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Research Article

Biochemical Composition Variation among Southern Ethiopian Arabica Coffee (*Coffea arabica L.*) Genotypes

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Coffee (*Coffea arabica* L.) provides several health benefits to users due to its strong medicinal and nutritional properties and caloric value. Green bean proximate composition diversity is unknown among the coffee genotypes now cultivated in southern Ethiopia. The study's major goals are to determine the variability in green bean proximate composition among coffee genotypes and to see if there are any relationships between green bean proximate attributes. Therefore, a nutritional laboratory experiment was carried out at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). Using the augmented design, a total of 104 entries were examined, including 100 accessions from southern Ethiopia and four standard checks. Each accession had data on 07 proximate composition parameters of green beans. The presence of significant ($P < 0.05$) differences among the examined accessions for most of the traits considered was revealed by analysis of variance, and a wide range of variation was detected for several traits, indicating that the coffee germplasm accessions have high genetic variability. According to the findings, coffee beans have crude protein (6.93 to 10.14%), total lipids (8.89 to 16.08%), crude ash (2.51–5.47%), crude fiber (6.79–22.25%), dry matter (89.08 to 91.63%), carbohydrate (40.65 to 59.38%), and caloric value (307.39–382.77 k/calories). One hundred four arabica coffee accessions were grouped into ten distinct groups by 20 (19.23%), 21 (20.19%), 39 (37.50%), 12 (11.54%), 04 (3.85%), 03 (2.88%), 02 (1.92%), 01 (0.96%), 01 (0.96%), and 01 (0.96%). The majority of intercluster distances were significantly varied, showing that diversity exists that can be utilized through selection and hybridization. Clusters III and *X* had the greatest intercluster distance ($D2 = 344.16$), followed by clusters II and *X* ($D2 = 236.33$), VII and *X* ($D2 = 199.04$), and clusters VI and I ($D2 = 106.25$). Clusters I and IV had the smallest intercluster distance ($D2 = 10.09$), followed by II and IV ($D2 = 10.66$), and I and VI ($D2 = 11.03$). The first three principal components with eigenvalues larger than one explained 71.84% of the overall variation. In general, genotypes differed in green bean proximate composition and might be used as gene sources to generate future green bean varieties with appropriate biochemical composition.

1. Introduction

Coffee (*Coffea arabica* L.) originated in Ethiopia and there is significant genetic diversity in the country. Ethiopia is the highest producer of coffee in Africa and the fifth major exporter in the world next to Brazil, Vietnam, Colombia, and Indonesia, contributing to 4.2% of the total world coffee production [1]. Coffee is one of the most widely consumed beverages on the planet. The species *Coffea arabica* (arabica) and *Coffea canephora* (robusta) are used to make the majority of coffee beverages consumed around the world. Because of its sensory characteristics, the former is deemed superior and commands greater pricing on the international market [2]. Green coffee beans are mature or immature coffee beans that have not been roasted. The exterior pulp and mucilage have been removed by wet or dry processing, and the wax coating on the outside surface is intact [3]. Caffeine is primarily responsible for the stimulant properties of coffee brew [4]. However, this beverage contains a vast variety of chemical components, some of which have numerous beneficial properties. Green coffee beans have a diverse spectrum of chemical components that react and

Table 1: Description of the genotypes.

Genotype	Districts	Specific location	Altitude (m.a.s.l)	Total no collected
Aw 05/06, Aw 59/06, Aw 94/06, Aw 111/06	Bensa	Tibiro	1750–1800	4
Aw 64/06, Aw 103/06	Bensa	Silinga	1740–1770	2
Aw 81/06, Aw 66/06, Aw 12/06, Aw 99/06	Bensa	Ware	1850–1210	4
Aw 92/06, Aw 96/06, Aw 106/06	Bensa	Bensha	1700–1930	3
Aw 79/06	Bensa	Hedamo	1800	1
Aw 58/06, Aw 29/06, Aw 107/06	Bensa	Segera	1750–1930	3
Aw 97/06, Aw 100/06, Aw 67/06, Aw 108/06, Aw 04/06	Bensa	Setamo	1790–2015	5
Aw 30/06, Aw 93/06, Aw 104/06	Bensa	Golisa	1800	3
Aw 71/06, Aw 98/06, Aw 89/06, Aw 78/06, Aw 73/06	Bensa	Shema lega	1790–2020	5
Aw 10/06, Aw 62/06, Aw 91/06, Aw 84/06, Aw 28/06, Aw 95/06	Bensa	Gungvma	1720–1790	6
Aw 27/06, Aw 68/06, Aw 83/06, Aw 72/06	Bensa	Hatese	1750–1810	4
Aw 02/06, Aw 88/06, Aw 90/06	Bensa	Micharo-2	1720–1800	3
Aw 67/06, Aw 112/06	Bensa	Mulke	1750–1760	2
Aw 60/06, Aw 61/06, Aw 109/06	Bensa	Abaye	1740–1750	3
Aw 08/06, Aw 22/06, Aw 26/06, Aw 74/06, Aw 76/06	Bensa	Leleno	1750–1830	5
Aw 14/06	Bensa	Mike	1780	1
Aw 105/06	Bensa	Agensa	1980	1
Aw 34/06, Aw 65/06	Dara	Chire	1800	2
Aw 16/06, Aw 75/06, Aw 80/06	Dara	Kisho	1770	3
Aw 01/06, Aw 07/06, Aw 41/06, Aw 51/06	Dara	Wachi cha	1800	4
Aw 24/06	Dara	Boreta	1750	1
Aw 21/06	Dara	Doke	1750	1
Aw 23/06	Dara	Olone	1750	1
Aw 19/06, Aw 57/06, Aw 85/06	Dara	HalelaDaka	1750	3
Aw 49/06, Aw 54/06, Aw 87/06	Dara	Buna Tawaba	1740	3
Aw 53/06, Aw 56/06, Aw 63/06, Aw 77/06	Dara	Loya	1750	4
Aw 11/06, Aw 25/06, Aw 42/06, Aw 55/06	Dara	Chiro	1800	4
Aw 06/06, Aw 39/06, Aw 52/06, Aw 70/06	Dara	Shilicho	180–1810	4
Aw 32/06, Aw 40/06, Aw 43/06	Dara	Babe Kombolcha	1830	3
Aw 31/06, Aw 38/06, Aw 46/06	Dara	AlemeKancha	1750–1800	3
Aw 17/06, Aw 18/06, Aw 45/06, Aw 82/06	Dara	Bango Markos	1750–1875	4
Aw 03/06, Aw 09/06	Dara	Dubancho	1760–1800	2
Aw 15/06, Aw 20/06, Aw 86/06,	Dara	Megenecho	1740–1760	3
Checks				
744, 7440, 75227, 1377				

interact during the coffee manufacturing process, resulting in a final product with even more structure diversity and complexity [3]. The nutritional contents and characteristics of coffee bean beverages are not well understood. In the literature, there is very little information on these characteristics of coffee's nutritional contents. However, there are still considerable knowledge gaps, and further research is needed to better identify the variation in nutritional contents of coffee arabica genotypes.

As a result, a detailed analysis of the nutritional and biochemical constituent compositions of commercially available arabica coffee beans from southern Ethiopia has been undertaken in this study. The goal of this study was to determine the proximate and bioactive chemical compositions of 104 coffee accessions collected in Ethiopia's southern regions. Green bean biochemical compounds can be used to forecast arabica coffee biochemical compound variability and provide a foundation for developing a coffee biochemical data library. The primary goal of this study was to define coffee accessions based on their biochemical composition and group them into clusters for breeding purposes. Using principal component analysis, the study also seeks to find the traits that contribute the most to the variation in the data. As a result, the purpose of this study was to identify the green bean proximate properties of arabica coffee genotypes collected from southern Ethiopia, as well as to assess the extent of biochemical heterogeneity among genotypes.

2. Methodology

2.1. Description of the Trial Site. The study was conducted at Awada Agricultural Research Subcenter. It is located in southern Ethiopia near Yirgalem, 315 kilometers from Addis Ababa. The subcenter is in southern Ethiopia's moderate to chilly semi-arid mid-highland agroecology. Geographically, it is situated in 6°3′N latitude and 38°E longitude, at a height of around 1740 meters above sea level. With an average precipitation of 1342 mm per year, the area has a semi-bimodal rainfall pattern with double wet and dry seasons. The average annual minimum and maximum air temperatures are 11 and 28.4 degrees Celsius, respectively, with an annual mean minimum and maximum rainfall of 858.1 and 1676.3 millimeters [5].

2.2. Genotypes. The investigation covered 100 *Coffea arabica* genotypes and involved four conventional checks. Coffee genotypes were gathered from promising and representative sites in Ethiopia's southern coffee-growing region. Table 1 shows the geographical origins of the genotypes that were gathered.

2.3. Trial Management and Experimental Design. Treatments consisted of 100 coffee accessions and fields established at the Awada Agricultural Research. Moreover, four released varieties (75227, 744, 7440, and 1377) were included as standard checks. The experiment was laid down in the field using augmented design, which is used with replicated controls (checks) to assess the performance of nonreplicated accession in complete block designs in five blocks [6]. A single treatment consisting of ten trees. The plant-to-plant spacing used was two meters by two meters, while the spacing between blocks was four meters. All the recommended agronomic practices were applied uniformly to all the plots [7].

2.4. Procedures for Coffee Harvesting and Processing. One treatment included ten coffee trees and a total of 5 coffee plants were used to prepare coffee samples for biochemical analysis from each treatment. Green cherries and foreign material were separated from healthy and red ripe cherries before pulping. The samples were properly processed for biochemical analysis utilizing the wet-processing method (pulping, fermentation, and drying). After the cherries were picked, each genotype was pulped separately using a single disc hand pulper. Pulped cherries were gathered in large plastic buckets, which were then cleaned of pulps and floater parchments. Wet parchment beans were then transferred to the other bucket, which was then filled with fresh water until the parchment beans were completely submerged in the water for fermentation. The wet parchment coffee was fermented for 40 hours before the first washing. According to Abrar et al. [8], samples were then immersed for 24 hours before being washed. When the mucilage had completely decomposed, the parchment coffee beans were thoroughly washed to remove all mucilage. The resulting green parchment beans were prepared and placed on mesh wire in direct sunlight until they were totally dried or their moisture content had reached 10.5–11.5%. Six kilograms of ripe, red coffee cherries from each treatment were used. For each treatment, 1.5 kilograms of clean coffee were made and used as biological samples. Sample parchment green beans were labeled and packed in white perforated plastic bags when they reached the appropriate moisture content.

2.5. Laboratory Analysis. An arbitrary code was assigned to all of the samples that were prepared (an identity letter and number). Green bean samples were labeled with an arbitrary code and brought to the lab. In order to investigate the level of variability among coffee (*Coffea arabica* L.) germplasm accessions based on biochemical features, a laboratory experiment was undertaken at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) nutrition laboratory. A total of 2 to 8 grams of dry, powdered coffee were used as technical samples for each treatment in the study of each biochemical parameter. The following are the biochemical examination methodologies for coffee beans:

2.6. Analyzing the Biochemical Makeup of Coffee Bean Samples. The procedure was used to assess the approximate composition (moisture content, crude protein, crude fat, crude fiber, crude ash, total carbohydrate, and calorie value) of coffee row beans [9].

2.6.1. Moisture Content Determination. The moisture content of a powdered coffee sample was tested in an oven using the drying method given in Ref. [9]. Weighing 2 grams of sample onto a preweighed dish and drying it in an air pressured draft oven at 105°C until the constant weight of dry matter was reached was used to assess the moisture content of the sample. The following formula was used to determine the moisture content of the sample:

$$\text{Moisture}\,(\%) = \left[\left\{\frac{(\text{Wt. of original sample} - \text{Wt. of dried sample})}{\text{Wt. of original sample}}\right\} \times 100\right], \quad (1)$$

2.6.2. Crude Protein Determination. The crude protein content of the powdered coffee sample was determined using Kjeldahl's method, as defined in Ref. [9], which involves protein digestion and distillation.

Digestion of protein: About 2 grams of the material was weighed and placed in 250 ml Kjeldahl flasks with an ash-free filter paper. Then 15–20 ml of 98% concentration sulfuric acid and 1 gram of digestion mixture (as a catalyst) were added. In the digesting chamber, the entire combination was heated until translucent residue contents were recovered. After that, it is allowed to cool. After chilling, the digest was transferred to 100 mL volumetric flasks and topped up with distilled water before being distilled with Markham distillation equipment.

Protein distillation: The Markham distillation apparatus was steamed for 15 minutes before use, following which a 100 mL conical flask containing 5 mL of 2% boric acid and 1 or 2 drops of the mixed indicator was placed under the condenser, with the condenser tip submerged in the liquid. A small funnel aperture was used to pipette around 5 ml of the digest into the apparatus's body. After washing the digest with distilled water, 3–4 drops of phenolphthalein and 5 ml of 40% (W/V) NaOH solution were added. The digest was steamed in the condenser until enough ammonium sulfate

was recovered. The color of the boric acid plus indicator solution changed from red to green, indicating that all of the ammonia had been released. The solution in the receiving flask was titrated with 0.1 N hydrochloric acid until it reached a purple endpoint. Along with the sample, a blank was run through. The percentage of nitrogen was estimated after titration using the formula:

$$\%\text{Nitrogen} = (\text{Vs} - \text{VB}) \times \text{MM acid} \times 0.01401 \times 100W. \quad (2)$$

Where, Vs = Volume (ml) of acid required to titrate the sample; VB = Volume (ml) of acid required to titrate the blank; M acid = Molarity of acid; W=Weight of sample (g).

Then, the percentage of crude protein in the sample was calculated from the % nitrogen as follows:

$$\%\text{Crude protein} = \%N \times F, \quad (3)$$

where, F (conversion factor), is equal to 6.25 [9].

2.6.3. Crude Fat Determination. Soxhlet extraction for 24 hours was used to evaluate the crude fat content of the powdered sample. A total of 3 grams of materials were correctly weighed into labeled thimbles. The 250 mL dried boiling flasks were weighed and filled with approximately 150 mL petroleum ether (boiling point 40–60°C). Cotton wool was stuffed into the extraction thimbles. The Soxhlet device was then put together and allowed to reflux for 24 hours. The thimble was carefully removed, and the petroleum ether from the top container was collected and emptied into another container for reuse. After that, the boiling flask was baked in a hot air oven until the petroleum ether was practically gone. It was dried, cooled in desiccators, and weighed [9].

$$\text{Fat}(\%) = \left(\frac{\text{Wt. of fat}}{\text{Wt. of original sample}}\right) \times 100. \quad (4)$$

2.6.4. Crude Fiber Determination. In a fiber flask, a 2 grams fat-free sample of powdered coffee was introduced to 100 ml of 0.255 N H_2SO_4. The mixture was then heated for one hour under reflux with a heating mantle/layer. A fiber sieve cloth was used to filter the heated mixture. The difference was discarded, and the residue was returned to the flask, which was then filled with 100 ml of 0.313 M NaOH and heated under reflux for another hour. To dissolve any organic constituents, the mixture was filtered through a fiber sieve cloth and 10 cc of acetone was added. The residue was rinsed twice on the sieve cloth with 50 mL of hot water before being put into the preweighted crucible. To remove moisture, the crucible with the residue was oven-dried overnight at 105°C. The residue-filled oven-dried crucible was chilled in a desiccator before being weighted (W1) and ashed at 550°C for 4 hours [9]. The crucible was cooled in a desiccator and weighted to get white and grey ash (free of carbonaceous particles) (W2). The crude fiber percentage was calculated as follows:

$$\text{Fiber}(\%) = \left[\left\{\frac{(W1 - W2)}{\text{Wt. of sample}}\right\} \times 100\right]. \quad (5)$$

Where: $W1$ = Oven dried crucible containing the residue; $W2$ = Crucible containing white and grey ash.

2.6.5. Ash Content Determination. After the material has been entirely burned at 550°C in a muffle furnace, ash is an inorganic residue that remains. It is the sum of all inorganic elements that are not volatile. In an ashing muffle furnace, approximately 8 grams of finely ground dried coffee powder sample was weighed into a porcelain crucible and cremated (burned) at 550°C for 6 hours until ash was recovered. Desiccators were used to chill the ash before reweighing it [9]. The following formula was used to determine the percent (%) ash content in the coffee sample:

$$\text{Ash}(\%) = \left(\frac{\text{Wt. of ash}}{\text{Wt. of sample taken}}\right) \times 100. \quad (6)$$

2.6.6. Total Carbohydrate Determination. The overall percentage carbohydrate content of the coffee sample was calculated by subtracting 100 from the total values of crude protein, crude lipid, crude fiber, moisture, and ash constituents of the sample. The result is the sample's % carbohydrate constituent [10]. Thus:

$$\%\text{carbohydrate} = [100\,(\%\text{ moisture} + \%\text{crude fiber} + \%\text{protein} + \%\text{lipid} + \%\text{ash})]. \quad (7)$$

2.6.7. Calculating the Calorie Content of Coffee Samples. By multiplying the protein amount by 4, the carbohydrate content by 4, and the fat content by 9, the calorie value of the samples was calculated [10].

$$\text{Caloric value}\left(\frac{kcal}{100g}\right) = [(\text{Crude protein} \times 4) + (\text{Total carbohydrate} \times 4) + (\text{Crude fat} \times 9)]. \quad (8)$$

3. Result and Discussion

3.1. Protein Analysis. The stated figures for green coffee protein content are mainly based on determining crude nitrogen and multiplying by 6.25 [11]. For average protein contents, no significant differences ($P < 0.05$) were found among the 104 genotypes (checks and accessions) evaluated (Table 2). The protein content of 104 coffee bean samples for different coffee genotypes ranged from 6.93% as a minimum value to 10.14% as a maximum value in the current study, with an average of 8.75% (Table 3). Differences in protein composition in coffee bean samples from different coffee genotypes could be attributed to genetic differences. Santos et al. [12] found that the protein level of several coffee

Table 2: ANOVA for proximate and biochemical components of coffee germplasm accessions.

Quality characters	Blocks (df = 4)	All entries Treatments (df = 103)	Test accessions (df = 99)	Checks (df = 3)	Checks vs. Accessions (df = 1)	Error (df = 12)
CP	0.013	0.601*	0.609*	0.108 ns	1.232*	0.227
EE	0.048	1.323***	1.240***	2.340***	5.124***	0.020
CF	0.829	14.050***	11.484***	28.510***	224.629***	0.631
CA	0.009	0.583***	0.588***	0.309***	0.826***	0.006
MC	0.370	0.357 ns	0.261 ns	0.350 ns	9.900***	0.278
TCH	2.248	17.971***	16.448***	23.079**	153.369***	1.455
CV	17.228	272.674***	244.359***	979.160***	956.521***	16.951
DM	0.370	0.356 ns	0.261 ns	0.349 ns	9.833***	0.277

MC = Moisture content, EE = Crude fat, CF = Crude fiber, CA = Crude ash, TCA = Total carbohydrate, CV = Caloric value, DM = Dry matter. ** = highly significant ($P < 0.01$), * = significant ($P < 0.05$), ns = nonsignificant, and df = degree of freedom.

Table 3: Mean, minimum, maximum, and range of 8 biochemical traits.

Variable	Mean	Range	CV (%)	Lsd (5%)
CP	8.75 ± 0.77	6.93–10.14	5.45	1.64
EE	11.30 ± 1.12	8.89–16.08	1.24	0.48
CF	16.29 ± 3.72	6.79–22.25	4.87	2.70
CA	4.33 ± 0.72	2.51–5.47	1.75	0.26
MC	9.56 ± 0.60	8.37–10.93	5.51	1.81
TCH	49.78 ± 4.21	40.65–59.38	2.43	4.20
CV	335.74 ± 15.77	307.39–382.77	1.23	14.20
DM	90.44 ± 0.60	89.08–91.63	0.58	1.81

MC = Moisture content, EE = Crude fat, CF = Crude fiber, CA = Ash content, TCA = Total carbohydrate, CV = Coefficient of variation, Lsd = Least Significant Difference, and DM = Dry matter.

samples ranged from 9.21–14.33%, which is consistent with the current figure for coffee beans. Alakali et al. [13] revealed the protein concentration of various tea samples ranging from 8.35–10.67%, which is consistent with the current finding. The protein level of several coffee bean samples was in the range of 7–16.16% according to Nogaim et al. [14], which agrees with the current data. Awika et al. [15] also recorded the protein level of various coffee samples ranging from 14.00–16.10%. Tessema et al. [16] found that the protein content of various coffee bean samples ranged from 3.69–5.24%, which is lower than the current figure.

3.2. Analyze the Ash (Total Minerals). Ash is the inorganic residue left after water and organic materials have been removed by heating in the presence of oxidizing agents, and it is used to calculate the total amount of minerals in food. The notion that minerals (the analyte) may be separated from all other components (the matrix) within food in some measurable way underpins analytical approaches for delivering information about the overall mineral content. Minerals are not damaged by heat and have low volatility compared to other food components, hence, the most generally used methods are based on this. The total mineral (ash) content of the coffee genotypes differed considerably ($P < 0.05$) (Table 2). The average ash level of 104 coffee bean samples for diverse coffee genotypes ranged from 2.51% to 5.47%, with a minimum of 2.51% and a maximum of 5.47% (Table 3). On a dry basis, mineral content accounts for (4.00 to 5.00%) of coffee weight [17]. The ash percentage of the present samples was greater than the ash content of coffee bean samples (3.90 to 4.42%) as stated by Risso et al. [18]. According to Santos et al. [12], the average ash content in different coffee bean samples is in the range of (4.00 to 4.90%), which is consistent with the current study. The average ash percentage in all coffee bean samples in this investigation was identical to the ash content in green tea samples (4.79%) reported by Akande et al. [19]. According to Nogaim et al. [14], the ash percentage of several coffee bean samples ranged from 3.40 to 6.51%, which is consistent with the current ash content data.

3.3. Lipid Analysis (Crude Fat). Lipid estimation is one of the most important aspects of any food material's nutritional evaluation [20]. The amount of lipids in coffee beans from different coffee genotypes varied significantly ($P < 0.05$) (Table 2). The lipid content of 104 coffee bean samples for various coffee genotypes ranged from 8.89% at the lowest to 16.08% at the highest, with an average of (11.30%) in the middle (Table 3). However, the range of these values was higher than the lipid content of green tea plants, which was reported as 6.09% by Akande et al. [19]. The present samples' lipid fraction was found to be in agreement with the averaged lipid fraction in coffee beans, which was around 15%, as stated by Ayaz et al. [20]. As reported by Modupe et al., the range of lipid contents of coffee bean samples was also found to be larger than the range of lipid contents of green tea (3.25 to 5.53%) [21]. However, the study sample data are consistent with the lipid content of green coffee beans, which was reported as 2.49 to 13.13% by Nogaim et al. [14]. Coffee has a fat content of 7 to 17%. Green *arabica coffee* beans have an average lipid content of 15%, but *robusta coffees* have a substantially lower lipid content, averaging approximately 10% [22]. The changes in the lipid composition of coffee bean samples from different coffee genotypes identified in this investigation could be related to the effect of genetic composition. The presence of a significant amount of lipids indicates that these beans have the potential to serve as a dietary supplement with promising nutritional properties.

3.4. Crude Fiber. Dietary fiber has lately acquired prominence due to its potential to lessen the prevalence of cardiovascular and digestive illnesses. The World Health Organization

Table 4: Correlation values between coffee biochemical traits of coffee germplasm accessions.

	CP	EE	CF	CA	MC	TCH	CV	DM
CP	1							
EE	0.103	1						
CF	0.135	0.144	1					
CA	−0.162	−0.173	0.036	1				
MC	0.088	−0.182	−0.099	0.099	1			
TCH	−0.316**	−0.357***	−0.932***	−0.142	−0.039	1		
CV	−0.073	0.281*	−0.878***	−0.295**	−0.141	0.779***	1	
DM	−0.089	0.182	0.010	−0.098	−1.00***	0.039	0.141	1

CP = Crude protein, EE = Crude fat, CF = Crude fiber, CA = Ash content, MC = Moisture content, TCA = Total carbohydrate, CV = Caloric value, and DM = Dry matter.

(WHO) recommends consuming 22 to 23 kg of fiber for every 1000 calories consumed [23]. The samples studied were found to be significantly different ($P < 0.05$) (Table 2). The fiber content of 104 coffee bean samples for various coffee genotypes ranged from 6.79% at the lowest to 22.25% at the highest, with an average of 16.29% (Table 3). Dietary fibers are nonstarch polysaccharides that bind minerals and speed their passage through the digestive system, reducing nutritional bioavailability and absorption. When fibers work along with other food ingredients like phytate, tannin, or oxalate, the whole process becomes more successful [24].

3.5. Carbohydrate Analysis. Coffee beans in different coffee genotypes exhibited significant ($P < 0.05$) variation in the amount of carbohydrate (Table 2) and the carbohydrate content in the study samples was in the range of 40.65–59.38% and the mean carbohydrate content for the coffee beans was 49.78% (Table 3). But, the range of these values was greater than that of carbohydrate contents for green coffee beans as 7.92 to 35.64%, which was reported by Nogaim et al. [14]. As reported by Bhattacharjee et al. [10], the carbohydrate content of different onion (*Allium cepa* L.) bulb samples was in the range of (14.15 to 14.77%) which is still very less than the present result. According to the result of the carbohydrate content in coffee beans, it was possible to conclude that coffee beans can be used as an enormous amount of energy source for consumers.

3.6. Caloric Value. The inherent chemical energy inherent in the bonds of the organic molecules of foods, such as their protein, carbohydrate, and fat constituents, as well as minor ingredients such as organic acids, are measured by the calorie value of a food. The quantity of calorific value in coffee beans from different coffee genotypes varied significantly ($P < 0.05$) (Table 2), and the calorific value content in the study samples ranged from (307.39 to 382.77 kcal/100 g), with the mean calorific value content for the coffee beans being (335.74 kcal/100 g) (Table 3). Caloric values of coffee genotype beans (307.39 to 382.77 kcal/100 g) observed in this study are better and comparable to those reported from energy-rich tubers such as cocoyam (*Colocasia esculenta*) 378.93 kcal/100 g, potato (*Solanum tuberosum)* 376.30 kcal/100 g, and water yam (*Dioscorea alata)*, 357.65 kcal/100 g [25].

Table 5: Eigenvalues, total variance, cumulative variance, and eigenvectors for 6 quantitative traits were studied on 104 coffee germplasm accessions.

Trits	Eigenvectors		
	PCI	PCII	PC III
CP	0.14016	−0.07431	0.42755
EE	0.05874	−0.19746	0.5998
CF	0.56881	−0.10396	0.02377
CA	0.12901	0.10412	−0.53346
TCH	−0.56908	0.11569	−0.15786
EV	−0.54268	−0.01698	0.29778
Eigenvalue	2.829	2.231	1.405
% of total variation	31.44	24.79	15.61
% of cumulative variation	31.44	56.22	71.84

CP = Crude protein, EE = Crude fat, CF = Crude fiber, CA = Ash content, TCA = Total carbohydrate, and CV = Caloric value, PC = Principal component.

3.7. Correlation Studies. The relationship between several proximate and bioactive chemicals is shown in (Table 4). The caloric value showed a very highly significant and positive association with total carbohydrates ($r = 0.779$) and a very highly significant and negative association with crude fiber ($r = -0.879$). Caloric value was weakly and positively associated with fat ($r = 0.281$) and dry matter ($r = 0.141$). Caloric value indicated no significant and negatively correlated with crude protein ($r = -0.073$), moisture content ($r = -0.141$), and crude ash ($r = -0.295$). Carbohydrates indicated significant and negative associated with protein ($r = -0.316$), crude fat ($r = -0.357$), crude fiber ($r = -0.932$), crude ash ($r = -0.142$), and moisture content ($r = -0.039$). Carbohydrates indicated weakly and positively related to dry matter content ($r = 0.039$).

3.8. Principal Component Analysis (PCA). Using 104 coffee (*Coffea arabica* L) genotypes/accessions and principal component analysis for 6 characters, the first three principal components with eigenvalues larger than one explained 71.84% of the overall variation (Table 5). Discriminatory characteristics such as crude protein, crude fiber, and crude ash accounted for the first main component, which accounted for 31.44% of the variability between accessions. Similarly, variance in crude ash and total carbohydrate accounted for 24.79% of the total diversity among the

Table 6: Inter (bottom) and intra (bold and diagonal) cluster distance among 104 coffee germplasm accessions for 6 quantitative traits.

Cluster	I	II	III	IV	V	VI	VII	VIII	XI	X
I	0									
II	40.77**	**0**								
III	94.64**	12.00	0							
IV	**10.09**	**10.66**	44.04**	0						
V	15.84	102.74**	182.38**	48.49**	0					
VI	**11.03**	71.33**	139.39**	29.86**	13.77	0				
VII	40.68**	17.48*	32.43**	19.51*	94.45**	83.90**	0			
VIII	42.57**	63.09**	100.69**	42.63**	66.91**	83.58**	21.35*	0		
XI	45.06**	102.01**	160.89**	64.02**	43.07**	71.58**	57.29**	20.31*	0	
X	84.46**	**236.33** **	344.16**	149.61**	43.82**	72.79**	199.04**	137.77**	98.54**	**0**

* Significant at $P < 0.05$ for $X^2 = 16.92$; ** Significant at $P < 0.01$ for $X^2 = 21.67$, ns = Significant. Total No. of accessions=104; total % of genotypes=100 The bold Number indicate the Maximum and the Minimum Value for cluster distance.

examined accessions. Similarly, the third major component, which accounted for 15.61% of the total variation in crude protein, crude fat, and caloric value, explained 15.61% of the entire variation (Table 5). Crude ash and crude protein both played a role in the variances in two of the three primary components (Table 5). The current study found that coffee genotypes/accessions have a lot of variances in the traits they looked at. This wide trait diversity among coffee genotypes/accessions suggests that there are numerous opportunities for genetic improvement through direct selection from genotypes/accessions and/or selection of diverse parents for hybridization programs, as well as germplasm conservation for future use. The discovery of biochemical compound composition variety in coffee (*Coffea arabica* L) is in line with previous research [3, 16, 26–28].

3.9. Divergence Analysis (D^2) for 6 Quantitative Characters. Theproc discrim of SAS procedure of pair-wise generalized squared distance was used to examine inter and intracluster distances for six quantitative characters. The results revealed significant and highly significant ($P < 0.05$ and $P < 0.01$) genetic distances between the majority of clusters, as well as nonsignificant variation within accessions grouped in the same cluster (Table 6). Clusters that are divergent in the intercluster distance study are good sources of genotypes that might be employed in the hybridization program to get a wide range of variance in the segregates and maximize heterosis from genetically varied parental lines.

The current study discovered that such information can be used and that there is a group of distantly related genotypes that can be used right away in the hybridization of a hybrid variety generation program. Clusters III and *X* had the greatest intercluster distance ($D2 = 344.16$), followed by clusters II and *X* ($D2 = 236.33$), VII and *X* ($D2 = 199.04$), and clusters VI and I ($D2 = 106.25$). Clusters I and IV had the smallest intercluster distance ($D2 = 10.09$), followed by II and IV ($D2 = 10.66$), and I and VI ($D2 = 11.03$). The intercluster distance with the highest value suggested that the accessions in these clusters were different. The lowest cluster distance, on the other hand, indicates a close link between the accessions.

Coffee accessions from cluster *X* and cluster I to XI and VI, as well as cluster XI and cluster I to VII, alongside cluster VIII and cluster I to VII, and cluster VII and cluster I to VI, could be possible parental lines for boosting heterotic value by crossing, based on the findings. Crossing germplasm accessions from different clusters of wide Mahalanobis distance (D2) could maximize opportunities for transgressive segregation, according to Peeters and Martinelli [29], because there is a high probability that unrelated genotypes will contribute unique desirable alleles at different loci. The degree of heterosis between populations, which reflects gene frequency differences, is proportional to their genetic divergence [30]. According to Singh [31], divergence analysis is used to discover varied genotypes for hybridization purposes, with genotypes grouped together being less divergent than genotypes in different clusters, especially clusters separated by the greatest statistical distance (D2).

3.10. Cluster Characterization Coffee Accessions Using Biochemical Traits. Biochemical similarities of 104 coffee genotypes were assessed by average linkage methods of cluster analysis using **6** proximate and biochemical characters with proc cluster of SAS. Based on the result of this analysis, the coffee accessions were classified into ten clusters with the numbers of accessions in each cluster I, II, III, IV, V, VI, VII, VIII, XI, and *X* being 20,21, 39, 12, 04, 03, 02, 01, 01, and 01, respectively, (Table 7). Cluster-III was the largest and consisted of 39 accessions (37.50%) followed by cluster-II consisted of 21 accessions (20.19%), cluster-I consisted of 20 accessions (19.23%), cluster-IV consisted of 12 accessions (11.54%), cluster-V consisted of 04 accessions (3.85%), cluster-VI consisted of 03 accessions (2.88%), cluster-VII consisted of 02 accessions (1.92%), and clusters VIII, XI, and *X* consisted of 01 accessions each (0.96%) (Table 7).

Mean performance of different clusters of the 6 traits studied (Table 8) showed that accession in cluster-VII was the high protein value (9.65) followed by cluster-X (9.41) and the least protein value was cluster-VI (7.90). Similarly, an accession in cluster-XI was the high fat value (16.80) followed by cluster-VII (15.80) and the least fat value was cluster-VI (10.21). Besides, an accession in cluster-VIII was the high fiber value (20.71) followed by cluster-VII (20.33) and the least fiber value was cluster-X (6.79). Also, an accession in cluster-X was the high ash value (4.83) followed by cluster-III (4.56) and the least ash value was cluster-VIII

Table 7: Clustering patterns of 104 coffee germplasm accessions based on 6 coffee proximate and biochemical traits.

Cluster	No. of accessions	% of genotypes	Name of accessions in each cluster
I	20	19.23	AW-44, AW-54, AW-45, AW-48, AW-34,AW-36,AW-11,AW-47,AW-51,AW-77,AW-70,AW-37,AW-86,AW-76,AW-85,AW-30,AW-31,AW-10,744, 1377
II	21	20.19	AW-08, AW-19, AW-102, AW-66,AW-71,AW-20,AW-38,AW-09,AW-21,AW-28,AW-33,AW-07,AW-49,AW-63,AW-83,AW-59,AW-92,AW-61,AW-50,AW-101,AW-95
III	39	37.50	AW-81, AW-100,AW-05,AW-06,AW-23,AW-14,AW-68,AW-88,AW-94,AW-90,AW-104,AW-18,AW-39,AW-32,AW-74,AW-27,AW-73,AW-42,AW-65,AW-13,AW-99,AW-35,AW-96,AW-67, AW-75,AW-82,AW-91,AW-17,AW-40,AW-52,AW-62,AW-84,AW-103,AW-58, AW-98,AW-60,AW-89,AW-93, 75227
IV	12	11.54	AW-22, AW-53, AW-41, AW-43, AW-46, AW-16, AW-72, AW-24, AW-26, AW-29, AW-97,7440
V	04	3.85	AW-15, AW-64, AW-69, AW-80
VI	03	2.88	AW-55, AW-56, AW-57
VII	02	1.92	AW-12, AW-87
VIII	01	0.96	AW-78
IX	01	0.96	AW-79
X	01	0.96	AW-25
Total	**104**	**100**	

Total No. of accessions=104; total % of genotypes=100.

Table 8: Mean values of 6 proximate and biochemical traits for ten clusters of 104 coffee germplasm accessions.

Cluster No.	Qualitative Traits					
	CP	EE	CF	CA	TCA	CV
I	8.81	11.55	12.77	4.17	53.36	352.63
II	8.42	10.95	17.24	4.35	49.50	330.24
III	8.85	11.19	20.17	4.56	45.66	318.73
IV	8.62	11.22	15.08	4.19	51.57	341.67
V	8.57	11.73	10.88	3.28	56.50	365.80
VI	7.90	10.21	9.27	4.50	58.60	357.87
VII	9.65	13.97	20.33	3.86	42.81	335.52
VIII	9.30	15.80	20.71	2.52	42.31	348.64
XI	8.42	16.08	18.83	2.92	45.05	358.62
X	9.41	13.77	6.79	4.83	55.31	382.77
Mean	8.79	12.65	15.21	3.92	50.07	349.25

**, * represents the maximum and minimum values, respectively, CP = Crude protein, EE = Crude fat, CF = Crude fiber, CA = Ash Content, TCA = Total carbohydrate, CV = Caloric value.

(2.52). As well, an accession in cluster-VI was the high total carbohydrate value (58.60) followed by cluster-V (56.50) and the least total carbohydrate value was cluster-VIII (42.31). Moreover, accessions in cluster-X was a high caloric value (382.77) followed by cluster-V (365.80) and the least caloric value was cluster-III (318.73).

4. Summary and Conclusions

The study's findings show that there is variability in proximate composition and biochemical characteristics among coffee germplasm collections. The observed divergence suggests that genetic gains for proximal and biochemical features are possible. According to the findings, coffee beans contain crude protein (6.93 to 10.14%), total lipids (8.89 to 16.08%), crude ash (2.51 to 5.47%), crude fiber (6.79 to 22.25%), dry matter (89.08 to 91.63%), carbohydrate (40.65 to 59.38%), and caloric value (307.39 to 382.77 kcal). The germplasm accessions were grouped into ten clusters after being characterized based on six attributes utilizing the linkage method of hierarchical cluster analysis. Using cluster analysis, one hundred and four arabica coffee accessions were divided into 10 separate groups based on nine traits, demonstrating a wide genetic variety of coffee genotypes 20 (19.23%), 21 (20.19%), 39 (37.50%), 12 (11.54%), 04 (3.85%), 03 (2.88%), 02 (1.92%), and 01 (0.96%). The majority of intercluster distances were significantly varied, showing that diversity exists that can be utilized through selection and hybridization. Clusters III and *X* had the greatest intercluster distance ($D2 = 344.16$), followed by clusters II and *X* ($D2 = 236.33$), VII and *X* ($D2 = 199.04$), and clusters VI and I ($D2 = 106.25$). Clusters I and IV had the smallest intercluster distance ($D2 = 10.09$), followed by II and IV ($D2 = 10.66$), and I and VI ($D2 = 11.03$). The first three principal components accounted for 71.84% of the overall variation, according to the PCA. These genotypes should be adequately preserved and could be utilized as a starting point for improving the genetics of the crop's distinguishing characteristics through selection and hybridization. Furthermore, the majority of the coffee qualities had favorable connections with one another. The nutritional and antinutritional content of distinct accessions was studied, and it was discovered that coffee genotypes differ significantly in terms of caloric value, carbohydrate, crude protein, crude fiber, crude fat, and crude ash. Furthermore, coffee could be used as plant food to help those with protein-energy malnutrition by adding essential nutrients to their diet. Furthermore, molecular investigations should be carried out to further characterize the germplasms in order to assure effective usage, conservation, and traceability of the country's vast coffee genetic heritage. As a result, this study offered quantitative

data on the biochemical contents of various coffee genotypes based on their inherent features and the alterations that may occur.

Data Availability

The biochemical data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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