Bull. Chem. Soc. Ethiop. **2024**, 38(4), 825-837.
DOI: https://dx.doi.org/10.4314/bcse.v38i4.1

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

PROXIMATE ANALYSIS AND MINERAL CONTENTS OF ATELLA FROM TRADITIONAL TELLA BREWERS IN JIMMA CITY, ETHIOPIA

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(Received August 24, 2024; Revised March 14, 2024; Accepted March 19, 2024)

ABSTRACT. Atella is a by-product of Tella, a local brewed beverage. It is used as a food source to promote weight gain, development, and milk yield in cattle and sheep. The aim of this study was to analyze the physicochemical properties, nutritional composition, and mineral content of Atella. The samples of Atella were purposively collected from 25 Tella vendors in Jimma City, Ethiopia. The physicochemical properties and proximate analyses were performed using the AOAC method. The mineral contents of the samples were measured using flame atomic absorption spectrometry (FAAS). The results showed that the pH value was between 3.36 - 4.23, temperature (19.27 - 20.20 °C), conductivity (124.10 - 153.50 µS/cm), dry matter content (15.87 - 20.17%), crude protein content (16.47 - 18.72%), crude fat content (3.73 - 5.43%), crude fiber content (15.52 - 19.73%), total ash content (3.58 - 4.47%), carbohydrate content (35.02 - 40.50%), and gross energy (247.42 -271.13 kcal/g). The average concentrations of Ca, Mg, Fe, Cu, Cr, and Pb were 656.71, 274.74, 79.16, 7.73, 1.55, and 0.41 mg/kg, respectively. The results of the study show that Atella is a nutrient-rich source of essential nutrients, minerals, and energy.

KEY WORDS: Tella, Atella, Physicochemical properties, Proximate analysis, Mineral content

INTRODUCTION

Tella is a traditional Ethiopian fermented drink made froma combination of different grains and a local herb called Gesho (*Rhamnus prinoides*) [1]. This particular alcoholic beverage is a locally produced drink that is often consumed during holidays, annual celebrations, special festivals, and church ceremonies in different regions of Ethiopia [2]. Tella has a number of variations, that are influenced by the specific ingredients and preparation methods [3]. Grains such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), teff (*Eragrostis tef*), and other ingredients such as powdered stems and leaves of Gesho (*Rhamnus prinoides*), malt and water are used to make Tella [4, 5]. Tella is classified as a low-alcohol beverage, with an alcohol content ranging from 4 - 6% (v/v) [3, 5, 6].

Tella goes through two different brewing phases. In the first phase, known as Tenses, the powdered leaves of the Gesho plant (*Rhamnus prenoide*) are used. These leaves are mixed with water in a small clay pot and left to ferment for four days [7]. The Tenses are transferred into large vessels called Gan or Insira, which can be made of clay, plastic, or metal barrels. It is then mixed with either Enkuro, a type of barley flour, or Kita, an unleavened bread. The mixture is then fermented for 96 hours. In the final stage, known as Difdif, a new fermentation process is initiated by adding water within a time frame of 96 hours [8]. The liquid mixture is filtered, producing Tella, the filtrate suitable for consumption, and Atella, the solid residue or by-product is discarded [5, 9]. The moisture content of Atella is higher than that of other spent grains, and it also has a relatively high protein value. The composition and nutritional value of Atella depends on the type of grain used and various process parameters such as temperature and fermentation time [10].

Atella is widely used by smallholder farmers as it is affordable and widely available among non-conventional feed resources [11]. It partially helps to address feed shortages, mitigates

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competition for food resources between humans and animals, reduces feed costs, and increases self-sufficiency in nutrients from locally accessible feed sources [12]. Atella has the potential to be a valuable addition to the rations of dairy cows in combination with other feed ingredients such as niger cake, wheat bran and barley straw to improve milk production [13]. In the dry season, it can be used to produce economic livestock feed and as a source of protein, as its crude protein content can reach up to 24% [12, 14]. However, a significant amount of Atella continues to be released into the environment in various rural and urban areas of Ethiopia [5]. In addition, there is little data on the proximate composition, nutritional value, and mineral content of Tella residue (Atella) to date. As far as we know, there is currently no report containing information on the nutritional value of Atella samples from Jimma City. Therefore, the aim of this study was to analyze the nutritional composition and mineral content of Atella samples from Jimma City.

EXPERIMENTAL

Study area

This study was conducted at Jimma University, Jimma City, Ethiopia (Figure 1). Jimma City is located 354 km from the capital, Addis Ababa. Its geographical coordinates are 7°40'26.01" N latitude and 36°50'8.85" E longitude. Jimma City lies between 1718 and 2000 meters above sea level and receives an average rainfall of between 800 and 2500 mm per year [15].

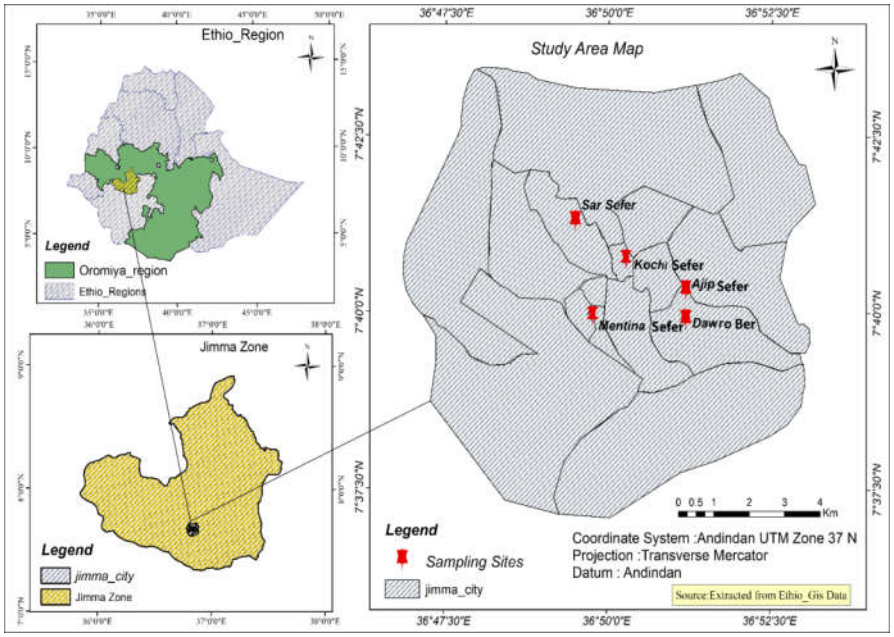

Figure 1. Map of study area and sampling sites.

Sampling technique and sample preparation

Atella samples were collected randomly from 25 Tella vendors in five different locations in Jimma City, namely Ajip Sefer, Kochi Sefer, Sar Sefer, Mentina Sefer, and Dawro Ber (Figure 1). The

samples were collected in polyethylene bottles that had been soaked overnight in a 10% HNO_3 solution. The bottles were then washed and rinsed several times with distilled water. Two liters of Atella samples were taken from each Tella vendor. The collected samples were then transported to the analytical chemistry laboratory at Jimma University and stored in a refrigerator at 4 °C for subsequent analysis. Targeted questioning of the tella sellers was also carried out during sampling using a structured questionnaire. Approximately 200 mL of each Atella sample was pooled into five composite samples corresponding to the sampling points. Finally, the composite samples were analyzed for selected heavy metals and proximate composition.

Chemicals, apparatus, and instruments

Apparatus and instruments used in this work include Kjeldahl flask (Gerhardt VAPODEST ®450, Germany), drying oven (Memmert, Germany), muffle furnace (D-6072 Driesch, Germany), FAAS (280FS, Malaysia), filter bag (Fiber Determinator SLQ6A, China), conductivity meter (Jiangsu DDS-307, China), electronic balance (Model AAA dam Co limited), digital pH meter, heating mantle, micropipette, desiccators, Soxhlet apparatus. Chemicals such as nitric acid (HNO_3 (70%)), sulfuric acid (H_2SO_4 (98%)), boric acid (H_3BO_3 (4%)), sodium hydroxide (NaOH (40%)), n-hexane (95%), pellets of $CuSO_4$ and K_2SO_4, hydrochloric acid (HCl (0.1N)), and methyl red were used.

Physicochemical properties

pH and temperature

The pH of the samples was measured using a digital pH meter after calibration with buffer solutions of pH 4, 7, and 10. First, the sample was diluted with distilled water at a ratio of 1:1 (w/v). The glass electrode of the pH meter was then immersed in the solution [16, 17]. The temperature of each sample was determined on-site using a thermometer. Each sample was measured in triplicate (n = 3), and the results were expressed as the mean ± standard deviation (SD).

Electrical conductivity

Distilled water and Atella were mixed in a ratio of 20:1 and then stirred. First, the probe was calibrated to a conductivity of 200 µS/cm. The conductivity of distilled water and Atella was then determined.

Proximate analysis

Moisture content

The moisture content was determined according to the methodology described in AOAC (2000) and Osman *et al.* [18, 19]. A predetermined amount of the sample was measured and placed in a crucible. The crucible was heated in an oven at 105 °C for about 3 hours. The crucible was cooled in a desiccator and then weighed. The heating and cooling cycle was carried out until a constant weight was reached. The moisture (%) content was calculated using Eq. 1. The dry matter (DM) content can be calculated by subtracting the percentage of moisture (%) from 100.

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (1)$$

where, W_1= weight (g) of the sample before drying; W_2=weight (g) of the sample after drying

Crude protein

The Kjeldahl wet digestion method was used to determine the total nitrogen content of the composite samples. The percentage of nitrogen and the corresponding crude protein (CP) content can be calculated using Eqs. 2 &3, respectively [19, 20].

$$\%N = \frac{V_1 \times N \times 0.014}{W_1} \times 100 \quad (2)$$

where, V_1 = volume of HCl, N = normality of HCl, W_1 = weight of sample.

$$\text{Crude protein } (\%) = \%N \times 6.25 \quad (3)$$

where, %N is the percentageof nitrogen in a sample and 6.25 is the protein-nitrogen conversion factor [19].

Analysis of crude fat

The Soxhlet extraction technique was used to determine the crude fat content using the ether extract of the samples according to the guidelines of AOAC (2000) and Puwastien *et al.* [18, 20]. A predetermined amount of the sample was placed in a Soxhlet extraction device, which had previously been thoroughly cleaned and dried. Then n-hexane was added to a round bottom flask and gently heated for 4 hours. The flask was then allowed to cool for 15 min. The solvent was removed with a rotavapor under reduced pressure at 40 °C. The crude fat obtained was then transferred to a beaker of known mass. The crude fat content was calculated using Eq. 4.

$$\text{Crude Fat } (\%) = \frac{W_2 - W_1}{W_s} \times 100 \quad (4)$$

where: W_2 = weight of beaker with crude fat; W_1 = weight of beaker; and Ws = weight of sample.

Ash content

The ash content was determined according to the protocol described in the AOAC [18]. A known quantity of the pulverized sample was carefully placed on a crucible that had previously been cleaned, dried in the oven and weighed. First, the sample was heated on a hot plate for 30 min. It was then incinerated in a muffle furnace at 550 °C for 8 hours. The sample was then left to cool in a desiccator and its weight was measured. The ash content of the samples (%) was calculated using Eq. (5).

$$\text{Ash } (\%) = \frac{(W_C + W_A) - (W_C)}{W_{DS}} \times 100 \quad (5)$$

where: W_C is weight of crucible; W_{DS} weight of dry sample; and W_A is weight of ash.

Crude fiber content

The determination of crude fiber was carried out according to the standard method of the AOAC [18]. This method includes digestion, filtration, washing, drying, and incineration. In brief, a pulverized sample weighing 2 g was placed in a filter bag. A hot H_2SO_4 solution (1.25%) was then added to a Pyrex glass jar containing the sample and the mixture was heated at 120 °C for 30 min. The sample contained in the bag was then boiled in hot NaOH (1.25%) at 20 °C for 30 min. The solution was then allowed to cool and the bag was rinsed with distilled water. The sample

was transferred to a crucible and dried in an oven at 130 °C for 2 hours. The dried sample was kept in the furnace at 550 °C for 5 hours. The mass of the crucible and the ash was recorded after cooling in the desiccator. The crude fiber content was calculated using Eq. 6.

$$\text{Crude fiber (\%)} = \frac{(W_C + W_{DST}) - (W_C + W_A)}{W_{ODS}} \times 100 \quad (6)$$

where: W_C is weight of crucible; W_{DST} is weight of dry sample after acid and base treatment; W_A is weight of ash; and W_{ODS} is weight of original dry sample.

Carbohydrate content

The carbohydrate content was determined by subtracting the combined values of moisture, ash, crude fat, crude fiber, and crude protein from 100, as described in Eq. 7 [17].

$$\text{Carbohydrate (\%)} = 100 - [(\text{M (\%)} + \text{A(\%)} + \text{CFa (\%)} + \text{CFb (\%)} + \text{Cp (\%)}] \quad (7)$$

where: M = moisture; A = ash; CFa = crude fat; CFb = crude fiber; and Cp = crude protein.

Gross energy

The gross energy value was estimated using the Atwater factors for protein (4), fat (9), and carbohydrates (4) [17]. The gross energy was calculated using Eq. 8.

$$\text{Gross Energy (\%)} = [(\text{Cp (\%)} \times 4) + (\text{CFa (\%)} \times 9) + (\text{CH (\%)} \times 4)] \quad (8)$$

where: Cp = crude protein; CFa = crude fat; and CH = carbohydrates

Minerals analysis

The wet ashing method was used to determine the presence of certain metals by FAAS [21]. In brief, 20 mL of 69% HNO_3 was mixed with 2 g of powdered composite sample in an Erlenmeyer flask. Then, 10 mL $HClO_4$ (70%) was added to the flask using a pipette and the mixture was heated at 120 °C for 1 hour. The resulting mixture was then filtered with Whatman No. 1 filter paper and the filtrate was collected in a cleaned 50 mL volumetric flask. The filter paper was thoroughly rinsed with double distilled water until the volumetric flask reached the mark. The blank sample was digested in the same way as the sample. Finally, using an appropriate wavelength for the metals of interest (Table 1), all required metals were successfully determined by FAAS.

Table 1. FAAS instrumental condition

Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Instrumental detection limit (IDL) (mg/L)
Ca	422.7	0.7	2	0.01
Mg	285.2	0.5	1	0.001
Fe	248.3	0.2	7	0.03
Cu	324.7	0.7	1.5	0.005
Cr	357.9	0.7	2	0.04
Pb	283.2	0.7	2	0.04

Construction of calibration curves

The intermediate standard solution for each metal was prepared from stock solutions containing1000 mg/L Ca, Mg, Fe, Cu, Cr, and Pb [22]. The working standard solutions (1.0, 2.0,

3.0, 4.0, and 5.5 mg/L) were prepared and then the absorbance was measured. Calibration curves were generated by plotting the relationship between concentration and absorbance.

Method validation

The method was validated by evaluating the accuracy, precision, limits of quantification (LOQ), limits of detection (LOD) and percent recovery for each metal analysis. The accuracy of the methods is evaluated by adding a known concentration of the standard analyte to the sample matrix and then analyzing the sample. The recovery percentage (R%) was calculated using Eq. 9.

$$\%R = \frac{\text{Conc. in spiked sample} - \text{Conc. in unspiked sample}}{\text{Conc. actual spiked sample}} \times 100 \quad (9)$$

Precision is measured by analyzing a series of samples from multiple samplings of a homogeneous lot. The relative standard deviation (%RSD) was then calculated using Eq. 10.

$$\text{RSD}\ (\%) = \frac{\text{SD}}{\text{Mean}} \times 100 \quad (10)$$

Limit of detection (LOD) and Limit of quantification (LOQ) were determined using Eqs 11 and 12.

$$\text{LOD} = 3.3 \times \text{SD/slope of intercept} \quad (11)$$

$$\text{LOQ} = 10 \times \text{SD/slope of intercept} \quad (12)$$

where: SD is standard deviation of the measurements.

Data analysis

All analyses were performed in triplicate and results were expressed as mean ± standard deviations. Correlations of data from physicochemical parameter analysis, and proximate analysis of Atella were processed with the Statistical Package for the Social Sciences (SPSS) software using one-way analysis of variance (ANOVA). The statistical analysis revealed a significant difference between the samples with respect to the parameters analyzed, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties of Atella

Atella is a semi-liquid residue left over from the preparation of a local alcoholic drink called Tella. The color of Atella varies from dark brown to black and depends on the specific techniques used in its preparation. The measurements of pH, temperature, and electrical conductivity of the Atella samples were carried out and the results are presented in Table 2.

Table 2. Physicochemical properties of Atella samples (mean±SD), where n = 3.

Sampling area	pH	Temperature (°C)	Conductivity (µS/cm)
Kochi Sefer	3.36 ±0.02	20.20±0.00	124.10 ± 0.70
Sar Sefer	3.86±0.04	19.83±0.29	130.67±0.38
Dawro Ber	4.13±0.00	19.60±0.20	153.50±0.26
Mentina Sefer	3.94±0.00	19.70±0.00	138.00±0.20
Ajip Sefer	4.23±0.00	19.27±0.23	130.67±0.12

The pH values of Atella were between 3.36 and 4.23, indicating an acidic nature. The acidity of Atella can be attributed to the production of acids by acid-producing microorganisms, especially *Lactobacillus spp.* during the fermentation process [7]. Several researchers reported that the pH of Tella was 3.80 ± 0.01, which is in the range of the current results for Atella [23]. The optimal pH range for beverages is normally between 3.5 and 4.0 [24]. The pH values of Atella were within the standard range, with the exception of the slightly elevated values observed in the Dawro Ber (4.13) and Ajip Sefer (4.23) samples. The measured pH values therefore indicate that the consumption of Atella does not pose any health risks to cattle. The pH values the Atella samples were lowest at Kochi Sefer (3.36) and highest at Ajip Sefer (4.23), in the order Kochi Sefer < Sar Sefer < Mentina Sefer < Dawro Ber < Ajip Sefer. The pH values of the samples collected from the five sites show significant variation. The variation in pH values can be attributed to differences in crop ingredients, preparation techniques, and storage methods of Tella Atella. The temperature range in Atella varied between 19.27 °C and 20.20 °C, which is favorable for the growth of psychrotrophic bacteria. Psychrotrophic bacteria have the ability to metabolize various organic waste substrates such as cellulose, lignin, and lipids at low temperatures, which promotes the decomposition of lignocellulose [25].

The electrical conductivity of the Atella ranged from124.10 µS/cm to 153.50 µS/cm. The electrical conductivity of the samples collected from five different locations showed significant variations. In particular, the sample from Kochi Sefer shows the lowest electrical conductivity (124.10 µS/cm), while the sample from Dawro Ber shows the highest electrical conductivity (153.50 µS/cm). The observed differences in electrical conductivity indicate variations in ion concentration between samples collected from different Tella venders. In general, Atella has low electrical conductivity values, which contribute to its pleasant taste and reduced salt content [23].

Nutritional value of Atella

The evaluation of the nutritional value of Atella as cattle feed was carried out, and the results are presented in Table 3. The dry matter (DM) content was determined by measuring the mass difference after drying at a temperature of 105 °C [19]. The DM content of the Atella samples was between 15.75% and 20.17%. This result is consistent with a previous study in which the dry matter content of Atella was between 20 - 30% [10]. The dry matter contents showed significant differences ($p < 0.05$) between the samples. The samples from Kochi Sefer have the highest DM content (20.17%), while the lowest DM content was found in the samples from Ajip Sefer (15.75%). The ascending order of their ranks was as follows: Ajip Sefer, Dawro Ber, Sar Sefer, Mentina Sefer, and Kochi Sefer. The variations in DM content can be attributed to differences in the production process of Tella and the variability of raw materials used in the preparation of Tella [8]. Compared to the other feeds, Atella has a relatively low dry matter content and a comparatively high moisture content [26].

Table 3. Proximate composition of the Atella samples (Mean ± SD; n = 3)

Sample location	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Carbohydrate (%)	Gross energy (kcal/g)
Kochi Sefer	20.17 ± 0.76	16.47 ± 0.62	5.43 ± 0.15	17.83 ± 0.52	3.58 ± 0.10	36.51 ± 0.54	255.45 ± 10.61
Sar Sefer	18.83 ± 1.53	17.50 ± 0.70	3.93 ± 0.25	15.52 ± 0.60	3.71 ± 0.15	40.50 ± 1.37	267.40 ± 6.80
Dawro Ber	18.72 ± 0.25	18.14 ± 0.22	3.73 ± 0.21	19.73 ± 2.00	4.21 ± 0.15	35.46 ± 2.28	247. 99 ± 7.92
Mentina Sefer	19.50 ± 0.50	17.96 ± 0.75	4.30 ± 0.21	19.20 ± 2.37	3.88 ± 0.08	35.02 ± 1.70	247.42 ± 8.21
Ajip Sefer	15.75 ± 0.35	18.72 ± 0.3	4.73 ± 0.15	16.73 ± 0.82	4.47 ± 0.08	39.15 ± 1.09	271.13 ± 3.36
Average	18.59 ± 0.68	17.76 ± 0.52	4.43 ± 0.19	17.80 ± 1.26	3.97 ± 0.11	37.33 ± 1.40	257.88 ± 7.38
%RSD	3.60	2.87	4.29	7.07	2.52	3.72	2.92

The crude protein content of Atella was between16.47% and 18.72%, which is comparable to the previous report on the crude protein content of Atella [26]. However, it is slightly lower than the value reported by Demeke (22%) [10]. The results also showed statistically significant differences in the protein content of the samples at a significance level of $p < 0.05$. The Kochi Sefer sample had the lowest protein content (16.47%), while the Ajip Sefer had the highest (18.72%). The crude protein values were arranged in the following order: Kochi Sefer < Sar Sefer < Mentina Sefer < Dawro Ber < Ajip Sefer. These variations in Atella can be attributed to the use of different amounts and types of ingredients in the preparation of Tella. Compared to other cattle feeds, Atella has a higher crude protein content (17.76%) than barley straw (4%), molasses cane (5%), oat straw (4%), rye straw (4%), corn silage (8%), sorghum silage (8%), corn and cob meal (9%), soybean straw (5%), and wheat straw (4%). However, its crude protein content is lower than that of alfalfa leaves (19%) and Brewers grain wet (26) [27]. On the basis of crude protein content, animal feeds are categorised into low (< 12%), medium (12–20%), and high protein content (> 20%), with Atella being considered a medium protein source (CP = 16.47% - 18.72%). Thus, Atella has the potential to serve as a protein supplement for the inferior tropical feeds [28].

The crude fat content of Atella was between 3.73% and 5.43%. The fat contents of the samples showed a statistically significant difference ($p < 0.05$). The sample from Dawro Ber had the lowest fat content (3.73%), while the sample from Kochi Sefer had the highest fat content (5.43%). The ranking of fat content in Atella is as follows: Dawro Ber < Sar Sefer < Mentina Sefer < Ajip Sefer < Kochi Sefer. Fats play an important role in animal nutrition due to their ability to provide energy and facilitate the absorption and storage of vitamins. High concentrations of fats in animal feed can have a detrimental effect on rumen fermentation and fiber digestibility. This phenomenon lead to an increased rumen filling and slower lipid passage [29]. Although Atella has a higher fat content (4.43%) compared to alfalfa leaves (2.3%), barley straw (1.9%), molasses cane (0.0%), oat straw (2.3%), rye straw (1.5%), sorghum stover (2.1%), soybean straw (1.4%), and wheat straw (1.5%), it remains lower than the fat content of wet brewers grains (6.5%) [27].

Dietary fiber is a non- digestible component of food that cannot be digested [11]. The crude fiber contents found in the Atella samples vary between 15.52% and 19.73%, with the following order: Sar Sefer < Ajip Sefer < Kochi Sefer < Mentina Sefer < Dawro Ber. The analysis indicates that the Atella samples from Dawro Ber have ahigher content of cellulose, hemicelluloses, and lignin than the other samples. However, no statistically significant difference ($p > 0.05$) in crude fiber content was found between the samples. The fiber content of Atella (17.80%) was higher compared to other cattle feeds such as molasses cane (0%), brewer's grain wet (15), and oats grain (12%). however, the fiber content of Atella was lower than that of barley straw (42%), oat straw (41%), rye straw (44%), sorghum stover (33%), soybean straw (44%) and wheat straw (42%) [27]. The lower dry matter, lower fiber content and higher crude protein content of Atella may possibly lead to an accelerated passage, which shortens the residence time for digestion [11].

The ash content refers to the mineral or inorganic residues that remain after combustion of the samples at temperatures above 500 °C [19]. The ash content of the samples ranged from 3.58% to 4.47%, and a statistically significant difference was found between the samples ($p < 0.05$). The ash content of Atella was found to be in the order of Kochi Sefer, Sar Sefer, Mentina Sefer, Dawro Ber and Ajip Sefer. The observed variations in the total ash content could possibly be due to the mineral composition of the ingredients used in the preparation of Tella. The ash values of the Atella samples (3.97%) were lower than those of molasses cane (10%), barley straw (7%), alfalfa leaf (8), oat straw (8%), rye straw (6%), sorghum stover (10%), soybean straw (6%) and wheat straw (7%). However, they were comparable to the ash values of oats grain (4%) and wet brewers grains (5%) [27]. The study showed that the carbohydrate composition of Atella was between 35.02% and 40.50%. The results indicate a significant difference between the samples ($p < 0.05$). The order of carbohydrate content in the Atella samples is as follows: Mentina Sefer, Dawro Ber, Kochi Sefer, Ajip Sefer, and Sar Sefer.

Energy is a basic requirement for the sustenance of living organisms. Gross energy refers to the total energy content of a substance. However, due to inadequacies in digestion and metabolism, living organisms cannot absorb all the energy from the food they consume [27]. The gross energy content of Atella was determined to be between 247.4 kcal/g and 271.1 kcal/g, in the following order: Mentina Sefer < Dawro Ber < Kochi Sefer < Sar Sefer < Ajip Sefer. However, there was no significant difference in the gross energy content of the samples ($p > 0.05$).

Correlations of physicochemical properties and proximate analysis of Atella

Correlation is a statistical measure that quantifies the degree of association between two or more variables and can be expressed as either a positive or negative correlation. The correlations between the physicochemical properties and proximate composition of Atella were analyzed using the Pearson correlation coefficient [27]. The results of the Pearson correlation analysis and their significance levels are presented in Table 4. The Pearson correlation coefficient (r) showed that there were weak, moderate, and strong positive and/or negative correlations between proximate composition and physicochemical properties of the Atella samples. The weak negative and weak positive correlations indicate less influence of a particular physicochemical property on the composition of the Atella sample. For example, the pH of the sample showed a significant positive correlation with electrical conductivity ($r = 0.597$, $p < 0.05$), crude protein ($r = 0.821$, $p < 0.01$), and ash content ($r = 0.839$, $p < 0.01$) and was negatively correlated with temperature ($r = 0.841$, $p < 0.01$), percentage of moisture ($r = 0.684$, $p < 0.01$), and percentage of crude fat ($r = 0.619$, $p < 0.05$) (Table 4). The strong positive correlation between the pH and electrical conductivity, crude protein and ash content indicates that any factor that increases the pH can increase the electrical conductance, crude protein and ash content of the feed, Atella.

Table 4. Correlations of physicochemical properties and proximate analysis of Atella significant at the 0.01 and 0.05 level (2-tailed)

Pearson correlation	pH	Tempera-ture	Electrical conduc-tivity	%Moisture	%Crude fat	%Crude protein	%Ash	%Crude fiber	%Carbo-hydrate	%Gross energy
pH	1	-0.841**	0.597*	-0.684**	-0.619*	0.821**	0.839**	0.087	0.098	0.133
Temperature		1	-0.357	0.751**	0.364	-0.873**	-0.866**	0.112	-0.164	-0.297
Electrical conductivity			1	-0.004	-0.739**	0.418	0.436	0.539*	-0.432	-0.492
% Moisture				1	0.015	-0.615*	-0.765**	0.202	-0.451	-0.638*
% Crude fat					1	-0.454	-0.284	-0.017	-0.059	0.149
% Crude protein						1	0.788**	-0.085	0.076	0.282
% Ash							1	0.073	0.060	0.225
% Crude fiber								1	-0.890**	-0.783**
% Carbohydrate									1	0.865**
% Gross energy										1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

The temperature of the sample was positively correlated with moisture ($r = 0.751$, $p < 0.01$), whereas it was negatively correlated with crude protein ($r = 0.873$, $p < 0.01$) and ash ($r = 0.866$, $p < 0.01$). The electrical conductivity of Atella exhibited a positive correlation with the percentage of fiber ($r = 0.539$, $p < 0.05$) but a negative correlation with crude fat ($r = 0.739$, $p < 0.01$). This implies that as the percentage of crude fat decreased, the electrical conductivity increased. An elevation in the fat composition of Atella could lead to a reduction in its electrical conductivity,

as fats typically exhibit nonpolar characteristics and possess low electrical conductivity. Moisture content was negatively correlated with protein ($r = 0.615$, $p < 0.05$), ash ($r = 0.765$, $p < 0.01$), and energy ($r = 0.638$, $p < 0.05$). This means that a decrease in moisture content in a given sample leads to an increase in the percentage of crude protein, ash, and gross energy. On the other hand, crude protein was positively correlated with ash ($r = 0.788$, $p < 0.01$). Carbohydrates exhibited a positive correlation with gross energy ($r = 0.865$, $p < 0.01$), whereas fiber was negatively correlated with carbohydrates ($r = 0.890$, $p < 0.01$) and gross energy ($r = 0.783$, $p < 0.01$). This implies that any factor increases the carbohydrate content in Atella, decreases its fiber content or increases the gross energy of Atella.

Mineral contents of Atella

Calibration curves were constructed for Ca, Mg, Fe, Cu, Cr, and Pb using five concentration points ranging from 1.0 to 5.0 mg/L. The values for LOD, LOQ, LDR, calibration curve equation, and coefficient of determination (R^2) are presented in Table 5.

Table 5. Analytical method performance of FAAS for selected metals

Analyte	LDR (mg/L)	SE	SD	LOD	LOQ	R^2	Calibration curve equation*
Cr	1.0 - 5.0	0.013	0.022	0.403	1.222	0.9986	A = 0.188C - 0.08
Cu	1.0 - 5.0	0.009	0.015	0.246	0.746	0.9995	A = 0.213C - 0.005
Pb	1.0 - 5.0	0.003	0.006	0.443	1.343	0.9984	A = 0.0476C - 0.031
Fe	1.0 - 5.0	0.005	0.008	0.491	1.487	0.998	A = 0.059C - 0.003
Ca	1.0 - 5.0	0.002	0.003	0.324	0.987	0.9991	A = 0.0382C - 0.0272
Mg	1.0 - 5.0	0.024	0.042	0.381	1.155	0.9988	A = 0.3707C - 0.0031

*In the calibration equation; A = absorbance intensity; and C = concentration (mg/L).

Recovery study

The percentage recovery of the elements investigated falls within the range of 89.00% to 96.00% (Table 6), which is considered acceptable for metal analysis. This suggests that the employed method demonstrates a good accuracy.

Table 6. Recovery study of the spiked Atella sample concentration in mg/L.

Metals	Un spiked sample (mg/L)	Spiked amount (mg/L)	Spiked sample (mg/L)	Recovery (%)
Cr	0.14	3.0	2.90	92.00
Cu	0.81	3.0	3.48	89.00
Pb	0.03	3.0	2.76	91.00
Fe	2.27	3.0	5.15*	96.00
Ca	12.90*	3.0	15.70*	93.00
Mg	5.39*	3.0	8.09*	90.00

*These values are multiplied by the corresponding dilution factors.

Minerals contents of Atella samples

The Ca content of Atella was 656.71 ± 5.79 mg/kg, as shown in Table 7. The Ca content of Atella was found to be the highest among the metals considered in this study, while Pb had the lowest

concentration of 0.41 ± 0.15 mg/kg, compared to Mg, Fe, Cu, and Cr. Some metals are toxic and mutagenic, even at very low concentrations, whereas others play important roles in chemical, biological, biochemical, metabolic, catabolic, and enzymatic reactions in living organisms [30]. The concentrations of toxic metals, specifically Cr and Pb, in cattle feed were below permissible levels for cattle and sheep [31]. The oxidation state of chromium affects its maximum tolerable level (MTL). Hexavalent chromium is much more toxic than trivalent chromium species [32]. Almost all chromium consumed orally is in the form of Cr(III), which is relatively non-toxic because of its poor intestinal absorption and limited entry of absorbed Cr(III) into cells. In contrast, cattle and sheep can tolerate up to 100 mg Pb/kg in their diet when the calcium level is high [31].

The concentrations of Ca and Fe in this study were lower, but that of Cu was slightly higher than that of previously reported for Atella (Table 7). Copper is an essential micronutrient that facilitates enzyme function, metabolic processes, and antioxidant defense systems. However, cattle, sheep, and goats are sensitive to high Cu concentrations [33]. In this study, the amount of Cu in Atella (7.73 ± 1.29 mg/kg) is below the MTL for cattle (40 mg/kg) and sheep (15 mg/kg) in feed [31]. Generally, the percentage of essential minerals (Ca, Mg, Fe, and Cu) in the Atella and its gross energy content are high enough to supplement cattle feed.

Table 7. Mineral content of Atella samples (Mean ± SD, mg/kg, n = 3).

Metals	Current study Atella (mg/kg DM)	Reported [26] Atella (mg/kg DM)	MTL in feed of cattle and sheep (mg/kg DM or %) [31]
Ca	656.71 ± 5.79	820.0	1.5 (%)
Mg	274.74 ± 4.98	-	0.6 (%)
Fe	79.16 ±1.54	3372.3	500
Cu	7.73 ±1.29	4.55	40 (15*)
Cr	1.55 ± 0.33	-	100
Pb	0.41 ± 0.15	-	100

*Only for sheep.

CONCLUSION

The current study assessed the physicochemical characteristics, nutritional profiles, and mineral composition of Atella. The findings indicate that Atella exhibits a low dry matter content (18.59%) and a relatively higher level of fat (4.43%), protein (17.76%), fiber (17.80%), carbohydrate (37.33%), ash (3.97%), and energy (257.88 kcal/g). The sample contained significant amounts of essential macroelements, namely, Ca and Mg, and microelements, such as Fe, Cu, and Cr. However, it also contains a minimal amount of toxic elements, specifically Pb. Atella is mildly acidic in nature. This acidity level has the potential to facilitate the mobility of metal ions in the feed. Therefore, the current practice of utilizing it as a feed source for grazing animals augments the energy and mineral needs of animals.

AKNOWLEDGEMENT

The authors would like to acknowledge the Chemistry Department of Jimma University, Ethiopia, for providing the necessary research facilities to conduct this study.

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