EFFECT OF AGRO-ECOLOGICAL ZONES ON THE NUTRITIONAL CONTENT OF BARLEY (*HORDEUM VULGARE* L.) ACCESSIONS CONSERVED IN THE ETHIOPIAN GENE BANK

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ABSTRACT: Barley (*Hordeum vulgare*) is one of conserved crop under cold storage in the Ethiopian Gene Bank, as it grows across a wide range of agro-ecological zones. This study utilized 33 barley accessions for nutrient content analysis to determine and compare the nutritional values of barley grown in different agro-ecological zones. Sample preparation and nutritional analysis were conducted following standard procedures defined by the Association of Official Analytical Chemists (AOAC). Descriptive statistical analysis and paired t-tests were employed to analyze the data. The findings revealed significant mean differences in the percentage of crude protein, moisture, and total carbohydrates content between barley sourced from Ethiopia's highlands and midlands. The results showed that the highest average crude protein (11.96%) and total carbohydrates (76.1%) were found in barley from midland agro-ecologies. Conversely, midland barley exhibited the lowest average moisture content (7.68%) and average crude fat content (1.86%). Both zones yielded an equal average mineral ash content of 2.4%.The study concluded that as altitude increases, the nutritional values of barley significantly decreased for all nutritional values except moisture and crude fat content. Based on the finding that higher altitude correlates with lower protein and carbohydrates but higher moisture and crude fat, it is recommended to prioritize barley accessions from midland agro-ecologies for breeding and food security programs aimed at maximizing crude protein and total carbohydrate content.

Key Words: Carbohydrates, Crude fat, Crude protein, Highlands, Midland,

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INTRODUCTION

Ethiopia is recognized as a center of genetic diversity and origin for numerous cultivated and wild plants (Vavilov, 1951; Harlan, 1969). However, this diversity, particularly among landraces is under severe threat from genetic erosion induced by modern agricultural practices (Melaku and Hailu, 1993). To protect this decline, the Ethiopian Biodiversity Institute (EBI), formerly the Plant Genetic Resources Centre of Ethiopia (PGRC/E) was established in 1976. The EBI focuses on preserving genetic diversity of crops, their wild relatives, and native species crucial to Ethiopia's agriculture. It has successfully collected over 90,000 accessions from more than 120 plant species, among these; barley is one of the most conserved crops, with 16,685 accessions (Angassa and Tesfaye, 2019).

Barley (*Hordeum vulgare* L.) is an economically significant and ancient crop in Ethiopia, with a cultivation history spanning at least 5,000 years (Kemelew and Alemayehu, 2011; Girma, 2014). According to Hadado et al. (2010), landraces account for over 90% of cultivated barley diversity, reflecting a deep-rooted relationship between the crop and Ethiopian farmers. Barley ranks fifth in acreage and production among Ethiopian cereals, following teff, maize, wheat, and sorghum (Teklemariam et al., 2022). It is grown across all regions of the country, primarily at altitudes ranging from 1,500 to 3,400 meters above sea level (masl) with adequate moisture (Yaynu, 2006). The majority of barley production is concentrated between 2,000 and 3,400 masl in northern and central Ethiopia (Asfaw, 2000).

This resilient crop is primarily cultivated by smallholder farmers for subsistence, especially in regions where modern agricultural methods are less feasible (Lakew et al., 1997). Barley is grown during two main seasons: the major rainy season ('meher') and a shorter one ('belg'), contributing to 20% of Ethiopia’s total cereal production (Wosene et al., 2015).

Ethiopia’s landscape includes three major agro-ecological zones: highlands (2,300–3,500 masl), midlands (1,500–2,300 masl), and lowlands (below 1,500 masl) (Dereje and Eshetu, 2011). While barley is predominantly a highland crop, it also adapts to certain midland regions (CSA, 2016). This cool-weather crop flourishes best between 2,000 and 3,500 masl (MoA, 1998). Notably, barley exhibits significant resilience, thriving in well-drained soils and tolerating higher salinity levels than most other crops (Bekele et al., 2005). "Food barley" is commonly cultivated in stressed areas where soil erosion, occasional drought, or frost limits the viability of other crops (Bekele et al., 2005). It serves as a reliable food source in the highlands, cultivated during both rainy seasons and under residual moisture conditions (Tilahun et al., 2015).

Barley types are generally categorized as food or malting barley based on their use; in Ethiopia, the vast majority of cultivation area is allocated to food barley. It is a staple in various traditional recipes and beverages, including Bread, Injera, Kolo, Genfo, Kitta, Kinche, Beso, Tella, and Borde. Additionally, barley straw is an essential component of animal feed, particularly during the dry seasons in the highlands (Getachew et al., 1996).

Nutritionally, barley possesses health-promoting functional properties (Cook, 2013; Idehen et al., 2017). Its primary components are carbohydrates, with low fat content, proteins, minerals, and vitamins (especially vitamin E). It is also rich in dietary fiber and antioxidants, predominantly polyphenols (Das et al., 2016).

Despite its importance, there remains a knowledge gap regarding how altitude, soil, and climate affect barley's nutritional content across different zones. While barley is vital for food security, existing research has not adequately compared the nutritional attributes of barley grown in distinct agro-ecological regions. This research gap presents an opportunity to investigate barley’s nutritional profile across diverse zones, considering ecological, economic, and societal dimensions. Addressing these gaps could strengthen the understanding of barley’s role in enhancing food security and improving agricultural practices in regions dependent on this resilient crop.

This study aims to bridge that gap by analyzing 33 accessions of conserved barley (15 from the midlands and 18 from the highlands). Therefore, the objective of this study was to evaluate and compare the nutritional value of barley cultivated in highland and midland regions, assessing the impact of agro-ecological conditions on its nutritional composition. These findings will enhance our understanding of barley's nutritional profile, benefiting both domestic consumption and future research efforts.

MATERIALS AND METHODS

Sample Size and Sample Preparations

A total of 33 barley accessions were utilized, comprising 15 accessions from the midland and 18 accessions from the highland region. Each sample accession, weighing 30 g, was sourced from the EBI cold storage facility, accompanied by complete passport data collection specific to each agro-ecological zone. Seeds were hand-sorted to remove foreign materials. Raw seeds (with seed coats) were processed into flour using an electric grinder (Retsch, ISO9001) and passed through a 0.5 mm mesh sieve. Flour was stored in airtight polyethylene bags. Analysis was conducted in duplicate (n=2) at the EBI laboratory.

Nutritional Content Analysis

Nutritional values were determined according to the Association of Official Analytical Chemists (AOAC. 2000) methods.

Determination of Moisture Content

The moisture content of the barley samples was determined by the oven drying method (AOAC, 2000). A clean, empty aluminum dish and its lids were dried in a drying oven (Moment, Germany) at 100°C for 1hr, and cooled in a desiccator (with granular silica gel) for about 30 min, and weighed. For each accession were conducted in 2 replicates. The samples prepared for each accession were mixed thoroughly, and about 3.0 g of flour was weighed in duplicate. The dishes and the samples were placed in the drying oven and dried for 3hr at 105°C. After drying, the samples were cooled in a desiccator for 30 min and weighed. The percentage of moisture calculates as follow:

$$\% \, Moisture = \frac{(wt \, of \, empty \, dishes + sample) - (wt \, of \, dishes + dry \, sample)) * 100)}{wt \, of \, sample}$$

Determination of Crude Protein

The protein content of the barley flour samples was determined by the Kjeldahl method (AOAC, 2000). 1g of barley sample was used for this process, the experiment was passed three steps, such as digestion, Distillation, and titration was involved. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and Kjeldahl tablet containing copper sulfate and potassium sulfate as a catalyst by 420°C temperature. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with 0.1N hydrochloric acid. Then the result is calculated based on the conversion factor from Nitrogen to protein.

Protein content (% N × 5.83) was determined by the Kjeldahl method described by (AOAC, 2000).

$$\% \, total \, Protein = (\% \, N \, x \, 5.83)$$

Where, % N = Percentage of Nitrogen,

5.83 = barley conversion factor

Determination of Crude Fat Content

The crude fat content of barley flour samples was determined according to AOAC (2000) by Soxtec using Petroleum ether (b. pt 40-60°C) solvent. About 3.00 g of barley flour sample was added into the extraction thimbles and covered with fat-free cotton. The thimbles with the sample content were placed into soxtec extraction chamber and then switched on the heater. The cooling water was switched on, and 25 ml of Petroleum ether was added to the extraction can and attached to the corresponding thimbles in soxtec extraction chamber. Overall extraction processes were conducted for about 1hr including solvent recovery and evaporate. Then the extraction can with their content were removed from the extraction chamber and placed in the drying oven at 100°C for about 30 min, cooled to room temperature in the desiccator for about 30 min and weighed. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction can before and after extraction as a percentage.

Calculate the fat content as follows: $Crude\ fat\ = \frac{((weight\ of\ can + fat) - (weight\ of\ can))*100}{weight\ of\ sample}$

Determination of Total Ash Content

The total ash content of barley flour samples was determined by the dry ash method in a muffle furnace according to AOAC (2000). The crucible was placed in the muffle furnace (Carbolite CSF 1200) at 550°C overnight to ensure that impurities on the surface of the crucible are burned off and the crucible cooled in the desiccator for 30 min. Then the crucible was weighed. About 3.0 g of barley flour of each sample in duplicate was added into each crucible, then put into the muffle furnace and heated at 550°C overnight.

Finally, taken out of the crucible from the Muffle Furnace and immediately place in a desiccator till cooled to room temperature, and each crucible with ash was weighed. The weight of total ash was calculated by difference and expressed as a percentage of samples.

Calculate the percentage ash content (wet weight basis) as follows:

$$\%\ \text{ash (wet)} = \frac{((weight\ of\ crucible +\ ash) - (weight\ of\ crucible)) * 100}{weight\ of\ sample}$$

Calculation of ash content on a dry basis (when the moisture content is known) is as follows:

$$\% \, ASH \, (dry) = \frac{\% \, ash \, (wet) * 100}{(100 - \% \, moisture)}$$

Total Carbohydrate Content

Total Carbohydrate content was obtained by subtracting the sum of the combined percent of moisture, protein, ash, and fat from 100 described by (AOAC, 2000). The result was calculated as follows;

Total Carbohydrate content = 100 – {moisture (g %) + crude protein (g %) + total fat (g %) + total ash (g %)}

Where (g %) = grams per 100 grams.

Statistical analysis

Experiments were conducted at the Ethiopian Biodiversity Institute laboratory, with each analyzed in duplicate (n=2). Summary statistics, tables, and line graphs were utilized to present the research data effectively. A two-sample t-test was employed to evaluate the mean differences in nutritional value of Ethiopian barley across various agro-ecological zones, with a significance level set at 5%.

The independent samples t-test was used to compare the means of two independent groups, assessing statistical evidence for significant differences between the population means, assuming equal variance. This assumption was tested using Levene's Test for homogeneity of variances at a 10% significance level. If this assumption was not met, and sample sizes differed across agro-ecological zones, the reliability of the p-value could be compromised. The output of the independent samples t-test also provides an approximate t-statistic that does not rely on the assumption of equal population variances.

RESULTS

Agro-ecological Zones

The study was conducted, on the barley accessions that preserved in the Ethiopian gene bank, were collected from altitudes ranging from 1,910 to 3,490 masl. In total, 33 accessions (15 midland, 18 highland) were gathered between June 6 and June 25, 1980 (E.C). Collections included samples from Debub Wello (8 accessions from Dessie Zuria, 8 accessions from Were-Ilu) and North Shewa (15 accessions from Gerar Jarso, 1 accession from Yaya Gulele and 1 accession from Sululta) different part of Ethiopia, as illustrated in Figure 1.

Figure 1. Agro- ecological Zone of Barley Accession Conserved in Gene Bank of Ethiopia

The Nutritional Analysis of Barley

The nutritional analysis of barley accession that was conserved at Gene Bank of Ethiopia was conducted based on dry weight for all agro-ecological zone of Ethiopian in 2010 E.C. As indicated in the Table 1, the minimum percentage of crude protein (10.59%) was observed in the barley that sampled from highland area of Ethiopian whereas; maximum (13.25%) was obtained in midlands, highlighting the nutritional superiority of midland barley. On contrary, the highest (2.38%) percentage of crude fat was recorded from barley grown in the highland and minimum (1.61%) percentage of crude fat were obtained in the barley that collected from midland while; both maximum (3.04%) and minimum (2.11%) percentage of mineral ash was obtained at highland areas. On contrary, the maximum (80.26%) and minimum (73.55%) percentage of total carbohydrates content were obtained in the barley that collected from midland. And also, the highest (9.55%) and lowest (4.64%) percentage of moisture were measured from barley gotten in highland and midland areas respectively. These nutritional variations underline the importance of agro-ecological zones in influencing the quality of barley, which has implications for agricultural practices and nutritional profiling of barley cultivars in Ethiopia.

Table 1. Experimental Results of Nutritional Analysis of Barely Based on Dry Weight

S/N	Accession No.	Crude Protein (%)	Crude Fat (%)	Mineral ash (%)	Moisture (%)	Total Carbohydrate (%)	Altitude	Agro-ecological Zone
1	4918	10.86	1.84	2.40	4.64	80.26	2250	Midland
2	4919	10.77	2.09	2.49	4.74	79.91	2250	Midland
3	4923	12.17	1.70	2.69	6.30	77.14	2250	Midland
4	4926	11.95	1.73	2.53	6.65	77.14	2200	Midland
5	4927	11.77	1.63	3.04	8.03	75.53	2540	Highland
6	4930	11.09	1.80	2.35	6.23	78.53	1910	Midland
7	4934	11.50	1.89	2.43	7.41	76.77	2300	Midland
8	4935	11.91	2.05	2.44	9.44	74.16	3050	Highland
9	4938	11.00	1.86	2.72	9.54	74.88	3160	Highland

10	4939	11.97	1.86	2.58	9.08	74.51	3350	Highland
11	4943	11.11	1.79	2.66	9.55	74.89	3490	Highland
12	4945	10.77	1.86	2.34	9.40	75.63	3450	Highland
13	4946	11.01	2.00	2.24	9.54	75.21	3330	Highland
14	4947	11.11	2.04	2.23	9.41	75.21	3420	Highland
15	4949	11.13	1.86	2.34	9.45	75.22	3300	Highland
16	4952	11.14	1.86	2.41	9.43	75.16	1910	Midland
17	4954	11.36	1.88	2.36	9.24	75.16	2200	Midland
18	4955	12.09	1.87	2.24	9.42	74.38	2250	Midland
19	4958	12.34	1.90	2.31	9.38	74.07	2250	Midland
20	4961	12.18	2.26	2.11	9.37	74.08	2970	Highland
21	4962	11.81	2.14	2.12	9.31	74.62	3120	Highland
22	4963	11.04	1.85	2.35	9.23	75.53	3100	Highland
23	4965	11.14	1.81	2.51	9.44	75.10	3040	Highland
24	4966	11.12	1.83	2.41	9.51	75.13	3040	Highland
25	4969	10.59	1.94	2.36	9.42	75.69	2500	Highland
26	4971	11.42	1.78	2.30	9.33	75.17	3100	Highland
27	4973	11.12	2.38	2.34	9.30	74.86	3100	Highland
28	4975	12.49	2.04	2.23	8.47	74.77	3050	Highland
29	4976	12.68	1.81	2.36	8.28	74.87	2100	Midland
30	4978	12.73	2.18	2.34	7.63	75.12	2100	Midland
31	4979	13.25	1.82	2.79	7.21	74.93	2300	Midland
32	4984	13.05	1.85	2.18	9.37	73.55	2250	Midland
33	4995	12.35	1.61	2.34	9.30	74.40	2250	Midland

Variation in Nutritional Value of Barley across Agro-ecological Zone of Ethiopia

The percentage of crude protein content of Ethiopian barley conserved at Gene Bank of Ethiopia was varied across different agro-ecological locations. As it can be depicted in Figure 2, the percentage of crude protein for barley cultivated in the midland was higher but also only in a few accession it got less as compared to highland areas. In contrast, the highest percentages of crude fat were predominantly observed in barley grown in the highland areas, as shown in Figure 3. This trend indicates that highland environments contribute positively to the fat content of barley, highlighting potential regional advantages for producing barley with enhanced lipid profile.

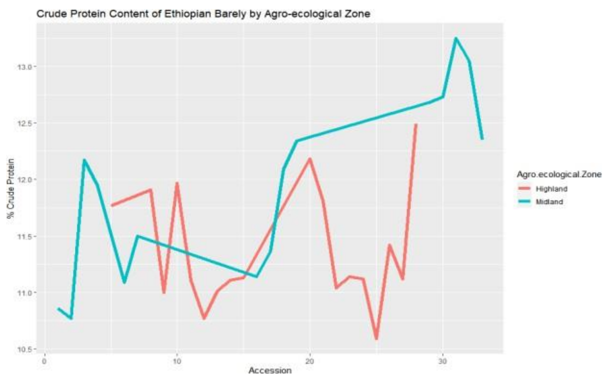

Figure 2. Crude Protein Content

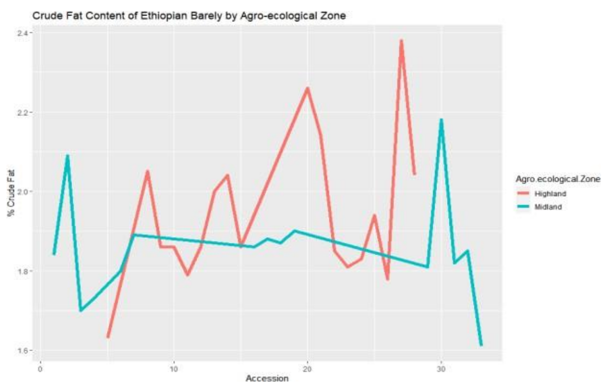

Figure 3. Crude Fat Content

The percentage content of mineral ash of barley for both highland and midland areas showed some sort of fluctuations across agro-ecological zone of Ethiopia (see Figure 4.) where; the highest moisture content was consistently observed in barleys from all highland agro-ecological zones, as shown in Figure 5. This trend suggests that highland environments may contribute favorable conditions for moisture retention in barley.

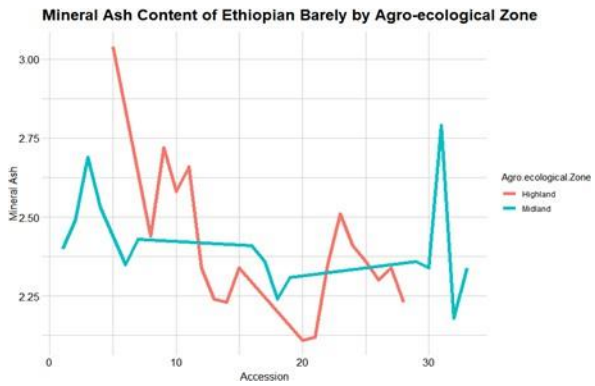

Figure 4. Mineral Ash Content

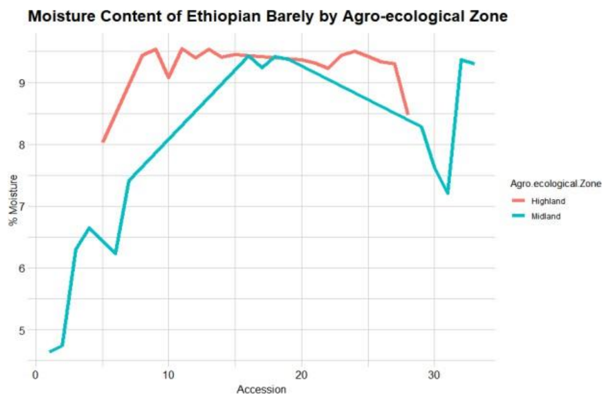

Figure 5. Moisture Content

The majority of the highest percentages of total carbohydrates were found in barley sourced from midland agro-ecologies. However, certain accessions from highland regions also exhibited elevated carbohydrate levels, as depicted in Figure 6. This finding highlights the potential for highland barley to possess competitive carbohydrate content, despite midland barley generally being superior in this regard.

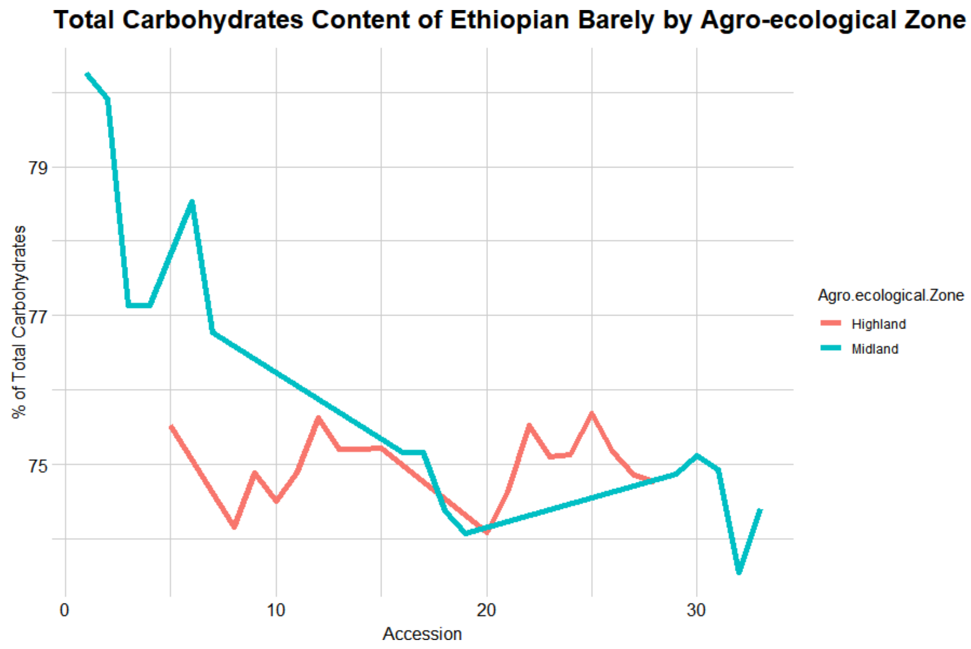

Figure 6. The Percentage of total carbohydrates Content of Barley

The Mean Difference of Nutritional Content of Barley across Agro-ecological Zone

To achieve the objectives of the current study, two independent t-tests were conducted under the assumption of homogeneity of variances, which posits that the variances of the percentage content for each nutritional component of barley are equal across agro-ecological zones.

Levene's Test for equality of variances was performed at a 10% significance level for all nutritional values, as detailed in Table 2. The test revealed significant homogeneity of variances for crude protein, moisture, and total carbohydrates, indicating comparable variance in these nutritional components of Ethiopian barley between midland and highland agro-ecological zones. Consequently, independent samples t-tests were conducted to assess the mean differences in these nutritional values of barley between the midland and highland regions. This analysis provides insights into the nutritional disparities that may exist due to environmental factors inherent to each region.

Table 2. Levene's Test for Homogeneity of Variances

Nutritional Components	Df	F-value
Crude Protein	1	3.32*
Crude Fat	1	0.98
Mineral Ash	1	0.8
Moisture	1	29.10*
Total Carbohydrates	1	9.37*
Total	31	

(*Significant at 10 % level)

Based on two independent samples t-test shown in the Table 3, a significant mean difference in the percentage content of crude protein, moisture and total carbohydrates was found between highland and midland agro-ecological locations of Ethiopian barley that conserved in the gene bank. As a result, the highest (11.96%) average percentage of crude protein was found in the barley that was taken from midland agro-ecology. Similarly, the highest (76.1%) mean percentage of total carbohydrates was obtained from barley gotten in the midland areas. In contrasts, the minimum (7.68%) average percentage of moisture was measured in the barley collected from midland agro-ecological zone.

Table 3. Independent t-test for Nutritional Contents of Barley across Agro-ecological Zone of Ethiopia

Nutritional Components	Agro-ecological Zone	Mean	SD	Df	T
Crude Protein	Highland	11.37	0.52	31	-2.52*
	Midland	11.96	0.80		
Crude Fat	Highland	1.94	0.18	31	1.52
	Midland	1.86	0.14		
Mineral Ash	Highland	2.41	0.23	31	-0.11
	Midland	2.41	0.16		
Moisture	Highland	9.27	0.40	31	3.84*

	Midland	7.68	1.70		
Total Carbohydrates	Highland	75	0.46	31	-2.12*
	Midland	**76.1**	2.11		

(*Significant at 5 % level)

DISCISSIONS

Barley is a crucial staple food crop and a cool-weather species predominantly grown in the highlands of Ethiopia, with an optimal altitude range of 2,000 to 3,500 meters above sea level. Understanding the impact of agro-ecological zones is vital for assessing the nutritional content of crops, as these values are heavily influenced by environmental factors (Dereje and Eshetu, 2011).

The highest crude protein percentage (11.96% ±0.80) was observed in barley from midland agro-ecologies, while the lower protein content (11.37% ±0.52) was recorded in highland areas. These findings align with previously reported values ranging from 7.50% to 12.12% (Jood and Kalra, 2001). Furthermore, it has been established that the protein content in barley is significantly influenced by the cultivar (Qi et al., 2006) and can vary based on growth conditions, particularly the rate and timing of nitrogen fertilization (Arendt and Zannini, 2013). This indicates that while midland barley tends to possess superior crude protein levels, there are exceptions that suggest adaptability and environmental influences on nutritional traits.

A statistically significant difference was also observed in the average crude fat content of barley. The highest crude fat percentage (1.94% ±0.18) was found in highland barley, while midland samples showed a slightly lower average (1.86% ±0.14). These results are consistent with the chemical composition reported by Makeri et al., (2013). This trend indicates that highland environments contribute positively to the fat content of barley, highlighting potential regional advantages for producing barley with enhanced lipid profile.

Additionally, the mineral ash content remained relatively uniform across all agro-ecological zones, although significant variations were noted in the moisture content of barley from different regions. Moisture percentages were recorded at 9.27% ±0.40 for highland barley and 7.68% ±1.70 for midland barley. Notably, moisture content increased with altitude, suggesting that it is influenced by both elevation and the hygroscopic capacity of the seed (Qi et al., 2006). This trend suggests that highland environments may contribute favorable conditions for moisture retention in barley.

The average difference in total carbohydrate content was found to be statistically significant as well. Midland barley exhibited the highest percentage of total carbohydrates (76.1% ±2.11), while highland barley showed a value of 75% ±0.46. This trend indicates that as altitude decreases, the percentage of total carbohydrates tends to increase, likely due to the reduction in certain nutritional values in the samples. Barley consistently demonstrates higher total carbohydrate levels compared to most other crops, as supported by various studies in the literature (Gashaw, 2021).

CONCLUSIONS AND RECOMMENDATIONS

This study established significant differences in the nutritional value of barley across Ethiopia's agro-ecological zones. As altitude increases, crude protein and total carbohydrates tend to decrease, while moisture content and crude fat increase. These variations can be attributed to factors such as temperature, soil composition, and annual rainfall, which differ across agro-ecological zones.

Recommendations:

- Focus on midland barley in agricultural practices for enhanced food security and dietary quality due to its higher nutritional values.

- Conduct further studies to investigate the mechanisms behind nutritional variations across agro-ecological zones and assess environmental impacts on barley quality.
- Policymakers should consider agro-ecological factors affecting barley production and nutrition when developing agricultural policies and food security initiatives.

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