Soil Fertility Assessment and Mapping of Spatial Variability at Amareganda-Abajarso Sub-Watershed, North-Eastern Ethiopia

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Abstract*:* Information on soil fertility assessment and mapping of arable land helps to design appropriate soil fertility management practices. Experiment was conducted at Amaregenda-Abajarso sub-watershed to assess the fertility status and mapping the spatial variability of selected soil fertility parameters. Based on land use type, soil color, altitude, slope gradient and aspect, and to a lesser extent soil management practices, the study area was divided into 8 land units (LUs). Then, a total of 24 composite surface soil samples were collected for laboratory analysis. All of the analyzed soil properties vary significantly ($P < 0.01$) among LUs except C: N ratio. The mean values of sand and clay fractions ranged from 67.33 (LU 8) to 43.4% (LU 3) and 40.93 (LU 1) to 12.67% (LU 8), respectively. Dominantly, sandy clay loam soil textural class was recorded. The mean soil bulk density varied from 1.15 to 1.38 g cm^{-3}. The lowest (6.05) and highest (6.74) values of soil pH were recorded for LUs 8 and 2, respectively. The organic matter content of soils ranged from 1.33 (LU 2) to 3.70% (LU 8). The total N content ranged from 0.09 (LU 2) to 0.30% (LU8). The available P content ranged from 9.31 (LU 8) to 19.53 mg/kg soil (LU 1). The exchangeable K content ranged from 97.48 (LU 2) to 357.70 mg kg^{-1} (LU8). The highest CEC (46.6) and lowest (33.47 cmol (+) kg^{-1}) values were recorded in LUs 8 and 5, respectively. Exchangeable Ca and Mg ranged from 9.25 (LU 4) to 23.35 cmol (+) kg^{-1} (LU 2) and 2.76 (LU 5) to 8.50 cmol (+) kg^{-1} (LU 3), respectively. The highest (76.86%) and lowest (50.61%) mean values of PBS were recorded for LUs 2 and 4, respectively. The EDTA extractable Fe, Mn, Cu and Zn, in mg kg^{-1}, ranged from 56.03 to 96.19, 65.30 to 226.48, 1.84 to 6.19, and 1.12 to 4.34, respectively. From the total LUs, 87.5 % were low in OM; 50%, deficient in total N and Fe, 25% were deficient in Cu and Zn and 12.5% were deficient in available K and Mn. In conclusion, integrated plant nutrient management practices that use organic inputs, mineral fertilizers, and improved crop varieties that can be adapted to local farming situations should be implemented to improve and sustain productivity of cultivated land in the area.

Keywords: Wollo; Soil; Cultivation; Crops; Maps; micronutrients; Macronutrients

1. Introduction

Soils underpin, literally and figuratively, all of the processes that support human societies and economies and, indeed, all other terrestrial life on earth (Steven *et al*., 2012). Ensuring food security for the ever-increasing world population has a direct relation with fertility and productivity of soils. However, soils of Sub-Sahara African (SSA) countries including those of Ethiopia are characterized by huge and widespread negative nutrient balance and low productivity (Chianu and Mairura, 2012). In these countries, agricultural productivity per unit area of land is declining through time and food production has not kept pace with the rapidly growing population (Roy *et al*., 2003). To feed the growing population in these countries, agricultural production has to increase. This can be achieved either by area expansion or by intensification. The first option has been less feasible due to land shortage. The remaining feasible option to increase productivity per unit area is through improved soil fertility management practices accompanied by the use of improved crop varieties and better agronomic practices (Sanchez *et al*., 1997).

In Ethiopia, agriculture, which is directly dependent on soil resource, is the backbone of the national economy. However, a FAO (1998) report shows that 24% of Ethiopia's soil face moderate to very severe fertility constraints. The problems of land degradation and low agricultural productivity in the country, resulting in food insecurity and poverty, are particularly severe in the rural highlands (Nedessa *et al*., 2005). Hence, soil fertility depletion is considered as the fundamental biophysical cause for declining per capita food production in SSA countries in general and Ethiopia in particular (Sanchez *et al*., 1997). Many approaches can be justified to mitigate these problems. However, to implement suitable management options, the fundamental element to start with is to identify the fertility status of the soils under the existing system of management practice.

Soil characterization in relation to evaluation of fertility status of the soils of an area is an important aspect in the context of sustainable agricultural production (Singh and Mishra, 2012). Periodic assessment of important soil properties and their responses to changes in land management is necessary to apply appropriate soil fertility management

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techniques, and to improve and maintain fertility and productivity of soil (Wakene and Heluf, 2003). Mapping the spatial variability of soil fertility by applying GIS is also the order of the day to elicit information for current and future uses. Spatial variability maps of different nutrients and its classification clearly show the specific locations of the areas, where attention is required with respect to management of plant nutrients (Jatav *et al.*, 2013).

In Ethiopia, the major soil fertility issues are understood only at the higher level with limited information. The widespread blanket fertilizer recommendation rate throughout the country is one indicator of the existence of little information about the fertility status of soils. Even the current fertilizer recommendations deal with Na and P dosage only. In fact, the Ethio-SIS-ATA in collaboration with stakeholders, is currently pursuing a rapid development program on assessment of the soil resources of the country to establish a national soil resources database, and assess the nutrient status of agricultural land to produce soil fertility maps of a number of districts in the country and come up with recommendations for fertilizer applications and other management interventions (ATA, 2013). However, without detailed soil related information at specific local level, sustainable crop production could not be achieved. Hence, prior to the recommending any soil management options, soil nutrient supply potential has to be assessed. Thus, more research needs to be carried out at a granular, actionable level and sustainable land use management options have to be set and applied. Despite these facts, the spatial fertility status of soils of Amaregenda-Abajarso sub-watershed has never been. Therefore, this study was conducted with the objectives of assessing the fertility status of soils and mapping the spatial variability of selected soil fertility parameters of the study area.

2. Material and Methods

2.1. Description of the Study Area

2.1.1. Location

The study was conducted at Amaregenda-Abajarso sub-watershed, which is located in Amhara National Regional State (ANRS), Ethiopia (Figure 1). It is situated at about 420 km north-east of Addis Ababa. Geographically, the site lies between11^0 03' 18" to11^0 05' 55.68" N and 39^0 30'10.08" to 39^{0}32'06" E, and at an altitude ranging from 2561 to 2996m meters above sea level.

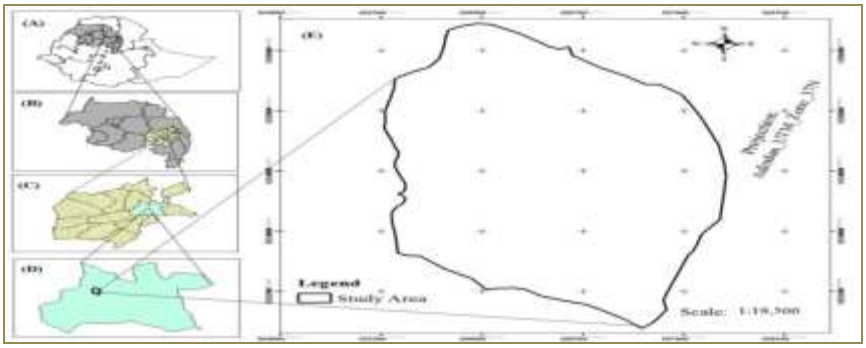

Figure 1. Map of the Study Area: (A) ANRS Zones in Ethiopia; (B) South Wollo Zone in ANRS; (C) Dessie Zuria District in South Wollo Zone; (D) Amaregenda-Abajarso sub-watershed in Dessie Zuria District; and (E) Amaregenda-Abajarso sub-watershed.

2.1.2. Climate

Based on 10-year (2004 - 2013) rainfall and temperature data obtained from Ethiopian National Meteorology Agency, Kombolcha station, the area is characterised by a uni-modal rainfall pattern with annual average rainfall ranging from 821.3 to 1010.0 mm. The maximum and minimum annual average temperatures are 27.69 and 12.21^{o}C, respectively (Figure 2).

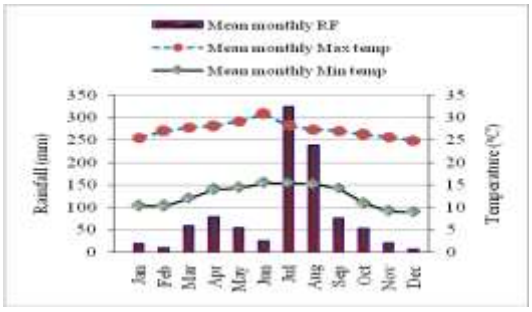

Figure 2. Mean monthly rainfall, monthly maximum and minimum temperatures of the study area.

2.1.3. Land Use, Vegetation and Soils

Mixed crop livestock production is the major farming system in the area. Crop production is entirely dependent on rainfall and land is plowed using oxen. The existing land use system consists of 54.74% cultivated land, 4.48% grazing land, 13.46% forest and woodlands, and 20.19% shrub and bush lands (Table 1). The major crops grown during the main rainy season are Teff [*Eragrostis tef* (Zucc.) Trotter], wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), field pea (*Pisum sativum* L.), vetch (*Vicia sativa* L.) and chickpea (*Cicer arietinum* L.). Teff and wheat are the dominant crops. The dominant tree species of the area are *Juniperus procera* Hochst. ex Endl and *Eucalyptus globulus* Labille. According to Ethiopian Mapping Agency, the dominant soils of the study area are Eutric Cambisols (57%) and Eutric Regosols (43%).

2.2. Site Selection, Soil Sampling, and Preparation

This study was carried out during 2013/2014 cropping season after crop harvest. Field data collection and soil sampling was carried out with the help of topographic map of the study area and satellite image of April 2013. Prior to the actual field work, tentative sampling sites were fixed on the satellite image on the basis of topography and land use types of the area.

The study area was divided into different land units (LUs) according to their differences in terms of land use type, surface soil color, altitude, slope gradient and aspect, surface land features, and to a lesser extent soil management practices such as type of fertilizers used, dominant previous, and current crop. Accordingly, a total of 8 land units were identified and demarcated. Once the representative LUs were identified, description of sampling site and soil sampling was carried out for each land unit (Table 1). Three replicated composite soil samples were collected from the depths of 0-20 cm for cultivated land units and 0-50 cm depth for forestland units. The samples were collected following a zig-zag pattern based on the complexity of topography and heterogeneity of the soil type. A total of 24 composite soil samples were collected from 8 land units. Depending on the size of the replications and their variability, 12 to 33 auger points were taken to make one composite sample. The soil samples were air-dried, crushed and passed through a 2 mm sieve for determination of most of the soil fertility indicators except total N and organic carbon for which the soil samples were passed through a 0.5 mm sieve.

2.3. Soil Properties

2.3.1. Physical Properties

The particle size distribution of the soils was analyzed according to the procedure outlined by Bouyoucos (1962) with the help of the hydrometer method. The bulk density of the soil was estimated from undisturbed soil samples which were collected by using a core sampler following the procedures used by Black (1965). Each core sample was oven-dried and the bulk density calculated by dividing the mass of the oven dry soil by the respective volume as it existed naturally under field conditions. The generally used average value of 2.65 g cm^{-3} for mineral soils was used for the particle density of the soil. Total porosity was estimated from the values of bulk density (BD) and particle density (PD) as:

$$\text{Total porosity (\%)} = \left(1 - \frac{BD}{PD}\right) \times 100$$

2.3.2. Chemical Properties

Soil pH was determined in H_2O using a 1:2.5 soil to water ratio using a digital pH-meter (Van Reeuwijk, 1993). The Walkley and Black (1934) wet digestion method was used to determine soil organic carbon content and percent soil organic matter was obtained by multiplying percent soil organic carbon by a factor of 1.724 following the assumptions that organic matter is composed of 58% organic carbon. Total N of the soil was determined by the Micro-Kjeldahl digestion, distillation and titration method (Bremner and Mulvaney, 1982). Available phosphorus was determined by Olsen method (Olsen *et al.*, 1954). Exchangeable cations were extracted with 1N NH_4OAC at pH7. Exchangeable Ca and Mg were measured by AAS and exchangeable Na and K were measured by a flame photometer (Rowell, 1994). Soil CEC was determined using ammonium acetate and ethanol as outlined by Chapman (1965). The percent base saturation of the soils was calculated as the percentage of the sum of exchangeable cations (Ca, Mg, K and Na) to the CEC (Bohn *et al.*, 2001). Extractable micronutrients (Fe, Mn, Zn and Cu) were extracted with EDTA method as described by Okalebo *et al.* (2002). The amounts of the micronutrients in the extract were determined by AAS.

2.4. Soil Fertility Mapping

Using topographic (1:50,000) map and satellite image as a reference, location map of the study area was developed using ArcGIS 10.1 by recording boundary coordinate points using GPS, delineation of sub-watershed was carried out. This sub-watershed was then divided into 8 land units. After that, the respective coordinate points marked using GPS were fed into the GIS environment; then, polygons for the sub-watershed and for each land unit were created by digitizing the recorded boundary points.

Based on the results of the laboratory analysis, soil fertility indices were generated and ratings made. Accordingly, the soils were classified into different fertility categories, i.e., very low, low, medium, high and very high on the basis of the content of each selected soil parameters. For each fertility class, different symbol, colors, and patterns were selected from symbol selector of Arc Map 10. Finally, the fertility status of the land units was mapped by using the respective legend symbols. Selected soil fertility parameters mapped were soil pH, organic matter (OM), total N, available P, CEC, exchangeable K, exchangeable Ca and Mg, and extractable micronutrient cations (Fe, Mn, Cu and Zn).

Table 1. Summary of properties of land units in Amaregnda- Abajarso sub-watershed.

Land units	Area (ha)	Altitude (m a.s.l)	Slope		Major soil type	Land use type	Dominant previous crop	Dominant current crop	Residue Mgt.	Dominant fertilizers used
			gradient (%)	aspect						
1	51	2612	3	South east	Eutric regosols	Cultivated	Wheat	Teff	Cleared	DAP, Urea
2	32	2602	2	West	Eutric cambisols	Cultivated	Vetch	Teff	Cleared	DAP, Urea
3	50	2600	2	North east	Eutric cambisols	Cultivated	Wheat	Teff	Cleared	DAP, Urea
4	102	2722	7	South west	Eutric regosols	Cultivated	Teff	Wheat	Cleared	DAP, Urea, compost
5	47	2707	6	North west	Eutric cambisols	Cultivated	Wheat	Wheat	Cleared	DAP, Urea,
6	48	2770	5	North west	Eutric cambisols	Cultivated	Barley	Wheat	Cleared	DAP, Urea
7	93	2661	4	South west	Eutric regosols	Cultivated	Wheat	Teff	Cleared	DAP, Urea, FYM
8	244	2785	13	South west	Eutric cambisols	Plantation forest	*Eucalyptus* and *Juniperus* trees		Remain in the field	None

Note: Mgt. = Management; FYM=Farmers Yield Manure; a. s. l = above sea level

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was applied using version 9.1.3 SAS software to analyze the selected soil physicochemical properties. Moreover, the least significant difference (LSD) test ($P < 0.05$) was used to compare the mean values of the selected soil physicochemical properties of the land units.

3. Results and Discussion

3.1. Soil Physical Properties

3.1.1. Soil Texture

Significant differences ($P < 0.01$) were perceived among the three soil separates (Table 2) in different land units (LUs). The highest mean values of sand (67.33%), silt (22.54%), and clay (40.93%) fractions were recorded for LUs 8, 7 and 1, respectively, whereas the lowest sand (43.4%), silt (11.93%), and clay (12.67%) fractions were observed in LUs 3, 1 and 8, respectively. According to USDA soil texture classification system, soils of LUs 1 and 2 have sandy clay textural class, whereas soils of LUs 3, 4, 5, 6, and 7 have sandy clay loam. Sandy loam and clay loam textural classes were observed for LUs 8 and 3, respectively. The most probable reasons for the variation in the study area may be differences in topography, slope gradient, and parent material. Consistent with this suggestion, Thangasamy *et al.* (2005) reported that variation in soil texture may be caused by variation in parent material, topography, *in situ* weathering and translocation of clay. From this study, it was found that soils of lower elevation site have higher clay content than higher elevation sites. In agreement with this finding, Sitanggang *et al.* (2006) reported that textural variations are mainly associated with variation in parent material and topography.

3.1.2. Bulk Density and Total Porosity

Statistically significant differences ($P < 0.01$) were observed among average soil bulk density values of the land units (Table 2). The highest (1.38 g cm^{-3}) and the lowest (1.15 g cm^{-3}) mean bulk density values were recorded for LUs 2 and 8, respectively. The variation in bulk density could be attributed to variation in soil organic matter content, soil texture, and intensity of cultivation (Sharma and Anil, 2003). Accordingly, the highest bulk density for LU 2 could be due to lower soil organic matter content and higher degree of soil compaction due to intensive cultivation since this LU has been cultivated for a long period of time. In contrast, the lower bulk density in LU 8 could be attributed to relatively higher soil organic matter content owing to trees litter fall and dieback of fine roots, higher total porosity and less frequent disturbance of the land, and the contribution of trees in loosening the soil structure through their roots.

According to Bohn *et al.* (2001), the acceptable range of bulk density is 1.3 to 1.4 g cm^{-3} for mineral agricultural soils. Most of the soil bulk density values of the different land units of the area were not very high, which signifies that the soils in the sub-watershed was too compact to limit root penetration and restrict movement of water and air in the soil. This indicates the existence of loose soil conditions in almost all land units and, therefore, the soils of the study area have good structure.

Percent total porosity values of the soils showed statistically significant ($P < 0.01$) differences among LUs. The highest (56.52%) and lowest (47.79%) mean total porosity was observed for LUs 8 and 2, respectively (Table 2). According to the rating suggested by FAO (2006b), the total porosity values of all LUs were very high (> 40%). This implies that there is better aggregation that can create conducive soil physical conditions for crop production in the area. Since total porosity values were derived solely from manipulating values of the bulk density, with a generally assumed particle density value of 2.65g cm^{-3}, those factors that affect bulk density have also a direct effect on percent total porosity. Therefore, the higher percent total porosity for LU 8 could be attributed to trapped air and higher organic matter contents of soil. The lower value of percent total porosity in LU 2 could be due to relatively higher bulk density observed and greater soil compaction as a result of intensive cultivation.

Table 2. Selected soil physical properties under different land units.

Land units	Particle size distribution (%)			Textural class	Bulk density (g cm^{-3})	Total porosity (%)
	Sand	Silt	Clay			
LU1	47.13^{c}	11.94^{b}	40.93^{a}	Sandy Clay	1.19^{e}	55.09^{ba}
LU2	47.93^{c}	13.07^{b}	39.00^{ab}	Sandy Clay	1.38^{a}	47.92^{e}
LU3	43.40^{d}	20.60^{a}	36.00^{b}	Clay Loam	1.21^{de}	54.34^{ba}
LU4	55.00^{b}	14.33^{b}	30.67^{c}	Sandy Clay Loam	1.32^{ba}	50.19^{de}
LU5	54.73^{b}	21.60^{a}	23.67^{d}	Sandy Clay Loam	1.23^{dc}	53.58^{bc}
LU6	54.27^{b}	20.73^{a}	25.00^{d}	Sandy Clay Loam	1.30^{bc}	50.94^{dc}
LU7	56.13^{b}	22.54^{a}	21.33^{d}	Sandy Clay Loam	1.33^{ba}	49.81^{de}
LU8	67.33^{a}	20.00^{a}	12.67^{e}	Sandy Loam	1.15^{de}	56.60^{a}
LSD (5%)	2.92	3.86	3.81		0.08	3.01
CV (%)	3.17	12.32	7.68		3.47	3.02

Note: Values followed by the same letter within a column are not significantly different

3.2. Soil Chemical Properties

3.2.1. Soil Reaction (pH)

Statistically significant differences ($P < 0.01$) were observed among soil pH values of the land units (Table 2). The lowest (6.05) and highest (6.74) pH values were recorded for LU 8 and 2, respectively. As per the ratings established by Tekalign Tadesse (1991), soils of LU 2 qualify for the neutral range, while the remaining land units fall under slightly acidic range (Figure 3). The variation in pH values among land units might be due to differences in parent material, topographic position, land use type, removal of basic cations by crop harvests, and prevailing weather conditions.

Relatively lower pH values were recorded for land unit 8, which might be attributed to a higher slope gradient (13%) that could result in reduction of basic cations from due to accelerated top soil erosion and leaching. Besides, the relatively higher organic matter content for land unit 8 also might have resulted in lower pH as it produces organic acids via oxidation and provides H^+ ions to the soil solution, thereby reducing soil pH. In agreement with this finding, (Abayneh Essayas *et al.*, 2001; Mohammed *et al.*, 2005) reported that soils in higher altitudes and slopes had lower pH values, probably suggesting washing out of basic cations from these parts. Similarly, Ahmed (2002) reported that continuous cultivation practices, excessive precipitation and steepness of topography could be some of the factors responsible for reduction of soil pH at the middle and upper elevations.

In contrast, relatively higher pH value (6.74) was observed for LU 2 (cultivated land in the lower position of the sub-watershed) which might be due to removal of bases from higher elevation sites by erosion and subsequent deposition at the lower elevation areas. According to Gazey and Davies (2009), pH value between 5.5 and 8.0 were considered as ideal for plant growth. Thus, the pH values of soils of the study area are ideal for plant growth and the availability of most of plant nutrients might not be limited within the observed pH range.

3.2.2. Soil Organic Matter (SOM)

Significant differences ($P < 0.01$) were observed among soil OM values of the land units (Table 3). The average organic matter content of soil in the area range from 1.33% (LU 2) to 3.70% (LU 8). According to the rating suggested by Tekalign Tadesse (1991), the soil organic matter content of all LUs except that of LU 8 in the study area can be categorized in the range of low soil organic matter content which qualifies for the category of medium range (Figure 4).

Table 3. Effect of land units on soil pH, electrical conductivity, contents organic matter, total nitrogen, available phosphorus, and C:N ratio.

Land units	pH (H_2O)	OM (%)	TN (%)	C:N	Av. P (mg kg^{-1})
LU1	6.52^{ba}	1.60^{cd}	0.10^{ed}	9.21	19.53^{a}
LU2	6.74^{a}	1.33^{d}	0.09^{e}	11.47	15.80^{bc}
LU3	6.50^{ba}	1.96^{cb}	0.18^{b}	7.49	15.82^{bc}
LU4	6.17^{d}	2.25^{b}	0.16^{cb}	8.06	16.00^{bac}
LU5	6.23^{dc}	1.66^{cd}	0.12^{ed}	8.33	15.32^{c}
LU6	6.18^{d}	1.86^{cd}	0.14^{cd}	8.15	17.62^{bac}
LU7	6.47^{bc}	1.73^{cbd}	0.11^{ed}	9.50	19.47^{ba}
LU8	6.05^{d}	3.70^{a}	0.30^{a}	8.70	9.31^{d}
LSD (0.05)	0.24	0.54	0.03	Ns	3.67
SEM (±)	0.05	0.15	0.01	0.59	0.67
CV (%)	2.17	15.79	13.39	35.61	13.05

Note: OM = organic matter; TN = total N; Av. = Available; Values followed by the same letter within a column are not significantly different

The most probable source of variation in soil organic matter contents among the land units might be variation in altitude, intensity of cultivation, cropping system and soil management practices. The highest organic matter content of LU 8 could be due to relatively continuous deposition of organic material as litter fall and root dieback from *Juniperus procera* Hochst. ex Endl and *Eucalyptus globulus* Labille plantations established on the site. Besides, lower rate of organic matter decomposition as a result of tree shade and high altitude, which in turn, lower soil temperature, also contribute to high OM content in LU 8. On the other hand, the lower organic matter content in the cultivated land units might be due to higher rate of OM decomposition aggravated by intensive cultivation, and also perhaps because of low rate of return of organic materials as crop residues due to a number of competing ends such as animal feed, fuel, construction, etc. Similarly, Wakene and Heluf (2003) and Alemayehu Kiflu and Sheleme Beyene (2013) reported that lower OM was recorded in cultivated field than other land uses; and this was because of the effect of continuous cultivation and OM oxidation.

3.2.3. Total Nitrogen and C:N Ratio

The total N was significantly ($P < 0.01$) affected by differences in land units (Table 3). The average percent total N content of the soils in the study area ranged from 0.09% (LU2) to 0.30% (LU8). According to the rating suggested by Tekalign Tadesse (1991), soils of

LUs 1, 2, 5 and 7 were found to be low; LUs 3, 4 and 6 were found to be moderate whereas LU 8 was found to be high in total N content (Figure 5). The contents of total N of soils in the area showed a similar trend with the contents of organic matter. These facts indicate that the source of total N and its ultimate source of variation is organic matter contents. This suggestion is consistent with that of Murage *et al.* (2000) who reported that soil organic matter is a surrogate for soil nitrogen content. Consequently, the lower total N content in LU 2 could be due to its lower organic matter content as a result of faster rate of degradation and consequent removal of the organic matter, coupled with limited application mineral nitrogen and organic fertilizers. In line with this finding, Yifru and Taye (2011) reported that total N contents of soils under cultivation were lower than the contents under forest soils. Land units 3, 4 and 6, had moderate contents of total N as compared to the remaining cultivated land units. These land units are found near settlements and they have better chances for addition of organic N sources due to anthropogenic activities.

The lower total N contents in most land units of the area could be ascribed to cereal-based continuous cropping system that could be attributed to rapid decomposition of OM following cultivation. Lower external N inputs (like plant residues, animal manures) and N (nitrate ions) leaching problem as a result of higher rainfall during summer could also contribute to lower total N content in soils of the study area. This finding is in agreement with that of Solomon Dawit *et al.* (2002) who reported that low levels of N in cultivated lands. Crop residues are continuously removed from the field. In the study area, farmers cut their crops during harvesting very near to the ground surface. As a result, with the short stubble left on the surface of the land, not much organic matter would be available as a source of total nitrogen in the field.

Statistically insignificant differences ($P > 0.05$) were observed for the C:N values of the different land units. However, the C:N ratios were relatively high and low in land units 2 and 3, respectively (Table 3). The average C:N ratio of the soils of the area ranged from 7.49 (LU 3) to 11.47 (LU 2). According to the rating suggested by Landon (1991) for soil C:N ratio, soils of land units 3 and 2 were categorized as very low (<8) and medium (10-15) range, respectively. The remaining land units were categorized as low (8-10) C:N range. In effect, the lower the value, the higher is the proportion of N in organic matter and the more the accumulation of NH_4^+ (Olowolafe, 2004). The variation in C:N values among LUs could be as a result of variation in intensity of cultivation, micro-climate, quality of organic material applied to the soil. In line with this, Saikh *et al.* (1998a) reported that cultivation of land results in reduction of soil organic matter and total N, and increase soil C:N as in the case of LU 2. Change in land use type and intensity of cultivation had more pronounced effect in soil N than organic carbon contents and this results in higher C:N ratio (Nega and Heluf, 2013). In contrast, soil cultivation which can encourage more aeration during tillage and increased temperature enhance mineralization rates of organic carbon more than organic N, which could probably be the causes for the lower C:N ratio in cultivated land (Achalu Chimdi *et al.*, 2012).

3.2.4. Available Phosphorus

The average contents of Olsen extractable P in the soils of the area ranged from 9.31 (LU 8) to 19.53 mg kg $soil^{-1}$ (LU 1) (Table 3). Based on the rating suggested by Cottenie (1980), the available P contents of LUs1, 6, and 7 are high while that of the remaining LUs are in the medium range (Figure 6). The variability in available P contents of soils might be due to different soil management practices, specifically, type and rate of organic fertilizers and inorganic fertilizer applied to the cultivated land units. Besides these factors, variations in parent material, soil texture, degree of P-fixation, soil pH and slope gradient may also contribute to differences in available P contents among the land units.

The lowest available P content in land unit 8 compared to the remaining LUs might be due to relatively higher proportion of retained and immobilized P r by microbes in the litter layers of the forest. Besides, owing to higher slope gradient of this LU, there might be severe erosion problem which can remove substantial amounts of available P from the top soil. The sandy nature of this land unit combined with the inherent characteristics of the parent material and relatively lower pH of this LU could be the other causes of lower available P content. The results of this study is in agreement with the findings of Sanchez *et al.* (1997), that P is a limiting nutrient in many sandy soils of the semi-arid tropics and in acid, weathered soils of the sub-humid and humid tropics. Similarly, Bewket and Strossnijder (2003), Gebeyaw (2007), and Nega and Heluf (2013), reported lower contents of available P in the forest soils than in cultivated soils.

In contrsat, relatively the higher P content in some cultivated land units (LU 1, 6 and 7) could be more probably due to the application of DAP fertilizer (residual P), conducive soil pH for P availability and the consequence of long-term manure and house refuse applications and the associated increase in microbial activity as they are found near to settlement areas especially, LU 7. Unlike the land units found in higher elevations, the relatively higher content of available P in LU 1, which was found at the low elevation, might be due to downward movement of P with soil in runoff water from high slope and accumulation at the lower slope site.

The lower organic matter content of these cultivated land units but higher available P content indicates faster rate of organic matter decomposition and mineralization than forest LU, (b) that OM is not necessarily the primary supplying source of available P in highly weathered tropical soils, rather mineral weathering has considerable importance as a source of

soil P (Juo *et al.*, 1996 and Havlin *et al.*, 1999) This finding is in agreement with the findings reported by Saikh *et al.* (1998b) and Gebeyaw (2007). However, contrary to this finding, low level of available P was recorded in the surface layers of the cultivated land soil in the Chercher highlands (Mohammed *et al.*, 2005). Wakene and Heluf (2003) also reported that low content of available P is a common characteristic of most Ethiopian soils.

Table 4. Cation exchange capacity, exchangeable cations and percent base saturation of soils.

and units	(cmol (+)/kg)					PBS (%)
	CEC	Ex. Ca	Ex. Mg	Ex. K	Ex. Na	
U1	42.93^{a}	20.02^{ba}	6.79^{b}	0.99^{ba}	0.91^{a}	66.88^{b}
U2	42.27^{a}	23.35^{a}	8.07^{ba}	0.44^{dc}	0.63^{cb}	76.86^{a}
U3	36.80^{b}	15.97^{cd}	8.50^{a}	0.42^{d}	0.43^{d}	68.80^{b}
U4	35.33^{b}	9.25^{e}	6.85^{b}	1.27^{a}	0.51^{cd}	50.61^{d}
U5	33.47^{b}	14.82^{d}	2.76^{c}	0.60^{bdc}	0.50^{cd}	55.81^{dc}
U6	34.53^{b}	16.03^{cd}	6.72^{b}	0.68^{bdc}	0.54^{cbd}	69.41^{b}
U7	34.87^{b}	16.94^{bcd}	7.19^{ba}	0.82^{bdac}	0.70^{b}	73.56^{ba}
U8	46.60^{a}	19.05^{bc}	6.74^{b}	0.89^{bac}	0.65^{cb}	58.65^{c}
SD (0.05)	4.92	3.51	1.44	0.46	0.17	7.48
V (%)	7.41	11.96	12.40	34.40	16.37	6.64

Note: Values followed by the same letter within a column are not significantly different.

3.2.5. Cation Exchange Capacity

Analysis of variance showed that the CEC of the soils in the study area varied significantly ($P < 0.01$) among the land units (Table 4). The highest (46.60 cmol (+) kg^{-1}) and the lowest (33.47 cmol (+) kg^{-1}) mean values of CEC were recorded in LUs 8 and 5, respectively. Based on the rating suggested by Hazelton and Murphy (2007), soils of LUs 1, 2 and 8 were categorized to very high range, whereas CEC values of the remaining land units were high (Figure 8).

The variation in CEC values of the studied soils may be because of variation in organic matter content, type and amount of clay, and intensity of cultivation. Intensive cultivation reduced the CEC under the cultivated land as reported by Mesfin (1998) and Gao and Change (1996). The relatively higher CEC value of soils of LUs 1 and 2 could be mainly due to relatively higher clay content and probably the predominance of 2:1 clay minerals, like semectites. The highest CEC value of land unit 8 is most probably due to its relatively higher organic matter content. Consistent with this suggestion, Oades *et al.* (1989) reported that organic matter is responsible for about 25-90% of the total CEC of surface mineral soils. Therefore, soil CEC is expected to increase through improvement in soil organic matter content.

Although there is variability in CEC values of the studied soils, the high to very high CEC values indicate that the soils can retain high amounts of cations such as K^{+}, Ca^{2+} and Mg^{2+} to support plant growth. Mohammed *et al.* (2005) reported that high CEC offers high buffering capacity to the soil. Furthermore, high CEC values have been implicated in high yields obtained from most agricultural soils.

3.2.6. Exchangeable Cations and Percent Base Saturation

Analysis of variance showed that all of the exchangeable basic cations varied significantly ($P < 0.01$), for Ca, Mg and Na; and ($P < 0.05$) for K among the land units (Table 4). The highest (23.35 cmol (+) kg^{-1}) and the lowest (9.25 cmol (+) kg^{-1}) mean values of exchangeable Ca were recorded for LUs 2 and 4, respectively. Soils of LUs 3 and 5 had the highest (8.50 cmol (+) kg^{-1}) and lowest (2.76 cmol (+) kg^{-1}) exchangeable Mg. The lowest exchangeable K (0.42 cmol (+) kg^{-1}) and exchangeable Na (0.43 cmol (+) kg^{-1}) were recorded for LU 3. However, the highest exchangeable K (1.27 cmol (+) kg^{-1}) and the highest exchangeable Na (0.91 cmol (+) kg^{-1}) were recorded for LUs 4 and 1, respectively (Table 3). The order of exchangeable basic cations in most agricultural soil is generally Ca > Mg > K > Na with a pH of 5.5 or more. The finding of this study also shows similar order of cations.

Based on the rating of exchangeable basic cations set by FAO (2006a), the mean values of exchangeable Ca in LUs 1 and 2 were very high; and medium in LU 4 (Figure 9). The remaining land units were classified as high in their status of exchangeable Ca. The mean value of exchangeable Mg and K for LUs 1, 6, 7 and 8 fall under high category (Figure 7 and 10). Similarly, LUs 4 and 5 had high category of exchangeable Mg and K, respectively. The exchangeable K and Mg qualify for medium and very high range, respectively in LUs 2 and 3. Land units 5 and 4 qualify for medium exchangeable Mg and very high exchangeable K contents. On the other hand, exchangeable Na is medium for all LUs except LUs 1 and 7 which qualify for high range.

The variation in exchangeable basic cation content among land units could be due to variation in OM content, amount of clay, parent material, cultivation intensity, leaching, erosion, elevation and soil management practices. Relatively, the higher exchangeable Na, Ca and Mg in LUs 1, 2 and 3, respectively, could probably be contributed by their relatively higher clay content. Exchangeable Ca and Mg

appeared to increase in the lower elevation sites of the study area. This might be attributed to removal of these exchangeable basic cations by erosion from higher topography and their subsequent accumulation in the lower elevations. From soil fertility point of view, exchangeable Ca, Mg, and K in all land units were in the range of medium. This implies that soils of the study area may not be deficient in exchangeable basic cations. Corroborating this result, Tuma (2007) also reported the same order of abundance of basic cations on the exchangeable complex of fluvial soils in Gamo Gofa zone, Ethiopia, and pointed out that such an order is favorable for crop production.

The highest (76.86%) and the lowest (50.61%) mean values of PBS were recorded for LUs 2 and 4, respectively (Table 4). Based on the rating suggested by Hazelton and Murphy (2007), soils of LUs 4, 5 and 8 qualified for moderate range in PBS, while the remaining LUs were in the range of high PBS status. The trends in PBS are similar to those observed in exchangeable basic cations, especially Ca and Mg, because factors and processes that affect the extent of basic cations also affect PBS. Thus, variability in PBS could also be because of variation in pH, OM content, soil texture, parent materials, and intensity of cultivation, leaching, slope and soil management practices. Slope factor may be significant for the variation as LUs, (4, 5 and 8), that have moderate PBS that is found in higher slope area than the other LUs. Soils with high PBS are considered relatively more fertile because many of the bases that contribute to higher PBS are essential macro plant nutrients (Havlin *et al*., 1999). Accordingly, the soils of the study area had moderate to high PBS and considered as fertile soils.

3.2.7. Extractable Micronutrients

The highest (96.19 mg kg soil) and the lowest (56.03 mg kg^{-1}) mean values of Fe were recorded in LUs3 and 5, respectively. The lowest mean values of Mn (65.30 mg kg^{-1}), Cu (1.84 mg kg^{-1}) and Zn (1.12 mg kg^{-1}) were observed for LU 8. The highest Mn (226.48 mg kg^{-1}) and Zn (4.34 mg kg^{-1}); and Cu (6.19 mg kg^{-1}) content were recorded in LUs 4; while the highest Cu (6.19 mg kg^{-1}) content was recorded in LU2 (Table 5).

According to the fertility classes suggested for EDTA extractable micronutrients by FAO (1982), soils of LUs 2, 5, 6 and 8 are low in Fe content whereas soils of the remaining LUs could be categorized into the medium range. On the other hand, the content of Mn is low for LU 8 and medium for the remaining LUs. Land units 4 and 8 have low Cu contents. The remaining LUs could be classified in the medium range in Cu content, except LU 2 which falls in the high range (Figure 11). Generally, in almost all LUs with the exception of LU 8, Mn content of the studied soils is not low; and even the numerical values of Mn are much higher than the other micronutrients. This finding is in agreement with that of Hue *et al.* (2001) who concluded that in humid tropics where most soils are highly weathered and leached, Mn toxicity is even more common than deficiency, and with that of Haque *et al.* (2000) who reported extractable Fe and Mn levels were usually adequate for Ethiopian soils.

The relative abundance of EDTA extractable micronutrients in the study area were found in the order of Mn > Fe > Cu > Zn for soils of all land units. Similar results were reported although there is variation in the method of extraction (Wondimagegne Chekol and Abere Mnalku, 2012). Teklu Erkossa *et al.* (2003) also reported that extractable Mn was higher than extractable Fe regardless of differences in altitudinal position and method of extraction. In contrast, previous studies in Ethiopia showed that relative abundance of extractable Fe was larger than extractable Mn. Tuma *et al.* (2013) reported that the concentration of available micronutrients in Abaya Chamo Lake basin were found to be Fe > Mn > Cu > Zn in almost all surface soils.

Table 5. Selected EDTA extractable micro nutrient cations of soils.

Land units	Extractable micronutrients (mg/kg soil)			
	Fe	Mn	Cu	Zn
LU1	93.33^{a}	136.53^{b}	5.37^{a}	1.90^{cb}
LU2	73.17^{dc}	156.62^{b}	6.19^{a}	2.03^{cb}
LU3	96.19^{a}	162.56^{b}	5.95^{a}	2.82^{b}
LU4	86.98^{ba}	226.48^{a}	1.87^{d}	4.34^{a}
LU5	56.03^{e}	114.16^{cb}	2.28^{cd}	1.63^{c}
LU6	66.51^{de}	111.87^{cb}	3.81^{b}	1.45^{c}
LU7	79.21^{bc}	157.53^{b}	3.44^{cb}	1.63^{c}
LU8	58.57^{e}	65.30^{c}	1.84^{d}	1.12^{c}
LSD (0.05)	11.80	57.21	1.37	1.11
CV (%)	8.94	23.38	20.55	30.35

Note: Values followed by the same letter within a column are not significantly different.

According to Anil *et al.* (2016), soil factors that affect the contents of soil micronutrients are organic matter, soil pH, sand, and clay contents. Besides, variation in slope, intensity of cultivation, soil drainage properties, soil type, leaching and erosion can also be responsible for the variation in soil micronutrient content.

Accordingly, the variation in each micronutrient contents among the studied land units may not be out of the above mentioned factors. Especially, variation in soil texture may probably be the main factors for the variation. The lowest Mn, Cu and Zn, and relatively the second lowest Fe content in LU 8 could be due to its higher sand and lower clay content. This is because of variation in intensity of leaching. Besides variation in sand and clay contents, probably sever soil erosion as a result of rugged topography (LU8) of the site and relatively higher rainfall in that particular micro-climate may also be responsible for low level of micronutrients content. Similarly, Wajahat *et al.* (2006) reported that most sandy soils are acutely deficient in micronutrients compared to clay soils.

3.2. Mapping of Spatial Soil Fertility Variability

The total area covered in the soil fertility mapping were 667 ha. The spatial variability maps showed that 635 ha (95.20%) of the study area has slightly acidic pH (H_2O) while 32 ha (4.80%) is neutral in soil reaction. Low OM content covers 423 ha (63.42%) whereas medium OM content takes 244 ha (36.58%). High total N (TN) content covers 244 ha (36.58%) of the study area, whereas moderate total N content takes 200 ha (29.99%) and 223 ha (33.43%) of the study area was low in TN content. The available P content of the studied soils covers 475 ha for medium and 192 ha for high status of the LUs.

From the total area, soils with high and very high CEC take 327 ha (49.02%) and 340 ha (50.08%), respectively. Medium, high and very high status of exchangeable K cover 82 ha (12.29%), 483 ha (72.41%) and 102 ha (15.29%), respectively (Figure 7). Medium levels of extractable Fe, Mn, Cu and Zn cover 540 ha (80.96%), 423 ha (63.42%), 289 ha (43.33%), and 375 ha (56.22%), respectively (Figure 11).

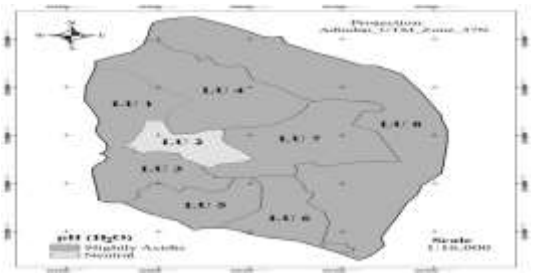

Figure 3. Spatial distribution of soil pH.

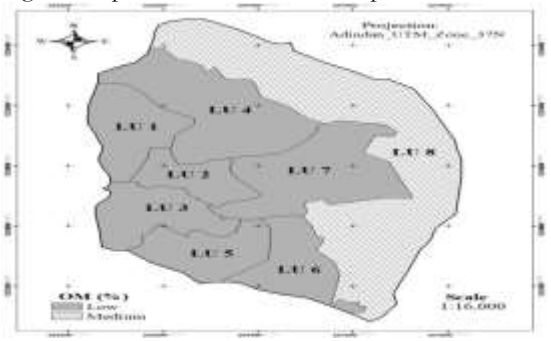

Figure 4. Spatial distribution of soil organic matter.

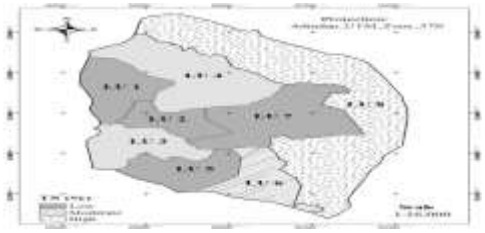

Figure 5. Spatial distribution of total N.

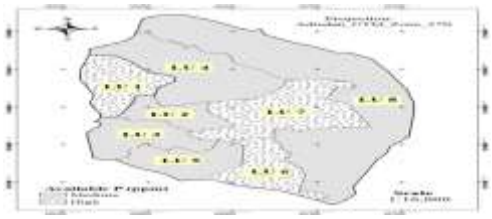

Figure 6. Spatial distribution of available P.

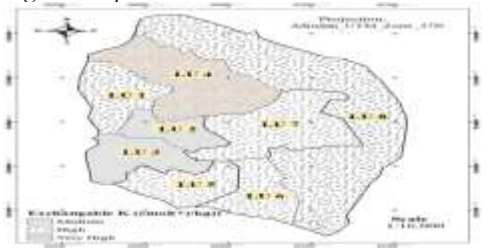

Figure 7. Spatial distribution of exchangeable K.

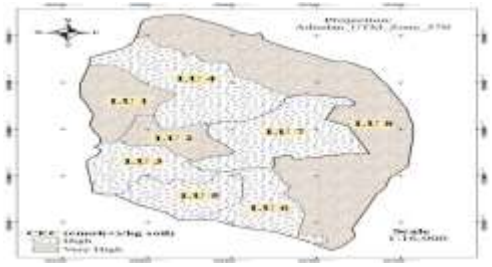

Figure 8. Spatial distribution of soil CEC.

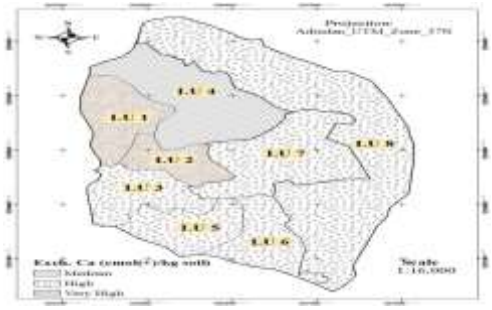

Figure 9. Spatial distribution of exchangeable Ca.

Figure 10. Spatial distribution of exchangeable Mg

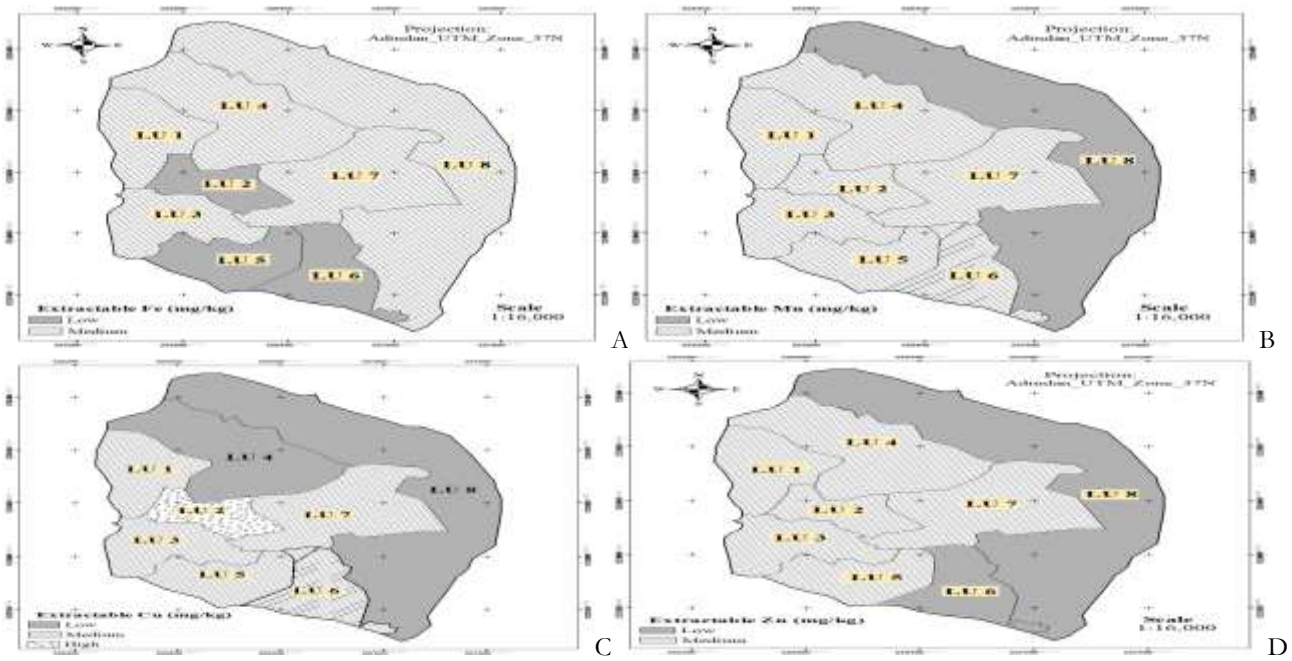

Figure 11. Spatial distribution of extractable micronutrients: A) Fe; B) Mn; C) Cu and D) Zn.

4. Conclusions

This study has demonstrated that most of the physical and chemical properties of the studied soils vary from land units to land units due to variation in slope gradient, elevation, parent material, land use type and soil management practices. Generally, soils of the study area have low to medium contents of OM, extractable Fe, Mn and Zn; low to high contents of TN and Cu; medium to very high contents of exchangeable K; medium to high contents of available P and PBS; medium to very high contents of exchangeable Ca and Mg; and high to very high CEC. Thus, the study area has no limitation in exchangeable cations specially K.

The lower value of soil chemical properties in some LUs indicates that nutrients should be replenished to increase their content for optimum and sustainable crop production in the area. Above all maintenance of soil OM should get much attention since lower values were recorded for about 63.42% (i.e. in all cultivated land units) of the study area although it plays a vital role for improvement of both physical and chemical soil properties. Therefore, creating public awareness about integrated and sustainable soil fertility management through maintenance of soil OM in particular has to be done. Well organized integrated watershed management practices have to be implemented. Environmentally and socially acceptable integrated nutrient management practices such as agroforestry systems, crop rotation, use of organic inputs (compost and FYM), chemical fertilizers, and improved crop varieties that can be adapted to local farming situations should be implemented for sustainable agricultural development in the study area.

However, soil analysis by itself cannot go further than the identification of soil nutrients status due to intricate nature of soil. Therefore, the nutrient supplying powers of the soils and demanding levels of the plants need further correlation and calibration work to come up with conclusive site-soil-crop specific fertilizer recommendation with appropriate rate.

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Stability Analysis in Bread Wheat (*Triticum aestivum* L.) Genotypes in North-western Ethiopia

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Abstract: Northwestern Ethiopian is one of the areas that has been experiencing weather variability both from season to season as well as from place to place in the same season over relatively small areas. In such weather conditions, the magnitude of genotype x environment interaction is more important than the average performance of crop genotypes. Therefore, experiments were conducted at Adet, Simada and Debretabor in 2014 and 2015 cropping seasons under rain-fed condition with the objectives of evaluating the stability of bread wheat genotypes for grain yield, and estimate the magnitude of genotype x environment interaction on grain yield. The treatment consisted of twelve bread wheat genotypes, namely, Bolo (G8), Dand'a (G5), Gambo (G4), Gassay (G6), Hidase (G1), Huluka (G3), Kubsa (G12), Menze (G10), Ogolcho (G2), Shorima (G11), Tay (G7), and Tsehay (G9). The experiments were laid out as a randomized complete block design with three replications per treatment at each site. The analysis of variance revealed the significant ($P \leq 0.01$) effect of genotype, growing season, location and all possible interactions of the three main factors. The results of AMMI analysis depicted significant ($P \leq 0.01$) differences among genotypes across the environments. According to the study, the performances of genotypes grain yield were highly affected by environment and the genotype. The highest variation was accounted for location (29 %) followed by genotype (18%) and location by year (18 %) and genotype by year (12%) effects. Based on Additive Main effects and Multiplicative Interaction (AMMI), genotype and genotype by environment (GGE) biplot and stability coefficient analyses Ogolcho (G2), Gambo (G4), Shorima (G11) and Tsehay (G9) were relatively stable genotypes across the test environments than the checks, TAY (G7) and Kubsa (G12). Therefore, based on the stability and overall mean grain yield of genotypes, recently released genotypes Gambo (G4), Ogolcho (G2) and Tsehay (G9) and relatively older genotypes Shorima (G11) and TAY (G7) could be recommended for production at the test environments in the Western Amhara Region.

Keywords: Additive Main effects and Multiplicative Interaction (AMMI*)*; Genotype and Genotype by environment (GGE); Grain Yield and Stability coefficient

1. Introduction

Ethiopia is frequently exposed to food shortages due to environmental variability, degradation of soil fertility, and ever increasing population (Ashenafi, 2008) and inappropriate use of improved technologies (Zerihun, 2016). However, the production and productivity of wheat in Ethiopia has increased in the last decades. The national average yield is 2.54 ton/ha (CSA, 2014). It is lower than the world's average yield/ha, which is about 3.3 ton/ha (FAO, 2014). This is due to factors such as use of low yielding cultivars; uneven distribution of rainfall, poor agronomic practices and serious wheat diseases like rusts (Dereje *et al*., 2000).

Therefore, Ethiopia's wheat production covers only 75% of the national demand; the remaining 25% of the wheat is obtained through imports (Eyob *et al*., 2014). So to overcome wheat yield imports and to cut down wheat national demand deficiency conducting considerable research works that contribute positive impact on wheat productivity and production are mandatory.

The process of variety development in the country is continuing year after year through various research institutes and universities. However, once released for production, the varieties are used for a long period of time continuously without considering their adaptation domain, grain yield stability and testing whether they are losing their yield potential or not. High yielding and rust disease resistant bread wheat varieties have recently been released in Ethiopia. However, farmers in Western Amhara Region commonly use relatively older bread wheat varieties such as Kubsa and Tay which were released in 1995 and 2005, respectively (MoA, 2013). Therefore, there is a need to evaluate the recently released bread wheat varieties across the environment and years. Hence, it is vital to evaluate grain yield stability of bread wheat genotypes used in the region with the objectives of evaluating the extent of grain yield stability of bread wheat varieties and cultivars, and estimate the magnitude of genotype x environment interaction on grain yield.

2. Materials and Methods

2.1. Description of the Experimental Sites and Materials

The experiment was conducted during the 2014 and 2015 cropping season under rain-fed conditions at Adet Agricultural Research Center, namely Adet, Simada and Debretabor. Twelve improved bread wheat genotypes were used for the study. The detail agro-ecological data of environments and the description of genotypes are listed in Tables 1 and 2, respectively.

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Table 1. Altitude, geographical location, and climate data of the research sites.

				Climate data for two cropping seasons			
		Geographical		2014		2015	
Testing site	Altitude (m.a.s.l)	Latitude	Longitude	RF (mm)	Average temp (^{0}C)	RF (mm)	Average temp (^{0}C)
Adet	2240	11^{0}16'N	37^{0}29'E	789.2	17.53	948.9	19.4
Simada	2460	11^{0}03N	37^{0}30'E	736.1	13.27	770.6	15.07
Debretabor	2591	11^{0}51'N	38^{0}01'E	1102.7	15.48	958.1	15.94

Note: RF (mm) = total amount of rain fall in the cropping season, and Average tem (^{0}C) = average temperature in the cropping season.
Source: AARC (2014) and ANRSMA (2014 and 2015).

Table 2. Description of bread wheat genotypes evaluated at three locations during 2014 and 2015 cropping seasons in Northwestern Ethiopia.

Genotype	Code	Breeder center	Year of release	Grain yield (t/ha) at time of release at		Recommended agroecology Zone	
				On station	On farm	Altitude(masl)	RF(mm)
Hidase (ETBW 5795)	G1	KARC	2012	4.4-7	3.5-6	2200-2600	>500
Huluka (Flag 5)	G3	KARC	2012	4.4-7	3.8-6	2200-2600	500-800
Ogolcho (ETBW 5520)	G2	KARC	2012	2.8-4	2.2-3.5	1600-2100	400-500
Shorima (ETBW 5483)	G11	KARC	2011	2.9-7	2.3-4.4	2100-2700	700-1100
Gambo (QUIAU#2)	G4	KARC	2011	3.5-5.7	4.5	750	NA
Tsehay (HAR 3837)	G9	DBARC	2011	3.8	2.8-3.5	2600-3100	>900
Danda'a (DANPHE#1)	G5	KARC	2010	3.5-5.5	2.5-5	2000-2600	>600
Bolo (HAR 3816)	G8	DBARC	2009	2.8-3.5	2.3-3.3	2580-3100	>904
Menze (HAR 3008)	G10	DBARC	2007	1.9-3.3	1.5-2.7	2800-3100	>904
Gassay(HAR 3730)	G6	ADARC	2007	4.4-5	3.5-4.7	1890-2800	>700
Tay (ET-12 D4/ HAR-604(1) (C)	G7	ADARC	2005	2.5-6.1	3.4-5.8	1900-2800	>700
Kubsa (HAR 1685)(C)	G12	KARC	1995	5.8-6.3	4-4.5	1850-2800	500-800

Note: ADARC = Adet Agricultural Research Center, DBARC = Debrebirhan Agricultural Research Center, KARC = Kulumsa Agricultural Research Center, C = Check and NA = Not available
Source: MoA, Crop Variety Register (1995-2012)

2.2. Experimental Procedures and Data Analysis

The land was ploughed three times and labeled manually at time of planting. The treatments were laid out as randomized complete block design with three replications per treatment at each site and six rows per plot. Planting was done in the first up to the second week of July with seeding rate of 150 kg/ha on the plot area of 1.2 x 2.5 m with a net plot area of 0.8 x 2.5 m. Urea and DAP fertilizers as a source of nitrogen and phosphorous were applied with at the rate of 74 kg N/ha and 46 kg P_2O_5/ha for Adet and 120 kg N/ha and 46 kg P_2O_5/ha for both Simada and Debretabor. Total amount of DAP and 1/3 of urea were applied at planting and the remaining 2/3rd of urea was applied at tillering after the first weeding. Weeding was done manually two times at tillering stage (three weeks - a month) and booting stage (50-60 days before heading) depending on the weed infestation of the trial site.

The grain yield data were analyzed using GenStat (17th Edn) software to compute genotype and environment main and interaction effects, seasonal variation effects and grain yield stability of genotypes. Whenever the results were found to be significant, Fisher's LSD test at 1% and 5% probability level was used, respectively, to separate the means of genotypes, environments and genotypes by environments interaction.

The AMMI analysis of variance summarizes most of the magnitude of genotype by environment interactions into one or a few interaction principal component axes (IPCA) (Zobel *et al.,* 1988; Crossa, 1990). The following AMMI model equation was used:

$$Y_{ger} - u - \alpha_g - \beta_e = \Sigma_n \lambda_n \tau_{gn} \delta_{en} + \rho_{ge} + \xi_{ger} \quad (1)$$

Where: Y_{ger} is the grain yield of genotype (g) in environment (e) for replicate (r), *u* is the grand mean, α_g are genotype mean, β_e are the environment mean deviations, λ_n is the singular value for IPCA axis n, τ_{gn} are genotype eigenvector values for IPCA axis n, δ_{en} are the environment eigenvector values for (PCA) axisn, ρ_{ge} are the residuals and ξ_{ger} is the error term.

GGE biplot analysis was carried out to identify high yielding and stable varieties as well as representative and discriminating environments as per Yan (2001).

$$Y\,ger - \beta e = \Sigma n^{\wedge} n\, \tau gn\delta en + pge + \xi ger \qquad (2)$$

Where: Y_{ger} is the grain yield of genotype (g) in environment (e) for replicate (r), β_e are the environment mean deviations, $^{\wedge}{}_n$ is the singular value for IPCA axis n, τ_{gn} are genotype eigenvector values for IPCA axis n, δ_{en} are the environment eigenvector values for (PCA) axis n, p_{ge} are the residuals and ξ_{ger} is the error term.

AMMI Stability Value (ASV) is the distance from the coordinate point to the origin in a two-dimensional plot of IPCA1 scores against IPCA2 scores in the AMMI model (Purchase, 2000). ASV was calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction sum of squares as follows:

$$ASV = \sqrt{[(SS_{IPCA1} \div SS_{IPCA2}) \times (IPCA1score)]^2 + (IPCA2score)^2} \qquad (3)$$

Lin and Binns (1988) defined the superiority measure (Pi) of the i^{th} test cultivar as the MS of distance between the i^{th} test cultivar and the maximum response as,

$$P_i = \left[n\,(\overline{X}_i - \overline{M}_j)^2 + \left(\sum_{j=1}^{n} (X_{ij} - \overline{X}_i - \overline{M}_j + \overline{M}_j)^2\right)\right]/2n \qquad (4)$$

Where: Xij=is the average response of the i^{th} genotype in the j^{th} environment, Xi=is the mean deviation of genotype i, Mj=is the genotype with maximum response among all genotypes in the j^{th} location, and n is the number of locations. The first term of the equation represents the genotype sum of squares and the second part represents the GE sum of squares.

According to Lin *et al.* (1986) the variance of genotype yields recorded across the test environments can be used as a measure of stability. For the genotype greatest stability is $S_i^2=0$.

The formula is:

$$S_i^2 = \Sigma R_{ij} - m_i)^2 / (e-1) \qquad (5)$$

Where: S_i^2= environmental variance, R_{ij} = observed genotype yield across environments, m_i = marginal means of genotypes, e=number of environments

3. Results and Discussion

3.1. Impact of Genotype, Location and Year on Grain Yield of Bread Wheat

The analyses of variances revealed highly significant ($P \leq 0.01$) differences among genotypes, locations, year and their interactions for grain yield (Table 3). The highest variation was accounted for by location (29%) followed by genotype (18%) and location by year (18 %) and genotype by year (12%) effects (Table 3). The grain yield of genotypes was highest at Adet in 2014 cropping season, and at Debretabor in 2015 cropping season. Similarly, grain yield of genotypes was lowest at Simada in 2015 (Table 4). Genotypes G4 (Gambo), G2 (Ogolcho), G11 (Shorima) and G9 (Tsehay) showed 31.4%, 25.8%, 10% and 8.9 % t/ha grain yield advantage over standard check (G7 = Tay) and 49.6%, 41.8%, 25.4% and 23.6% t/ha grain yield advantage over standard check (G12 = Kubsa), respectively (Table 4). The significant influence of genotype, location, growing season and all possible interactions of these on grain yield has been reported by Fetien Abay and Asmund Bjornstad (2009), Hintsa Gebru and Fetien Abay (2013) and Mohammed (2013). As a result, screening and development of wide adaptable and relatively stable genotypes are determinant factor to increase bread wheat productivity and production.

Table 3. Analysis of variance for grain yield of 12 bread wheat genotypes at three locations during 2014 and 2015 cropping seasons in Northwestern Ethiopia.

Source of variation	DF	SS	MS	% total sum square explained
Genotype	11	9187.87	835.26**	18
Location	2	14393.4	7196.7**	29
Year	1	2764.46	2764.46**	5.6
Genotype x Location	22	3798.1	172.64**	7.6
Genotype x Year	11	5809.92	528.17**	12
Location x Year	2	8998.73	4499.37**	18
Genotype x Location x Year	22	2591.46	117.79**	5.2
Error	142	2247.1	15.82	4.5
Total	213	49791.04		

Note: DF=Degree of freedom, SS= Sum square, and MS=Mean square

Table 4. Mean grain yield (t/ha) of twelve bread wheat genotypes at three locations during the 2014 and 2015 cropping seasons in northwestern Ethiopia.

	Adet		Simada		Debretabour		
Genotypes	2014	2015	2014	2015	2014	2015	Mean
G1	4.67	4.26	4.29	1.85	1.84	5.45	3.73^{cde}
G2	6.47	5.82	5.19	2.59	4.80	4.91	4.97^{a}
G3	5.70	5.32	2.46	2.12	2.80	4.34	3.79^{cd}
G4	7.09	6.01	5.69	2.64	4.94	4.70	5.18 ^{a}
G5	5.29	3.09	4.49	1.67	3.70	3.65	3.65^{de}
G6	4.49	3.81	4.27	2.06	3.25	3.91	3.63^{de}
G7	5.92	5.22	2.79	1.78	3.09	4.87	3.95^{c}
G8	5.06	1.39	3.49	1.47	3.59	3.37	3.01^{f}
G9	6.68	4.55	4.59	1.77	3.53	4.61	4.29^{b}
G10	5.27	1.19	3.66	1.40	2.77	3.38	2.95^{f}
G11	5.61	5.16	4.61	2.35	3.82	4.51	4.34^{b}
G12	4.97	5.57	1.28	2.20	1.71	5.09	3.47^{e}
Yr Mean	5.60	4.29	3.90	1.99	3.32	4.39	
Loc mean	4.94		2.95		3.86		3.92
LSD	0.76		0.44		0.69		0.64
CV (%)	9.4		9		11		10.2
P level	<0.001		<0.001		<0.001		

Note: G1= Hidase, G2= Ogolocho, G3= Hulluka, G4= Gambo, G5= Danad'a, G6= Gassay, G7=Tay, G8= Bolo, G9= Tsehay, G10=Menze, G11=Shorima, G12=Kubsa, Yr=Year, LOC=Location, LSD= Least significant difference and CV=Coefficient of variation

3.2. The Main and Interaction Effects of Genotypes and Environments on Grain Yield

The AMMI analysis of grain yield showed highly significant differences among genotypes, environments and their interactions (Table 5). Environment depicted the highest variation on grain yield performance of genotypes which accounted for 41.91% followed by genotype by environments interaction (19.55%) and genotype (14.72%) (Table 5). Consistent with the results of this study, Misganaw *et al.* (2015) reported higher contribution of environment followed by genotype and genotype by environment interaction to the total sum squares for grain yield of bread wheat genotypes.

The partitioning of the genotype-environment interaction in AMMI model analysis showed that four of the Interaction Principal Component Axes (IPCAs) were highly significant ($P \leq 0.01$). However, the first two IPCAs accounted for the largest proportion of 82.26% interaction sum of square while the other two IPCAs only accounted for 16.95%, indicating the first two were sufficient to explain the interactions (Table 5). Approximately as much variation in grain yield was explained by the interaction term captured by IPCA1 (66.54 %) as by the genotypic main effect. This showed that interaction is as important as genotypic main effect, implying that both specific and wide adaptations are important. In the biplot axes system, either main effects and IPCA1, or IPCA1 and IPCA2 are commonly used as abscissa and ordinates (Zobel *et al.*, 1988; Gauch, 1992).

Table 5. AMMI analysis of variance for grain yield of 12 bread wheat genotypes at six environments (three locations over two years) in the 2014 and 2015 cropping seasons.

Source of variation	DF	SS	MS	Sum of square explained	
				% total	% G x E
Genotype	11	9188	835.3**	18.3	
Environment	5	26157	5231.3**	52.1	
Block	12	718	59.8**	1.42	
G x E interaction	55	12199	221.8**	24.3	
IPCA 1	15	8117	541.1**		66.54
IPCA 2	13	1918	147.5**		15.72
IPCA 3	11	1476	134.2**		12.10
IPCA 4	9	592	65.8**		4.85
Error	132	1943	14.7	3.87	
Total	215	50205			

*Note: **=significant at P ≤ 0.01, DF = degree of freedom, SS = sum squares, MS = mean squares, G x E = genotype by environment, and IPCA = interaction principal component axes.*

The AMMI biplot showing the main and IPCA1 effects of both genotype and environment on bread wheat grain yield is depicted in Figure 1. In such a system, distances along the abscissa (horizontal line) shows main effect differences whereas the ordinate (vertical line) shows differences in interaction. Akter *et al.* (2014), genotypes that group together have similar adaptation and also environments which group together influences the genotypes in the relative way. Thus, G1 (Hidase), G3 (Hulluka), G5 (Danda'a) and G6 (Gassay) had more or less similar genotypic main effects, differing in interaction while G2 (Ogolcho) and G11 (Shorima), and G4 (Gambo) and G9 (Tsehay) had nearly similar interaction effects, only differing in genotypic main effects. In the same manner, E4 (Adet-2015) and E2 (Simada-2014) had higher interaction effect; whereas E5 (Simada-2015) and E1 (Adet-2014) had minimum interaction effect, but they had higher differences in environmental main effect.

In Figure 2, the distances from the origin indicate the magnitude of interaction exerted by environments on genotypes, or vice versa (Voltas *et al.*, 2002; Fetien Abay and Asmund Bjornstad, 2009; Misra *et al.*, 2010; Akter *et al.*, 2014). In other words, genotypes near the origin are not sensitive to environmental interaction, whereas genotypes distant from the origin are sensitive and have large interaction effects. Hence from this study, genotypes G11 (Shorima), G9 (Tsehay), G6 (Gassay), G7 (Tay) and G3 (Hulluka) were weakly influenced by environmental factors. That is to say, the grain yield response of each genotype was relatively similar across environments while G10 (Menze), G8 (Bolo), G12 (Kubsa) and G4 (Gambo) were strongly affected by environmental factors. That means the grain yield response of each genotype varied across environments.

According to Yan *et al.* (2000) and Yan and Rajcan (2002), ideal genotypes are those having large PC1 scores (high grain yield) and small absolute PC2 scores (high stability). Accordingly, G11 (Shorima), G6 (Gassay), G2 (Ogolcho) and G9 (Tsehay) were better stable genotypes and genotypes G4 (Gambo), G2 (Ogolcho), G11 (Shorima) and G9 (Tsehay) were high yielder in that order of importance. Though G6 (Gassay) was relatively stable genotype, it is not preferable for production due its low-yielding capacity.

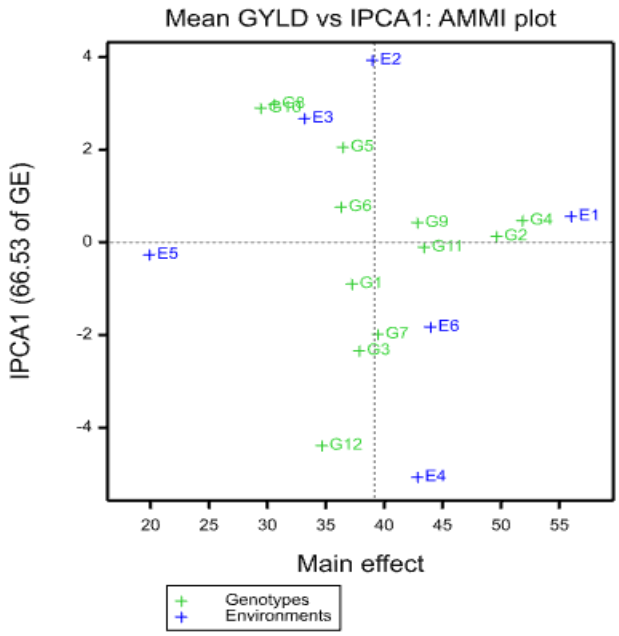

Figure 1. AMMI biplot main effects and IPCA1 of genotypes and environments using symmetrical scaling (G1 = Hidase, G2 = Ogolocho, G3 = Hulluka, G4 = Gaambo, G5 = Danda'a, G6 = Gassay, G7 = Tay, G8 = Bolo, G9 = Tsehay, G10 = Menze, G11 = Shorima, G12 = Kubsa, E1 and E4 = Adet E2 and E5 = Simada and E3 and E6 = Debretabor, IPCA = Interaction Principal Component Axes).

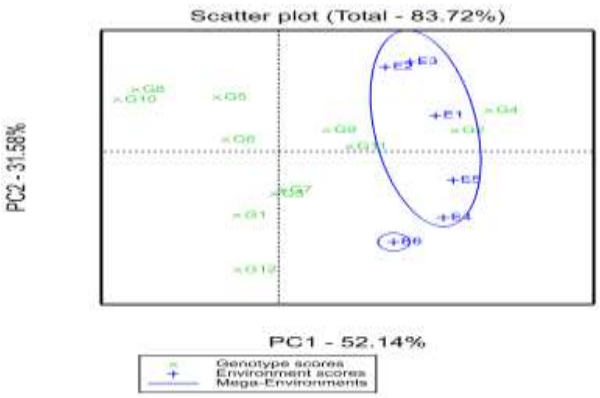

Figure 2. GGE biplot analysis of genotypes and environments using environment scaling (G1 = Hidase, G2 = Ogolocho, G3 = Hulluka, G4 = Gaambo, G5 = Danda'a, G6 = Gassay, G7 = Tay, G8 = Bolo, G9 = Tsehay, G10 = Menze, G11 = Shorima, G12 = Kubsa, E1 and E4= Adet E2 and E5 = Simada and E3 and E6= Debretabor, PC = Principal Component).

3.3. Genotypes Stability Analysis for Grain Yield

AMMI biplot analyses showed adaptable and stable genotypes in graphical forms. As a matter of fact, to know the stability of genotypes in numerical terms, further stability analysis work is pertinent to explore stable genotypes using different stability analysis methods. In terms of different stability coefficient analysis methods, AMMI, Cultivar superiority and static stability values G11 (Shorima), G4 (Gambo) and G6 (Gassay) were ranked 1st and G12 (Kubsa), G10 (Menze) and G12 (Kubsa) were ranked least, respectively (Table 6). According to this study, grain yield stability of genotypes ranks varied with the methods used.

Table 6. Grain yield stability of bread wheat genotypes based on different methods of analyses.

Genotype	Mean	Stability coefficient analysis method					
		ASV	R	Cultivar Superiority	R	Static stability	R
G1	37.26	4.28	6	176.9	9	230.4	7
G2	49.65	1.99	3	8.2	2	174	4
G3	37.89	9.91	9	157.9	6	236.7	9
G4	51.84	3.34	5	4.6	1	228.3	6
G5	36.48	8.7	8	159.8	7	152.8	3
G6	36.33	3.23	4	160.9	8	77.8	1
G7	39.49	8.4	7	123.7	5	261.7	11
G8	30.61	12.7	11	318.6	11	198.3	5
G9	42.88	1.89	2	59	4	257.3	10
G10	29.47	12.4	10	346.1	12	231.5	8
G11	43.43	1.17	1	52.9	3	132.1	2
G12	34.68	18.9	12	291	10	376.2	12

Note: G1 =Hidase, G2 = Ogolocho, G3 = Hulluka, G4= Gaambo, G5 = Danda'a, G6 = Gassay, G7 = TAY, G8= Bolo, G9 = Tsehay, G10 = Menze, G11 = Shorima, G12 = Kubsa, ASV = AMMI stability value, R = Rank

According to Lin *et al.* (1986), Becker and Leon (1988) and Lin and Binns (1988) stability statements, Static stability analysis had a drawback which implies both higher and lower grain yielding genotypes as stable, AMMI stability value only shows consistency of genotypes contribution to genotype by environment interactions and cultivar superiority analysis only shows mean performance of genotypes across environments, nonetheless it is difficult to know consistency of genotypes yield response across environments. This shows the necessity of combined use of different stability analysis methods to properly evaluate stable genotypes both in potential and consistency of grain yield over environments. In general, G4 (Gambo), G2 (Ogolcho), G11 (Shorima) and G9 (Tsehay) were relatively high yielding and stable genotypes based on AMMI stability, cultivar superiority and static stability values. Therefore, these genotypes are preferable as a source of material for bread wheat improvement and production across the tested environments.

4. Conclusions

This study has demonstrated that the grain yields of genotypes were significantly influenced by environment and by genotype x environment interactions, which together accounted for more than 76% of the variations observed. Thus, according to the AMMI biplot, GGE biplot and stability coefficients analyses, G4 (Gambo), G2 (Ogolcho), G11 (Shorima) and G9 (Tsehay) were relatively more stable genotypes in all the test environments than the two checks G7 (Tay) and G12 (Kubsa). Therefore, in view of the mean grain yield and stability of genotypes, G4 (Gambo), G2 (Ogolcho), G11 (Shorima) and G9 (Tsehay) could be used as alternative varieties at the test environments. However, it

is better to study varieties including many locations as much possible for consecutive years to identify stable genotypes and mega environments that represent the area where genotypes can be tested in the process of variety development for the region because of the northwestern Ethiopia is covering large area of the country known with wide range of variations in changing climate conditions year after year within small areas. In addition to this, In the Western Amhara Region due to evolving of epidemic rust races through weather variability and/or mutation most of the varieties break down their resistance to rust disease after few years of production. Hence Development Agents, Wheat Commercial Producers and Farmers thoroughly explore bread wheat genotypes that have high grain yielding and disease resistance potential per year for bread wheat production.

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Isolation and Characterization of *Lactobacillus* Species from Head *Cabbage (Brassica oleracea var. capitate*) and its Potential Application as a Probiotic Agent

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Abstract: Lactic acid bacteria (LAB) are one of the most important groups of microorganisms used in food fermentation, contribute to extended shelf life of the fermented products, and are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products. The purpose of this study was to isolate and characterize *Lactobacillus* species from head cabbage (*Brassica oleracea var. capitate*) and to evaluate their probiotic properties under conditions simulating human gastrointestinal (GI) tract. For this purpose, a total of 15 head cabbage samples were collected randomly from Haramaya district during the period from May to August, 2014. Based on cultural and biochemical characteristics, *Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus cellobiosus,* and *Lactobacillus brevis* were isolated from the cabbage heads. The probiotic effect of each isolate was evaluated for antimicrobial activity against human pathogenic bacteria, resistance to bile acids, resistance to low pH, antibiotic resistance, and haemolytic activity. Results showed that all isolates had antagonistic effects against pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia*) with different degree of inhibition zone but *L. brevis* shown the highest inhibition zone followed by *L. cellobiosus* and *L. plantarum*. According to resistance to bile acid, *L. plantarum, L. cellobiosus* and *L. brevis* retained their viability with a negligible reduction. Regarding antibiotics resistance, *L. plantarum, L. cellobiosus* and *L. brevis* were resistant strains to Streptomycin, Gentamycin, and Tetracycline antibiotics. According to a haemolytic test, all isolates did not exhibit β–haemolytic activity. The growth of *Lactobacillus* species recorded under all the pH values were viable. In conclusion, the present study indicated that *L. brevis* and *L. cellobiosus* possess potential probiotic properties but further *in-vivo* tests are required to elucidate their particular effects on human health.

Keywords: Antimicrobial susptibility; Gastrointestinal (GI) Tract; Pathogenic Bacteria; Probiotic.

1. Introduction

Lactic acid bacteria (LAB) are a broad group of Gram positive, non-spore forming, and catalase-negative, facultative anaerobic and nutritionally fastidious organism. They are widespread in soil, vegetables, meat, milk and the human body. LAB are among the most important groups of microorganisms used in food fermentation where they play an essential role and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products (Noopur *et al*., 2010; Hassanzadazar and Ehsani, 2013). One of the most important contributions of these microorganisms is the extended shelf life of the fermented products. Growth of spoilage and pathogenic bacteria in these foods is inhibited due to competition for nutrients and the presence of starter-derived inhibitors such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins (Noopur *et al*., 2010; Noordiana *et al*., 2013).

Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). It is well established that probiotics confer a number of beneficial health effects to humans and animals. Intake of probiotics stimulates the growth of beneficial microorganisms and reduces the amount of pathogens, thus, improving the intestinal microbial balance of the host and lowering the risk of gastro-intestinal diseases (Chiang and Pan, 2012), alleviatinglactose intolerance, the enhancement of nutrients bioavailability, and prevention or reduction of the prevalence of allergies in susceptible individuals (Isolauri, 2001; Chiang and Pan, 2012). Probiotics are reported to have also anti-mutagenic, anti-carcinogenic, hypo-cholesterolemic, antihypertensive, anti-osteoporosis, and immune-modulatory effects (Chiang and Pan, 2012). They relieve the symptoms of inflammatory bowel diseases, irritable bowel syndrome, colitis, alcoholic liver disease, constipation and reduce the risk for colon, liver and breast cancers (Prado *et al.,* 2008).

The selection of probiotic bacteria includes several criteria: safety, viability, resistance to acid and bile salts, adherence to gut epithelial tissue, ability to colonize the gastrointestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose reduction (Tkhruni *et al.,* 2013). In the past years, a lot of work has been done to isolate and characterize lactic acid bacteria from different sources but the probiotic properties of these lactic acid bacteria strains were not well determined. Therefore, the

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purpose of this research was to isolate and characterize *Lactobacillus* species from head cabbage (*Brassica oleracea var. capitata*) and to evaluate its probiotic properties under conditions simulating the human gastrointestinal (GI) tract.

2. Materials and Methods

2.1. Description of the Study Area

Haramaya district is one of the woreda in the Oromia Region of eastern Hararghe Zone, Ethiopia. The altitude of this district ranges from 1400 to 2340 meters above sea level. Haramaya district is located at the distance of out 500 km from Addis Ababa in the easterly direction. The Latitude and Longitude of Haramaya is 9.4062 and 42.0014 respectively.

2.2. Treatments and Experimental Design

The treatments consisted of examination comprised isolation of *Lactobacillus* species, physiological and biochemical characterization, and probiotic feasibility tests. The experiment was designed as a complete randomized design and replicate three times.

2.3. Sample Collection

A total of 15 samples of head cabbage were collected randomly during the period from May to August, 2014. The cabbage samples were collected from Haramaya University's student cafeteria (HUSC), Haramaya town market (HaTM) and also from Bate town market (BaTM). The cabbage samples were taken in accordance with the instructions given in Health Protection Agency (HPA, 2004) and the Food and Drug Administration (FDA, 2003). The cabbage samples were packaged into sterile plastic containers, transported to Microbiology Laboratory, Haramaya University and experiment was done immediately to prevent deterioration.

2.4. Isolation of *Lactobacillus* Species

Osmotic pressure stabilization was done by taking one gram of each sample and homogenizing it in nine ml of a sterile salt solution (0.85% NaCl) using the vortex. Then, sequential decimal dilutions of the homogenate were obtained. One ml of each aliquot dilution was spread plated on MRS agar for the isolation of *Lactobacillus* species and incubated anaerobically for 48 h at 37°C. The colonies were randomly picked from plates and purified by successive streaking on MRS agar media before being subjected to characterization. Gram-positive, catalase-negative and rod and/or bacilli isolates were considered as *Lactobacillus* as described by (Harrigan and McCance, 1990). The cultures were stored and maintained at -20°C on MRS agar slants supplemented with 10% (v/v) glycerol for further studies.

2.5. Physiological and Biochemical Characterization of *Lactobacillus* Isolates

Growth of *Lactobacillus* isolates was examined in MRS broth at the temperatures of 15, 37 and 45°C for 24 h anaerobically according to (Briggs, 1953). CO_2 and lactic acid production from glucose was tested in citrate lacking MRS broths media containing inverted Durham tubes. Salt tolerance was tested in MRS broth by incorporating 2, 4 and 6.5 % (w/v) sodium chloride and incubating it at 37°C according to the method described by (Briggs, 1953). A catalase test was performed by adding 3% of hydrogen per oxide (H_2O_2) in a test tube containing an overnight culture of *Lactobacillus* species. Ammonia production in MRS broth (containing 0.3% arginine and 0.2% sodium citrate instead of ammonia citrate) was detected using Nessler's reagent according to method described by (Briggs, 1953). The isolates were further characterized by their carbohydrate fermentation pattern using different sugars (lactose, raffinose, sucrose, salicine, cellobiose, gluconate, arabinose, and mellibiose). Gram positive, catalase negative, and bacilli colonies were taken as *Lactobacillus* species and stored in a glycerol culture at -20°C for further investigation.

2.6. Probiotic Feasibility Test of *Lactobacillus* Isolates

All *Lactobacillus* isolates were examined for their probiotic properties under conditions simulating human gastrointestinal (GI) tract (resistance to low pH and resistance to bile acids), antibiotic resistance, haemolytic activity and antimicrobial activity against human pathogenic bacteria. Resistances to low pH and bile acids were determined according to reduction in cell viability (log cfu/ml) comparing with the reference microbial count. Accordingly, 2.80 log cfu/ml was used as a reference to all *Lactobacillus* isolates.

2.6.1. Antibiotic Resistance

All *Lactobacillus* isolates were evaluated for their antibiotic resistance by the disk diffusion method using three different antibiotics (streptomycin, tetracycline and gentamycin). Six milliliters of overnight grown cultures were inoculated in to de Man Ragosa and Sharpe (MRS) broth and mixed thoroughly using vortex and spread plated over MRS agar in triplicates. After solidification of the media, the antibiotic disks were kept on the solidified agar surface and the plates were left over for 10 minute at 4°C for diffusion of antibiotics and then anaerobically incubated at 37°C for 48 h. Zone of inhibition was measured using calipers in millimeter (mm) according to the method described by (Todorov and Dicks, 2004).

2.6.2. Resistance to Low pH

Lactobacillus isolates were assessed for their resistance to pH 2.0, 3.0, and 4.0 incubated anaerobically at 37°C for 3 h. All overnight grown *Lactobacillus* cultures were harvested by centrifugation (5000 rpm for 10 min at 4°C). Pellets were washed once in phosphate-saline buffer (PBS at pH 7.0). Then cell pellets were suspended with different pH ranges and incubated at 37°C. Enumeration of viable colonies on MRS agar was carried out using a colony counter at 3 h gap.

2.6.3. Resistance Against Bile

Resistance to bile acids was carried out using all overnight grown *Lactobacillus* cultures and spread plated on MRS agar containing 0.3% (w/v) bile concentration in triplicates and incubated anaerobically at 37°C for 4 h according to the method described by (Kumar, 2012). Enumeration of viable colony on MRS agar was carried out for every hour using a colony counter.

2.6.4. Haemolytic Activity

Lactobacillus isolates were screened for haemolytic activity on Colombia human blood agar plates incubated anaerobically at 37°C for 48 h. Haemolytic activity of the cultures were evaluated for signs of β-haemolysis, γ-haemolysis and α-haemolysis on Colombia human blood agar plates (Nour-Eddine, 2006).

2.6.5. Antibacterial Activity of *Lactobacillus* Isolates

Antimicrobial effects of *Lactobacillus* isolates were determined by the agar diffusion method as described by (Todorov and Dicks, 2004). The *Lactobacillus* isolates were evaluated for antimicrobial activities against human pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)* obtained from Haramaya University, Microbiology Laboratory. The tested *Lactobacillus* species were inoculated in to MRS broth and incubated at 37°C for 48 h anaerobically. Cell free supernatant and cell mass were screened for antibacterial activities against the pathogenic bacteria. A cell-free supernatant was obtained by centrifugation of the liquid culture at 8000 rpm for 20 minutes at 4°C. Wells with a diameter of six milliliters were loaded with 15µl of the cell free supernatant. The wells were prepared in the Mueller Hinton agar previously seeded with the test isolates. The plates were then incubated at 37°C for 24 h after which the diameter of inhibition zones was measured using a caliper in millimeter (mm).

3. Data Analysis

Analysis of variance was done in accordance with the complete randomized design. Significant differences among the means were separated using the least significant difference (LSD) test at 5% level of significance.

4. Results and Discussion

4.1. Isolation of *Lactobacillus* Species

A total of 65 lactic acid bacteria colonies were isolated from the MRS agar medium; 22 of the isolates shared characteristics of *Lactobacillus* species which are gram positive, catalase negative, non-motile, non-sporulating, anaerobic, and bacilli (Table 1). According to biochemical characterization, eleven isolates were *Lactobacillus cellobiosus,* eight isolates were *Lactobacillus plantarum*, and three isolates each were *Lactobacillus salivarius, Lactobacillus fermentum* and *Lactobacillus brevis*. All isolates were well identified based on their sugar fermentation profile. *Lactobacillus cellobiosus* and *Lactobacillus plantarum* was the dominant bacteria isolated in all the head cabbage samples. Similar reports by (Hernández *et al.,* 2005) indicated isolation of *L. plantarum* from cucumber fermentation. Similarly, (Daeschel *et al*., 1990), isolated *L. plantarum* from Italian ewe cheeses, and (Hassanzadazar and Ehsani, 2013) reported that the dominant isolated bacillus genus from Koopeh Cheese was *Lactobacillus plantarum* (58% of the lactobacilli population).

4.2. Physiological and Biochemical Characterizations of *Lactobacillus* Species

All *Lactobacillus* isolates were able to grow at the temperatures of 37 and 45°C (Table 1). Similar reports by (Iman *et al.* 2014) indicated that two isolates *L. plantarum* K3 and *L. plantarum* SH4 and *L. fermentum* C12 were able to grow at 45°C, while 12 isolates were not able to grow at the same temperature in isolated Syrian fermented foods. All isolates were able to grow in NaCl at the concentrations of 2 to 4% (Table 1). The growth of two *L. plantarum* isolates isolated by (Pal *et al,* 2004) from cabbage showed very weak growth in the presence of 4% NaCl concentration. All tested isolates did not produce ammonia from arginine. This is comparable with the results reported by (Estifanos Hawaz, 2014) that all isolates of lactic acid bacteria isolated from cow milk curd of dairy products did not produce ammonia. In this study, *L. plantarum* and *L. fermentum* isolates produced CO_2 gas from glucose, and all isolates are catalase negative (Table 1).

4.3. Probiotic Feasibility Test of *Lactobacillus* Species

4.3.1. Resistance to Low pH

All selected *Lactobacillus* isolates were examined for pH resistance (2.0, 3.0, and 4.0). Results showed that the counts of *Lactobacillus* species recorded under all the pH values were viable. *L. plantarum, L. cellobiosus,* and *L. salivarius* were able to survive at pH ranging from 2 to 4 with significant reduction in viability but *L.brevis* retained its viability with negligible reduction. *L. fermentum* species was able to survive only at pH 4 (Table 2).

4.3.2. Antibiotic Resistance

According to the antibiotic resistance test, *Lactobacillus plantarum, Lactobacillus cellobiosus* and *Lactobacillus brevis* were potent antibiotic resistant strains (Table 3). *Lactobacillus salivarius* and *Lactobacillus fermentum* were not resistant to Gentamycin and Tetracycline, respectively. This is in agreement with (Halami *et al.,* 2000) reported that *Lactobacillus* species are resistant to β-lactam, cephalosporin, aminoglycosides, quinolone, imidazole, nitrofurantoin and fluoroquinolines. Belletti *et al.* (2009) also reported that *Lactobacilli* are generally resistant to aminoglycosides.

Table 1. Cultural, physiological and biochemical characteristics of the *Lactobacillus* isolates.

	Lactobacillus isolates				
Tests	*L. brevis*	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*
Growth at					
15°C	-	-	-	-	+
37°C	+	+	+	+	+
45°C	+	+	+	+	+
Growth at pH					
2.0	+	+	-	+	+
3.0	+	+	-	+	+
4.0	+	+	+	+	+
Production of					
CO_2	-	-	+	+	-
Ammonia	-	-	-	-	-
Lactic acid	+	+	+	+	+
Growth at NaCl					
2%	+	+	+	+	+
3%	+	+	+	+	+
4%	+	+	+	+	+
Catalase test	-	-	-	-	-
Cell shape	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Motility test	-	-	-	-	-
Aerobicity	f.a	f.a	f.a	f.a	f.a
Spore forming	-	-	-	-	-
Capsule formation	-	-	-	-	-
Fermentation of					
Lactose	+	+	+	-	+
Raffinose	-	-	+	-	+
Sucrose	+	-	+	v	+
Salicine	+	+	-	-	-
Cellobiose	-	-	-	-	-
Gluconate	-	-	+	+	-
Arabinose	-	-	v	-	-
Mellibiose	-	-	+	-	v

Note: *+ = positive, - = negative , v = variable, and f.a = facultative anaerobic*

Table 2. Resistance to low pH of *Lactobacillus* isolates (log cfu/ml).

	Means ± SD Resistance to low pH (log cfu/ml)				
pH range	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*	*L. brevis*
2.0	1.15 ± 0.11[a]	N	1.12 ±0.00[a]	1.00 ±0.00[a]	2.63 ±0.00[a]
3.0	1.22 ± 0.12[a]	N	1.22 ±0.50[a]	1.20 ±0.22[a]	2.75 ±0.21[a]
4.0	1.30 ± 0.10[a]	1.34±0.00[a]	1.28 ±0.00[a]	1.22 ±0.03[a]	2.80 ±0.01[a]
Average	1.22 ±0.11	1.34±0.00	1.21±0.16	1.14±0.08	2.73±0.07

Note*: a = Means bearing similar superscripts in the same column differ insignificantly (p>0.05); N = negative*

Table 3. Antibiotic resistance of *Lactobacillus* isolates.

	Lactobacillus isolates				
Antibiotics	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*	*L. brevis*
Streptomycin	R	R	R	R	R
Gentamycin	N	R	R	R	R
Tetracycline	R	N	R	R	R

Note*: R = resistance; N = negative*

4.3.3. Resistance to Bile Acids

All *Lactobacillus* isolates were screened for bile salt tolerance. *Lactobacillus salivarius* and *Lactobacillus fermentum* were survived with significant reduction in viable count but *L. plantarum, L. cellobiosus* and *L. brevis* were retaining their viability with negligible reduction after 4 h of exposure to 0.3 % (w/v) bile acid (Table 4). This is in agreement with (Jensen *et al.,* 2012) who reported that *Lactobacillus* species tolerate gastric juice well with no reduction in viability. Similarly (Vitali *et al.,* 2012) determined the probiotic potential of large number of lactic acid bacteria isolated from fruit and

vegetables were survived in gastric and intestinal conditions.

Table 4. Resistance to bile acids 0.3 % (w/v) of *Lactobacillus* isolates (log cfu/ml).

	Means ± *SD* Resistance to bile acids 0.3 % (w/v)				
Time	*L. salivarius*	*L. fermentum*	*L.plantarum*	*L. cellobiosus*	*L. brevis*
0 h	1.42±0.11[a]	1.50±0.00[a]	2.51±0.00[b]	2.53±0.00[b]	2.80±0.00[b]
1 h	1.00±0.00[a]	1.25±0.03[a]	2.41±0.00[b]	2.51±0.02[b]	2.63±0.00[b]
2 h	0.78±0.02[a]	1.01±0.01[a]	2.40±0.00[b]	2.50±0.00[b]	2.50±0.03[b]
3 h	0.35±0.00[a]	0.98±0.02[a]	2.35±0.00[b]	2.45±0.04[b]	2.48±0.00[b]
Average	0.89±0.03	1.19±0.02	2.42±0.00	2.50±0.12	2.60±0.08

***Note**: a, b = Means bearing similar superscripts in the same column differ insignificantly (p > 0.05); N = negative*

4.3.4. Haemolytic Test

All isolates were tested for hemolytic activity and gave negative results. These results agree with the findings of (Sandra *et al.* 2012) who reported that none of the fifteen putative probiotics was found to be β-hemolytic. This is also comparable with (Estifanos Hawaz, 2014) who reported that all examined *Lactobacillus* strains isolated from cow milk curd did not exhibit β-hemolytic activity.

4.3.5. Antibacterial Activity of *Lactobacillus* Species

The *Lactobacillus* species cell free supernatants (CFS) were tested for their antimicrobial activities against human pathogenic microorganisms (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)*. Results showed (Table 5) that all the CFSs were found to produce inhibition zone against pathogenic bacteria but *L. brevis* produced a wider inhibition zone against the tested microorganism followed by *L. cellobiosus*, *L. plantarum* and the lowest antimicrobial effect was recorded for *L. salivarius* and *L. fermentum* isolates. This may be due to production of short-chain organic acids (lactic, acetic, propionic), bacteriocins (nisin, acidolina, acidofilina, lacatcyna, lacocydyna, reutryna, laktoline, entrocine) and hydrogen peroxide. Bacteriocins have a high antibacterial activity against *Escherichia coli, Salmonella, Staphylococcus aureus, Clostridium perfringers,* and *Campylobacter* (Patil and Ajay, 2010; Karimi *et al.,* 2012). A similar result on antagonistic activity of LAB was reported by (Hernandez *et al.* 2005) in that, the CFS of the selected *Lactobacillus* isolates from vegetables inhibited the growth of *K. pneumoniae, S. aureus*, and *E. coli.* Vitali *et al.* (2012) isolated *lactobacillus* strains from olives which had antimicrobial effect against *S. aureus, E. facalis,* and *Salmonella enteric.* Messaoudi (2012) also reported that *Lactobacillus* strains isolated from chicken showed inhibition against *L. monocytogenes, S. aureus* and *Salmonella.*

Table 5. Antimicrobial activity of *Lactobacillus* isolates (CFS).

	Means ± *SD* zone of inhibition zone (mm)			
Lactobacillus isolates	*P. aeruginosa*	*S. aureus*	*E. coli*	*K. pneumonia*
L. salivarius	12±0.01[a]	9±0.00[a]	10±0.02[a]	12±0.00[a]
L. fermentum	13±0.00[a]	10±0.03[a]	12±0.00[a]	12±0.01[a]
L. plantarum	14±0.10[a]	15±0.04[a]	14±0.01[a]	15±0.00[a]
L. cellobiosus	22±0.00[b]	20±0.01[b]	21±0.00[b]	22±0.01[b]
L. brevis	25±0.02[b]	24±0.00[b]	25±0.00[b]	23±0.01[b]

***Note:** a, b = Means bearing different superscripts in the same column differ significantly (p < 0.05)*

5. Conclusion

Cabbage is one of the potential sources of lactic acid bacteria due to its nutritional composition. In the present findings; *L. salivarius, L. brevis, L. plantarum, L. cellobiosus,* and *L. fermentum* were isolated from cabbage (*Brassica oleracea var. capitate*). Probiotic properties of all isolates were investigated under conditions simulating human gastrointestinal (resistance to low pH and resistance to bile acids), antibiotic resistance, haemolytic activity and antimicrobial activity against pathogenic bacteria. Results showed that, all *Lactobacillus* strains shown antagonistic effect against all human pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)* with different degree of inhibitory effect but *L.brevis* shown high inhibition zone followed by *L. cellobiosus* and *L. plantarum.* Regarding to resistance to bile acid, *L. plantarum, L. cellobiosus* and *L. brevis* were retaining their viability with negligible reduction and did not exhibited β–haemolytic activity. The growth of *Lactobacillus* species recorded under all the pH values were viable. In conclusion, the current *in-vitro* study indicated that, *L. brevis* and *L. cellobiosus* are potential probiotic candidate but further *in-vivo* test is required for its specific outcome on human health.

6. Acknowledgements

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Sun-Dried Bovine Rumen Content (SDRC) as an Ingredient of a Ration for White Leghorn Layers

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Abstract: Due to increasing price of conventional feeds, alternative locally available non-conventional feed ingredient is required for layers' production. Rumen contents are abundantly available as slaughterhouse by-product and mainly considered as waste material creating environmental pollution. Therefore, a study was conducted for 90 days to evaluate effects of sun-dried rumen content (SDRC) inclusion in layer rations on egg laying performance, egg quality parameters, fertility, and hatchability, chick quality and blood parameters. Treatment diets contained T1, T2, T3, and T4 at 0, 5, 10, and 15 percent SDRC, respectively. On chemical analysis, the rumen content contained 11.18% crude protein, 1.22% ether extract, 22.99% crude fibre, 21.54% ash and 1099.32 cal/kg of DM of metabolizable energy. The daily DM intake value increased ($P < 0.05$) with the increase in level of rumen content. The bird fed with 10 percent SDRC diet had a high DM intake. The weight gain and egg production of the laying hens reduced significantly ($P < 0.05$) as the level of rumen content increased. The average egg weight increased significantly ($P < 0.05$) with the increase in the level of rumen content. The feed to gain ratio increased as the level of rumen content increased in the diet. The hen fed T2 (5 percent SDRC) had the best feed to gain ratio. Most external and internal egg quality parameters, especially yolk color, were improved when the diet contained sun-dried rumen content (SDRC). Fertility, hatchability, early and mid embryonic mortality showed no significant differences among treatments. However, chick quality parameters increased ($P < 0.05$) with the increase in the level of rumen content. Mortality rate was not influenced by treatments. All blood parameters studied were within the normal range. It is concluded that under the condition of this experiment, diets up to 10% SDRC in the ration did not affect DMI, daily weight gain, egg production performances and blood parameters in white leghorn layers.

Keywords: White leghorns; SDRC; Egg mass; Egg quality; Fertility; Hatchability

1. Introduction

Feeding, which constitutes up to 70% of the total cost, is a major factor limiting poultry production in most developing countries. Poultry are most often dependent on cereal such as maize grain, which is stable food for human beings. Prices of these cereal crops are usually high and increases invariably. There is, therefore a need to explore, identify and utilize cheaper non-conventional alternative ingredients which attracts less competition. If properly processed and harnessed, one of such non-conventional feed source could be rumen content which is a waste material from abattoir and slaughter houses. Sun-dried rumen content (SDRC), a potential alternative feed source obtained from the rumen of ruminant animals consists of fermented and non-fermented dietary feeds that passed various stages of digestion in the rumen (Adeniji and Balogun, 2002). Rumen content contains the end products of microbiales metabolic activities such as microbial protein, amino acids, vitamins, volatile fatty acids (VFA) and contains no anti-nutritional factors (Okpanachi *et al*., 2010). It is fairly rich in crude protein (10-25 %) (Javanovic and Cuperloric, 1977). But, high percent of fibre content (25 %) has limited its use in poultry nutrition (Ricci, 1977). Rasyid *et al.* (1981) reported that an addition of 10% rumen content to broiler chicken feed did not affect its performance. Accordingly, its utilization as animal feed will increase the flexibility of ration formulation and reduce environmental pollution. Several relevant trials have been conducted on the suitability of dried rumen contents as feedstuff for livestock and fish such as rabbit (Okpanachi *et al*., 2010), broiler chickens (Colette *et al.,* 2013), Catfish (Agbabiaka *et al*., 2011), Nile Tilapia (Abdel-Hakim *et al*., 2008), feedlot Lambs (Salinas-Chavira *et al*., 2007) and Cattle (Cherdthong *et al*., 2014; Rios *et al*., 2010). There is currently no research based information on the use of rumen content for production of layers in Ethiopia. Therefore, this study was aimed at establishing the optimum inclusion level of sun-dried rumen content as feed ingredient in layers and on the subsequent laying performance of the hens.

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2.Material and Methods

2.1. Ingredients and Experimental Rations

The experiment was conducted on the main campus of Haramaya University's Poultry Farm. The feed ingredients used in the formulation of the different experimental rations in this study were rumen contents, maize grain, wheat short (WS), soybean meal (SBM), salt, vitamin premix, dicalcium phosphate and limestone. The rumen content was collected fresh from the abattoir of Haramaya University and sun dried for 3-4 days depending on the intensity of the sun. The remaining feed ingredients were purchased from the surrounding market. All the ingredients except wheat short, vitamin premix and dicalcium phosphate were hammer milled to pass through 5 mm sieve size and stored until required for chemical analysis and formulation of the experimental rations. The ingredients were then mixed based on their chemical composition to prepare the compound experimental rations. The four treatment rations used in this study were formulated on an isocaloric and isonitrogenous basis in such a way to consist 2800-2900 kcal ME per kg DM and 16-17% CP for layers (NRC, 1994).

2.2. Management of Experimental Hens

The existing concrete floor type poultry experiment house of Haramaya University poultry farm was used for the experiment. Pens, watering, feeding troughs and laying nests of the experimental house were properly cleaned, disinfected and sprayed before the placement of the experimental animals. The pens had the dimension of 2.5 * 2m with a stocking density of 3 hens per m^2. The floor of each pen was covered with saw-dust litter. The experimental hens were obtained from Haramaya University's poultry farm. Hens with no defects, uniform in size, and the same age were selected from a flock of hens in growers' house. A total of 204 hens of five months of age comprising 180 females and 24 males of White Leghorn breed were used for the study. The animals were given leg band numbers. These hens were weighed and randomly distributed into four treatments. The hens were acclimatized to the four treatment rations for 7 days and then fed for 90 days (experiment duration). All health precautions and disease control measures were taken throughout the study period according to the procedure followed by the poultry farm. Feed was measured and provided to the animals every day. The daily feed offer was divided into two equal parts and given to experimental hens in a group per pen at 08:30 and 14:30 hours *ad libitum* throughout the experimental period. Feed refusals were collected, weighed and recorded every next morning at 07:30 hours. The feeding and watering troughs were cleaned every morning before the daily meal is offered. Clean, fresh water was available to the animals every time. The hens were weighed individually at the beginning and at the end of the experiment in group using sensitive balance.

2.3. Experimental Design and Treatments

The design of the experiment was a completely randomized design (CRD) with 4 dietary treatments each with three replications. A total of 180 point of lay white leghorn hens (15 per pen) were randomly distributed to the 12 pens, each pen having 2 males of the same breed and age. The control diet was layers ration prepared without sun dried rumen content. The treatment ration to be used was formulated as indicated in Table 1.

2.4. Chemical Analysis

Except for dicalcium phosphate, salt, vitamin premix and limestone, representative samples feed ingredients used in the experiment were taken and analyzed before formulating the actual dietary treatments. The results of the analysis were used to formulate the ration. Samples were taken from each treatment ration bulked over the experimental period and sub sampled for chemical analysis at the end of the experiment. Thus, the total samples analyzed were 5 feed ingredients and 4 treatment rations. Dry matter (DM), ether extract (EE), crude fiber (CF) and ash were analyzed according to AOAC (1990). Nitrogen (N) content was determined by Kjeldahl method and crude protein (CP) was calculated as Nx6.25. Calcium (Ca) and total phosphorous (P) were determined by atomic absorption spectrometry (FAO, 1980). All the chemical analysis were analyzed in nutrition laboratory at Haramaya University. Metabolisable energy (ME) of the experimental diets was determined by indirect method according to Wiseman (1987) as follows:

$$ME\ (Kcal/kg\ DM) = 3951 + 54.4\ EE - 88.7\ CF - 40.8\ Ash \quad (1)$$

2.5. Measurements and Observations

Feed intake: The hens in each replicate were group fed, feeding being *ad libitum* for the entire length of the experiment. A weighed amount of feed was offered twice a day. Refusal was collected daily before offering fresh feed and weighed after removing external contaminants by visual inspection and hand picking. The feed offered and refused were recorded for each replicate and multiplied by respective DM contents. The amounts of feed consumed were determined as the difference between the feed offered and refused on DM basis. Similarly, the daily CP and ME intakes were calculated from the feed offered and refused and the differences were multiplied by the respective CP and ME concentrations.

Body weight measurement: The experimental hens were weighed individually on the first day of the commencement of the experiment and at the end of the experiment using sensitive balance. Average body weight gain for each replicate was computed by subtracting the initial weight from the final weight and dividing by the number of experimental days. The pen means were used for data analysis.

Egg production parameters: Egg number and egg weight were recorded daily to calculate percent egg production and egg mass. Eggs were collected three times a day from each pen at 1000, 1400 and 1800 hours. The sum of the three collections along with the number of hens alive on each day were recorded and summarized at the end of the period. Rate of lay for each treatment expressed as the average percentage hen-day egg production (HDEP) and hen-housed egg production (HHEP) were computed by taking the average values from each replicate following the method of Hunton (1995) as:

$$\% \text{ HDEP} = \frac{\text{Number of eggs collected per day}}{\text{Number of hens present that day}} * 100 \quad (2)$$

$$\% \text{ HHEP} = \frac{\text{Sum of daily egg counts}}{\text{No. of hens' originally housed}} * 100 \quad (3)$$

Egg weight and egg mass: Eggs collected daily was weighed immediately after collection for each replicate in the treatment. The average weight of daily collected eggs from each replicates was calculated as weight of all eggs divided by the number of eggs laid. After mean weight had been determined, the following formula was used to calculate the egg mass on daily bases (North, 1984).

Average egg mass = % Hen-day egg production * Average Egg weight in gram (4)

Feed conversion ratio: Feed conversion ratio per replicate was determined as a ratio of the total weight of feed consumed on DM basis and egg mass according to the following formula.

$$\text{FCR} = \frac{\text{Mean dry matter intake (g/hen/d)}}{\text{Average egg mass (g/hen/d)}} \quad (5)$$

Egg quality measurements: Internal egg quality parameters were measured for each replicate. For internal quality egg measurement, eggs were randomly picked and weighed once every week from each replicate. The weighed eggs were broken on a flat tray to measure shell weight, shell thickness, albumen height, haugh unit, albumen weight, yolk weight, yolk color, yolk diameter, yolk height and yolk index. The measurements were done by taking five eggs per replicate and a total of 15 fresh eggs per treatment were used for quality analysis.

Procedures followed to determine egg quality parameters were as shown below:
Eggs were broken on flat mirror and the different components were taken for internal and external quality parameters analysis. After breaking eggs, egg membrane was carefully removed from the shells and shell thickness was measured using micrometer gauge. The measurements were taken from three sites; the top (pointed part), bottom (round part) and the middle of the egg. Finally, the average of the three measurements was taken as shell thickness of each egg. Egg shell weight was measured using sensitive balance with 0.01g sensitivity. The albumen of the broken egg was carefully separated from the yolk. Tripod micrometer was used to measure the albumen height. Albumen quality was evaluated by calculating Haugh unit (HU). Haugh unit was determined according to the formula suggested by Standelman and Cotterill (1986).

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}) \quad (6)$$

Where: HU = Haugh unit; H= albumen height (mm); W= egg weight (g)

Albumen weight was calculated as the difference between the weight of the whole egg, and the weight of yolk and egg shell. The proportion of the albumen to egg weight was calculated by using the following formula.

$$\text{Albumen \%} = \frac{\text{Albumen weight (g)}}{\text{Weight of whole egg (g)}} * 100 \quad (7)$$

After the separation of the yolk and albumen, the quality of yolk was determined by taking the weight, diameter, height and its color. Yolk diameter was measured using graduated caliper. The yolk height of the broken egg was measured directly (without removing it from the albumen) using tripod micrometer and recorded to the nearest 0.1mm. Yolk weight was determined by using sensitive balance. After taking all the necessary measurements, first yolk membrane was removed; the yolk was stirred thoroughly to mix all parts. Then sample was taken on a piece of white paper and yolk color was determined by comparing the yolk sample with Roche color fan measurement strips which consists of a series of 1-15 colored plastic strips with one rated as very pale yellow to 15 representing a deep intense reddish orange color. Yolk quality was expressed also in terms of yolk index. It was determined as the ratio between yolk height and diameter according to the formula;

$$\text{Yolk Index} = \frac{\text{Yolk Height (mm)}}{\text{Yolk Diameter (mm)}} * 100 \quad (8)$$

Percentage of yolk to egg weight was determined by the following formula:

$$\% \text{ of yolk} = \frac{\text{Yolk weight (g)}}{\text{Weight of whole egg (g)}} * 100 \quad (9)$$

Fertility and hatchability: Medium size eggs and normal shape were selected by visual inspection for incubation. Before the commencement of incubation, the eggs collected from different treatments and their respective replicates were selected, weighed, coded and stored for not more than 5 days at a temperature of 10°C to l2°C. A total of 180 eggs (45 eggs from each treatment and 15 from each replicate) were incubated for fertility and hatchability estimation. Incubation was carried out between 8th to 11th weeks of the experiment period. Eggs were transferred to hatchery unit after 18th day of incubation. During incubation period, eggs were

candled to identify fertile and non fertile eggs and embryo mortality. Candling was conducted three times, at 9th, 14th, and 18th day of incubation. Those eggs that appeared relatively opaque were considered fertile and those that appeared clear were considered non-fertile. To confirm its clearness, break out analysis was done for the eggs identified as infertile. Chicks were hatched out after 21st day of incubation. The hatched chicks were then counted and weighed. The average percentage fertility per pen was calculated by dividing the total number of eggs found to be fertile at candling with total number of eggs set and multiplied by 100.

$$\% \text{ of fertility} = \frac{\text{Total fertile eggs}}{\text{Total eggs set}} * 100 \quad (10)$$

Hatchability as a percentage of fertile eggs was calculated by dividing the number of chicks hatched by the number of fertile eggs.

$$\% \text{ of hatchability on FEB} = \frac{\text{Number of chicks hatched}}{\text{Total fertile eggs}} * 100 \quad (11)$$

Where: FAB= fertile egg basis

2.6. Breakout Analysis

Breakout analysis was done to identify stages of embryo mortality of the incubated eggs. Eggs failed to hatch was removed from the hatchery tray, placed on egg flats and the exterior of the egg were examined first for piping and location of the air cell. The shell was cracked at the large end, over the air cell, and a hole opened in the shell and membranes to observe the interior of the egg. When the egg appeared to be infertile or contains a very early dead embryo, germinal disc was observed. When the embryo was relatively small, the egg was broken into a dish for further examination. Eggs with late stage embryos were observed for piping into the air cell, and then opened from large end to small end without disturbing the position of the embryo. According to Butcher (2009), the stages of embryo development were classified into early, mid, and late. Early embryonic development signs characterized by eye development, but without limb buds. The mid embryonic development signs characterized by limb development. The presence of feather in the embryo indicates late embryo development. In addition to this, live piped and dead piped identification was also done. Eventually, embryonic mortality of fertile eggs as early, mid, late, pip alive and pip dead was analyzed using logistic regression.

Chick quality: The quality of chicks was measured by visual observation, weighing and measuring the length of the chicks. Based on the quality standard stated by North (1984), chicks that are not malformed, with no unhealed navels, not dehydrated, physically active, stand up well and look lively were recorded good quality chicks. The visual observation was conducted by the researcher and experts of poultry farm. In order to measure the weight and length, one day old chicks were selected randomly from each replicate and their average was taken. The weight was taken using a sensitive balance and the length was measured by stretching the chick on the table and taking the length from the tip of the beak to tip of the middle toe using a ruler.

$$\text{Chick quality}^{\beta} = \frac{\text{Total No. of quality chicks}}{\text{Total No. of hatched chicks}} * 100 \quad (12)$$

$^{\beta}$= visual observation

Blood parameters: Blood samples on the first day before starting the experiment and at the end of the experiments were obtained from 4 randomly selected hens from each pen for all treatments. Blood samples were taken by inserting a sterile needle into the wing vein and extracting 1 ml of blood. The samples were then placed inside test tubes containing EDTA, properly shaken to mix with the EDTA to prevent coagulation. The blood samples were taken to the laboratory for analysis. Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haemocytometer. Packed Cell Volume (PCV) was calculated using the standard formula described by Dacie and Lewis (1991).

2.7. Data Analysis

Data on body weight, feed intake, egg production, feed conversion efficiency, egg mass, fertility, hatchability, egg quality and chick quality were subjected to statistical analysis using SAS version 9.1.3 (2002) with one-way ANOVA. The least significance difference (LSD) test was employed for separating means on when the F-tests were found to be significant. The following model was used for the analysis:-

$$Y_{ij} = \mu + T_i + e_{ij} \quad (13)$$

Where: Y_{ij} = the j observation taken under i treatment; μ = overall mean; T_i = treatment effect; e_{ij} = error term

Data on the early embryo mortality, mid embryo mortality, late embryo mortality, live pipped and dead pipped were analyzed by logistic regression.

3.Results and Discussion

3.1. Laboratory Analysis of Feed Ingredients and Treatments

The results of the laboratory analysis of the different feed ingredients used and the four experimental diets are shown in Tables 1 and 2, respectively. From the results, it could be seen that ruminal contents were lower in DM, CP, CF, ME and ether extract (Table 1) than the values given by Agbabiaka *et al.* (2011) and Colette *et al.* (2013). This may be due to vegetation diversity and selectivity of pasture by different ruminants in different locations. This result is consistent with the proposition that the composition of the rumen content is influenced by the pre-slaughter feeding regimen and the length of the holding period between feeding and slaughter (Abouheif *et al.*, 1999). Variation could also be due to the chemical

composition of the type of pasture grazed by the slaughtered animal and species differences (Dairo *et al.,* 2005). The CP value (11.18%) of the rumen contents obtained in our experiment was within the ranges (10-25%) as reported by Javanovic and Cuperloric (1977). The crude fiber content increased linearly with the increasing levels of rumen content (SDRC) in the diets. This could be attributed to the fibrous nature of the SDRC compared to other feed ingredients in the diets. The CF values obtained in the treatment diets were within a range (6 to 10%) that was reported to be optimum for poultry (NRC, 1994). Corroborating this result, Deaton *et al.* (1977) showed that diets with a CF content as high as 8.07% did not affect layer performance.

Table 1. Chemical composition of feed ingredients used to formulate the experimental rations[1].

Nutrient	Maize grain	SDRC	Wheat short	NSC	SBM
DM (%)	88.59	85.36	87.75	90.87	92.54
CP (% DM)	6.86	11.18	13.93	32.34	36.36
CF (% DM)	1.85	22.99	7.96	19.40	2.60
EE (% DM)	5.35	1.22	4.20	10.03	11.42
Ash (% DM)	2.20	21.54	4.96	8.14	6.49
ME (kcal/kg of DM)	3988.55	1099.32	3271.06	2443.74	4076.836
Calcium (% of DM)	0.03	0.20	0.25	0.38	0.56
Phosphorus (% of DM)	0.25	0.45	0.51	0.95	0.61

Note: [1]SDRC = sun dried rumen content; NSC = noug seed cake; SBM = soybean meal. DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; Ca= calcium; P= phosphorus; ME =metabolizable energy.

On the other hand, the control diet had the highest metabolizable energy level which may have resulted from the variation in the ingredients used in the other diets. The low metabolizable energy values of SDRC containing diets could be due to the lower energy value of SDRC (1099.32 kcal/kg of DM) in the ration (Table 2).

Table 2. Proportion of ingredients used in formulating the layers ration and chemical composition of the treatment ration.

Item[1]	Treatment[2]			
Ingredient (%)	T1	T2	T3	T4
Maize grain	46	45	42	40
SDRC	0	5	10	15
Wheat short	16	13	14	12
NSC	24	23	20	19
SBM	5	5	5	5
Vitamin premix	0.8	0.8	0.8	0.8
Salt	0.5	0.5	0.5	0.5
Limestone	7	7	7	7
Dicalcium phosphate	0.7	0.7	0.7	0.7
Total	100	100	100	100
Nutrient content				
DM (%)	90.73	90.26	90.34	89.86
CP (% DM)	17.84	16.55	16.18	16.09
CF (% DM)	6.44	7.86	9.53	9.32
EE (% DM)	9.09	9.24	7.99	7.65
Ash (% DM)	11.82	13.94	13.70	13.88
Ca (% DM)	3.88	3.81	3.24	3.31
P (% DM)	0.40	0.37	0.43	0.42
ME (kcal/kg of DM)	3244.15	3187.40	2981.10	2974.45

Note: DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; P = phosphorous; Ca = calcium; ME = metabolizable energy.
[1]SDRC = Sun dried rumen content; NSC = noug seed cake; SBM = soybean meal..
[2]T1 = Diet containing 0% SDRC; T2 = diet containing 5% SDRC; T3 = diet containing 10% SDRC; T4 = diet containing 15% SDRC.

The crude protein and metabolisable energy contents of the rations slightly decreased with the increasing levels of SDRC (Table 2). However, the CP and ME levels were within the ranges of the recommended levels of 16-18% and 2500-3300 kcal/kg, respectively for white leghorn layers (Tadel1e, 1997 and Leeson and Summers, 2001). Generally, the four treatment rations used in the current study were nearly comparable in their CP and ME contents.

Dry matter intake: The DM intake of layers is presented in Table 3. The dietary treatments had significant ($P < 0.05$) effect on the DM intake of the laying hens. The DM intake values obtained increased ($P < 0.05$) with the increase in the level of rumen content in the diets. DM intake was significantly lower in the control diet-fed groups as compared to the rumen content-fed group, which might be due to better utilization of nutrients in the former. In our experiment, the rumen content diets were readily accepted by the laying hens at the first offer which showed there was no palatability problem with the SDRC diets.

The increase in average DM intakes of the hens as SDRC increased in the diets may be attributed to the dilution of nutrients by the dietary fiber thereby reducing the nutrient composition and consequently the energy. Hens must, therefore, eat more to meet their energy requirement to sustain rapid growth and development, hence the increased DM intake. This observation is in agreement with the findings of Juvanovic *et al.* (1977) who reported that rumen content containing diets induced more feed intake as compared to the control diet. Similar results were found by Esonu *et al.* (2006) who reported higher feed consumption in groups fed with diet containing rumen content than the control. Consistent with the results Adeniji (2008) also reported that rumen content containing diet induced more feed intake than the control treatment. However, our results disagree with the findings of Abouheif *et al.* (1999), who observed a decrease in DM intake in rumen content based diet groups as compared to other diets.

Table 3. Dry matter intake, BW change, and egg laying performances of hens fed on a ration containing sun dried rumen contents at different levels.

	Treatment[1]					
Parameter	T1	T2	T3	T4	SEM	SL
DM intake (g/hen per day)	81.47b	83.87ab	86.40a	84.19ab	1.01	*
CP intake (g/hen per day)	14.89a	13.88b	13.98b	13.58b	0.28	*
ME intake (g/hen per day)	282.84a	267.31b	257.56bc	250.43c	7.01	*
Initial BW (g)	977.56	995.87	950.58	987.87	9.87	NS
Final BW (g)	1157.63a	1155.42a	1119.85b	1122.42b	10.24	*
BW gain (g/hen)	180.08a	159.56ab	169.27ab	134.55b	9.72	*
AD gain (g/hen)	2.00a	1.77ab	1.88ab	1.50b	0.11	*
Total egg/hen	54.93a	54.62a	49.71ab	44.87b	2.38	*
HDEP[2] (%)	61.30a	62.36a	56.59ab	50.44b	2.72	*
HHEP	61.01a	60.69a	55.24ab	49.85b	2.64	*
Egg weight (g)	46.92b	48.66a	48.88a	47.79ab	0.45	*
Egg mass (g/hen per day)	28.87a	30.40a	27.70a	24.28b	1.30	*
FE (g of egg/g of feed DM)	3.06b	2.96b	3.35b	3.88a	0.21	*
Mortality rate	2.22	8.89	8.89	4.44	1.67	NS

Note: [a, b]Means within a row with different superscripts differ significantly ($P < 0.05$).
*[2]T1 = diet containing 0% SDRC; T2 = diet containing 5% SDRC; T3 = diet containing 10% SDRC; T4 = diet containing 15% SDRC. *=$P > 0.05$. [2]HDEP = hen-day egg production, SL = significant level, * $P < 0.05$, NS = not significant*

Body weight gain: There were positive responses in the weight gain and final weight of hens up to 10% level of SDRC inclusion. Although, there were no significant differences between the 0 and 10% SDRC diets, significant ($P < 0.05$) differences occurred between the 0 and 15 percent SDRC diets. The slight decrease in weight gain indicated the effect of the fibrous nature of the rumen content. Sonaiya *et al.* (1989) reported that fiber causes depression in the proportion of energy digested and retained for metabolism. Awotoye (1991) also reported a gradual decrease in weight gain of experimental broiler hens when dietary level of dried rumen content increased. But our results disagree with the results of Esonu *et al.* (2006) who observed general increment in growth rate as dietary inclusion of dried rumen content increased.

Egg production: Hen-day egg production (HDEP) and hen-housed egg production (HHEP) of White leghorn hens consumed diet containing different levels of sun dried rumen content is presented in Table 3. The mean number of eggs produced per bird during the experiment period, HDEP, HHEP, and egg mass were significantly different between treatments. Total eggs produced per hen, HDEP (%), HHEP and egg mass were significantly lower ($P < 0.05$) for 15 percent SDRC diets (T4) as compared to the other treatment (Table 3). The percentage hen day egg production values obtained for T1, T2 and T3 were similar ($P > 0.05$), indicating uniformity in the laying pattern and quantity of egg laid by the hens. Better egg production parameters recorded for control diet is due to the better nutrient balance. The findings in this trial agree with the observations of Adeniji (1995) who recorded a stepwise decrease in egg production for fed diets containing high levels of a mixture of blood and rumen content. Concurrently, the intake of major nutrients like protein and energy would be adversely affected in

fed 15 percent SDRC diets (T4) (Table 3), hence the poor laying performance recorded. Layers on diets 4 performed more poorly than those fed diets 2 and 3 possibly as a result of nutrient imbalance.

Egg weight and egg mass: The effect of varying levels of SDRC in layers' ration on egg weight and egg mass is presented in Table 3. The mean egg weight seemed comparable for hens fed on SDRC containing diets, except for the eggs laid by the hens on control diet that were significantly ($P < 0.05$) smaller (46.92 g) as compared to T2 and T3. It is suggested that an inverse relationship exists between the rate of lay and egg size. This might have caused the very low egg weight from hens on the control diet which had the highest rate of lay in this study. Egg mass was significantly lower ($P < 0.05$) for hens fed 15 percent SDRC diets. Fakhraei *et al.* (2010) noted that egg mass followed the same trend as egg production, and the same trend has been seen in the present study as well.

Feed conversion ratio: The effect of including varying levels of SDRC in layers' ration on feed conversion ratio expressed as feed consumed per egg mass is presented in Table 3. Differences were observed in the efficiency with which the feeds were converted into egg. The 5 percent SDRC diet though, proved a little bit more efficient than the control although, it was not statistically better ($P > 0.05$) than the control or 10 percent SDRC diet. The 15 percent SDRC diet proved to be the least efficient. This situation is in accordance with the differences in egg production. T1 and T2 produced higher percentage of eggs; as a result, they are efficient in nutrient utilization.

Table 4. Egg quality characteristics of hens fed a ration containing sun dried rumen contents at different levels.

	Treatment[1]					
Parameter	T1	T2	T3	T4	SEM	SL
Sampled egg weight (g)	49.26b	50.03ab	50.88a	50.96a	0.40	*
Shell thickness (mm)	0.32	0.32	0.31	0.31	0.00	NS
Shell weight (g)	5.713ab	5.82a	5.31ab	5.17b	0.16	*
Albumen height (mm)	8.98	8.38	8.70	8.77	0.12	NS
Albumen weight (g)	28.77	29.03	28.90	29.64	0.19	NS
Haugh unit	96.72a	93.53b	94.56ab	95.23ab	0.67	*
Yolk weight (g)	14.42	14.65	14.38	14.79	0.09	NS
Yolk height (mm)	15.19	15.46	15.25	15.51	0.08	NS
Yolk diameter(cm)	38.10 b	38.83a	38.43ab	38.51ab	0.15	*
Yolk index	0.40	0.40	0.39	0.40	0.00	NS
Yolk color (RCF)[2]	1.81c	2.79b	4.80a	5.17a	0.80	*

Note: [a–c]Means within a row with different superscripts differ significantly ($P < 0.05$).
*[1]T1 = diet containing 0% SDRC; T2 = diet containing 5% SDRC; T3 = diet containing 10% SDRC; T4 = diet containing 15% SDRC, [2]RCF = Roche color fan, SL = significant level, * $P < 0.05$, NS = not significant.*

Egg quality characteristics: An increasing trend of sample egg weight was observed as the inclusion level of SDRC increased. Significantly smaller egg was laid by hens fed the control diet as compared to hens consumed 10 percents of SDRC diet. However, egg shell weight significantly differed only between hens fed with 5 percent SDRC diet and 15 percent SDRC diet, being lower in the 15 percent SDRC diet. But, egg shell thickness was not significantly affected by dietary treatments. The mean values for the shell thickness observed across the treatments in this study were similar. The similarity in these values showed that SDRC has no adverse effect on the metabolism of either calcium or phosphorous of the diets. Values observed in this study agreed with the report (0.33-0.36 mm) given by Sakunthala and Reddy (2004) for white leghorn eggs. Egg albumen weight and albumen height were not significantly affected ($P > 0.05$) by dietary treatments. However, there was significant difference ($P < 0.05$) in percentage of Haugh unit.

Haugh unit was greater ($P < 0.05$) for control diet than 5 percent SDRC diet and was similar ($P > 0.05$) among other treatments. Haugh unit determines albumen quality and the highest HU refers to good quality albumen. In this study, all treatments scored HU within the recommended range of 70-100, which is an indication of good egg quality (Lewko and Omowicz, 2009). There was no significant ($P > 0.05$) difference among dietary treatments in yolk weight, yolk height and yolk index (Table 4), despite the expected positive association between egg weight and yolk weight (Suk and Park, 2001). Yolk height and yolk index was greater ($P < 0.05$) for the diet with no SDRC but was similar ($P > 0.05$) among the SDRC containing diets. Contrary to the current result, Sekerodlu and Ebubekr (2009) reported that yolk height and yolk index increased with egg size. Therefore, the results regarding effects of nutrition on yolk index appeared to be inconclusive. There was significant ($P < 0.05$) difference among dietary treatments in yolk diameter and yolk color (RCF) (Table 4). The Roche color fan values showed higher ($P < 0.05$) yolk color score for T4 (15 percent SDRC diet) and T3 (10 percent SDRC diet) and lighter yolk color for T1 (0 percent SDRC diet). Smith (1996) reported that the color of the yolk is determined by the presence and absence of xanthophylls, some of which are precursor of vitamin A. Therefore, the color of the yolk is influenced to a

large degree by nutrition of the hens. The present result showed that as the level of SDRC increased, the intensity of yolk color increased. Xanthophyll of rumen content (mainly consists of greens) is expected to fulfill the needs for egg yolk pigment or poultry skin pigment (Wizna *et al.*, 2008).

Fertility, hatchability, chick quality and embryo mortality: Fertility, hatchability, chick quality, early, mid embryo mortality parameters and pip embryo mortality % appeared to be not negatively affected (P > 0.05) by the dietary inclusion of SDRC in the present study (Table 5). However, fertility and hatchability of eggs were actually improved in the diets containing SDRC as compared with the control diet. Correspondingly, embryo mortality also decreased as the level of SDRC increased. As documented by Hocking *et at.* (2002) poor hatching results occur when nutritionally deficient feeds are used for layers. Odunsi *et al.* (2002) also stated that inadequacy of nutrients in the breeder diets resulted in poor hatchability of fertile eggs. Thus, the present result indicated that inclusion of rumen content did altered nutrients that enhance fertility of particularly males, and hatchability of eggs.

Table 5. Fertility, hatchability, embryonic mortality, and chick quality of hens fed a ration containing sun dried rumen contents at different levels.

	Treatment[1]					
Parameter	T1	T2	T3	T4	SEM	SL
Fertility (%)	95.56	90.00	94.44	97.78	1.64	NS
H on total eggs set basis (%)	63.33	73.33	72.22	78.89	3.22	NS
H on fertile egg basis (%)	66.05	82.38	76.35	80.77	3.67	NS
Early embryo mortality (%)	8.19	2.38	4.80	2.22	1.39	NS
Mid embryo mortality (%	6.99	2.22	5.91	2.22	1.24	NS
Late embryo mortality (%)	10.49a	7.38b	5.99b	4.44b	1.29	*
Pip embryo mortality (%)	2.22	4.52	4.72	2.22	0.69	NS
Chick weight (g)	30.39b	31.46ab	31.62a	32.05a	0.35	*
Chick length (cm)	14.50b	14.49b	14.63ab	14.85a	0.084	*
Chick quality Visual score	96.64ab	94.41b	100.00a	98.81a	1.23	*

Note: [a,b]Means within a row with different superscripts differ significantly (P < 0.05).
*[1]T1 = diet containing 0% SDRC; T2 = diet containing 5% SDRC; T3 = diet containing 10% SDRC;T4 = diet containing 15% SDRC, SL = significant level, * P < 0.05, NS = not significant.*

Durmus *et al.* (2004) noted increased hatchability with increasing zinc concentration in the diets of Brown parent stock layers. The study of Brown and Pentland (2007) showed that zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus, a structure important for successful fertilization. Rumen content contains significant amount of iron, phosphorus, calcium, and is relatively rich in vitamin C (Agbabiaka *et al.*, 2012). Adesola *et al.* (2012) reported improved hatchability as a result of ascorbic acid supplementation to diets of indigenous Venda hens. However, the relatively poor hatchability and higher embryonic mortality observed in the control group might be happening due to a deficiency in critical nutrients, such as zinc, vitamin E, and so on, which are important for better hatchability (Park *et al.*, 2004; Mahmood and Al-Daraji, 2011). In the present study, the feed ingredients, as well as the formulated experimental rations, were not analyzed for such nutrients. Gabreil *et al.* (2006) reported that level of dietary protein significantly affected egg fertility and hatchability. The similarity in fertility and hatchability among treatment of the present study may be related to similarity in nutrient use or the efficiency of nutrients absorbed from the egg. Thus, the result indicated that replacing malted barley for maize up to 30% did not negatively affect fertility and hatchability.

There was significant (P < 0.05) difference among dietary treatments in chick weight; length and visual score (Table 5). As compared with the control diet, chick quality parameters were actually improved in the diets containing SDRC. Differences in chick weight observed in our study appeared to be attributable to comparable variations in egg weight. This observation is in agreement with the findings of Abiola *et al.* (2008) and Malago and Baitilwake (2009) who noted a positive correlation between egg size and chick weight at hatching.

Table 6. Blood parameters of hens fed a ration containing sun-dried rumen contents at different levels.

Parameter	Treatment[1]				SEM	SL
	T1	T2	T3	T4		
PCV (%)						
Before the experiment	29.68ab	31.83a	26.92b	28.67b	1.03	*
At the end of experiment	34.08	32.33	35.17	33.67	0.59	NS
RBC (10^6/µl)						
Before the experiment	3.32a	2.94b	3.13ab	3.29a	0.09	*
At the end of experiment	3.77ab	3.31b	3.93a	3.78ab	0.13	*
WBC(10^3/µl)						
Before the experiment	22.3a	18.65b	17.93b	18.59b	0.58	*
At the end of experiment	18.97	18.0	17.02	24.0	1.51	NS

Note: RBC = red blood cell, WBC = white blood cell, HB = haemoglobin, PCV = packed cell volume. SL = significant level
** P < 0.05, NS = not significant.*

Blood parameters: Blood analysis (Table 6) revealed that treatment had significant effect ($P < 0.05$) on red blood cells (RBC) before and after experiments. Though at the end of experiment blood analysis showed no significant effect of dietary treatments on PCV values and WBC total count, there were significant difference ($P < 0.05$) in PCV and WBC before the experiment. Generally, inclusion of SDRC to layer diets significantly improved PCV and RBC of the hens as it indicated in mean values shown before and after the experiments. The results showed that the values for all the parameters were within the normal range (Schalm *et al.*, 2010), though there were differences within treatments. Thus, dietary treatments had no negative effect on the health status of the hens. Laying hens fed high level of SDRC diets had better WBCs and RBCs. This showed that these hens have enhanced immune-competence to handle physiological stress.

4.Conclusion

Results of the present study have demonstrated that up to 10% sun-dried rumen content can be included in the diets of white leg horn layers without compromising production performance and the health status of the layers. The results implied the rumen content is a potential feedstuff for layers; it is economical and simple to practice. The SDRC is a promising feedstuff particularly during the periods of scarcity and high cost of conventional feeds. Its utilization also alleviates the problem of environmental pollution and disposal of rumen content in abattoirs.

To fully explore the potential of rumen contents as a feedstuff in layers' nutrition long-term feeding trials should be performed. In addition, the impact of different preservation methods (e.g. drying and ensiling) on the nutritional value of rumen contents needs further investigations. Moreover, the economic aspects of sun-dried rumen content feeding, which include feed costs, animal performance and carcass traits, need to be evaluated.

5.Acknowledgements

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6.References

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Strength and Keeping Quality of Abomasum Rennet and its Influence on Yield and Quality of Halloumi Cheese made from Cow Milk

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Abstract: Halloumi cheese was made from cow milk using commercial rennet, and bovine and ovine rennet. But in Ethiopia commercial rennet is expensive and unavailable for smallholder dairy farmers. To solve this problem an experiment was conducted to evaluate strength, quality and suitability of locally available bovine and ovine abomasum rennet for halloumi cheese making in comparison with commercial rennet. The treatments consisted of bovine rennet, ovine rennet and commercial rennet (control) arranged in a completely randomized design (CRD). Rennet was extracted from bovine and ovine abomasa and comparisons were made with commercial rennet. Shelf life and clotting activity of the extracted rennet were determined using Halloumi cheese making. The result of the study indicated that commercial rennet (0.189 ± 0.02) had a higher clotting activity and short clotting time than bovine (0.179 ± 0.02) and ovine (0.023 ± 0.02) rennet. Bovine rennet was active up to nine weeks of storage while ovine rennet was active only up to six weeks of storage after which both types of rennet start losing their clotting activity. A highly significant difference was observed between locally and commercially produced rennet in terms of rennet and curd pH. The use of bovine and ovine rennet in the Halloumi cheese making was acceptable to the sensory panelist and scored more than the average for overall acceptances (Score >3.5). In conclusion, bovine rennet showed better strength and quality compared to ovine rennet and could be used for halloumi cheese making where commercial rennet is not available.

Keywords: Bovine rennet; Clotting activity; Curd strength; Ovine rennet; Shelf life

1. Introduction

Milk-clotting enzyme is the first active agent in cheese-making and an essential ingredient for the production of various cheese types. Clotting of milk is usually an enzyme-driven step during which rennet enzymes bring about cleavage of Met105– Phe106 of κ-casein. Today, nearly all types of cheese are manufactured by clotting the milk with a milk clotting enzyme and then processing the curd in various ways (Moschopoulu, 2011) Considering the nutritional, economic and value addition importance of cheeses as well as the unaffordable price of commercially available rennet to most resource poor producers, it is essential to search for locally available and affordable rennet. Moreover, a significant number of livestock are slaughtered for domestic meat consumption as well as export (CSA, 2013). This means that, a substantial volume of liquid rennet can be prepared from adult livestock. However, there is very limited information available on production and efficiency of rennet making from locally available sources. Therefore, the objectives of this research were to compare the strength and shelf life of bovine and ovine rennet with commercial ones and to evaluate their suitability of liquid rennet extracted from mature bovine and ovine abomasum for Halloumi cheese making.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted in the Dairy Laboratory of Holetta Agricultural Research Center (HARC) of the Ethiopian Institute of Agricultural Research (EIAR). It is located 34 km to the West of Addis Ababa in the central highlands of Ethiopia (2400 m above sea level, 38.5°E longitude and 9.8°N latitude). The average maximum and minimum daily temperatures are 21°C and 6°C (http://www.eiar.gov.et/index.php/holeta-agricultural-research-center).

2.2. Experimental Materials and Preparation of Liquid Rennet

Fresh abomasums of adult cattle and sheep were collected from one abattoir and one slaughter house in the vicinity of Holetta town. Commercial pepsin powder was purchased from Addis Ababa market. Rennet was prepared according to the method described by O'Connor (1993) as shown in Figure 1. After removing the internal contents, abomasal tissues was cleaned with tap water, and veins and fat contents were removed. Then they were inflated with air and dried in a wooden-wire mesh cabinet placed in an open air. After drying, each abomasum was cut into very thin strips separately, weighed and soaked in 1000 ml solution of 150 g NaCl, 40 g calcium chloride and 10 ml

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acetic acid solution individually. The solution was stored at 24-25 ^{o}C. The extract was filtered with muslin cloth. After filtration a yellowish clarified solution (approximately 950 ml) was obtained.

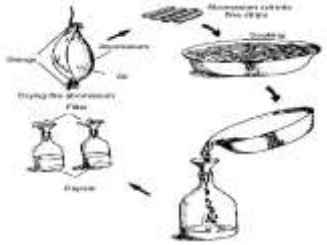

Figure 1. Schematic diagram of rennet making (O'Connor, 1993).

2.3. Treatments and Experimental Design

The treatments consisted of bovine rennet, ovine rennet, and commercial rennet. The experiment was designed as completely randomized design (CRD).

2.4. Experimental Procedures

2.4.1. Halloumi Cheese Making

Halloumi cheese was produced by slightly modifying the process indicated by O'Connor (1993) from cow milk. 120 L of cow milk was collected (at different time) from the dairy farm of Holetta Agricultural Research Center. Milk was pasteurized at 72^{o}C for 20 seconds and cooled immediately with cold water to 32^{o}C. Then, the crude abomasal rennet was added to the cheese milk. Curd was expected in 40-45 minutes. Then the curd was cut in to a 3 – 4 cm cube with a sharp cutting knife. Stirring of curd and whey removal was then done by heating at 38-42^{0}C. The curd was left to settle to decant off the remaining whey. Once the curd was separated from the whey, the content was scooped into a mould lined with cheese cloth and pressed for about four hours. After the whey was removed, it was heat treated and the curd removed from the press cut into the desired size and placed in heated whey. After 20 minutes, the curd pieces were transferred into the draining table and allowed to cool. Finally, the cold curd was placed in clean, dried, and closed cup for quality and microbiology examination.

2.4.2. Minimum Rennet Concentration for Curd Formation

Crude rennet extract had unknown pepsin content and it was, therefore, necessary to establish the strength of the rennet before applying it to milk coagulation. This is important to calculate the appropriate dosage required for optimal milk curdling and to produce a desired quality cheese. The correct amount of rennet to be added into the cheese milk was determined based on the standard developed by O'Connor (1993). Different rennet concentrations were set to identify the minimum curd forming concentration. Based on this procedure, the minimum curd forming bovine rennet concentration was 2 ml/lt of milk and that of ovine rennet concentration was 30 ml/lt of milk. These rennet concentrations were selected and used for cheese making.

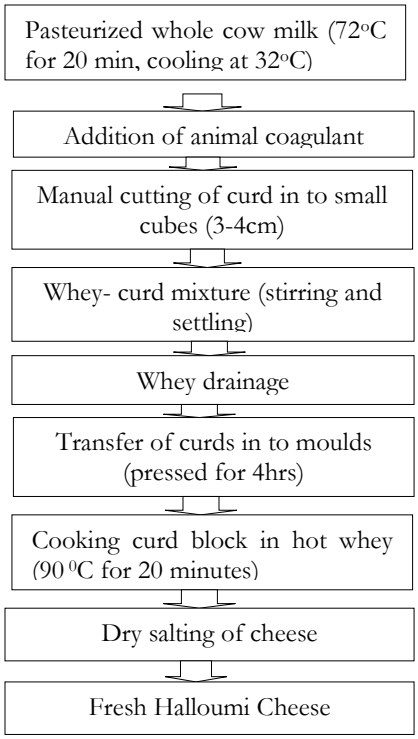

Figure 2. Halloumi cheese making flow chart
Source: O'Connor, 1993.

2.5. Observations

2.5.1. Rennet Strength

Rennet strength is conventionally measured by determining the time required to coagulate milk under a standardized condition. The clotting activity (strength) of rennet types was measured and reported in clotting strength according to Berridge (1952). In addition, the time length (days) rennet maintains its strength was determined by testing the extracted rennet samples for strength every week. Rennet pH was measured in each analysis.

2.5.2. Measurement of Rennet Clotting Time

The method of Berridge (1952) was used to measure clotting time as follows. The standard substrate was prepared from cow milk heated at 32 ^{0} C at 10% (w/v) solution of $CaCl_2$ (0.01M) solution. The prepared animal rennet of 1ml/10ml of standard substrate was added and mixed manually and incubated in a water bath at 32°C. After thoroughly mixing three times, the "zero" clotting time started. The milk clotting activity of each extract was measured, with the assumption that all the soluble proteins from the extract were enzymes which coagulate milk at 32°C. The clotting activity equation as reported by Berridge (1952) in rennet units (RU) was used.

$$RU = 10 \times V/Tc \times Q \quad (1)$$

Where: RU = rennet unit; V= volume of standard substrate (milliliter); Q = volume of animal rennet (milliliter); Tc = time of clotting (second)

2.5.3. Clotting Strength

The clotting activity of rennet types is reported in clotting strength of Soxhlet (F) based on the equation of Bourdier and Luquet (1981).

$$F = RU/0.0045 \quad (2)$$

In the experiment, the major chemical composition of Halloumi cheese was evaluated for determination of fat, protein, moisture, total solids, ash, pH and acidity.

Fat Content: the fat content of halloumi cheese was determined by Gerber method following the procedures outlined by O'Connor (1994). Three gram of cheese sample was weighed in a piece of greaseproof paper. Then 10 ml of sulfuric acid was dispensed in to the butyrometer followed by carful addition of water so that it rests on the acid. The cheese sample was then wrapped from cylinder that fits in to the butyrometer. Then additional 4-5 ml water was added. One ml amyl alcohol was then added into the cheese samples. Then the butyrometer was securely closed with a stopper and shaken to dissolve the cheese. The butyrometer was placed in the heated water bath and removed periodically for mixing until the cheese was fully dissolved. Finally the butyrometer was centrifuged and reading was recorded as for milk and cream.

pH: The pH of each cheese sample was measured following the procedures outlined by O'Connor (1994) using electronic digital pH meter. The electrode was immersed directly into the cheese sample (curd) until the pH sensitive bulb was covered. The temperature of the sample was measured and the pH meter was activated and pH reading was recorded.

Acidity: Acidity was measured following the procedures outlined by O'Connor (1994) by titration. A ten gram of cheese sample was prepared, into which 105 ml of water was added at 40° C and the content was shaken vigorously. 25 ml portion (2.5 g) of the filtrate was titrated with standard 0.1 N NaOH solution (until definite pink color persisted) using a phenolphthalein indicator. The result was expressed as percent lactic acid.

$$\text{Lactic acid (\%)} = \left[\frac{\text{ml } \frac{N}{10} \text{NaOH} \times 0.009}{\text{ml of sample used}}\right] \times 100 \quad (3)$$

Total Solid: Five grams of Halloumi cheese samples were weighed on a crucible in a duplicate and kept in a constant temperature oven set at 105°C for 12 hrs until a constant weight was achieved. The loss in weight was calculated as percent moisture and the total solids was calculated subtracting percent moisture from 100 (Kirk and Sawyer, 1991).

$$\text{Total solid} = \frac{\text{crucible weight+oven dry sample weight-crucible weight}}{\text{sample weigh}} * 100 \quad (4)$$

Ash: A fresh Halloumi cheese sample was ignited at 600°C for 3 hours and weighed after cooling under room temperature. Loss in weight was calculated as percent organic matter and the ash content was determined by subtracting the organic matter percentage from 100 (Kirk and Sawyer, 1991).

2.5.4. Cheese Quality

Cheese quality was determined through a sensory evaluation based on appearance, odor, and texture and consistency of the cheese samples. A panel of fifteen experienced volunteering cheese tasters was selected. They included mainly researchers and graduate students in the Dairy Science Department of Holetta Agricultural Research Center. Clean water held at room temperature was served for cleaning mouth during testing the samples. The panel evaluated the appearance, odor, texture, and consistency of Halloumi cheese. Five scale hedonic scales was used to evaluate the halloumi cheese in which, 5 refers to excellent, 4 to good, 3 to fair, 2 to poor and 1 for unacceptable quality of Halloumi cheese (Lawless and Heymann, 1999).

2.5.5. Statistical Analysis

All treatments were replicated four times. A repeated measure analysis was applied to assess differences among the ovine rennet, bovine rennet, and the commercial enzymes by using SAS Software version 9.1 (SAS, 2005). Failure data analysis was run to fit the expiry date data to Weibull survival distribution to model the shelf-life data (rennet) as used by Schmidt and Bouma (1992).

Data for chemical composition of Halloumi cheese were subjected to analysis of variance (ANOVA) to test for significant differences at $P < 0.05$. For each treatment, mean comparisons were done using the least significant difference (LSD) test for variables of which F-values showed significant differences at 5 % significance level.

Models

Observation Model:

$$Y_{ijkl} = \mu + R_i + S_j + \delta_{ij} + \varepsilon_{ij} \quad (5)$$

Where: Y = processing parameters, quality parameters or shelf-life; μ = Overall mean; R_i = effect of coagulant rennet source (bovine, ovine, commercial rennet); S_i = effect of storage periods (weeks); δ_{ij} = Interaction of rennet source and storage period; ε_{ij} = random error

Weibull Model:

The model used for failure analysis was Weibull distribution with the general function;

$$f(x; k, \lambda) = \frac{k}{\lambda}\left(\frac{x}{\lambda}\right)^{k-1} e^{-(x/\lambda)^k} \quad (6)$$

Where: x > 0. (x = failure day), $k > 0$ is the *shape parameter* and $\lambda > 0$ is the scale parameter of the distribution. $K < 1$ indicates that the failure rate decreases over time. If the failure rate is constant over time, then $k = 1$. $K > 1$ indicates the failure rate increases over time

3.Results and Discussion

3.1. Clotting Activity

Clotting time, activity and strength of Soxhelt (F) for the ovine, bovine and commercial rennet is presented in Table 1. At individual observation level, the clotting activity of the milk coagulants used ranged from the lowest 0.0074 RU for ovine rennet produced locally to the highest 0.196 RU for commercial rennet with the overall average value being 0.13 RU. On average, the highest clotting activity (0.189 RU) was observed for the commercial rennet, while the lowest value (0.023 RU) was recorded for ovine rennet. No apparent differences ($P > 0.05$) were observed between commercial and bovine rennet in their clotting activity, while ovine rennet showed significantly lower ($P < 0.05$) clotting activity compared with the other two types of rennet. It was observed that the average clotting activity increased with increasing Soxhelt strength and decreasing clotting time. Accordingly, the highest clotting activity recorded for the commercial rennet corresponded with the highest Soxhelt strength and shortest clotting time.

Variation in milk clotting activity (MCA) and strength might be related to differences in the nature of the clotting ability of raw materials, the proteolytic efficiency and/or the concentration of the rennet types on the milk substrate used. For instance, as indicated by Guinee and Wilkinson (1992), the MCA of rennet relies on its ability to degrade casein micelles, the action being dependent on the chymosin and pepsin content of the rennet complex. As a critical parameter in the progress of curd formation, clotting time can be usefully related to curd firmness and curd yield. The weak clotting ability of ovine rennet observed in the present study might be attributed to the weak property of pepsin present in the rennet solution. This observation is in agreement with that of Vairo Cavalli *et al.* (2005), who indicated that the breakdown of cow milk by ovine caseinate took place much more slowly than that of bovine counterpart. The weak clotting power of ovine pepsin on cow milk might be related to species specificity. Consistent with this suggestion, Calvo and Fontecha (2004) also indicated that the clotting ability of each rennet proteolyses is stronger for their species specific casein compared with that of other species.

Table 1. Mean (± SD) clotting time, change in clotting activity (rennet unit: RU) and strength of Soxhelt (F) for bovine, ovine and commercial rennet.

Milk Coagulants	No. of observations	Clotting time (min)	Clotting activity (RU)	Strength of Soxhelt (F)
Bovine rennet	4	9.50^{a}	$0.179^{a} \pm 0.02$	$39.9^{a} \pm 2.42$
Ovine rennet	4	22^{b}	$0.023^{b} \pm 0.02$	$16.9^{b} \pm 2.42$
Commercial rennet (C)	4	8.80^{a}	$0.189^{a} \pm 0.02$	$42.2^{a} \pm 2.42$
Overall Mean	12	13.4	0.130 ± 0.14	33.3 ± 1.57

Note: ^{abc} *Means in the same column without common letter are significantly different at P < 0.05*

3.2. Stability of Rennet from Bovine, Ovine and Commercial Source

The cumulative failure days (risk condition) of different rennet sources is shown in Figure 3. The stability of all rennet sources was not affected during the 6 weeks of storage i.e. the failure probability (hazard) of losing stability was zero. Due to the short period of the experiment, the highest shelf life (stability) was observed for commercial rennet which shows no failure date during the ten weeks of storage while ovine rennet started to lose its stability after six weeks of storage. On the other hand, bovine rennet was active up to the 9th weeks of storage. The changes that take place during storage may depend on manufacturing process, temperature of storage and extent of exposure of the rennet to light. Arvanitoyannis (2009) indicated that rennet can resist even very high temperatures when it is dry. However, liquid rennet solutions are less stable and the substance decomposes in various speeds (Pometto *et al.,* 2006). In the current study, rennet was stored in a dark closed container that may help the enzyme to retain its stability. However, temperature of storage (room temperature) may affect their stability by creating variations in heat. The efficiency of liquid rennet reduces at room temperature, the intensity of which is related to the strength of the solution, and the rennet strength decreases when the substance is handled and stored improperly, with light, heat and shaking exerting clear detrimental effects on the rennet (Arvanitoyannis, 2009).

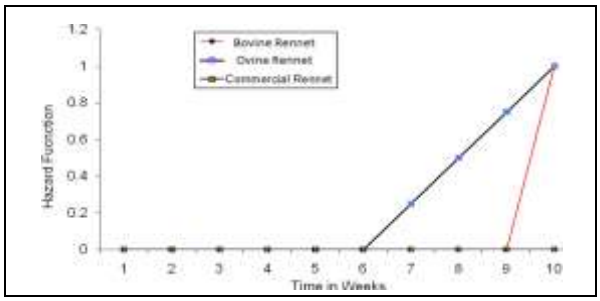

Figure 3. Cumulative failure plots of rennet extracted from bovine and ovine abomasums and commercial rennet.

The pH value of stored rennet ranged from 3.45 to 4.21. The repeated measure analysis revealed that a significant difference ($P < 0.05$) was observed between pH values of locally produced and commercial rennet. Commercial rennet showed no variation in terms of rennet pH. pH plays a significant role in the stability of coagulant enzyme. During the storage period, the pH value of locally produced liquid rennet decreased (Figure 4). The decline in pH values of bovine and ovine rennet might be because of lack of purification methods and storage temperature used in the processing. As reported by De Caro *et al.* (1995) high recorded loss of rennet may be related to conventional ways of manufacture and lack of purification processes. In the current study, bovine and ovine rennet were crude extracts and stored at room temperature. Hooydonk and Van Den Berg (1988) further proposed that the strength of natural rennet could not be maintained at a constant level. When rennet is kept at high temperatures, pepsin activity increases in whey proteins (Hooydonk and Van Den Berg, 1988). In addition, lactic acid bacteria may contribute to the development of acidity there by reducing stability and pH.

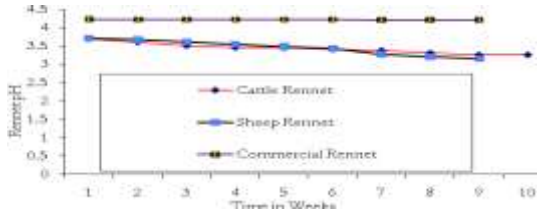

Figure 4. pH of stored rennet from bovine, ovine and commercial rennet sources.

Curd pH value of stored rennet ranged from 5.34 to 6.12. Mean curd pH value of repeated measure analysis revealed significant differences ($P < 0.05$) among bovine, ovine, and commercial rennet. During the storage period, curd was formed even though the pH declined (Figure 5). The result showed a consistent decline in curd pH with storage period indicating its diminishing shelf life. Milci *et al.* (2005) revealed that average pH values of Halloumi cheeses manufactured from bovine milk ranged from 5.37 - 6.45 which is in agreement with the current findings.

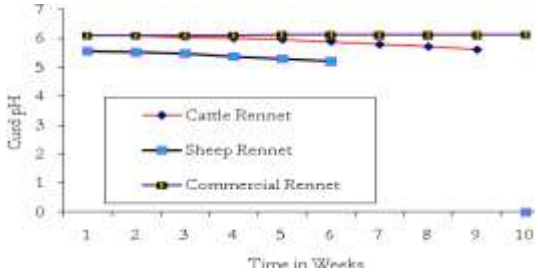

Figure 5. pH of curd obtained from bovine, ovine and commercial rennet sources.

3.3. Effect of Locally Produced Rennet on Halloumi Cheese Yield and Quality

3.3.1. Chemical Composition of Milk

The fat, protein, and pH value of the milk used in the manufacture of Halloumi cheese were 4.28, 3.25 and 6.22, respectively. Fat and protein are the two primary milk components that are recovered in the cheese making process and are directly related to cheese yield (Fox *et al.*, 2000). These constituents influence the casein to fat ratio, total solids, lactose, mineral content, consequently moisture levels and extent of acid development in the finished cheese (Traordinary Dairy, 2001). The casein fraction of milk protein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately affecting cheese quality and yield (Lawrence, 1993).

3.3.2. Chemical Composition of Halloumi Cheese

Chemical composition of Halloumi cheese manufactured using bovine, ovine and commercial rennet is presented in Table 2. The composition of cheese has a marked influence on all aspects of quality, including sensory properties, texture, and cooking properties (Guinee and Fox, 2004; Amenu and Deeth, 2007; Tunick *et al.*, 2007). The yield of Halloumi cheese sample ranged from 124 to 134 g per a liter of milk. The average yield was 129 g. All treatments had a significant ($P < 0.05$) effect on the yield of Halloumi cheese. Halloumi cheese manufactured using fresh bovine rennet had higher yield compared to Halloumi cheese manufactured using ovine and commercial rennet (Table 2). Differences in the cheese yield among the different types of coagulants might be due to their proteolytic specificity, as highly specific coagulants provide higher cheese yields. This is substantiated in numerous cheese yield trials and quality studies comparing various rennet and coagulant types (Emmons *et al.*, 1990; Emmons and Binns, 1991; Banks, 1992; Guinee and Wilkinson, 1992; Quade and Rudiger, 1998) and reviewed by Emmons and Binns (1991) and Garg and Johri (1994). Bovine and commercial rennet had higher clotting and short coagulation time. Milk with favorable coagulation properties (short coagulation

and curd firming times and firm curd) is expected to give more cheese yield (Kubarsepp *et al.*, 2005). The weak clotting power and non-specificity of ovine rennet to bovine milk results in lower cheese yield.

The moisture content of Halloumi cheese samples ranged from 46.8 to 51.6%. The average moisture content was 49.2% (Table 2). There were no significant ($P > 0.05$) differences between cheeses manufactured using bovine and commercial rennet with respect to moisture attributes. These values are in agreement with the results of Pezeshki *et al.* (2011) where cheeses manufactured with *Withania coagulant* had no significant difference in moisture content compared to the moisture contents of cheeses produced using different rennet preparations. Raphaelides *et al.* (2006) reported moisture contents of 47.4% for Halloumi cheese manufactured from bovine milk. In producing cheese using ovine rennet, a large amount of rennet (30ml/1Lt) was added to the cheese milk which significantly reduced the moisture content of the cheese. This could be ascribed to the large amount of enzyme extracting salts that increases synersis due to acidity that facilitates squeezing out of the moisture from the cheese. This observation is in agreement with the findings of Guven *et al.* (2008) who reported that moisture content of cheese decrease as rennet concentration increases. The value of the moisture content of Halloumi cheese observed in this study suggests that it is an intermediate moisture cheese and is much lower than the corresponding value (79%) reported for the traditional Ethiopian cottage cheese(Mogessie, 1992).

Table 2. Proximate composition of Halloumi cheese made using different rennet sources.

Constituents	Treatment		
	Bovine rennet	Ovine rennet	Commercial rennet
Yield	134 ± 5.68^{a}	125 ± 5.68^{b}	127 ± 5.68^{ab}
Fat	23.8 ± 4.24^{a}	21.5 ± 4.24^{a}	22.5 ± 4.24^{a}
Protein	18.9 ± 1.12^{a}	19.7 ± 1.12^{a}	18.8 ± 1.12^{a}
Moisture	51.6 ± 1.71^{a}	47.6 ± 1.71^{b}	49.8 ± 1.71^{a}
Total solid	53.2 ± 1.71 ^{a}	53.4 ± 1.71^{a}	48.4 ± 1.71^{b}
pH	6.12 ± 0.07^{a}	5.72 ± 0.07^{b}	6.16 ± 0.07^{a}
Acidity	0.027 ± 0.01^{b}	0.049 ± 0.01^{a}	0.026 ± 0.01^{b}
Ash	5.13 ± 1.49^{a}	5.05 ±1.49^{a}	5.01 ±1.49^{a}

Note: ^{abc} *Means in the same row without common letter are significantly different at* $P < 0.05$

The pH value of Halloumi cheese ranged from 5.60 to 6.22 with the average pH values of 6.0. No significant ($P > 0.05$) difference was observed between Halloumi cheese manufactured using commercial and bovine pepsins in pH while Halloumi cheese manufactured using ovine rennet showed significantly lower pH. The acidic features of the rennet and the minimum requirements of ovine rennet to form curd (30 ml/l) could be a factor for this significant difference. Hynes *et al.* (2001) indicated that reduced pH causes the protein matrix in the curd to contract and squeeze out moisture and finally affect cheese yield. According to the findings of Milci *et al.* (2005) and Raphaelides *et al.* (2006), the average pH values of Halloumi cheeses manufactured from bovine milk ranged from 5.37- 6.45. The pH value of the cheese determines microbial stability, and susceptibility to mould and spoilage microorganisms.

The average titrable acidity of Halloumi cheese ranged from 0.0495 to 0.0261 and was significantly ($P < 0.05$) affected by the treatments. The LSD test showed that the titrable acidity of Halloumi cheese made using bovine and ovine rennet was differed significantly which could be attributed to the pH and the concentration of rennet. In the current study, bovine and ovine rennet differed in their minimum curd forming concentration. Thus, the high concentration of ovine rennet significantly lowers the pH of the cheese.

The fat content of Halloumi cheese (Table 2) ranged from 21.3% to 23.8%. Proximate analysis revealed that the fat content of Halloumi cheese was not affected ($P > 0.05$) by different rennet types. The fat content of Halloumi cheese obtained in the present study was lower than the values reported in earlier studies 44.5% (Fasakin and Unokiwedi, 1992) and 25.4% (Milci *et al.*, 2005).

The Protein content of Halloumi cheese was not also affected ($P > 0.05$) by the rennet type used (Table 2). The protein content of Halloumi cheese obtained in the present study ranged from 18.8 to 19.7%, which was slightly lower than the value reported by Milci *et al.* (2005), 22.8%. Similarly, the Halloumi cheese samples analyzed have higher crude protein content than the Ethiopian traditional cottage cheese *Ayib* which was reported to be 15 g/100 g protein (O'Mahony, 1988; Mogessie, 1992; Binyam, 2008).

Gross compositions of total solids and ash value in the present study were not affected ($P > 0.05$) by different rennet types (Table 2). The total solid value of Halloumi cheese ranged from 48.4 to 53.4. An ash content of the Halloumi cheese ranged from 5.01 % to 5.1%. This is slightly lower than the value reported by Milci *et al.* (2005) who reported that the average ash content of the Halloumi cheese produced by using bovine, ovine milk was 6.52 %. The ash content of the

Halloumi samples analyzed in the present study was also higher than the ash content (1.16%) of Ethiopian cottage cheese, *Ayib,* reported by Binyam, (2008). The high ash content of the Halloumi cheese could serve as a good source of minerals.

3.4. Sensory Quality of Halloumi Cheese Made using Different Rennet Sources

Mean scores of sensory attributes of Halloumi cheese made using different rennet sources are illustrated in Table 3. All the sensory scores of Halloumi cheese made using bovine , ovine, and commercial rennet were found to be within the acceptable sensory score (score > 3.5) implying that treatments had no significant effect on the sensory quality of cheese. The average value of odor and consistency was 3.96 and 4.05, respectively. This is in agreement with the findings of Gaborit *et al.* (2001) and Martinez-Cuesta *et al.* (2001) who reported that cheeses elaborated with animal rennet and powdered vegetable coagulants had no significant ($P > 0.05$) differences in any of the odor and consistency characteristics studied. No significant ($P > 0.05$) difference was observed between Halloumi cheese manufactured using commercial and bovine pepsins in their taste score, while Halloumi cheese manufactured using ovine pepsin showed a significantly ($P < 0.05$) lower taste score compared to the other two. This could be related to the high amount of ovine rennet (30 ml/lt) added to the cheese milk which gives the cheese a characteristic salty taste.

Table 3. Mean Sensory scores of Halloumi cheeses treated with different rennet sources.

Treatments	Appearance	Odor	Taste	Consistency
Bovine rennet	4.1 ± 0.56^{a}	4.0 ± 0.31^{a}	4.2 ± 0.37^{a}	4.15 ± 0.53^{a}
Ovine rennet	4.35 ± 0.56^{a}	4.1 ± 0.31^{a}	3.65 ± 0.37^{b}	3.95 ± 0.53^{a}
Commercial rennet	4.2 ± 0.56^{a}	3.8 ± 0.31^{a}	3.95 ± 0.37^{a}	4.05 ± 0.53^{a}

Note: ^{abc} Means in the same column without common letter are significantly different at $P < 0.05$

4.Conclusions

The results of this study have demonstrated that a better cheese yield and lower minimum coagulant cost were recorded for cheese made using bovine rennet than ovine rennet. However, considering rennet quality and strength, commercial rennet was stronger in making cheese with shorter rennet coagulation time. All the sensory scores of Halloumi cheese made using bovine, ovine and commercial rennet were found to be within the acceptable sensory score (score > 3.5). In general, in areas where commercial rennet is unavailable and expensive, bovine rennet could be the best alternative for Halloumi cheese making with regard to cost effectiveness, better clotting strength, shelf life, availability and better cheese yield. Production of bovine rennet in the country could also create employment opportunities.

5.Acknowledgments

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Characterization of Lactic Acid Bacteria from Camel Milk and their Technological Properties to Use as a Starter Culture

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Abstract: Proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavor. The objective of this study was to characterize lactic acid bacteria (LAB) from camel milk and elucidate their properties to use as a starter culture. Twenty-one lactic acid bacteria species were isolated from 30 samples of camel milk collected from Babile Woreda, eastern Ethiopia. Isolates were characterized phenotypically and their technological properties such as acidification, exopolysaccharide production (EPS), proteolytic and antimicrobial activities were studied following standard procedures. The results revealed that the isolated LAB strains belonged to *Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus* genera. All lactic acid bacteria strains showed proteolytic activity with different degrees of clear zones. The lactic acid bacteria strains exhibited either high to low acidification activities. About 85% of the lactic acid bacterial strains had significant exopolysaccharide production (EPS). Three LAB strains showed maximum antagonistic properties against indicator organisms (*Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*). It could be concluded that *Lactobacillus plantarum* HUM19, *Lactobacillus acidophilus* HUM20, and *Streptococcus cremoris* HUM8 had high acidifying, antimicrobial and proteolytic activities, and EPS production among all other lactic acid bacteria isolates.

Keywords: Acidifying activity; Antimicrobial activity; Exopolysaccharide production; Fermentation; Proteolytic activity; Technological properties

1. Introduction

Fermented foods and beverages constitute a major portion of people's diets in Africa (Oyewole, 1997). Microorganisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria (LAB) which are used to prepare starter culture for different dairy products. The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavor (Temmerman *et al.*, 2002).

Currently, LAB are a focus of intensive international research for their essential role in fermented foods as a starter culture and for their ability to produce various antimicrobial compounds promoting probiotic properties (Temmerman *et al.,* 2002), various production of metabolic and enzymatic substances, which contribute to flavor, aroma and texture developments (Kleerebezemab *et al.*, 2000), for their ability to transform lactose and improve the digestibility of fermented dairy products (Soukoulis *et al.,* 2007; Weinberg *et al.,* 2007) as well as for their preservative quality (Abdelbasset and Djamila, 2008).

The microbiological quality of milk and milk products is influenced by the initial flora of raw milk (Ritcher and Vadamuthu, 2001). When camel milk is left to stand, its acidity rapidly increases due to the presence of LAB (Ohris and Joshi, 1961). It has also been recognized that LAB are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other microorganisms (Daeschel, 1989).

Ethiopia is a country where vast arid regions exist. In these regions, long spells of dry period without any rain are common. Under such conditions, the only livestock, which can successfully survive and produce substantial quantities of good quality milk is the camel. But accessing market for camel milk is low for producers due to remoteness from towns and roads, and cannot be used in a fresh state and goes wasted. During peak production season, it can be saved and effectively utilized through converting it into fermented camel milk product by using starter culture of lactic acid bacteria (Seifu *et al.*, 2012). Therefore, the present study was aimed at characterizing lactic acid bacteria obtained from camel milk and elucidates their technological properties to use as a starter culture for prolonging the shelf life of the milk by preserving its taste and flavour.

2. Materials and Methods

2.1. Description of Study Area

The study was carried out in Eastern Ethiopia, Babille Woreda (district), which is predominantly an agro-pastoral Woreda (Tofik, 2014). The town is situated at the latitude of 09°13'N 42°20'E and longitude of 07°16'N 32°13'E and elevation of 1648 meters above sea level. The town is located at the distance of 30 km east of the town of Harar and 535 km from Addis Ababa, eastward. The study sites of the pastoral areas are located at the distance of about 15 km from the

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town of Babile. The rainfall pattern is bimodal but unpredictable and erratic in distribution. The mean annual temperature is between 34°C and 38°C (www.weather-forecast.com).

2.2. Sample Collection

A total of 30 samples of camel milk were collected aseptically from the pastoral households of Babille Woreda in Ethiopia. Camel milk samples were collected using sterile bottles and transported to Haramaya University, Microbiology Laboratory in an icebox for analysis. Aseptic sampling was followed as described by the Health Protection Agency HPA (2004) and the Food and Drug Administration FDA (2003). In the laboratory, the camel milk samples were kept at the temperatures below 4°C and analyzed within 48 h of collection (HPA, 2004).

2.3. Isolation of Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) were isolated from the camel milk samples using de Man Ragosa and Sharpe (MRS) and M17 agar according to the method described by Harrigan and McCance (1976) by pour plate method in triplicates. Ten (10) ml of unpasteurized camel milk samples were homogenized with a 90 ml sterile saline solution (0.85%, w/v NaCl) to make an initial dilution (10^{-1}). The suspension was used for making suitable serial dilutions up to 10^{-8} by incorporating 1 ml into 9 ml of sterile NaCl solution (0.85%, w/v) in sterile tubes. From appropriate dilutions of 10^{-2} to 10^{-4}, 1 ml of the samples were pour plated into MRS medium, incubated anaerobically for 72 hrs at 37°C, for isolation of *lactobacilli* and M17 medium, incubated anaerobically for 48 hrs at 30°C, for isolation of *Lactococci*. After incubation, colonies were enumerated and recorded as colony forming units (cfu) per ml of milk. Then desired glistening colonies were picked up from the MRS and M17 agar plates by a sterile platinum loop and sub culturing was continued until the pure culture was obtained.

The presumptive lactic acid bacteria (LAB) isolates were inoculated in MRS/M17 broth, incubated at 30°C and checked for purity by streaking on their respective isolation media until only a single type of colony was present. The preliminary isolation of lactic acid bacteria was made on the basis of Gram staining and catalase reaction followed by microscopic examination to observe cell arrangements and morphological characteristics as described by Harrigan and McCance (1976). Only Gram positive, catalase negative, non-motile, cocci and bacilli shaped isolates were considered as presumptive lactic acid bacteria according to Savadogo *et al.* (2004). The cultures were stored and maintained at -20 on MRS and M17 agar slants supplemented with 10% (v/v) glycerol for further studies.

2.4. Characterization of LAB Isolates

Presumptive isolates that showed the general characteristics of lactic acid bacteria were selected randomly and subjected to different tests that included growth at different temperatures (10, 15 and 45°C), at different NaCl concentration (2%, 4%, and 6.5%), gas production from glucose and arginine hydrolysis was carried out according to the method described by Harrigan and McCance (1976). Identification to species level was conducted by subjecting isolates to various carbohydrates fermentation (Starch, Amygdalin, Arabinose, Cellobiose, Fructose, Galactose, Glucose, Lactose, Maltose, Mannitol, Mannose, Melizitose, Melibiose, Raffinose, Rhamnose, Ribose, Sucrose, Salicin, Sorbitol, Trehalose and Xylose) in MRS/M17 broth containing 1% solution of carbohydrate and added to 0.025% bromocresol purple as indicator according to Schillinger and Lucke (1987). Results were recorded after 48 h of incubation at 37°C. Based on the results, the isolates were then identified to species level using the species identification procedure of Bergey's manual of Determinative Bacteriology (Holt *et al.,* 1994), and by comparing the result with previously published scientific research work of Bettache *et al.* (2012).

2.5. Technological Properties

2.5.1. Acidifying Activity

The acid production by the isolated lactic acid bacteria species was determined after inoculating the isolates into sterile reconstituted skim milk powder (10% w/v) at a rate of 1-2% inoculums /100 ml milk in sterile bottles of 200 ml capacity according to the method described by Attia *et al.* (2001). The inoculated skim milk medium was incubated at 30°C for mesophilic and at 38°C for thermophilic lactic acid bacteria (Farah *et al.,* 1990; De Vuyst and Degeest, 1999; Attia *et al.,* 2001). Change in pH was monitored at different intervals by taking samples at 0 h (initial), 12 h, 24 h, 48 h and 72 h until the pH of the medium reached 4.6 (iso-electric point) (Patrignani *et al.*, 2007). The isolated lactic acid bacteria species were characterized as fast acid producers (less than12 h to reach pH 4.6), medium acid producers (12-48 h to reach pH 4.6) or slow acid producers (more than 48 h to reach pH 4.6) based on their acid production potential according to Seifu *et al.* (2012).

2.5.2. Proteolytic Activity

To determine the proteolytic activity of lactic acid bacteria isolates, MRS/M17 agar supplemented with 10% skim milk was poured, solidified, and then dried. Sterile Whatman paper discs were deposited on the surface of the agar. Each disc received a volume of 20 µl of a young culture. After incubation at 37°C for 24 h, proteolysis was indicated by clear zones around discs (Vuillemard *et al.*, 1986), which were recorded as positive activity. All strains with positive reaction in

MRS/M17 with 1% skimmed milk were considered as strains with a slight proteolytic activity (Lasagno *et al.,* 2002).

2.5.3. Antimicrobial Activities of LAB Isolates

2.5.3.1. Indicator Strains

The indicator strains including *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa* were used for the antimicrobial test of the lactic acid bacteria isolates. All indicator strains were obtained from the Pasture Institute in Addis Ababa.

2.5.3.2. Preparation of Cell-Free Supernatant (CFS)

Each LAB isolate was inoculated in 10 ml of MRS broth and incubated at 30°C for 48 hrs. After incubation, a cell-free supernatant was obtained by centrifuging the bacterial culture at 6000 rpm for 15 minutes, followed by the filtration of the supernatant through 0.20 µm pore size filters.

2.5.3.3. Screening for Antimicrobial Activities

The agar-well diffusion method was employed in the screening of LAB for antimicrobial activities. Indicator microorganisms were prepared by inoculating 20 ml of molten agar media seeded with $1x10^7$ cfu/ml of an overnight culture of each indicator organism and allowing them to solidify in a Petri dish. 50 µl of the filtered cell-free supernatant of test strains was separately placed into the wells. The plates, prepared in triplicate, were kept at 4°C for 24 h according to Bonade *et al.* (2001) to allow pre-diffusion of the CFS into the agar and then incubated at 37°C for 24 h. They were then observed for possible clearing of zones (inhibition zones). The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the well using a caliper in mm.

2.5.4. Exopolysaccharide (EPS) Production

The screening of the isolates for EPSs production was carried out according to the method described by Guiraud (1998). The isolates cultured on MRS agar were streaked onto LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) potassium nitrate, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80, and 1.7% (w/v) agar, pH 7.1± 0.2) Sawadogo *et al.* (2007) and incubated at 30°C for 48 h. The sticky aspect of the colonies was determined by testing them for slime formation using the inoculated loop method as described by Knoshaug *et al.* (2000). The isolates were considered positively slimy producer if the length of slime diameter was above 1.5 mm.

The positive isolates were confirmed by growing them on MRS sucrose broth and incubating them at 30°C for 24 hrs. A volume of 1.5 ml of the 24 h culture was centrifuged at 5000 rpm for 10 minute at 4°C and 1 ml of the supernatant was put in a glass tube and an equal volume of ethanol 95% was added. An opaque link formed at the interface of the tube indicated the presence of EPSs.

2.6. Data Analysis

Obtained experimental data of the study were analyzed using descriptive statistical methods.

3. Results and Discussion

3.1. Isolation and Identification of LAB

In the present study, a total of 95 lactic acid bacteria colonies were isolated, of which 51 and 44 colonies were obtained from MRS and M17 agar media, respectively. A total of 9 isolates selected from MRS agar plates were found to belong to the genus *Lactobacillus*. According to the biochemical test, *Lactobacillus brevis* HUM14, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus casei subsp. casei* HUM18 and *Lactobacillus plantarum* HUM19 produced gas from glucose. This is in agreement with the results of Seifu *et al.* (2012) that all *Lactobacillus* strains isolated from *ititu* were able to produce gas from glucose. All *Lactobacillus* isolates did not produce ammonia from arginine, catalase negative, and are non-motile. Regarding growth at different temperature, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus delbrueckii subsp. bulgaricus* HUM16, *Lactobacillus amylophilus HUM17, Lactobacillus casei subsp. casei* HUM18, and *Lactobacillus plantarum* HUM19 grew at 15°C. The results also showed that *Lactobacillus delbrueckii subsp. lactis* HUM13, and *Lactobacillus acidophilus* HUM17 *grew* at 45°C whereas *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 and *Lactobacillus helveticus* HUM21 were able to grow at 15 and 45°C. According to pH resistant test, all *lactobacillus* isolates were able to grow at pH 5.0, while *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 and *Lactobacillus delbrueckii subsp. lactis* HUM13 were able to grow at pH 4.0. Regarding growth of isolates at different NaCl concentration, *Lactobacillus delbrueckii subsp. lactis* HUM13, *Lactobacillus brevis HUM14,* and *Lactobacillus paracasei subsp. tolerans* HUM15, grew at 2% NaCl. The growth of isolates in a medium containing 2% NaCl observed in the present study is similar to the findings of Hutkins *et al.* (1987), where all *lactobacilli* isolated from camel milk were able to grow at 2% NaCl. The results also showed that *Lactobacillus amylophilus* HUM17, *Lactobacillus casei subsp. casei* HUM 18, *Lactobacillus plantarum* HUM19 and *Lactobacillus helveticus* HUM21, grew at 2 and 4% NaCl, whereas *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 grew at 4 and 6.5% NaCl. Different *lactobacilli* strains isolated from camel milk are shown in Table 3, while the results of different physiological and biochemical tests are given in Table 2.

The other isolates were referred to the genus *Lactococcus*. They were identified by their morphological, cultural, physiological and biochemical characteristics. Ten isolates picked from M17 agar plates were found

to belong to the genus *Lactococcus*. All *Lactococcus* were unable to produce gas from glucose, show arginine hydrolysis by some strains, catalase-negative and non-motile which is indicator of *Lactococcus* strains. Arginine hydrolysis indicated that *Lactococcus lactis subsp. lactis* HUM1, *Pediococcus acidilactici* HUM4, *Pediococcus pentosaceus* HUM5, and *Lactococcus garviae* HUM6 were able to produce ammonia from arginine. Regarding growth at different temperature, *Lactococcus lactis subsp. cremoris* HUM2 was able to grow at 15°C, whereas *Lactoccoccus raffinolactis* HUM3, *Pediococcus acidilactici* HUM4, *Lactococcus garviae* HUM5, *Pediococcus pentosaceus* HUM6, *Streptococcus cremoris* HUM8, *Streptococcus lactis* HUM9, *Pediococcus damnosus* HUM10, and *Streptococcus salivarius subsp. thermophilus* HUM12 grew at 45°C. According to growth at different pH range; *Lactoccoccus raffinolactis* HUM3 was able to grow at pH 2.0 and 4.0. On the other hand, *Lactococcus garviae* HUM5, *Streptococcus cremoris* HUM8, *Streptococcus lactis* HUM9, and *Streptococcus salivarius subsp. thermophilus* HUM12 were able to grow at pH 4.0 and 5.0, whereas *Lactococcus lactis subsp. lactis* HUM1, *Lactococcus lactis subsp. cremoris* HUM2, *Pediococcus acidilactici* HUM4, *Pediococcus pentosaceus* HUM6, and *Pediococcus damnosus* HUM11 were able to grow at pH 5.0. Regarding growth at different NaCl concentration, *Lactococcus lactis subsp. lactis* HUM1, *Lactococcus lactis subsp. cremoris* HUM2, *Lactoccoccus raffinolactis* HUM3, and *Lactococcus garviae* HUM5 grew at 2 and 4% NaCl whereas *Pediococcus acidilactici* HUM4 and *Pediococcus pentosaceus* HUM6 were able to grow at 4.0 and 6.5% NaCl. Similar observations were reported by Togo et *al.* (2002) who indicated that Lactococcus isolates were able to grow at higher NaCl (4% and 6.5%). The different *Lactococci* strains isolated from camel milk are shown in Table 3 while the results of different physiological and biochemical tests are given in Table 1.

Two isolates selected from M17 agar plates were identified as *Enterococcus* strains, which included *Enterococcus faecalis* HUM7. This isolate was able to grow at 15 and 45°C, pH 4.0, and in a medium containing 6.5% NaCl, whereas *Enterococcus casseliflavus* HUM2 was able to grow at 15°C, pH 5.0, and in a medium containing 2% and 4% NaCl. *Enterococcus* was observed to be the only genera that showed growth at a high NaCl concentration (6.5%) which is similar with an earlier finding by El-Hadi *et al.* (2006), Gram-positive and catalase negative bacteria that are capable of growing at 15 and 45°C, and in a medium containing 6.5% NaCl were considered to be *Enterococci* (Table1).

In the current study, more growth of *Lactobacillus* species in camel milk as compared to others isolates were observed on their selective culture media and comprised 72.60% of the total lactic acid bacteria (Table 5). These findings are in accordance with Khedid *et al.* (2006), who reported that *Lactobacillus* species isolated from camel milk was the dominant genus with 37.5% of the total lactic acid bacteria isolates. Bettache *et al.* (2012) indicated that members of the genus *Lactobacillus* isolates dominated in all Dhan samples as well as in the traditional butter. Consistent with the results of this study, Abu-Tarboush (1994) reported that camel milk provided support to the growth of *L. acidophilus*.

Table 1. Physiological and biochemical characteristics of *cocci* strains.

Characteristics	*Lactococci* isolates											
	HUM 1	HUM 2	HUM 3	HUM 4	HUM 5	HUM 6	HUM 7	HUM 8	HUM 9	HUM 10	HUM 11	HUM 12
Gas from glucose	-	-	-	-	-	-	-	-	-	-	-	-
Cell shape	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci
Ammonium from arginine	+	-	v	+	+	+	v	-	-	-	v	-
Motility	-	-	-	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-
Aerobicity	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a
Growth at different temperature												
10°C	-	-	-	-	-	-	-	-	-	-	-	-
15°C	-	+	-	-	v	-	+	-	-	-	+	-
45°C	+	-	+	+	+	+	+	+	+	+	-	+
Growth at different pH												
2.0	-	-	+	v	-	-	-	-	-	-	-	-
4.0	-	-	+	v	+	-	+	+	+	-	-	+
5.0	+	+	-	+	+	+	-	+	+	-	+	+
Growth in the presence of NaCl												
2%	+	+	+	-	+	-	-	-	-	-	-	-
4%	+	+	+	+	+	+	-	-	-	-	-	-
6.5%	-	-	-	+	v	+	+	v	-	-	v	v

Note: + = positive reaction; - = negative reaction; v = variable reaction; f.a = facultative anaerobic; HUM1 = *Lactococcus lactis subsp. lactis*; HUM2 = *Lactococcus lactis. subsp. cremoris*; HUM3 =*Lactococcus. raffinolactis*; HUM4 = *Pediococcus acidilactici*; HUM5 =*Lactococcus.garviae*; HUM6 = *Streptococcus lactis*; HUM7 = *Enterococcus faecalis*; HUM8 = *Streptococcus cremoris*; HUM9 = *Streptococcus salivarius subsp. Thermophiles*; HUM10 = *Pediococcus damnosus*; HUM11 = *Enterococcus casseliflavus*; HUM12 = *Pediococcus pentosaceus; n = 2*

Table 2. Physiological and biochemical characteristics of *Lactobacillus* strains.

Characteristics	*Lactobacilli* isolates								
	HUM 13	HUM 14	HUM 15	HUM 16	HUM 17	HUM 18	HUM 19	HUM 20	HUM 21
Gas from glucose	-	+	+	-	-	+	+	-	-
Cell shape	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli
Ammonia from arginine	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-
Aerobicity	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a	f.a
Growth at different temperature									
10°C	-	-	-	-	-	-	-	-	-
15°C	-	-	+	-	+	+	+	-	+
45°C	+	-	-	+	-	-	v	+	+
Growth at different pH									
2.0	-	-	-	-	-	-	-	-	-
4.0	+	-	-	-	-	-	-	+	-
5.0	+	+	+	+	+	+	+	+	+
Growth in the presence of NaCl									
2%	+	+	+	-	+	+	+	-	+
4%	-	-	-	-	+	+	+	+	+
6.5%	-	-	-	-	-	-	-	+	v

Note: + = Positive reaction; - = Negative reaction; v = Variable reaction; f.a = Facultative anaerobic; HUM13 = *Lactobacillus delbrueckii subsp. Lactis*; HUM14 = *Lactobacillus brevis*; HUM15 = *Lactobacillus paracasei subsp. Tolerans*; HUM16 = *Lactobacillus delbrueckii subsp. Bulgaricus*; HUM17 = *Lactobacillus amylophilus*; HUM18 = *Lactobacillus casei subsp. Casei*; HUM19 = *Lactobacillus helveticus*; HUM20 = *Lactobacillus acidophilus*; HUM21 = *Lactobacillus plantarum; n =2*

Table 3. Carbohydrates fermentation profile of lactic acid bacteria (LAB) species.

Isolates number	Carbohydrates																				Species identification
	Lac	Mal	Glu	Gal	Mos	Man	Mlz	Sal	Mel	Cel	Rha	Suc	Rib	Xyl	Str	Amy	Ara	Fru	Sor	Tre	Raf	
HUM 1	+	+	+	+	+	-	-	+	-	+	-	-	+	v	+	-	-	+	-	v	-	*Lactococcus lactis subsp.lactis*
HUM 2	+	-	+	+	+	-	-	-	-	+	-	-	-	v	+	-	-	+	-	-	-	*Lactococcus lactis subsp.cremoris*
HUM 3	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	-	+	+	*Lactococcus raffinolactis*
HUM 4	v	-	+	+	+	-	-	-	-	+	+	+	+	+	-	-	v	+	-	+	-	*Pediococcus acidilactici*
HUM 5	+	-	+	+	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	*Lactococcus garviae*
HUM 6	v	+	+	+	+	-	-	+	-	+	+	+	+	v	-	+	+	+	-	+	-	*Pediococcus pentosaceus*
HUM 7	+	+	+	v	+	+	+	+	+	-	-	+	+	-	+	-	-	-	+	+	-	*Enterococcus faecalis*
HUM 8	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	v	*Streptococcus cremoris*
HUM 9	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	v	*Streptococcus salivarius subsp.thermophilus*
HUM 10	-	v	+	-	+	-	v		v	+	-	v	-	-	-	-	-	+	+	-	-	*Pediococcus damnosus*
HUM 11	+	+	+	-	+	+	-	+	+	-	+	+	+	+	-	-	-	-	+	+	+	*Enterococcus casseliflavus*
HUM 12	+	-	-	+	+	+	-	v	v	-	-	+	+	+	+	-	-	-	+	v	-	*Streptococcus lactis*
HUM13	+	+	+	v	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	*Lactobacillus delbrueckii subsp.lactis*
HUM 14	v		+	+	-	-	-	v	v	v	-	-	-	-	-	-	+	+	-	-	-	*Lactobacillus brevis*
HUM 15	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lactobacillus paracasei subsp.tolerans*
HUM 16	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	*Lactobacillus delbrueckii subsp.bulgaricus*
HUM 17	-	-	+	+	-	v	-	-	-	-	-	v	-	-	+	-	-	-	-	-	-	*Lactobacillus amylophilus*
HUM 18	-	v	+	+	+	+	v	+	+	+	-	-	-	-	-	+	-	-	+	+	-	*Lactobacillus casei subsp.casei*
HUM 19	+	+	+	+	+	+	+		-	+		-	-	+	-	+	-	+	+	+	-	*Lactobacillus plantarum*
HUM 20	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	*Lactobacillus acidophilus*
HUM 21	+	-	+	+	v	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lactobacillus helveticus*

Note: Tre = Trealose; Suc = sucrose; Amy = Amygladin; Str = Starch; Mlz = melizitose; Sor = Sorbitol; Ara = Arabinose; Gal = Galactose; Rib = Ribose; Rha = Rhamnose; Xyl = Xylose; Mal = Maltose; Cel = cellubiose; Mel = melibiose; Sal = Salicin; Man = Mannitol; Mos = Mannose; Fru = Fructose; Glu = Glucose; Lac = Lactose; Raf = Raffinose.

3.2. Acidifying Activity

To select a potential candidate starter culture, the lactic acid bacteria strains isolated from camel milk were characterized on the basis of acid production capability. The acidity was increased during the fermentation, and there was variability in acidification rate between the different strains used to inoculate the milk.

According to acidifying activity, *Lactobacillus acidophillus* HUM20*,* and *Lactobacillus plantarum* HUM19, were considered to be fast acid producer among *Lactobacilli* strains as they reached to a final pH value of 4.33 and 4.50 from an initial value of 6.75 and 6.77, respectively within 8 h of incubation. This is in agreement with the results of Fguiri *et al.* (2016) that *Lactobacillus plantarum* was selected as fast acid producer *Lactobacillus* isolate from camel milk. On the other hand, *Lactobacillus brevis* HUM14, *Lactobacillus helveticus* HUM16, *Lactobacillus delbrueckii subsp. bulgaricus* HUM2*,* and *Lactobacillus casei subsp. casei HUM18* were found to be medium acid producers from an initial value of (6.77, 6.73, 6.67, and 6.79 to a final pH value of 4.44, 4.43 4.34 and 4.40, respectively) within 48 h of incubation. This is in accord with the findings of Seifu *et al.* (2012) who reported that *Lactobacillus delbrueckii subsp. bulgaricus* showed medium acidification activity and reduced the pH of the skim milk powder from an initial values of 6.77 to a final pH values of 4.57 within 48 h of incubation. On the other hand, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus delbrueckii subsp. lactis* HUM13 and *Lactobacillus amylophilus* HUM17 reduced the pH of the skim milk from an initial value of 6.75 to a final pH value of 4.42, 4.46, and 4.6, respectively, within 72 h of incubation.

Among *Lactococcus* strains, all *Lactococcus* isolates were slow acid producers except, *Pediococcus pentosaceus* HUM6, and *streptococcus cremoris* HUM8 reduced the pH of the skim milk fast from an initial value of 6.78 and attained a final pH value of 4.56, and 4.38 respectively, within 10 h of incubation. This is in agreement with the results of Fguiri *et al.* (2016) who reported that *pediococcus pentosaceus* was selected as fast acid producer among *Lactococcus* isolate from camel milk.

The difference observed in acidifying activities between each strain of lactic acid bacteria species may be associated with specific capacity to break down the carbon and nitrogen substrates in the medium and the capability to assimilate the nutrients essential for growth (Badis *et al.*, 2004). On occasions, differences are also due to the presence or absence of nutrient transport systems (Albenzio *et al.,* 2001).

3.3. Proteolytic Activity

According to proteolytic test all investigated, lactic acid bacteria isolates showed different diameter of a clear zone around the discs. According to Vuillemard *et al.* (1986), a strain is called proteolytic if it has a zone of lysis of diameter between 15 and 21 mm. Compared to these data, our strains revealed that proteolytic zone diameters were between 15 and 21 mm. The results obtained during the investigation of Proteolytic test are shown in Table 4.

Table 4. Proteolytic activities of lactic acid bacteria species.

Isolates	Diameter of inhibition zone (mm)
Lactococcus lactis subsp. lactis HUM1	16±0.03
Lactococcus lactis subsp. cremoris HUM2	16±0.00
Lactococcus raffinolactis HUM3	15±0.10
Pediococcus acidilactici HUM4	15±0.31
Lactococcus garviae HUM5	17±1.40
Pediococcus pentosaceus HUM6	17±3.00
Enterococcus faecalis HUM7	15±0.00
Streptococcus cremoris HUM8	21±0.01
Streptococcus salivarius subsp. thermophilus HUM9	15±0.00
Pediococcus damnosus HUM10	16±0.00
Enterococcus casseliflavus HUM11	15±0.00
Streptococcus lactis HUM12	15±0.01
Lactobacillus delbrueckii subsp. lactis HUM13	15±0.40
Lactobacillus brevis HUM14	18±0.03
Lactobacillus paracasei subsp. tolerans HUM15	15±0.00
Lactobacillus delbrueckii subsp. bulgaricus HUM16	18±0.00
Lactobacillus amylophilus HUM17	15±1.30
Lactobacillus casei subsp. casei HUM18	16±0.02
Lactobacillus plantarum HUM19	19±0.10
Lactobacillus acidophilus HUM20	20±0.00
Lactobacillus helveticus HUM21	17±2.30

Note: The value indicated is means ± SD; n = 4

Among all lactic acid bacteria isolates, *Lactobacillus acidophilus* HUM20, *Lactobacillus plantarum* HUM19, and *Streptococcus cremoris* HUM8 had a high proteolytic activity with different diameters of clear zones. Proteolytic activity is essential for the development of organoleptic properties of different fermented milk products (Axelsson 1998; Christensen *et al.,* 1999). The production of high-quality fermented dairy products is dependent on the proteolytic systems of starter bacteria, as the peptides and amino acids formed have a direct impact on flavour or serve as flavour precursors in these products (Axelsson, 1998; Christensen *et al.,* 1999). Several peptidases with different specificities have been identified in lactic acid bacteria. All peptidases have been found to be intracellular and liberated in fermented milk products after cell lysis (Law and Haandrikman, 1997; Axelsson, 1998).

3.4. Antimicrobial Activities

The antimicrobial properties of lactic acid bacteria isolates from camel milk are shown in Table 5. The LAB strains were able to inhibit the selected indicator organisms to varying degrees of the zones of inhibition. Similar to our findings, Kivanc (1990) and Tadesse *et al.*

(2005) observed varying degrees of inhibition of various food borne pathogens by cell-free filtrates of LAB. Afolabi *et al.* (2008) showed that antimicrobial producing microorganisms had the ability to inhibit the growth of other bacteria which included both Gram-negative and Gram positive bacteria. Such antimicrobial activities were also demonstrated in the works of other researchers such as Adesokan *et al.* (2008) where LAB species were tested against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli,* and *Proteus vulgaris*. It has been also demonstrated that, the antimicrobial compounds produced by LAB can inhibit the growth of pathogenic bacteria of possible contaminants in fermented products (Raccah *et al.,* 1979; Smith and Palumbo, 1983; Cintas *et al.,* 1998).

The ability to inhibit other organisms is due to the fact that LAB produces substances which are injurious to the indicator organisms depending on the concentration or quantity produced (Axelsson, 1998; Christensen *et al.,* 1999). These substances serve as competitive advantage to LAB when in mixed culture especially during fermentation and hence the dominance of LAB during fermentation of milk, cereals and vegetables (Afolabi *et al.*, 2008). Wakil and Osamwonyi (2012) indicated that LAB isolates showing antimicrobial activity were discovered to produce antimicrobial substances like lactic acid, hydrogen peroxide, and diacetyl, showing that the ability to inhibit other organisms was directly related to the ability of these organisms to produce these substances. Daeschel (1993) reported the ability of LAB to produce lactic acid, thereby reducing the pH of the fermenting medium. The lactic acid produced serves to reduce the pH of the medium, thereby making it acidic which is not conducive for the survival of spoilage bacteria which may have found their way into the fermenting substrate during spontaneous fermentation. Lactic acid is a natural preservative that inhibits putrefying bacteria and is responsible for the improved microbiological stability and safety of the food (Raccah *et al.,* 1979).

Table 5. Antimicrobial activities of lactic acid bacteria species against pathogenic microorganisms.

LAB isolates	Indicator strains			
	S. aureus	*S. typhi*	*E. coli*	*P. aeruginosa*
Lactococcus lactis subsp. lactis HUM1	+	+	+	+
Lactococcus lactis subsp. cremoris HUM2	+	+	+	++
Lactococcus raffinolactis HUM3	++	+	+	+
Pediococcus acidilactici HUM4	+	++	+	+
Lactococcus garviae HUM5	++	+	+	++
Pediococcus pentosaceus HUM6	+	++	+	+
Enterococcus faecalis HUM7	+	+	+	+
Streptococcus cremoris HUM8	+++	+ +	+++	++
Streptococcus salivarius subsp. thermophilus HUM9	+	+	+	++
Pediococcus damnosus HUM10	++	++	+	++
Enterococcus casseliflavus HUM11	+	+	+	+
Streptococcus lactis HUM12	++	+	+	++
Lactobacillus delbrueckii subsp. lactis HUM13	+	++	+	+
Lactobacillus brevis HUM14	++	+	+	++
Lactobacillus paracasei subsp. tolerans HUM15	++	++	+	+
Lactobacillus delbrueckii subsp. bulgaricus HUM16	++	+	+	+
Lactobacillus amylophilus HUM17	+	+	+	+
Lactobacillus casei subsp. casei HUM18	++	+	+ +	++
Lactobacillus plantarum HUM19	++	+ +	+++	+ ++
Lactobacillus acidophilus HUM20	+++	+ +	+++	++
Lactobacillus helveticus HUM21	+	+	+	+

Note: + = [1–4 mm]; ++ = (4–8 mm]; +++ = (8–12 mm]
Source: Akabanda *et al.*, 2014

3.5. Exopolysaccharide Production (EPS)

The results showed that all the groups of LAB strains tested produced exopolysaccharide (EPS) with slime length diameter above 1.5 mm. This finding is in agreement with the findings of Patel and Prajapati (2013), who reported most of the LAB producing EPS belonged to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc,* and *Pediococcus.* According to Bridget and Lordsday (2011) a total of 77% of LAB strains produced exopolysaccharides under the experimental condition from Nigerian yoghurt.

Exopolysaccharide production is a desirable feature of bacteria applied in dairy products because EPSs act as natural biothickener leading to higher consistency and

viscosity of the product and reduced syneresis Ruas-Madiedo *et al.* (2006). However, most of them are chemically or enzymatically modified in order to improve rheological properties (e. g. cellulose, starch, pectin and alginate) (Ruas-Madiedo *et al.* (2006), and therefore, their use is strongly restricted for food applications. The EPSs of microbial origin have unique rheological properties because of their capability of forming very viscous solutions at low concentration and their pseudoplastic nature (Becker *et al.,* 1998). Some strains of LAB have been reported to produce EPS and gain increasing attention over the last few years because of their contribution to the rheology and texture of fermented milk and food products (Cerning and Marshall, 1999). EPS-producing LAB has a greater ability to withstand technological stresses and survive the passage through the gastrointestinal tract compared to their nonproducing bacteria (Stack, 2010). Hence, the choice of EPS-producing starter culture seems to give several advantages over nonproducing ones.

Table 6. Frequency distribution of lactic acid bacteria of different genera based on their carbohydrate fermentation profile.

Genus	Species	Number of isolates	% of total isolates
Lactobacillus	*Lactobacillus delbrueckii subsp. lactis*	12	8.22
	Lactobacillus brevis	2	1.37
	Lactobacillus paracasei subsp. tolerans	5	3.42
	Lactobacillus delbrueckii subsp. bulgaricus	8	5.48
	Lactobacillus helveticus	2	1.37
	Lactobacillus casei subsp. casei	9	6.16
	Lactobacillus plantarum	18	12.33
	Lactobacillus acidophillus	35	23.97
	Lactobacillus amylophilus	15	10.27
Lactococcus	*Lactococcus lactis subsp.lactis*	5	3.42
	Lactococcus garviae	3	2.05
	Lactococcus raffinolactis	4	2.74
	Lactococcus lactis subsp. cremoris	2	1.37
Streptococcus	*Streptococcus cremoris*	4	2.74
	Streptococcus lactis	3	2.05
	Streptococcus salivarius subsp.thermophilus	5	3.47
Enterococcus	*Enterococcus faecalis*	2	1.37
	Enterococcus casseliflavus	2	1.37
Pediococcus	*Pediococcus pentosaceus*	2	1.37
	Pediococcus damnosus	5	3.42
	Pediococcus acidilactici	3	2.05
Total		146	100

4. Conclusion

This study has demonstrated that 21 species of lactic acid bacteria were isolated from camel milk. The most dominant lactic acid bacterial species was *Lactobacillus* that comprised 72.6% of the total lactic acid bacteria isolates. Based on the overall technological properties, *Lactobacillus acidophilus* HUM20, *Lactobacillus plantarum* HUM19, and *Streptococcus cremoris* HUM8 were high in acidifying and proteolytic activities, exopolysaccharide production (EPS), and antimicrobial activities, implying that these bacteria could be used as starter cultures for the industrial processing of camel milk under controlled environments in the future. However, further research should be conducted to elucidate performance in mixed cultures, EPS quantification and lipolytic activities, aroma production and other desirable characteristics of the isolates as well as their molecular attributes to determine their suitability for commercial production of fermented camel milk products.

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Climate Variability and Livelihood Strategies Pursued by the Pastoral Community of the *Karrayu People,* Oromia Region, Central Ethiopia

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Abstract: Variability in seasonal rainfall and precipitation as a result of climate change has been threatening the already fragile livelihoods of pastoral communities in Ethiopia. The current study was conducted to elucidate climate variability, perception, and livelihood strategies in the *Karrayu* pastoral area of Fenatale district, in the Oromia region of the country. A semi-structured questionnaire, key informant interviews, focus group discussions were used to elicit data climate variability and livelihood strategies in the community. One hundred twenty randomly selected sample respondents were used for the study. Assessment of the climate variability was based on reports of Metehara (1968-1984) and Awash (1985-2007) Stations of the National Meteorological Service Agency and primary data collected from the area. Rainfall coefficient (RC), least square regression models (rho), standardized rainfall anomalies (SRA) and coefficient of variation (CV) were used for analysis of the data. The findings identified eight rainy months and four dry months with rainfall coefficients of > 0.6 and < 0.6, respectively. However, March, April and September had a moderate concentration (RC 1.0 - 2.0) while July and August had higher rainfall concentration (RC > 2.0). There was an increase in the maximum and annual average temperature and a decline in the minimum annual temperature from 1965-2007, which was also augmented by the perception of 66% of the respondents. The seasonal rainfall variability was significantly higher (CV ranged from 0.25 to 0.80) than the annual rainfall variability (CV = 0.18). Thus, a large proportion of *Karrayu* pastoralists perceived less water and food (76%), migrated to other areas (73%), became dependent on Participatory Safety Net Program (72%), and eked out a living by collecting and selling firewood and charcoal (57%) as major livelihood strategies. In conclusion, the results of this study have revealed that there have been such persistent increments in temperature and seasonal rainfall variability that the pastoralist community in the area has been markedly resorting to various livelihood strategies to adapt to climate variability and change. However, to complement the community efforts, integrated livelihood development mechanisms better be devised with active involvement of different stakeholders for sustainable development of the pastoral and agropastoral communities of the *Karrayu* including early warning systems, collection of reliable climate data, disaster risk reduction and risk sharing strategies to avert the risk of vulnerable groups of the community from the hazards of climate variability in particular and change in general. Likewise, further critical studies are recommended regarding trend analysis and variability of rainfall of the study area using other parameters to substantiate these findings.

Keywords: Climate Variability; *Karrayu*; Livelihood Strategies; Pastoral Community

1. Introduction

Pastoralism has a great share in national and regional economies for several poorest countries of the world. It serves as a base for local livelihoods and provides multi-directional ecological services (Nori *et al.,* 2008). Pastoral and agro-pastoral societies inhabit the arid and semi-arid environments, which are characterized by high probability of the occurrence of frequent and intense drought and flood (WISP, 2007; Birch and Grhan, 2007). Africa is one of the most vulnerable continents to climate change/variability, and 350–600 million Africans will be at risk of drought and increased water stress by 2050s IPCC (2007). The pastoral and agro-pastoral production systems are mostly vulnerable to increased climate variability (Stige *et al*., 2006; Sithole and Murewi, 2009 cited in Kgosikoma and Batisani, 2014).

Drought occurs anywhere in the world but its damage is not as severe as in Africa in general and in Ethiopia in particular (NMA, 2007). Ethiopia is very vulnerable to the harmful effects of climate extremes primarily drought and flood. Due to climate change/variability, the country has suffered from severe poverty, low adaptive capacity to drought, highly sensitive livelihood to weather, and low access to education, information, technology, and health services (Senait *et al*., 2010).

The adverse effect of climate variability and change is accelerating the already existing pastoral livelihood insecurity (Tagel and Veen, 2015). Knowledge of people's perceptions and adaptation measures are crucial to inform future actions and thereby minimize impacts of climate change. Improving resilience of communities and households to the effects of impacts of climate change

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requires understanding of the existing local practices (Smucker and Wisner, 2008).

The pastoral areas of Ethiopia cover over 60% of the total landmass inhabiting 10 million people (Beruk, 2008). These areas are known for erratic rainfall, which is usually inadequate to meet the physiological water requirements of biotic system. This phenomenon is aggravated by high temperature and strong dry winds, leading to high evapo-transpiration, which in turn accelerates water scarcity to its climax (Mesfin, 2000). There is increased frequency of meteorological drought episodes, unseasonal flash floods and outbreaks of diseases in the pastoral regions of Ethiopia (Debay, 2010). Unlike others, livestock represents a householder's source of income, savings, social status and security of pastoral community and any losses of livestock through droughts and/or flooding results in economic and social disaster at the household level (Orindi *et al.,* 2007). Hence, pastoralists are among the most vulnerable groups of society as they are increasingly being characterized by poverty, food insecurity, environmental risk, and political, economic and social marginalization (Hogg, 1997 cited in Dula, 2013).

Few local level studies have been done on climate change and variability regarding the arid and semi-arid areas of Ethiopia (Gebre *et al.,* 2013; Nega *et al.,* 2015). As there are spatio-temporal variations in the degree of climate change and variability as well as their resilience capacities, informative studies at local level are crucial for reliable data and for decision actions. The current study has been done on *Karrayu* pastoral area in Fentale district of Oromia region where there have been frequent climate-induced disasters, such as drought, flood, and resultant livelihood insecurity. The study was aimed at evaluating the trend and variability of major climatic element; assessing the perception of local pastoral community; and identifying major livelihood strategies to tackle the adverse effects of climate change and variability.

2. Materials and Methods

2.1. Descriptions of the Study Area

Karrayu area (administratively under Fentale district) is located between $8^{0}42'$ to $9^{0}00'$N latitudes and $30^{0}30'$ to $40^{0}11'$E longitudes in East Shewa Zone of Oromia, Central Ethiopia. The capital town of the district, Metehara, is located at the distance of about 200 km south east of Addis Ababa, along the Addis Ababa-Djibouti road and rail ways. The neighbours of Fentale district are the Afar Debine in the north, Arsi Oromo in the south, Awash National Park in the east, the *Ittu* in west Hararghe and the *Argoba* in the west, and Bereket and Shenkora and Minjar districts in the south west (Fig. 1). The study area is situated in tectonically active Main Ethiopian Rift (Goerner *et al.,* 2009) whose topography is characterized by flat, undulating plains, hills and mountain ridges. The altitude ranges from 900 to 2700 meters above sea level (FWARDO, 2007).

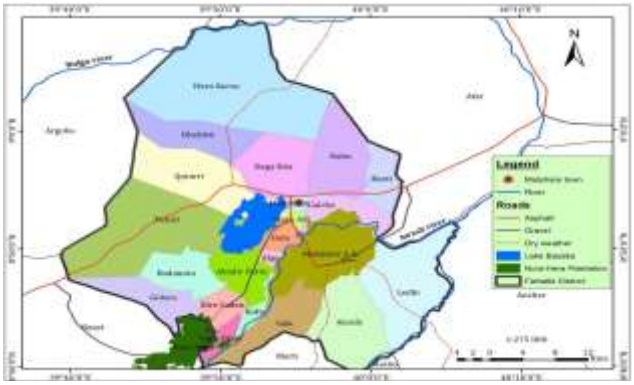


Figure 1. Location map of *Karrayu* area in Fantalle District Source: Dula, 2013.

The average monthly and annual temperatures of the area are 25^{0}C and 26^{0}C, while the average annual minimum and maximum temperatures are 17.3^{0}C and 32.5^{0}C, respectively. The highest rainfall occurs in the Ethiopian summer (*Kiremt*) season (Jun to September) followed by four to eight consecutive months (October through May) that are water deficit (Haji *et al.,* 2015). The total annual rainfall ranges between 400 and 700 mm, which is associated with semi-arid (*kolla)* agro ecological zones or dry/arid climatic areas (lower *kolla*) (Ayalew, 2001; MOA/FAO/UNDP, 1983). Awash River and Lake Beseka are the main hydrologic elements in the *Karrayu* area.

The soils in *Karrayu* area are dominated by Andosols and Leptosols, having reddish colour, silt clay to sandy loam texture, and low organic matter content. The dominant tree species are *Acacia senegali* (vernacularly known as *Burquqqee)*, *Acacia tortolis* (*Xaddacha*) and *Acacia etaica (Ajo*). Among these, *Acacia senegali* and *Acacia tortilis* are highly harvested for fuel wood and charcoal production (OIDA, 2007; FWARDO, 2007). The *Karrayu* area is a habitat of a variety of birds, fishes, crocodiles and other aquatic species from Lake Beseka and plays an important role in the wildlife ecology as it is located in the northern vicinity of Awash National Park (Eleni, 2009).

According to FWARDO (2007), the area is one of the most drought-prone areas in the East Showa Zone of Oromia region that is inhabited largely by *Karrayu* pastoralists. The total population of Fentale district was 82255, of which 53% and 47% were male and female, respectively. The rural and urban ratio of total population is about 75% and 25%, respectively (CSA, 2009). *Karrayu* has two major tribal divisions, namely, *Dullacha* and *Basso* (Haji *et al.,* 2015) and is inhibited as a single dominant group in Fentale district until the 1950s and the 1960s. Since then, *Ittu* Oromo and the Somali community continually have permanent settlements in *Karrayu* area (Ayalew, 2001). Pastoralism is the dominant livelihood base of the area, including rearing cattle, goats, sheep and camels. Agro-pastoral livelihood system began after the 1980s following the severe scarcity of feed, grazing land and water. Agro-pastoral households cultivate maize, sorghum, groundnut, and onion (FWARDO, 2007).

Climate variability is mainly manifested through decreasing trend in rainfall and increasing trend in temperature in the *Karrayu* like in other parts of the country in particular and the world at large. As a result, pastoral people inhabiting this area have suffered frequently from climate related hazards, particularly drought. The much variable climatic condition that is reflected by erratic and low rainfall amounts enhanced vulnerability of these pastoralists to climate variability and change (that require proper interventions (Dula, 2013).

2.2. Data Collection and Analysis

Three sample *kebeles*: Gelcha, Kobo, and Banti Mogassa were purposively selected based on their accessibility, vulnerability to drought and water scarcity, and representativeness of the features of pastoral and agro-pastoral livelihood system of the area. The stratified random sampling technique was used to select 120 sample respondents by taking into account their settlement, location, and wealth rank. Primary data were gathered through semi-structured questionnaire interviews, focus group discussions (FGDs), key informant interviews, and field observations. The key issues of the theme were demographic, livelihood, perception on climate variability and drought, water use and management and livelihood strategies. For the sake of showing the features of water stress and drought in association with rainfall pattern of *Karrayu* area, three seasons: *Bega* (*Bonna*), *Belg* and *Kiremt* seasons were considered. The secondary data were obtained from archives of the National Meteorological Service Agency, Fentale district, regional and national governmental and non-governmental institutions. In addition, consecutive meteorological data (1985-2007) of Awash and Metehara (1965-84) Stations were used from the National Meteorological Service Agency.

Quantitative data analysis was undertaken by using SPSS software. The rainfall variation and distribution were analyzed and interpreted on the basis of daily, monthly, seasonally and annual time duration. The number of wet days and corresponding amounts and probability of wet days were calculated by taking a daily rainfall amount of > 0.1 mm as a threshold to identify a rainy day in accordance with Bewket and Conway (2007). The probability of wet day was computed as:

$$P(w) = \frac{n}{N} \quad (1)$$

Where: P (w) = probability of wet day; n = number of wet days; N = total number of days (both wet and dry days)

Following Bewket and Conway (2007), inter-annual variability was evaluated by calculating standardized rainfall anomalies using:

$$SRA = (Pt-Pm)/\sigma \quad (2)$$

Where: SRA= Standardized Rainfall Anomaly; Pt = rainfall amount of a given period; Pm=is Long term mean rainfall over a period of consideration; σ=is standard deviation of rainfall over a period of observation.

Moreover, linear trend regression analysis, coefficient of variation, rainfall coefficients were used to quantify trends and their statistical significance, respectively; while patterns and trends of climate change and variability were presented using graphs, tables and charts.

3. Results and Discussion

3.1. Trend and Variability of Major Climatic Elements

3.1.1. Temperature Variation and Distribution

The study area experienced a decreasing average monthly minimum temperature for almost all months of a year during 1985-2007 as compared to that of 1965-1984. The least average annual minimum temperatures were recorded in December, January, and November from 1965-2007. November was the second coldest month preceding December (Fig. 2). Similarly, local community identified December as the coldest month.

The mean monthly minimum temperature for the period of 1985-2007 was relatively lower than that for the period of 1965-1984, which implies a gradual decrease in the minimum temperature. Conversely, the mean maximum monthly temperature indicates increasing trend for both periods, with the exception of the months of July and August (Fig. 2). With regard to annual linear trend, there was increase in maximum and annual average temperature for the periods of 1965-84 and 1985-2007, while a declining trend was observed in the minimum annual temperature from 1965-2007 (Table 2).

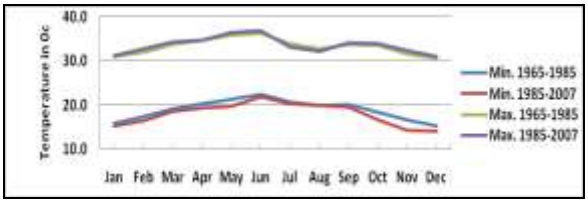


Figure 2. Mean monthly minimum and maximum temperature variation

Table 2. Annual mean, standard deviations and linear trend (LT) of minimum (Min), mean and maximum (Max) temperature (°C) variations.

Years	Annual Min.		Annual Mean		Annual Max.		Linear Trend Value		
	Mean	STD	Mean	STD	Mean	STD	Min.	Max	Mean
1965-1984	18.8	0.7	26.1	0.5	33.3	0.6	0.76	0.46	0.61
1985-2007	17.9	0.6	25.7	0.4	33.6	0.5	0.46	0.4	0.43
1965-2007	18.34	0.8	25.89	0.5	33.44	0.5	-0.11	0.12	0.01

3.1.2. Rainfall Variation and Distributions

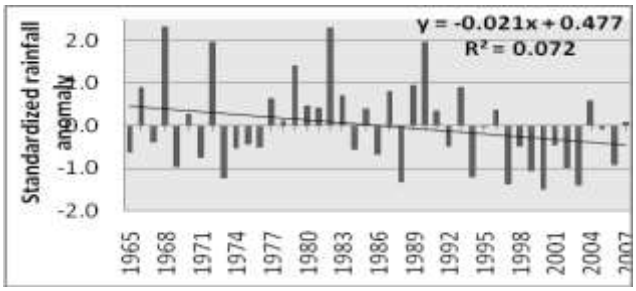
3.1.2.1. Annual and Seasonal Rainfall Variability and Trends

For the period between 1965-2007, the mean annual rainfall was 512.5 mm, with over half (58%) being received during the *Kiremt* season, followed by nearly a third (33%) being received in the *Belg* season. The rainfall of the area has a bimodal distribution (Table 3). The mean seasonal rainfall *Bega* (dry season*)* followed by *Belg* (small rainy season) indicated variability. As indicated in Fig 3 (A–D), the rainfall of annual total and all three seasons showed a decreasing trend. Likewise, the pastoralists also perceived that in the season of *Belg* (*Arfaasaa*) period alone, the rainfall showed a decreasing tend. This can also be verified by slight decrease in the mean annual rainfall during the aforementioned period by about 0.26 mm every decade. Despite this fact, the linear trend values and associated regression results showed insignificant decrease in both the seasonal and annual rainfall (as R<0.5 Table 3 and Fig 3). This seems against the report for the eastern and south eastern Ethiopia from 1982 to 2003 by Yilma and Zanke (2004) and for the Borana pastoral areas of southern Ethiopia (Wassie and Fekadu, 2015). Hence, further work is required to substantiate this fact.

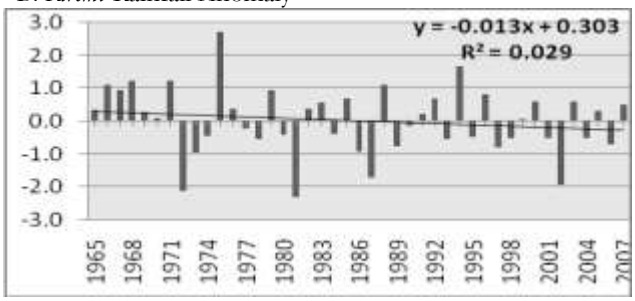
Table 3. Annual and seasonal rainfall amount and percentage and linear trend (1965 -2007).

Variables	Mean Rainfall		Decadal Linear Trend Equation	Linear Trend	Regression Trends	
	Amount (mm)	(%)			R^2	R
Kiremt	321.22	58.16	Y= -0.13x + 0.303	-0.13	0.029	0.17
Belg	184.78	33.46	Y= -0.21x + 0.477	-0.21	0.072	0.27
Bega	46.30	8.38	Y= -0.09x + 0.207	-0.09	0.014	0.19
Annual	552.3	100	Y= -0.26x + 0.507	-0.26	0.106	0.33

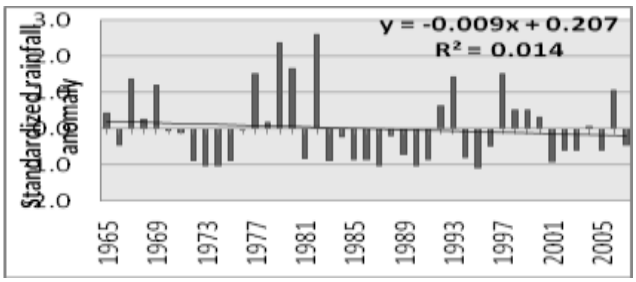
A. *Belg* Rainfall Anomaly



B. *Kiremt* Rainfall Anomaly



C. *Bega* Rainfall Anomaly



D. Annual Rainfall Anomaly

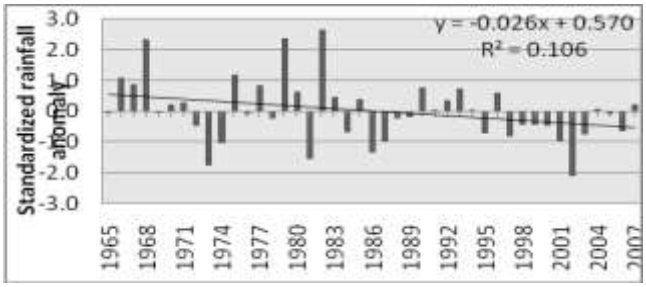


Figure 3. Annual and seasonal rainfall standardized anomalies and trends (1965 – 2007).

3.1.2.2. Monthly Rainfall

The mean monthly rainfall received in almost all months of the years from 1965 – 2007 were almost uniformly distributed among the months whereby August and July were with the highest levels (119.9mm and 117.7mm), respectively (Table 4). The annual mean total rainfalls for 1965-1984 and 1985-2007 were 598.1mm and 512.50mm, respectively (Table 4). According to the values of rainfall coefficient, eight months (Feb. through Sept.) were rainy as their RC values were > 0.6 while the remaining four months (Oct. to Jan.) were dry with rainfall coefficient (RC < 0.6). Three of the wet months (March, April and September) were with moderate concentration (as RC ranges from 1.0 to 2.0 while July and August had intense or big rainfall concentration with RC above 2.0). The monthly rainfall variability from 1965 through 2007 can be detected from the CV value (Table 4). According to Hare (1983), CV below 20% imply less variability and hence monthly rainfall

experienced very less variability. Yet, proper use of water harvesting technology should be devised to use and manage the intense rainfall of July and August. Moreover, early warning systems and integrated environmental management measures are required to minimize/avoid disaster and design possible remedial actions.

Table 4. Monthly and annual mean rainfall, coefficient of variation (CV) and rainfall coefficient (RC) for the decades 1965- 1984, 1985 -2007 and 1965- 2007.

Variables	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Period of 1965-1984													
MRF[1]	20.2	48.7	57.6	52.7	46.0	41.1	114.2	121.1	50.8	23.7	15.9	6.0	598.1
C.V.[2]	1.9	1.4	0.9	0.8	0.6	0.9	0.4	0.5	0.5	1.2	1.5	1.8	0.3
RFC.[3]	0.4	1.0	1.2	1.1	0.9	0.8	2.3	2.4	1.0	0.5	0.3	0.1	
Period of 1985- 2007													
MRF[1]	8.3	29.5	52.0	50.7	30.2	25.8	120.6	118.9	42.3	21.8	3.4	8.9	512.5
C.V.[2]	1.5	1.5	0.7	0.7	1.0	0.8	0.4	0.3	0.5	1.4	1.5	1.9	0.2
RFC.[3]	0.2	0.7	1.2	1.2	0.7	0.6	2.8	2.8	1.0	0.5	0.1	0.2	
Period of 1965-2007													
MRF[1]	13.8	38.5	54.6	51.6	37.5	32.9	117.7	119.9	46.3	22.7	9.2	7.6	552.3
C.V.[2]	2.03	1.48	0.78	0.75	0.83	0.90	0.41	0.42	0.51	1.28	1.94	1.94	0.24
RFC.[3]	0.3	0.8	1.2	1.1	0.8	0.7	2.6	2.6	1.0	0.5	0.2	0.2	

Notes: [1]MRF = Mean Rainfall; [2] CV = Coefficient of Variation; [3]RFC = Rainfall Coefficient

3.1.2.3. Amount, Variation, and Trends of Rainy Days

From the points of view of hydrology, and soil and water conservation, the duration, intensity and amount of rain that falls within a given area is very significant (Daniel, 1977). Accordingly, the mean annual number of rainy days was 84.93 with 23.27% probability of wet days. This implies that about 76.73% of the annual mean days from 1993-2007 were dry (Table 5). As stated in Hare (1983), CV < 20% are less, 20% to 30% moderate and > 30% highly variable, the number of rainy days are less variable for *Kiremt* while moderately variable for *Belg* and annual while *Bega* season has highest variability from 1993 to 2007 (Table 5). Concerning the amount of the rainy days, there was highest variability in *Bega* and *Belg* seasons as their CV was > 30% (i.e., 45% and 80%, respectively as compared to *Kiremt* seasons and annual values (with CV = 25%, and 18%, respectively (Table 5). However, CV above 25% is taken as significant value (Snedecor and Cochran, 1989) and hence it can be concluded that *Belg* and *Bega* are with significantly highest variability in amount of rainy days.

Both numbers of rainy days and amount for annual and all season's rainy days show decreasing trend and negative anomalies that was found significant at 0.05 levels for the aforementioned years (Table 5 and Fig. 5 A-D). This finding can be substantiated from the findings of Yilma and Zanke (2004) who reported that the rainy days of *Kiremt* in the eastern Ethiopia showed a significant decline (at 5% level) since 1982 compared to that of 1965-81.

Generally, the annual, seasonal and monthly and daily rainfall of the study area was characterized by reduced amounts during the study periods. Moreover, there were continued increases in the minimum and maximum temperatures of the study area. Yet, this requires proper meteorological data to come up with sound recommendations vis-a-vis climate change adaptive and coping strategies for the life and livelihood of the *Karrayu* community in particular and the region at large.

Table 5. Mean probability of wet days (W), coefficient of variation (C.V.), linear trend (LT) of number of rainy days and rainfall amount for *Belg, Bega* and *Kiremt* (1993-2007).

Periods	Number of Rainy Days				Rainfall Amount of Rainy Days			
	Mean	P(w)	C.V. (%)	LT(day/decade)	Mean	% of annual	C.V. (%)	LT (mm/decade)
Belg	21.13	17.61	28	- 0.57	141.03	27.98	45	- 0.17
Kiremt	46.60	38.20	18	- 0.60	306.67	60.85	25	- 0.32
Bega	17.2	13.98	43	-0.40	56.31	11.17	80	- 0.35
Annual	84.93	23.27	32	- 0.93	504.01	100.00	18	-0.56

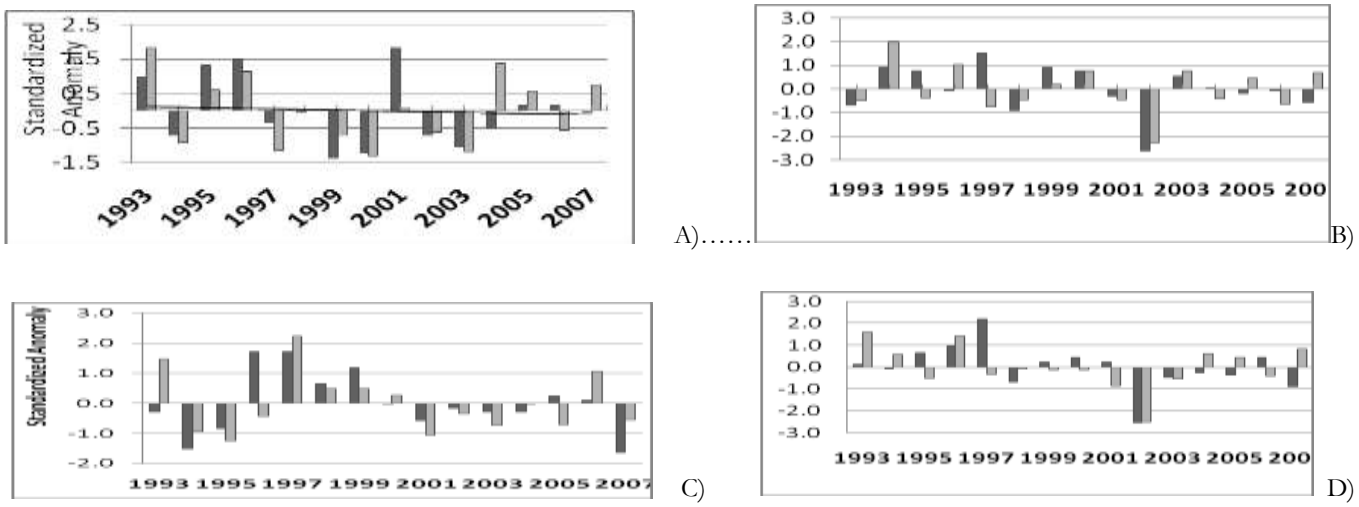


Figure. 5: Standardized seasonal and annual anomalies of number of rainy days (black bar) and rainfall amounts on wet days (white bar), (1993-2007). A) *Belg* Anomalies; B. *Kiremt* Anomalies; C. *Bega* Anomalies; D. Annual Anomalies

3.2. Pastoralists' Perception of Climate Change/Variability and Drought

As revealed in Table 6, about 68.3% and 42.5% of the respondents perceived increment in the mean maximum temperature and decrement in the mean minimum temperature, respectively. Similarly, the annual total rainfall was perceived to decrease by 65% of the respondents while about 57.5% of them indicated a decrement in rainfall variability. Moreover, nearly 2/3rd of them perceived decrease in rainfall intensity (Table 6). Furthermore, participants of FGDs and key informants' interviews indicated the presence of variations in the average temperature and rainfall. As per their perceptions, the periods of high and low temperatures were clearly known and demarcated. For instance, very high daily temperature was in June and the low temperatures were in December and January. Hence the local pastoral community is expected to adapt accordingly. But, recently there are variations in low temperature that started as of September while high temperature occurred from April through July on irregular basis and that exacerbated the resiliencies efforts of the community.

Table 6. Pastoralists' perception of temperature and rainfall variations by sample respondents.

Climatic Elements	Perceptions by Sample respondents of *Karrayu* Pastoral Community									
	Increases		No changes		Decreases		Unknown		Total	
	No	%	No	%	No	%	No	%	No	%
Max. Temperature	82	68.3	21	17.5	10	8.3	7	5.8	120	100.0
Min. Temperature	35	29.2	14	11.7	51	42.5	20	16.7	120	100.0
Rainfall Intensity	27	22.5	16	13.3	77	64.1	-	-	120	100.0
Rainfall Variation	30	25.0	16	13.3	69	57.5	5	4.2	120	100.0

In the case of rainfall during the past years, pastoralists underlined that the rainfall was relatively ample and less variable as it occurred during two rainy seasons: small rainy season (March to May) (*Arfaasa*) and big rainy season (June to September) *(Bonna)*. The former extends from March to May while the latter covers from June through September. Often, the rainfall period may extend from March to September. Within those months, the amount of rainfall was ample and harvestable in different ponds and used throughout several months. But, recently the *Karrayu* area received rainfall either in July and/or in August for only one to two months. Unlike past years, water collected and stored in ponds and surface depressions got dry up and that in turn resulted in severe water scarcity.

3.3. Livelihood Strategies by Pastoralists towards Climate Change/Variability and Drought

During drought period, pastoralists encounter shortage of food and/or finance to purchase food. The price of livestock on local market decreased and that of grain increased. In addition, the product of livestock like meat, milk, etc get deteriorated. Under such scenario, pastoralists developed numerous livelihood strategies including strategies concerning food, income, family and livestock related affairs (Table 7). This goes in accordance with Wassie and Fekadu (2015) who reported with shift from pastoral to non-pastoral livelihood activities in the *Borana* pastoral areas.

According to Table 7, over half percent of sample respondents pastoralists in the *Karrayu* area identified major livelihood coping and survival strategies to climate change and resultant drought including use of less amount of water (76%), settling around Metehara Sugar Factory (73%), dependence on Productive Safety Net Program (PSNP) (72%), decrement in amount of food consumption (64%), migration with their camels to other areas (62%), and sale of fire wood and charcoal (57%). Those livelihood strategies with least percentage of respondents include preparation of food storage devices, destocking by selling old/weak livestock, borrowing cash from 'the haves' or relatives, family splitting and migration to urban areas.

Table 7. Major livelihood strategies to climate change and variability by No. (%) respondents.

Major Livelihood Strategies		No. (%)
Coping/ Adaptive Strategies		
Food		
1	Use less amount of water	91 (75.8)
2	Eat less amount of food	77 (64.2)
3	Chewing chat	57 (47.5)
4	Borrowing food grain	52 (43.0)
5	Generating food store	18 (15.0)
Livestock		
1	Settling around Metehara Sugar Factory	87 (72.5)
2	Selling old/weak livestock before drought	46 (38.0)
Livelihood Diversification of Income		
1	Depending on Productive Safety Net Program	86 (72.0)
2	Sale fire wood and charcoal	68 (57.0)
3	Storage of butter until the price is high	57 (47.5)
4	Selling skins and hides of dead animals due to drought	54 (45.0)
5	Borrowing cash from 'the haves' or relatives	41 (34.0)
Survival Strategies		
Family		
1	Migration to other areas with their camel	74 (62.0)
2	Splitting family	39 (32.0)
3	Migration to urban areas	37 (31.0)

Note: Each option is out of 100% respondents.

As per discussion with key informants of *Fentale* district, the beneficiaries of the PSNP were identified by the representative committees selected by the community and the beneficiaries are rhetorically selected based on the degree of food-insecurity and other socio-economic criterion. Children, elders and pregnant women are given priority of getting access to the PSNP. The selected beneficiaries get access to the program during dry months (from January to June). Most of the food aid is given according to their participation in the development works identified in the area: namely, participation in public work like de-silting and digging ponds, constructing flood protection dams, road etc. Such beneficiaries are required to work 5 times per month and are given 40 birr/head/month for three months (Jan to March) and 15 kg of grain/head/month for the next three months (April to June). In most predominantly drought periods, beneficiary pastoralists in the study area rely on the PSNP during the dry period as their main coping strategies but there are numerous pitfalls in the implementation of the program as reported by Dula (2013).

As the traditional livelihood strategies have become increasingly insufficient to sustain local livelihoods during times of drought (Muller-Mahn *et al.,* 2010 cited in Dula, 2013), the importance of PSNP should be underlined here. Regarding the dependence on PSNP, it is variable among percentage of population of the pastoral communities in different *kebeles* that benefited from the program. It ranges from < 20% Haro Kersa *Kebele* to > 50% by pastoralists in Tututi, Kobo, Debiti and Dire Saden *Kebeles* (Fig.6).

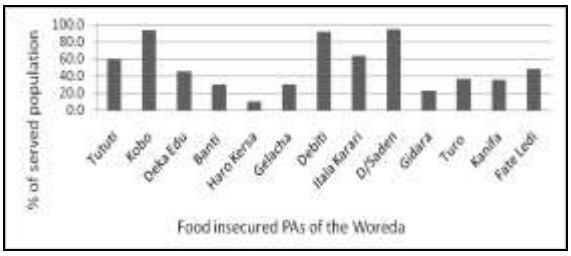


Figure 6. Percentage distribution of people served by PSNP.

According to the opinion of participants of FGDs and key informants during past years, community in *Karrayu* area relied on only livestock products like milk, meat, butter, etc. They do not have permanent settlement areas but migrate together with their cattle between "*Ona Gannaa*" (wet grazing area) and "*Ona Bona*" (dry grazing area). In olden times, there was no scarcity of grazing land and hence pastoralists did not know about farming; and culturally it was not accepted to be a farmer. But, now there is dynamism in the situation in the *Karrayu* area. Owing to all these dramatic changes since 1980s, the *Karrayu* have exercised two major livelihood modifications against recurrent drought: from mobile to sedentary and from pastoral to agro-pastoral livelihood system (this is also clearly mentioned by Dula (2013) like the one reported for Borana pastoralists by Wassie and Fekadu (2015). This is in response to the challenges faced by the pastoralists to modify their livelihoods according to the ongoing changes, to search for new alternative strategies, to diversify their livelihoods and to maintain their adaptive capacities with regard to future changes (Dula, 2013; Wassie and Fekadu, 2015).

4. Conclusion

The analysis of meteorological data of Awash and Metahara stations and the perception of pastoralists in *Karrayu* area revealed steady increments as well as decrements in the amount and patterns of temperature and rainfall, respectively. Eight distinct months of dry periods were known for water deficit and resultant drought as extreme weather events in the area. Cognizant of the climate variability and extreme weather events, the largest portions of the pastoral community perceived that numerous livelihood strategies including the use of fewer amounts of water and food, migrating to settle around Metehara Sugar

Factory, dependence on PSNP, migration with their camels to other areas, and sale of fire wood and charcoal were found to be essential to sustain the lives of humans as well as livestock.

However, to complement the community efforts, integrated livelihood development mechanisms better be devised with active involvement of different stakeholders for sustainable development of the pastoral and agropastoral communities of the *Karrayu*. These may include but not limited to early warning systems, collection of reliable climate data, disaster risk reduction and risk sharing strategies to avert the risk of vulnerable groups of the community from the hazards of climate variability in particular and change in general. Likewise, further critical studies are recommended regarding temperature and rainfall variability of the study area to substantiate the current findings and endorse very sound and applicable recommendations that can be implemented for the sustainable food, feed and water availability to the human and livestock population of *Karrayu* pastoralist area in particular and similar agroecology at large.

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Registration of 'Gudatu' Finger millet (*Eleusine coracana* sub spp*. coracana*) Variety

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Abstract: 'Gudatu', finger millet (*Eleusine coracana* (L.) Gaertn) variety, with the pedigree of Acc. 215990 was developed and released by Bako Agricultural Research center for western Oromia and similar agro-ecological areas of Ethiopia. This variety was evaluated against two standard checks (Boneya and Wama) for grain yield, disease reaction and other agronomic traits across three locations (Bako, Gute, and Diga) during the 2010, 2011 and 2012 main cropping seasons. Genotype and genotype by environment interaction (GGE) analysis revealed that Gudatu was stable and high yielder with yield advantage of 10.56 % over the standard check, Wama. Consequently, Gudatu was released in 2014 for its high grain yield potential and moderately resistant against blast disease, the major constraint of finger millet production in western Ethiopia.

Keyword: Genotypes; Finger millet (*Eleusine coracana*); *Magnaporthe oryze*

1. Introduction

Finger millet (*Eleusine* coracana) is an annual allotetraploid cereal belonging to the family Poaceae, widely grown in the arid and semiarid areas under varied agro-climatic conditions (Hilu and de Wet, 1976). Finger millet is adapted to a wide range of environments and is known for withstanding harsh environmental conditions (Lenne *et al.,* 2007). Limited availability of stable high yielding and disease tolerant finger millet variety is one of the major production constraints in the country. Therefore, development of adaptable, stable high yielding and disease resistant variety under different environments is the first and foremost steps in plant breeding.

2. Varietal Origin and Evaluation

Gudatu (Acc. 215990) was introduced from Ethiopian Institute of Bio-diversity (IBC). The variety and other pipeline genotypes were evaluated against the standard checks, Boneya and Wama, across three locations (Bako, Gute & Diga) for three consecutive years (2010-2012).

3. Agronomic and Morphological characteristics

This released variety, Gudatu (Acc. 215990) is characterized by light brown seed color, average 1000 seeds weight of 3.33 grams and has an average plant height 91.97 cm. The detailed agronomic characteristics of the variety are indicated in Table 1 below.

4. Yield Performance

Gudetu (Acc. 215990) gave seed yield ranging from 2.0 to 2.1 t h^{-1} on a farmer's field and 2.1 to 2.3 t h^{-1} on research field. The variety showed yield advantage of 10.6% over the then recent check, Wama.

5. Stability and Adaptability Performance

Eberhart and Russell (1966) model revealed that Gudetu variety showed regression coefficient (bi) closer to unity stable and widely adaptable variety than the remaining genotypes. Besides, genotype and genotype by environment (GGE) biplot analysis showed that Gudetu (Gn2) fell in the central circle, indicating its high yield potential and relative stability compared to the other genotypes (Fig 1). Generally, Gudatu (Acc 255990) was more stable and high yielder than other pipeline genotypes and standard checks.

6. Reaction to Major Diseases

Gudatu is moderately resistant to major diseases particularly blast (*Magnaporthe oryzea*), a devastating disease that affect all above ground parts of the plant.

7. Conclusion

The finger millet variety Gudatu, gave high grain yield, showed better adaptability and stable performance than the standard checks. The variety is also tolerant to blast disease. Therefore, it was released and recommended for Bako, Gute, Diga and areas with similar agro-ecology in the country.

8. Acknowledgments

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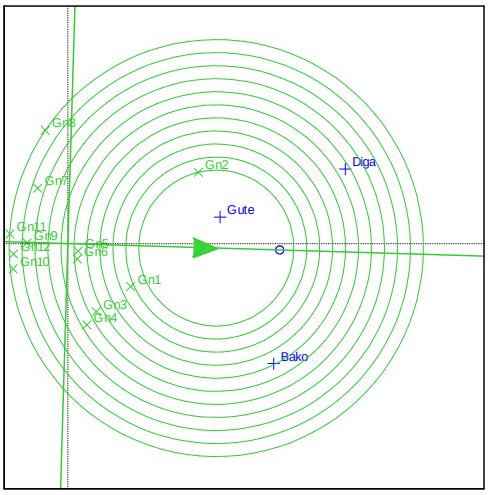
Table 1. Agronomic and Morphological characteristics of Gudatu Finger millet variety (Acc. 215990).

Agronomic characteristics of Finger millet variety Gudatu	
Variety name	Gudatu
Adaptation area	Bako, Gute, Diga and other areas with similar agro- ecology
Altitude (m.a.s.l)	1400- 1900
Rain fall (mm)	1200 – 1300
Days to heading	70-80
Days to maturity	145-150
1000 seed weight (g)	3.33
Plant height (cm)	91.97
Seed color	Light brown
Growth habit	Erect
Grain yield (ton/ha)	
On farmer's field:	2.0 – 2.1
On research field:	2.1 – 2.3
Blast disease (*Magnaporthe oryzea*) disease reaction	moderately resistant
Year of release:	2014
Breeder/Maintainer:	Bako Agricultural Research Center (BARC/OARI)



Key: Gn = Genotype

Figure 1. GGE biplot analysis depicting the stability of tested genotypes and test environment.