ | 0.0260 | 100 |
| | | River (Erba-Household) | 5 | 1100.00 | 1101.00 | $1100.80^{a}\pm0.200$ | <0.0001 | 100 |
| | Barraq | Tapped | 4 | 23.000 | 1101.00 | $593.750^{abc}\pm294.006$ | <0.0001 | 100 |
| | | Pond (Haro Sora) | 8 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | | Pond (Haro Wanji) | 3 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | Gongoma | Tapped | 4 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | | Spring | 2 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |
| | | River (Gongoma) | 9 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | 100 |

*Fecal coliform count*

A maximum faecal coliform count of 107.183 MPN/100 ml was obtained from tested tap water samples of Waltai Gobu kebele. Samples from spring in the same kebele had the lowest faecal coliform count of 9.197 MPN/100 ml. Whereas, tap water samples from Garre and Chafe Mana kebeles had showed a faecal coliform contamination of 26.075 and 56.693 MPN/100 ml, respectively (Table 3). Desalegn *et al*., (2013) reported that all water samples were found to be contaminated by faecal coliforms.

On the other hand, tests of water samples obtained directly from Tegona and Bamo rivers and households in Waltai Sura and Waltai Tosha kebeles, respectively. The communities in the area depend on the two rivers for drinking water in which the result indicated that the highest MPN of 1101 and above was obtained. Whereas, the highest faecal coliform value of 54 CFU/100 ml was reported from protected well (Desalegn *et al*., 2013) Samples collected from hand pump and bore hole in Aloshe kebele showed the lowest faecal coliform contamination of 14.677 and 2.99 MPN, respectively (Table 3). But the lowest value of faecal coliform (0.34 CFU/100 ml) was obtained from tap water samples in Dire Dawa.

There was a significant statistical difference between the sources in faecal coliform count in Dallo Manna District with a range stretching from 14.1 to 1101.0 MPN across the three kebeles. Tap water samples from Gongoma and Barraq recorded lowered faecal coliform counts of 14.050 and 21.000 MPN (Table 3). Samples from Haro Sora pond, Erba river and Haro Wanji pond were found to have 725.125, 972.600 and 1100.67 MPN, respectively.

In general, according to IRC (2002) risk classification for thermotolerant coliforms or *E.coli* for rural water supplies, drinking water sources such as Bamo and Tegona rivers at Goba district, Erga and Yarod rivers at Delo Mana, some piped supplies in Goro district, ponds like Haro Wanji and Haro Sora at Dallo Mana have been found to be classified under “high” to “very high” risk categories. On the contrary, only samples from bore hole in Aloshe kebele of Goba district seemed to conform to WHO guidelines.

Table 3. Faecal coliform count 100 $ml^{-1}$ of sample

| District | Kebele | Drinking water source | N | Minimum | Maximum | Mean | p(<0.05) | Risk category |
|---|---|---|---|---|---|---|---|---|
| **Goro** | Waltai Gobu | Tapped | 12 | 2.990 | 1100.00 | $107.183^{ef}\pm90.343$ | 0.2605 | HR |
| | | Spring | 3 | 2.990 | 21.000 | $9.197^{f}\pm5.904$ | 0.2596 | LR |
| | Garre | Tapped | 15 | 7.300 | 240.000 | $59.693^{f}\pm15.962$ | 0.0022 | IR |
| | Chafe Mana | Tapped | 15 | 2.990 | 210.000 | $26.075^{f}\pm13.609$ | 0.0760 | IR |
| **Goba** | Waltai Sura | River (Tegona) | 4 | 1100.00 | 1101.00 | $1100.75^{a}\pm0.250$ | <0.0001 | VHR |
| | | River (Tegona-Household) | 4 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | VHR |
| | | River (Micha-Household) | 5 | 43.000 | 1101.00 | $515.600^{cd}\pm241.17$ | 0.0993 | HR |
| | | Bore hole (Burgullo) | 2 | 460.000 | 460.000 | $460.000^{cde}\pm0.000$ | 0.000 | HR |
| | Waltai Tosha | River (Bamo) | 8 | 1100.00 | 1101.00 | $1100.80^{a}\pm0.133$ | 0.000 | VHR |
| | | River (Bamo-Household) | 7 | 1101.00 | 1101.00 | $1101.00^{a}\pm0.000$ | 0.000 | VHR |
| | Aloshe | Hand pump | 13 | 2.990 | 150.000 | $14.677^{f}\pm11.282$ | 0.2177 | IR |
| | | Bore hole | 2 | 2.990 | 2.990 | $2.990^{f}\pm0.000$ | 0.000 | IC |
| **Dallo M** | Haya Oda | River (Yadot-Household) | 6 | 93.000 | 210.000 | $151.000^{def}\pm33.778$ | 0.0466 | HR |
| | | River (Erba) | 4 | 93.000 | 1100.00 | $633.250^{bc}\pm271.14$ | 0.1016 | HR |
| | | River (Erba-Household) | 5 | 460.000 | 1101.00 | $972.600^{ab}\pm128.15$ | 0.0016 | HR |
| | Barraq | Tapped | 4 | 15.000 | 23.000 | $21.000^{f}\pm2.000$ | 0.0018 | IR |
| | | Pond (Haro Sora) | 8 | 240.000 | 1101.00 | $725.125^{abc}\pm144.80$ | 0.0016 | HR |
| | | Pond (Haro Wanji) | 3 | 1100.00 | 1101.00 | $1100.67^{a}\pm0.333$ | <.0001 | VHR |
| | Gongoma | Tapped | 4 | 9.100 | 23.000 | $14.050^{f}\pm3.292$ | 0.0236 | IR |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Spring | 2 | 20.000 | 210.000 | $115.000^{ef}\pm95.000$ | 0.4396 | HR |
| | River (Gongoma) | 9 | 11.000 | 1101.00 | $356.778^{cdef}\pm148.387$ | 0.0429 | HR |

**Source:** IRC (2002) **Note:** IC=In conformity with WHO guidelines; LR=low risk; IR=intermediate risk; HR=high risk; VHR=very high risk

*Escherichia coli count*

Samples from each of the different sources were also tested for the detection of *E. coli.* It is in the spring water samples that the lowest MPN/100 ml of *E. coli* cells (8.863) was recorded in Waltai Gobu kebele of Goro district. Tap water samples from the same kebele had an *E. coli* count value of 12.098 CFU/100 ml. With non-significant variability, 11.055 CFU/100 ml were counted in samples from the similar source from Chafe Mana district. The maximum MPN/100 ml (42.607) was recorded in tap water samples from Garre district (Table 4). A comparably lower *E. coli* value of 6.0±0.54 was reported in Kenya in tap water samples (Abok *et al*., 2018).

However, in Goba district, the highest MPN of *E. coli* of 972.800 was obtained from samples collected from households who fetched the water from Bamo river for drinking. Unprotected bore hole (Burgullo) and Bamo river samples took the 2$^{nd}$ (780.500) and the 3$^{rd}$ (696.800) places in terms of MPN of *E. coli*. Similar report showed that the highest contamination level of 160.0±14.14 CFU/ml was detected in rain water samples in a study published in Kenya (Abok *et al*., 2018). Water samples with almost no *E. coli* (2.99 cells/100 ml) were obtained from protected bore hole in Aloshe kebele of the district (Table 4). Similarly an equivalent value of 3.554 cells was recoded in samples from hand pumps in the same kebele. Drinking water samples from Tegona and Micha rivers (both household and source) showed relatively lower MPN of *E. coli* ranging from 328.5 to 514.0 (Table 4).

Water samples from Erba river drinking households were found to host the highest number of *E. coli* cells (884.400) per 100 ml. However, samples directly taken from the river showed a significantly lower MPN of *E. coli* (398.250). At Barraq kebele, samples from Haro Wanji pond and tap showed a lower *E. coli* cell population of 12.663 and 20.000 per 100 ml, respectively. Gongoma and Yadot rivers exhibited an MPN of 60.566 and 84.333, in Gongoma and Haya Oda kebeles, respectively (Table 4).

Based on IRC (2002) risk classification for thermotolerant coliforms or *E.coli* for rural water supplies, all the rivers in Goba being used as sources of drinking water for the local society were under “high risk” category. Similarly, Erba river, Haro Sora pond and spring at Dallo Mana have fallen under “high risk” classification (Table 4). Bore hole sourced drinking water have conformed with WHO guideline. Samples from spring in Goro and hand pump in Goba were under “low risk category”. Almost none of the samples have complied with WHO guidelines and Ethiopian standards for drinking water quality (ESA, 2013; WHO, 2017) except that of bore hole in Goba district.

Table 4. *E. coli* 100 ml$^{-1}$ of water sample

| District | Kebele | Drinking water source | N | Minimum | Maximum | Mean | p (<0.05) | Risk category |
|---|---|---|---|---|---|---|---|---|
| Goro | Waltai Gobu | | 12 | 2.990 | 43.000 | $12.098^{g}\pm3.227$ | 0.0032 | IR |
| | | Spring Tapped | 3 | 2.990 | 20.000 | $8.863^{g}\pm5.571$ | 0.2526 | LR |
| | Garre | Tapped | 15 | 6.200 | 240.000 | $42.607^{fg}\pm19.329$ | 0.0447 | IR |
| | Chafe Mana | Tapped | 15 | 2.990 | 43.000 | $11.0553^{g}.571$ | 0.0079 | IR |
| Goba | Waltai Sura | River (Tegona) | 4 | 28.000 | 1101.00 | $328.500^{defg}\pm259.0143$ | 0.2942 | HR |
| | | River (Tegona- | 4 | 35.000 | 1101.00 | $514.000^{bcde}\pm219.818$ | 0.1014 | HR |
| | | River (Micha- | 5 | 3.600 | 1101.00 | $443.660^{cdef}\pm268.360$ | 0.1736 | HR |
| | | Bore hole (Burgullo) | 2 | 460.000 | 1101.00 | $780.500^{abc}\pm320.500$ | 0.2481 | HR |
| | Waltai Tosha | River (Bamo) | 8 | 93.000 | 1101.00 | $696.800^{abcd}\pm139.014$ | 0.0007 | HR |
| | | River (Bamo-Household) | 7 | 460.000 | 1101.00 | $972.800^{a}\pm128.200$ | 0.0016 | HR |
| | Aloshe | Hand pump | 13 | 2.990 | 9.100 | $3.554^{g}\pm0.466$ | <.0001 | LR |
| | | Bore hole | 2 | 2.990 | 2.990 | $2.990^{g}\pm0.000$ | 0.000 | IC |
| Dallo Manna | Haya Oda | River (Yadot-Household) | 6 | 15.000 | 210.000 | $84.333^{fg}\pm62.945$ | 0.3123 | IR |
| | | River (Erba) | 4 | 93.000 | 1100.00 | $398.250^{cdefg}\pm237.896$ | 0.1927 | HR |
| | | River (Erba-Household) | 5 | 20.000 | 1101.00 | $884.400^{ab}\pm216.100$ | 0.0149 | HR |
| | Barraq | Tapped | 4 | 11.000 | 23.000 | $20.000^{g}\pm3.000$ | 0.0069 | IR |
| | | Pond (Haro Sora) | 8 | 35.000 | 460.000 | $267.500^{efg}\pm63.259$ | 0.0039 | HR |
| | | Pond (Haro Wanji) | 3 | 2.990 | 20.000 | $12.663^{g}\pm5.047$ | 0.1289 | IR |
| | Gongoma | Tapped | 4 | 9.100 | 23.000 | $12.575^{g}\pm3.475$ | 0.0363 | IR |
| | | Spring | 2 | 11.000 | 210.000 | $110.500^{fg}\pm99.500$ | 0.4667 | HR |
| | | River (Gongoma) | 9 | 2.990 | 210.000 | $60.566^{fg}\pm28.388$ | 0.0654 | IR |

**Source:** IRC (2002) **Note:** IC=In conformity with WHO guidelines; LR=low risk; IR=intermediate risk; HR=high risk; VHR=very high risk

***Microbial contamination across the different agro ecologies***

Microbial contamination of the drinking water samples looks different for the different agro ecologies across the zone. Standard plate count showed an increasing trend with decreasing altitude (Fig. 1A). However, total coliform was lower at mid altitude as compared to high and low lands with still being highest at lowlands (Fig. 1B). Feacal coliform contamination of drinking water followed an opposite looking trend with the highest contamination being at the highland agro ecologies. Similar to that of coliforms, the lowest population was observed at the mid altitude districts (Fig. 1C). Similarly, *E. coli* distribution followed the same trend as that of faecal coliforms with the highest level being highlands and the second pick being observed at lowlands and the least reported at mid altitude areas (Fig. 1D)

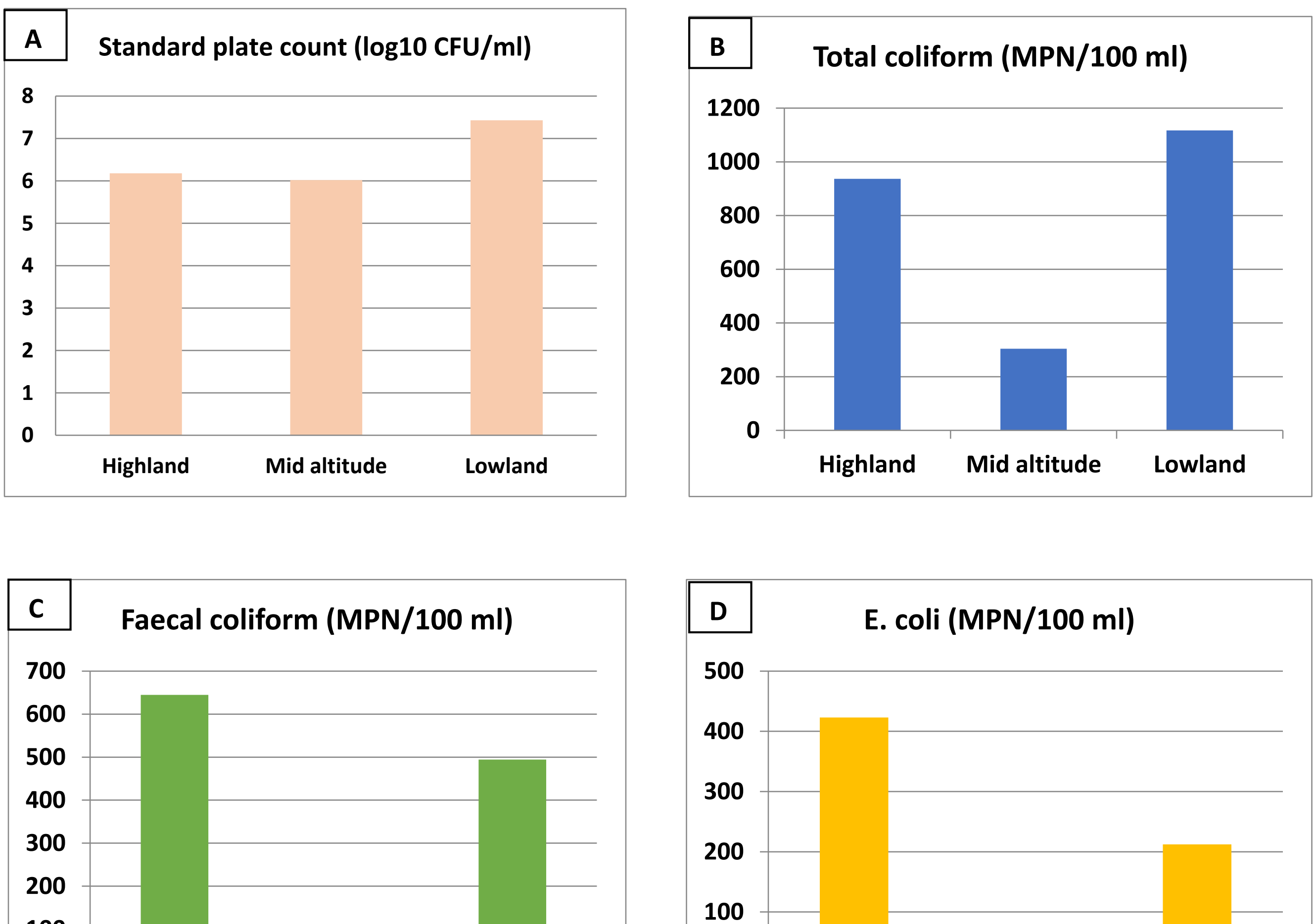


*Figure 8. Microbial contamination level of drinking water across different agro ecologies of Bale Zone in the study districts*

## Conclusion and recommendation

Most of the samples from river are seriously contaminated by total coliform, faecal coliform and *E. coli* showing that there is high sewage discharge and disposal of the community refuse as well as washing of human excreta and animal dung from the bank into the rivers. The finding showed that almost all tap water samples were found to be positive for total coliform which probably arise from poor management and inadequate periodic maintenance of the entire system starting from the reservoir, distribution system and/or point of use. Inadequate hygienic procedures followed during maintenance of the distribution system might have also contributed to the detection of the indicator microorganisms in the drinking water samples.

Hand pumps supplying drinking water to the society in Aloshe Kebele of Goba district are well protected by fences and lockable gates restricting unauthorized intrusions which undoubtedly lowered the *E. coli* population and made them safer for drinking in relative terms.

The highly turbid and stagnant ponds in Delo Mana district which are being currently used by the local community for drinking, food preparation and hygienic purposes are among those sources with maximum population of total and faecal coliforms. Even though those sources are fenced by thorny shrubs for protection from animal entry, most probably people fetching the water take faecal materials from around by their foot to the point of pouring the water as there is no structure restricting inappropriate human contact with the water.

Thus, the society, as an emergency action, should take actions like filtration, boiling and treatment with some commercially available antimicrobial agents following manufacturers' instructions. Furthermore, the water sector should take an immediate action in performing appropriate treatments (chlorination) at the source/reservoirs for piped distribution. In addition, the distribution lines should be periodically inspected and maintained for proper functionality.

For majority of our farmers/pastoralists who depend on polluted river and highly turbid ponds for drinking water, the sector should make great endeavor to establish facilities for the supply of safe drinking water to avoid the likely health burden to be posed on the society from the highly contaminated water sources.

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