



Nutritional composition and *in vitro* fermentation of avocado fruit fractions from different varieties †

[Composición nutricional y fermentación *in vitro* de fracciones de fruto de aguacate de diferentes variedades]

Selamawit Terefe*, Ajebu Nurfeta and Adugna Tolera

School of Animal and Range Sciences, Hawassa University, Hawassa, P.O. Box 05, Ethiopia. Email: s.terefe@yahoo.com

*Corresponding author

SUMMARY

Background: The rising feed shortages in livestock production have increased interest in exploring alternative resources. Avocado by-products, often discarded, may serve as a nutrient-rich and sustainable feed option for ruminants. **Objective:** To evaluate the chemical composition, mineral content, *in vitro* dry matter digestibility (IVDMD), and *in vitro* gas production of morphological fractions of ripe fruits from seven avocado varieties (Bacon, Ettinger, Fuerte, Hass, Local, Nabal, and Pinkerton) grown in the Wondo Genet district, Sidama region, Ethiopia. **Methodology:** Chemical analyses were conducted using Near-Infrared Reflectance Spectroscopy, and mineral analyses were conducted using Atomic Absorption Spectroscopy, while *in vitro* gas production was measured following the Menke and Steingass protocol using sheep rumen fluid. Statistical analysis was carried out using the Proc Mixed procedure in SAS software (version 9.4). **Results:** The results revealed significant effects ($P < 0.0001$) of variety and morphological fraction on all measured parameters. The Nabal variety had the highest fruit, pulp, seed, and peel weights. Seeds showed the highest dry matter and *in vitro* dry matter digestibility, while pulp fraction had the highest ether extract and crude protein contents. Pinkerton and local varieties had the highest protein content, whereas coat was richest in neutral detergent fiber (NDF) and acid detergent fiber (ADF). Peel fractions exhibited high ADL. The highest calcium content was found in the Fuerte variety and coat fraction, while the Pinkerton variety and pulp fraction had the highest phosphorus content. Significant differences were also observed in gas production kinetics, metabolizable energy (ME), organic matter digestibility (OMD), and short-chain fatty acid (SCFA) production. Local variety and seed fraction exhibited the highest IVDMD and SCFA, while pulp fraction had the highest ME contents. **Implications:** Avocado fruit by-products, especially pulp and seed, can serve as alternative ruminant feed resources, supporting sustainable livestock production and reducing agro-industrial waste; however, appropriate processing and preservation strategies are required to overcome limitations associated with long-term storage. **Conclusion:** Avocado fruit waste has high crude protein, ether extract, digestibility, and energy values, with significant variation across varieties and fractions, indicating their nutritional potential in ruminant feeding when suitable storage and handling practices are applied.

Key words: Avocado fruit fractions; Avocado varieties; *In vitro* gas production; Chemical composition; Mineral content.

RESUMEN

Antecedentes: La creciente escasez de alimento en la producción ganadera ha incrementado el interés en explorar recursos alternativos. Los subproductos del aguacate, a menudo desechados, pueden servir como una opción de alimento sostenible y rica en nutrientes para rumiantes. **Objetivo:** Evaluar la composición química, el contenido mineral, la digestibilidad *in vitro* de la materia seca (IVDMD) y la producción de gas *in vitro* de fracciones morfológicas de frutos maduros de siete variedades de aguacate (Bacon, Ettinger, Fuerte, Hass, Local, Nabal y Pinkerton) cultivadas en el distrito de Wondo Genet, región de Sidama, Etiopía. **Metodología:** Los análisis químicos se realizaron mediante espectroscopia de reflectancia del infrarrojo cercano y los análisis minerales se realizaron mediante espectroscopia de absorción atómica, mientras que la producción de gas *in vitro* se midió siguiendo el protocolo de Menke y Steingass utilizando fluido ruminal de oveja. El análisis estadístico se realizó utilizando el procedimiento Proc Mixed en el software SAS (versión 9.4). **Resultados:** Los resultados revelaron efectos

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ORCID = S. Terefe: <https://orcid.org/0000-0002-6923-7749>; A. Nurfeta: <https://orcid.org/0000-0003-3286-4789>; A. Tolera: <https://orcid.org/0000-0002-1693-775X>

significativos ($P < 0.0001$) de la variedad y la fracción morfológica en todos los parámetros medidos. La variedad Nabal presentó los mayores pesos de fruto, pulpa, semilla y cáscara. Las semillas mostraron la mayor materia seca y digestibilidad *in vitro* de la materia seca, mientras que la fracción de pulpa presentó los mayores contenidos de extracto etéreo y proteína cruda. Las variedades Pinkerton y locales presentaron el mayor contenido proteico, mientras que la cubierta fue más rica en fibra detergente neutra (FDN) y fibra detergente ácida (FDA). Las fracciones de cáscara exhibieron alta LAD. El mayor contenido de calcio se encontró en la variedad Fuerte y la fracción de cubierta, mientras que la variedad Pinkerton y la fracción de pulpa presentaron el mayor contenido de fósforo. También se observaron diferencias significativas en la cinética de producción de gas, energía metabolizable (EM), digestibilidad de materia orgánica (DMO) y producción de ácidos grasos de cadena corta (AGCC). La variedad local y la fracción de semilla exhibieron la mayor DIVMS y AGCC, mientras que la fracción de pulpa presentó los mayores contenidos de EM. **Implicaciones:** Los subproductos del fruto del aguacate, especialmente la pulpa y la semilla, pueden servir como alimento alternativo para rumiantes, apoyando la producción ganadera sostenible y reduciendo los residuos agroindustriales. Sin embargo, se requieren estrategias adecuadas de procesamiento y conservación para superar las limitaciones asociadas con el almacenamiento a largo plazo. **Conclusión:** Los residuos del fruto del aguacate presentan altos valores de proteína cruda, extracto etéreo, digestibilidad y energía, con una variación significativa entre variedades y fracciones, lo que indica su potencial nutricional en la alimentación de rumiantes cuando se aplican prácticas adecuadas de almacenamiento y manejo.

Palabras clave: Fracciones del fruto del aguacate; Variedades de aguacate; Producción de gas *in vitro*; Composición química; Contenido mineral.

INTRODUCTION

Livestock productivity in Sub-Saharan Africa is constrained primarily by shortages of affordable and nutritionally adequate feed resources. Feed accounts for up to 70 % of livestock production costs, and increased reliance on conventional feed ingredients often exacerbates competition between human food and animal feed uses (Makkar, 2018). The supply and demand for animal feed can be balanced by utilizing by-products and co-products from the food and agro-industrial sectors. Pulp, peel, seeds, skin, pomace, husks, pods, and stems are examples of by-products produced by the fruit and vegetable processing industry, which together constitute the majority of agri-food by-products (Raṭu *et al.*, 2023).

Avocado (*Persea americana*) is a nutritious tropical and subtropical fruit (Araújo *et al.*, 2018) cultivated in Ethiopia. It contains the seed (endocarp), peel (epicarp), pulp (mesocarp), and coat (Rosero *et al.*, 2019). Commercial avocado processing generates large quantities of by-products, primarily pulp waste, peels, and seeds, which are typically discarded but have potential value as alternative animal feed resources. Peel and seed account for up to 30 % of the fruit's total weight (Shaltout *et al.*, 2024). These by-products differ in nutritional composition across avocado varieties, which vary in agronomic traits (Janick and Moore, 1996), antioxidant capacity, and oil content (Wang *et al.*, 2010, Yahia, 2012). Studies have shown that the avocado pulp contains high levels of lipids (15–30 %) (Hamzah, 2012) but exhibits limited *in vitro* digestibility for organic matter and fiber (Eliyahu *et al.*, 2015). The seed, often treated as waste, contains antioxidants and bioactive compounds that could contribute to

livestock health and performance (Mensah and Golomeke, 2015).

In Ethiopia, six avocado varieties viz. Hass, Fuerte, Pinkerton, Nabal, Bacon, and Ettinger are cultivated (FAOSTAT, 2010). By-products derived from these varieties differ in nutritional composition across avocado varieties and are further influenced by factors such as ripeness, climate, soil, and fertilizer use, which collectively affect the feed value of their morphological fractions and justify their nutritional evaluation for livestock feeding (Ahmed *et al.*, 2010). Therefore, the present study aimed to determine the NIR-estimated chemical and mineral composition, evaluate the *in vitro* dry matter digestibility, and assess the *in vitro* gas production of morphological fractions of selected ripe avocado varieties cultivated in Ethiopia. We hypothesized that these parameters would differ among avocado varieties and their morphological fractions.

MATERIALS AND METHODS

Description of Study Area

The samples were collected from Wondo Genet, Sidama, Ethiopia (7°19'N, 38°38'E, 1876 m above sea level). (Abewoy *et al.*, 2022). The mid-highland agro-climatic zone supports mixed farming, characterized by bimodal rainfall (January–April, July–September) and a dry season (October–February). Annual rainfall averages 1200 mm, with temperatures of 17–19 °C (Bizuwerk *et al.*, 2004). The soil type is predominantly Andosols, derived from volcanic ash, known for their fertility and good water retention, and the soil pH of the study area varies between 5.6 and 6.5 (Molla, 2022).

Sample Collection and Preparation

Commonly grown seven avocado varieties, Hass, Fuerte, Pinkerton, Nabal, Bacon, Ettinger, and a local variety, were collected from Wondo Genet Agricultural Research Centers, Wondo Genet, Ethiopia, at the mature green stage. For each variety, 20 fruits were randomly collected from five trees (four fruits per tree to ensure adequate biological representation and minimize within-tree variation). Upon arrival in the laboratory, fruits from each variety were pooled and homogenized, and three composite samples were prepared to serve as analytical replicates. The use of composite sampling and three analytical replicates follows established international protocols for *in vitro* digestibility and gas production studies (Tilley and Terry, 1963, Menke and Steingass, 1988) and is widely adopted to reduce within-fruit variability while maintaining reliable analytical precision. After transportation to Hawassa University, the fruits were cleaned, weighed, and kept at room temperature (6-14 days) until ripe, depending on each variety, for morphological assessment. The morphological fractions of the whole fruit, pulp, seed, coat, and peel were determined by weighing each component. The pulp of the fruit was cut into halves, mashed, and oven dried at 60 °C for 72 h due to the high-moisture content and then ground. The seed and peel were removed and weighed; care had been taken to free the peel from adhering to the pulp. The seed was separated from the coat and washed, broken into smaller fragments and dried. The peel, seed and coat were also oven dried at 60 °C for 48 h, ground to a particle size of 1mm for analysis. All samples except pulp were stored at room temperature and the avocado pulp was frozen until analysis to prevent spoilage and oxidation, as it is more moisture- and oil-rich.

Chemical Analysis

The dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ether extract (EE), and *in vitro* DM digestibility were analyzed using a calibrated Near InfraRed Spectroscopy (NIRS) (Foss 5000 apparatus) at Holeta Agricultural Research Center. Samples were dried at 60°C overnight, and 3g was scanned (1108-2492 nm, 8 nm step) for average spectra (Shenk and Westerhaus, 1993). Mathematical and statistical treatments of the NIRS spectra were first treated using Infra soft International (ISI) software. The Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potassium (K), Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn) were analyzed using Atomic Absorption Spectroscopy (AAS) (Thermo Electron Corp., UK), while Sodium (Na) was determined using a UV-spectrophotometer.

In vitro gas production

Fresh rumen fluid was collected from two slaughtered sheep from the butcher house who are involved in selling sheep meat. The donor animals were fed grass hay for 7 days before slaughter. Rumen fluid was collected early in the morning and collected into a pre-warmed thermos flask (39 °C) and immediately transported to the laboratory. Upon arrival, the rumen fluid was mixed, filtered through two layers of cheesecloth, flushed with CO₂, and added to the buffer solution (1:2 v/v) under constant stirring. Gas production (GP) was determined according to the procedure of Menke and Steingass (1988). A buffer solution was prepared and maintained in a water bath at 39 °C under continuous flushing with CO₂. Fermentation buffer solution was prepared from bicarbonate buffer (NaHCO₃ and (NH₄)₂CO₃), macro mineral solution (KH₂PO₄, Na₂HPO₄, MgSO₄·7H₂O), micro mineral solution (MnCl₂·4H₂O, CaCl₂·2H₂O, CoCl₂·6H₂O), resazurin solution, distilled water, and reducing solution (Na₂S·9H₂O, NaOH).

Prior to incubation about 200 mg of avocado sample was weighed and transferred into 100 mL calibrated glass syringes in triplicate in two separate runs/replications. Three syringes with only buffered rumen fluid, termed as blanks, was included in each run. The syringes containing samples, pre-warmed at 39 °C overnight and their pistons lubricated with vaseline to ease movement and prevent gas from escaping. Thirty mL of buffered rumen fluid was injected into each syringe and incubated at 39 °C in a water bath and shaken manually every hour for an initial eight hours (including zero hour) of incubation. The gas production of samples and blanks was recorded at 3, 6, 9, 12, 24, 32, 48, 72 and 96 hours of incubation. Total gas values were corrected for blank incubation which contains only rumen fluid. The purpose of correcting the gas values of feed samples using the blanks was to avoid recording gas produced by the rumen liquor, so that the result reported would be from the incubated feed samples only (Menke and Steingass, 1988). The plunger of the syringe was reset to 30 mL if gas production exceeds 70 mL.

The volume of gas production characteristics was estimated using the equation $Y = a + b(1 - e^{-c(t-L)})$ (Ørskov and McDonald, 1979). Where, Y= volume of gas produced at time t; a= gas production from the immediately soluble fraction (mL); b= gas production from the insoluble fraction (mL); c= gas production rate constant (mL h⁻¹); a + b= potential gas production (mL); t= incubation time (h) and a lag time (colonization time, h) in the onset of GP.

For metabolizable energy (ME) estimation, the gas production of the feed samples was calculated at 24h gas production on 200 mg DM using results from the

blanks, the sample weight and its DM concentration. Organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) contents were estimated according the equation of Menke and Steingass (1988).

$$\text{ME (MJ/Kg DM)} = 1.06 + 0.157 \times \text{G24} + 0.084 \times \text{CP} + 0.22 \text{ EE} - 0.081 \text{ Ash}$$

$$\text{OMD (\%)} = 14.88 + 0.8893 \times \text{G24} + 0.448 \times \text{CP} + 0.651 \times \text{Ash}$$

$$\text{SCFA (mmol/0.2gDM)} = 0.0239 \times \text{G24} - 0.0601$$

Where: GP, CP, EE and Ash are 24 h gas production (mL/0.2g DM), crude protein, ether extract and ash (% DM) of the incubated feed samples, respectively. The volume of gas production was expressed as mL per mL/0.2g DM, following the standard substrate weight used in the *in vitro* gas production technique. Reporting gas volume relative to 0.2 g DM follows the standardized procedure established by Menke and Steingass (1988), which has since become the conventional substrate amount used in *in vitro* gas production assays (Blümmel *et al.*, 1997, Getachew *et al.*, 1998).

Statistical Analysis

Descriptive statistics were used to summarize fruit weight and morphological fraction distribution across avocado varieties. The results are presented as mean values for each variety and corresponding proportions (%) of coat, peel, pulp, and seed relative to total fruit weight. Analysis of variance (ANOVA) was performed using the Proc Mixed procedure in SAS 9.4 to assess the effects of avocado varieties and fractions on chemical composition, minerals, and *in vitro* DM digestibility. The model included variety, fraction, and their interactions. The statistical model used was:

$$Y_{ijl} = \mu + V_i + F_j + (VF)_{ij} + \varepsilon_{ijl}$$

Where:

Y_{ijl} = Response variable μ = Overall mean V_i = Effect of the *i*-th avocado variety F_j = Effect of the *j*-th morphological fraction $(VF)_{ij}$ = Interaction between variety and fraction ε_{ijl} = Random error term.

Means of avocado variety and fractions were compared using Tukey's test and significance was declared at $p < 0.05$.

RESULTS

Proportion of different fractions of avocado varieties

Table 1 shows the fruit weight and the proportion of different morphological fractions (coat, peel, pulp, and seed) of seven avocado varieties collected from Wondo Genet. Among the varieties, Nabal recorded the highest average fruit weight (412.5 g), whereas the local variety had the lowest (117.4 g). The Hass variety had the highest peel proportion (14.2 %) and Ettinger and Nabal had the highest pulp proportions (76.9 % and 77.0 %, respectively). The Local and Bacon varieties showed relatively higher seed proportions (23.0 % and 21.0 %). The data presented in Table 1 are descriptive and are intended to illustrate the distribution of fruit weight and morphological fractions among avocado varieties; no statistical comparison was performed for these parameters.

Chemical composition of different fractions of avocado fruit varieties

Table 2 summarizes the chemical composition of avocado fruit fractions across different varieties. Significant interactions were detected between variety and fraction for all measured parameters. Variety and fraction had a significant effect on all components ($P < 0.0001$). Seed had the highest ($P < 0.05$) DM content whereas the lowest was for pulp. Among the varieties, greater ($P < 0.05$) DM content was observed for Hass compared with the others, the least being for Nabal. The pulp had the highest ash content. Among the variety Bacon and Ettinger had the highest ($P < 0.05$) ash content. Among the fractions, pulp had the highest ($P < 0.05$) crude protein while the lowest was for coat. Pinkerton and local variety had the greatest ($P < 0.05$) CP content. Coat had the highest NDF and ADF content while peel had the highest ADL content. Pulp had the highest ($P < 0.05$) ether extract value and Ettinger also had the greatest EE value. Seed had the highest ($P < 0.05$) IVDMD while pulp had the lowest value. Among the varieties, local and Pinkerton had the highest ($P < 0.05$) IVDMD value.

Table 1. The weight of different varieties of avocado fruits and the weight and proportion of the fruit parts of the different avocado varieties in Wondo Genet.

Avocado Variety	Fruit wt (g)	Fraction weight (g)				Proportion (%)			
		Coat	Peel	Pulp	Seed	Coat	Peel	Pulp	Seed
Bacon	242.9	2.5	14.8	172.4	51.0	1.0	6.1	71.0	21.0
Ettinger	253.5	2.3	15.1	194.8	39.1	0.9	5.9	76.9	15.4
Fuerte	262.8	1.9	18.9	195.9	44.4	0.8	7.2	74.6	16.9
Hass	185.0	1.1	26.3	128.3	27.8	0.6	14.2	69.3	15.0
Local	117.4	1.8	7.9	78.8	27.0	1.5	6.7	67.1	23.0
Nabal	412.5	2.2	36.7	317.4	52.3	0.5	8.9	77.0	12.7
Pinkerton	273.8	1.2	36.6	201.8	32.0	0.5	13.4	73.7	11.7
STD	90.4	0.5	11.2	73.8	10.6	0.4	3.5	3.8	4.2

Note: Values are descriptive means; no statistical comparison was performed for fruit weight and fraction proportions.

Mineral composition of avocado fruit

Table 3 summarizes the mineral content avocado fruit fractions across different varieties. Significant interactions were detected between variety and fraction for all measured minerals. Both variety and morphological fraction had a significant effect on all mineral concentrations ($P < 0.0001$). Calcium content was highest in the Fuerte variety and the coat fraction. Phosphorus was the greatest ($P < 0.05$) in the Pinkerton variety and pulp. Potassium was greatest ($P < 0.05$) Fuerte variety while the lowest was in Pinkerton. The highest ($P < 0.05$) K content was in the pulp fraction. Sodium and magnesium were the highest in Bacon variety. Among the fractions pulp contained greater ($P < 0.05$) Na content while Na was greater in coat compared with other fractions. Iron was most abundant in the Hass variety and in the peel fraction. Zinc concentration was greater ($P < 0.05$) in

the local variety and in the coat fraction. Copper content was highest ($P < 0.05$) in the Nabal variety and the pulp fraction.

In vitro gas production of Avocado fruit

Total gas production from avocado fruit fractions increased steadily across incubation periods (Figure 1-4), with the most rapid rise observed during the early to mid-incubation stages (3–48 hours), followed by a slowing in increase toward 96 hours. This trend suggests that the majority of fermentable substrates were utilized within the first 48 hours. Among the morphological fractions, the seed and coat showed consistently higher gas production trends, indicating greater fermentability. The peel fraction exhibited a moderate rise, while the pulp showed the lowest gas accumulation and a relatively flat line across time points.

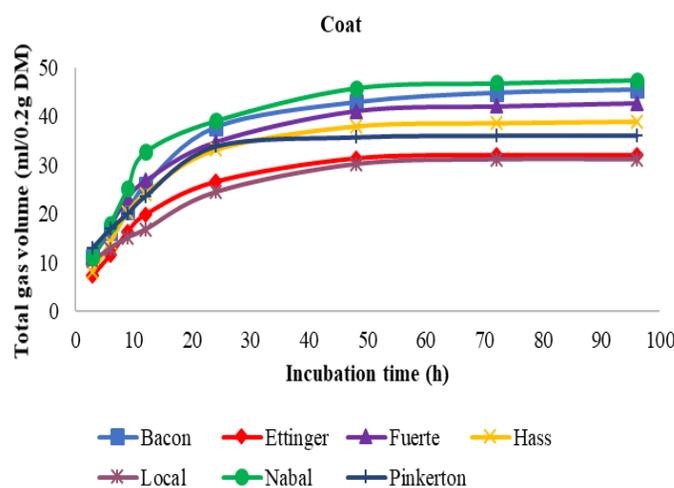


Figure 1. Coat gas production.

Table 2. Chemical composition (% DM, unless specified) and IVDMD of avocado varieties across fractions.

Parameters	Fractions	Variety							Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton			F	V	F × V
Dry matter (%)	Coat	32.2	38.2	28.5	29.9	30.8	27.9	33.9	31.6 ^b	0.03	****	****	****
	Peel	19.3	18.3	16.3	18.8	21.5	28.5	17.3	19.9 ^c	0.03	****	****	****
	Pulp	13.8	14.4	15.6	18.7	16.1	8.9	15.9	14.8 ^d	0.03	****	****	****
	Seed	36.9	33.5	34.4	38.6	27.0	28.7	32.4	33.1 ^a	0.03	****	****	****
	Mean	25.6 ^c	26.1 ^b	23.7 ^c	26.5 ^a	23.8 ^c	23.5 ^f	24.9 ^d					
	SEM	0.04	0.04	0.04	0.04	0.04	0.04	0.04					
Ash	Coat	5.3	5.5	3.3	6.0	5.5	4.4	4.9	5.0 ^c	0.08	****	****	****
	Peel	6.9	7.4	4.2	4.6	6.1	3.6	5.2	5.4 ^b	0.08	****	****	****
	Pulp	9.2	9.6	7.1	7.9	6.4	9.5	8.8	8.4 ^a	0.08	****	****	****
	Seed	3.5	3.8	3.1	3.1	2.9	3.2	3.2	3.2 ^d	0.08	****	****	****
	Mean	6.2 ^a	6.6 ^a	4.4 ^c	5.4 ^b	5.2 ^b	5.2 ^b	5.5 ^b					
	SEM	0.11	0.11	0.11	0.11	0.11	0.11	0.11					
Crude protein	Coat	2.6	1.8	5.5	6.1	12.6	4.2	6.3	5.6 ^d	0.10	****	****	****
	Peel	9.8	14.9	10.6	8.8	17.0	3.7	8.7	10.5 ^b	0.10	****	****	****
	Pulp	20.5	15.4	18.7	17.6	13.9	16.6	28.7	18.8 ^a	0.10	****	****	****
	Seed	7.7	6.7	6.8	7.9	8.8	7.3	8.6	7.7 ^c	0.10	****	****	****
	Mean	10.2 ^{bc}	9.7 ^c	10.4 ^b	10.1 ^{bc}	13.1 ^a	7.9 ^d	13.1 ^a					
	SEM	0.13	0.13	0.13	0.13	0.13	0.13	0.13					
Neutral detergent fiber	Coat	72.1	75.3	66.7	63.4	58.1	74.0	60.7	67.2 ^a	0.09	****	****	****
	Peel	50.1	33.6	42.5	54.6	40.5	70.8	53.2	49.3 ^b	0.09	****	****	****
	Pulp	38.8	42.8	39.5	36.5	41.2	28.4	41.3	38.4 ^c	0.09	****	****	****
	Seed	27.9	32.4	31.1	28.5	38.9	35.9	27.7	31.8 ^d	0.09	****	****	****
	Mean	47.3 ^b	46.0 ^c	44.9 ^d	45.8 ^c	44.7 ^d	52.3 ^a	45.7 ^c					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					
Acid detergent fiber	Coat	36.6	37.6	32.5	32.3	22.9	38.5	27.9	32.6 ^a	0.09	****	****	****
	Peel	15.6	11.4	13.1	23.9	10.8	36.3	16.9	18.3 ^c	0.09	****	****	****
	Pulp	17.7	37.8	28.0	25.1	26.9	24.4	39.2	28.5 ^b	0.09	****	****	****
	Seed	8.2	8.1	7.2	6.9	10.3	7.9	7.6	8.0 ^d	0.09	****	****	****
	Mean	19.5 ^f	23.7 ^b	20.2 ^c	22.1 ^d	17.8 ^g	26.8 ^a	22.9 ^c					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					
Acid detergent lignin	Coat	11.5	11.8	11.1	11.6	12.3	9.1	11.6	11.3 ^b	0.09	****	****	****
	Peel	13.4	6.1	11.6	14.9	7.9	15.5	15.3	12.1 ^a	0.09	****	****	****
	Pulp	2.9	3.6	1.2	3.4	3.0	0.7	4.5	2.8 ^d	0.09	****	****	****
	Seed	7.5	7.2	6.1	6.1	8.6	6.9	6.8	7.0 ^c	0.09	****	****	****
	Mean	8.8 ^b	7.2 ^c	7.5 ^{de}	8.9 ^b	7.9 ^{cd}	8.1 ^c	9.5 ^a					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					
Ether extract	Coat	5.7	6.4	6.1	7.3	12.3	4.7	10.4	7.6 ^c	0.09	****	****	****
	Peel	17.3	25.3	19.7	14.4	20.3	7.7	16.3	17.3 ^b	0.09	****	****	****
	Pulp	59.9	61.9	64.4	57.7	58.0	51.9	59.3	59.0 ^a	0.09	****	****	****

Parameters	Fractions	Variety							Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton			F	V	F × V
<i>In vitro</i> dry matter digestibility	Seed	4.1	5.7	5.2	4.1	5.0	6.9	4.5	5.1 ^d	0.09	****	****	****
	Mean	21.8 ^d	24.8 ^a	23.8 ^b	20.9 ^c	23.9 ^b	17.8 ^f	22.6 ^c					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					
	Coat	51.2	51.4	54.1	54.3	53.1	57.4	52.3	53.4 ^b	0.09	****	****	****
	Peel	48.1	54.3	48.8	45.9	57.5	39.9	46.9	48.8 ^c	0.09	****	****	****
	Pulp	26.5	26.3	21.9	24.9	28.8	39.6	38.0	29.4 ^d	0.09	****	****	****
	Seed	80.3	80.3	80.6	82.9	77.1	76.9	80.1	79.7 ^a	0.09	****	****	****
	Mean	51.5 ^{cd}	53.1 ^b	51.4 ^d	52.0 ^c	54.1 ^a	53.4 ^b	54.3 ^a					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					

SEM= Standard error of mean; ^{a,b,c,d} Means with one or more similar superscripts within a row or within a column are not significantly different ($P>0.05$)
 F=morphological fraction effect; V= Avocado variety effect; ns: not significant. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Table 3. Mineral composition (mg/100g) of avocado varieties across fraction.

Parameters	Fractions	Variety							Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton			F	V	F×V
Calcium	Coat	521.8	705.2	580.6	352.4	542.9	584.4	550.8	548.3 ^a	0.01	****	****	****
	Peel	461.8	407.2	582.9	305.5	610.7	384.5 ⁿ	481.3	461.9 ^b	0.01	****	****	****
	Pulp	334.9	275.9	354.3	195.3	304.7	282.8	371.9	302.8 ^d	0.01	****	****	****
	Seed	442.5	318.2	315.8	253.2	336.0	353.4	405.1	346.3 ^c	0.01	****	****	****
	Mean	440.2 ^d	426.6 ^c	458.4 ^a	276.6 ^g	448.6 ^c	401.3 ^f	452.3 ^b					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Phosphorus	Coat	55.5	105.0	101.5	92.5	172.0	55.0	66.5	92.6 ^d	0.49	****	****	****
	Peel	124.5	53.5	118.5	134.5	32.0	63.5	155.5	97.4 ^c	0.49	****	****	****
	Pulp	182.0	105.5	152.5	174.0	224.0	207.5	211.5	161.0 ^a	0.49	****	****	****
	Seed	134.5	130.0	125.0	152.5	94.0	71.5	165.0	143.2 ^b	0.49	****	****	****
	Mean	124.1 ^d	98.5 ^c	124.4 ^d	138.4 ^b	130.5 ^c	99.4 ^c	149.6 ^a					
	SEM	0.64	0.64	0.64	0.64	0.64	0.64	0.64					
Potassium	Coat	785.6	465.7	1360.5	1567.5	1109.2	678.2	892.6	979.9 ^d	0.09	****	****	****
	Peel	1291.6	1444.8	1973.5	1665.6	1786.5	750.8	1421.7	1476.3 ^b	0.09	****	****	****
	Pulp	1611.5	1487.6	1680.7	1744.3	1351.7	2329.7	1078.4	1611.9 ^a	0.09	****	****	****
	Seed	980.7	865.6	971.7	936.5	1174.2	1167.5	1066.8	1023.3 ^c	0.09	****	****	****
	Mean	1167.3 ^c	1065.9 ^g	1496.6 ^a	1478.5 ^b	1355.4 ^c	1231.6 ^d	1114.9 ^f					
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12					
Sodium	Coat	22.0	8.9	8.7	12.3	11.7	21.8	28.5	12.2 ^c	0.01	****	****	****
	Peel	23.2	15.2	19.6	8.2	17.5	19.6	20.0	17.6 ^b	0.01	****	****	****
	Pulp	97.1	52.8	41.9	96.8	45.5	100.9	21.5	65.2 ^a	0.01	****	****	****
	Seed	19.0	5.3	6.4	6.3	6.7	6.2	6.7	8.1 ^d	0.01	****	****	****
	Mean	40.3 ^a	20.5 ^d	19.2 ^f	30.9 ^c	20.3 ^c	37.2 ^b	19.2 ^f					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Magnesium	Coat	247.9	232.0	262.5	211.4	188.7	305.2	245.1	241.8 ^a	0.01	****	****	****
	Peel	225.4	207.2	289.5	163.7	347.2	105.2	176.5	216.4 ^b	0.01	****	****	****
	Pulp	181.9	142.4	121.6	139.1	150.9	197.0	96.7	147.1 ^c	0.01	****	****	****
	Seed	180.1	85.9	83.2	110.2	92.2	163.7	120.8	119.5 ^d	0.01	****	****	****
	Mean	208.8 ^a	166.9 ^c	189.2 ^d	156.1 ^g	194.7 ^b	192.8 ^c	159.8 ^f					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Iron	Coat	15.6	11.6	14.2	19.6	11.1	16.9	28.4	16.8 ^b	0.01	****	****	****
	Peel	20.5	16.1	20.5	36.8	29.1	17.0	17.4	17.9 ^a	0.01	****	****	****
	Pulp	13.8	9.6	11.4	26.2	14.8	22.1	16.5	16.4 ^c	0.01	****	****	****
	Seed	7.7	4.6	9.1	4.3	10.1	5.9	7.7	11.7 ^d	0.01	****	****	****
	Mean	14.4 ^c	10.4 ^g	13.8 ^f	21.8 ^a	16.2 ^c	15.5 ^d	17.5 ^b					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Zinc	Coat	2.5	3.4	5.9	4.2	5.9	2.0	2.1	3.7 ^a	0.01	****	****	****
	Peel	3.2	3.7	1.8	0.8	6.5	1.2	1.7	2.7 ^b	0.01	****	****	****
	Pulp	2.1	3.1	1.8	1.0	3.0	5.4	1.8	2.6 ^c	0.01	****	****	****

Parameters	Fractions	Variety							Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton			F	V	F×V
Copper	Seed	1.8	3.3	0.9	1.5	3.5	1.4	1.6	2.0 ^d	0.01	****	****	****
	Mean	2.4 ^e	3.4 ^b	2.6 ^c	1.9 ^f	4.7 ^a	2.5 ^d	1.8 ^g					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
	Coat	0.5	0.3	0.4	1.1	0.5	1.3	1.1	0.72 ^b	0.01	****	****	****
	Peel	0.5	0.5	0.5	0.3	0.6	0.5	1.0	0.55 ^d	0.01	****	****	****
	Pulp	0.4	0.7	0.5	0.7	0.3	4.6	1.1	1.19 ^a	0.01	****	****	****
	Seed	0.5	0.6	0.5	0.5	0.7	0.4	1.1	0.61 ^c	0.01	****	****	****
Manganese	Mean	0.45 ^e	0.52 ^d	0.46 ^c	0.65 ^c	0.52 ^d	1.71 ^a	1.07 ^b					
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
	Coat	3.2	4.7	5.6	8.6	5.0	7.3	5.5	5.7 ^a	0.01	****	****	****
	Peel	4.1	4.6	5.5	2.9	8.3	1.9	8.4	5.1 ^b	0.01	****	****	****
	Pulp	1.4	2.0	0.6	1.2	1.5	2.2	2.1	1.6 ^c	0.01	****	****	****
	Seed	1.1	1.4	0.8	0.7	1.3	2.9	1.2	1.3 ^d	0.01	****	****	****
	Mean	2.5 ^f	3.2 ^e	3.1 ^e	3.4 ^d	4.0 ^b	3.6 ^c	4.3 ^a					
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01						

SEM= Standard error of mean; ^{a,b,c,d} Means with one or more similar superscripts within a row or within a column are not significantly different ($P>0.05$)
 F=morphological fraction effect; V= Avocado variety effect; ns: not significant. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

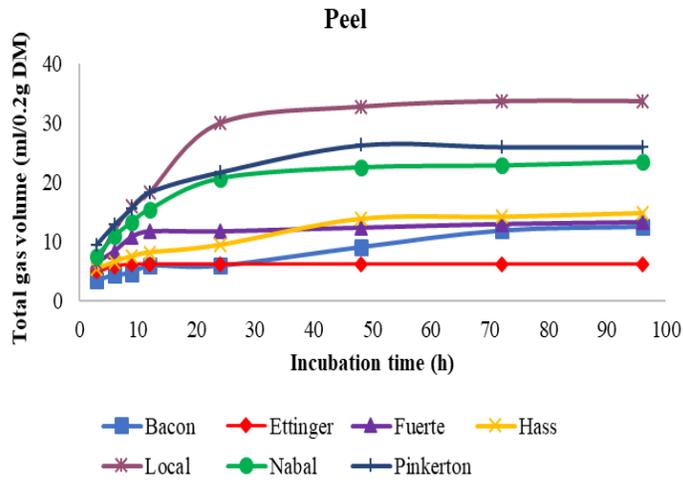


Figure 2. Peel gas production.

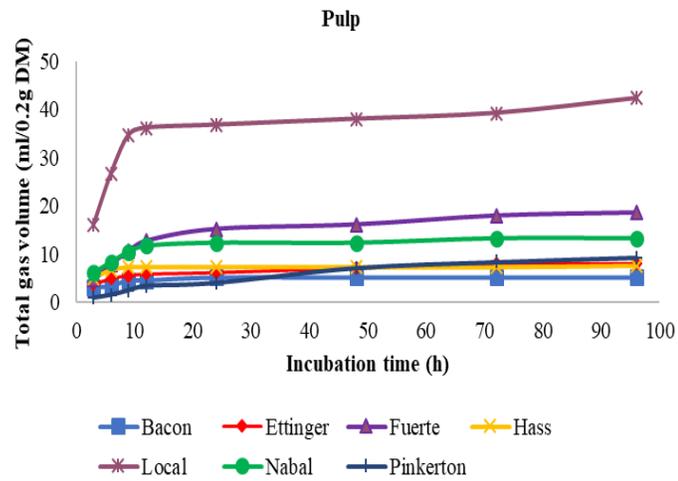


Figure 3. Pulp gas production.

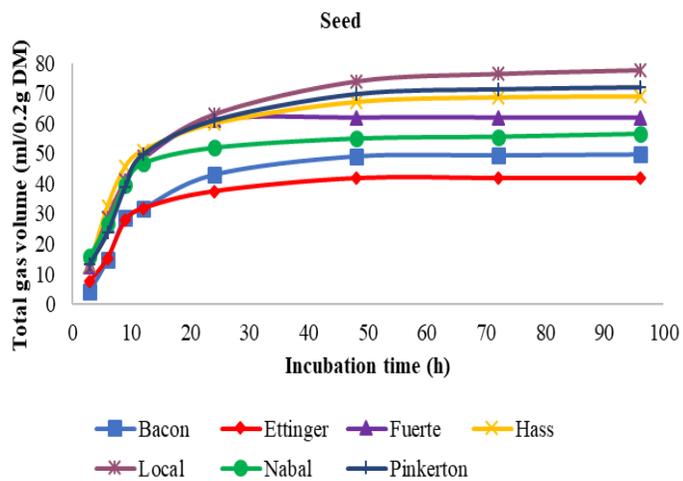


Figure 4. Seed gas production.

***In vitro* gas characteristics of avocado fruit morphological fractions**

In vitro gas production characteristics were significantly influenced by avocado variety and morphological fraction (Table 4), except for the “a” and lag time parameters, where variety had no significant effect. There were interaction effects except for the a+b. Seed fractions showed the highest gas production (63.0 mL/0.2 g at 24 h). Among the varieties, gas production from the local variety was greater ($P<0.05$) than that of the other varieties, except Nabal. The “b” fraction for Ettinger was lower than that of local and Pinkerton, while the other varieties had an intermediate value. The “a” fraction for the coat was greater than that of pulp and seed. Seed had the highest “b” value. Ettinger had a greater ($P<0.05$) “c” value compared with other varieties except Hass and Nabal. Among the fractions, the coat had a lower “c” value than peel and pulp. The gas production potential (a+b) for local was higher than that of Ettinger, while the other varieties had an intermediate value. Among the fractions, the highest a+b was for seed. The lag time for seed was higher ($P<0.05$) than for coat and peel.

Metabolizable Energy, Organic Matter Digestibility, and Short-chain Fatty Acids of Avocado Fruits

Avocado variety and morphological fraction significantly affected ($P<0.0001$) the estimated ME, OMD, and SCFA values derived from *in vitro* gas production (Table 5). Significant interaction effects were observed between variety and fraction. Pulp had the highest estimated ME, while local variety had the highest estimated ME content. The highest ($P<0.05$) estimated OMD was for seed. Among the varieties, local had the greatest estimated OMD of the others, except Pinkerton. Estimated SCFA was highest in local variety except for Nabal. Seed was the highest in estimated SCFA.

DISCUSSION

Proportion of different fractions of avocado varieties

The Nabal variety showed higher fruit weight compared to values reported by Abebe *et al.* (2022) for the same variety in Raya Azebo, northern Ethiopia, whereas the weights of Hass and Fuerte were comparable to those in the same study. However, both varieties showed lower values than those reported by Rodríguez-Carpena *et al.* (2011) under different growing conditions. The higher fruit

weight of the Nabal aligns with Melke (2024), who noted its positive response to the favorable climate and fertile soils of the Sidama region, particularly Wondo Genet. From a feed-use perspective, fruit weight is relevant only insofar as it determines the quantity of by-products generated during fruit processing or from cull fruits not suitable for human consumption. Larger fruits tend to yield higher pulp proportions, thereby increasing the availability of usable by-products for livestock feeding without intensifying competition between human food and animal feed (Zapata-Luna *et al.*, 2025). Heavier fruits with high pulp proportion, such as Nabal, may yield more usable biomass. This highlights the need to evaluate each fraction’s nutritional value and digestibility, rather than focusing solely on fruit size. Pinkerton, Ettinger, and Bacon also fell within reported ranges (Crane *et al.*, 2013) supporting the influence of varietal potential and local environment. Differences across studies likely result from variations in altitude, soil fertility, genotype, and management practices (Abbas *et al.*, 2020, Abebe *et al.*, 2022), with soil type also playing a key role in productivity (Dubrovina and Bautista, 2014).

The pulp, peel, and seed weights of Hass and Fuerte were lower than those reported by Rodríguez-Carpena *et al.* (2011), but comparable to the values by Ly *et al.* (2021) for pulp and peel weight and Abebe *et al.* (2022) for seed weight. These variations are consistent with genotype–environment interactions known to influence fruit composition (Tango *et al.*, 2004, Bressani *et al.*, 2009). From a feed utilization point of view, the relative contribution of each fraction is important because it directly affects the overall nutritive value and potential digestibility of the fruit (Marcos *et al.*, 2020, Ly *et al.*, 2021). In the present study, the pulp fraction represents whole, non–oil-extracted avocado pulp, which is naturally rich in crude protein and lipids. These nutrients may contribute to rumen microbial growth by supplying nitrogen and readily available energy sources (Wanapat *et al.*, 2024). However, the ether extract content of avocado pomace, a by-product of avocado oil extraction, is considerably lower because most of the oil is removed during processing. In contrast, the seed fraction contributes fermentable carbohydrates that can enhance *in vitro* dry matter digestibility (Duguma *et al.*, 2024). Therefore, the observed variation in fraction proportions among varieties not only reflects morphological diversity but also provides a basis for explaining consequent differences in chemical composition, *in vitro* digestibility, and gas yield discussed later in this paper.

Table 4. The *in vitro* gas production constants of avocado varieties across fractions.

Parameters	Fractions	Variety								Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton	F			V	F×V	
24 hr. Gas Production (mL/0.2g)	Coat	37.7	26.6	34.7	33.1	24.5	39.1	33.8	32.8 ^b	1.33	****	****	****	
	Peel	5.9	6.2	11.8	9.5	30.1	20.7	21.8	15.1 ^c	1.33	****	****	****	
	Pulp	5.2	6.2	15.2	7.3	36.8	12.3	4.1	12.4 ^c	1.33	****	****	****	
	Seed	42.9	37.5	60.9	59.9	63.0	51.9	60.9	53.9 ^a	1.33	****	****	****	
	Mean	22.9 ^{cd}	19.1 ^d	30.7 ^b	27.5 ^{bc}	38.6 ^a	30.9 ^{ab}	30.1 ^{bc}						
	SEM	1.76	1.76	1.76	1.76	1.76	1.76	1.76						
a	Coat	2.9	0.9	3.2	-0.9	6.3	-0.2	5.8	2.6 ^a	1.01	****	ns	*	
	Peel	3.4	-4.9	2.1	4.2	-1.1	3.2	3.8	1.5 ^{ab}	1.01	****	ns	*	
	Pulp	1.1	3.5	0.8	-6.7	-9.5	1.3	-0.7	-1.5 ^b	1.01	****	ns	*	
	Seed	-13.8	-10.9	-14.7	-17.7	-7.4	-8.1	-9.5	-11.7 ^c	1.01	****	ns	*	
	Mean	-1.6	-2.9	-2.2	-5.3	-2.9	-0.9	-0.1						
	SEM	1.33	1.33	1.33	1.33	1.33	1.33	1.33						
b	Coat	42.4	31.3	39.2	39.8	25.8	47.0	30.7	36.6 ^b	3.39	****	**	*	
	Peel	16.0	11.2	10.7	11.7	35.4	20.1	22.8	18.2 ^c	3.39	****	**	*	
	Pulp	4.2	4.9	16.8	14.1	48.7	11.6	52.6	21.8 ^c	3.39	****	**	*	
	Seed	63.1	52.4	77.1	85.4	83.3	63.9	80.7	72.3 ^a	3.39	****	**	*	
	Mean	31.4 ^{ab}	24.9 ^b	35.9 ^{ab}	37.7 ^{ab}	48.3 ^a	35.7 ^{ab}	46.7 ^a						
	SEM	4.49	4.49	4.49	4.49	4.49	4.49	4.49						
c	Coat	0.06	0.07	0.08	0.09	0.05	0.09	0.08	0.08 ^b	0.02	***	***	****	
	Peel	0.01	0.71	0.17	0.04	0.07	0.09	0.11	0.17 ^a	0.02	***	***	****	
	Pulp	0.19	0.06	0.10	0.54	0.25	0.21	0.05	0.19 ^a	0.02	***	***	****	
	Seed	0.11	0.14	0.14	0.16	0.10	0.16	0.11	0.13 ^{ab}	0.02	***	***	****	
	Mean	0.09 ^b	0.25 ^a	0.12 ^b	0.21 ^{ab}	0.12 ^b	0.14 ^{ab}	0.09 ^b						
	SEM	0.03	0.03	0.03	0.03	0.03	0.03	0.03						
a+b	Coat	45.3	32.2	42.4	38.8	32.0	46.8	36.5	39.2 ^b	3.53	****	**	ns	
	Peel	19.4	6.2	12.8	15.9	34.2	23.3	26.5	19.8 ^c	3.53	****	**	ns	
	Pulp	5.3	8.3	17.6	7.4	39.3	12.9	51.9	20.4 ^c	3.53	****	**	ns	
	Seed	49.3	41.5	62.4	67.7	75.9	55.9	71.3	60.6 ^a	3.53	****	**	ns	
	Mean	29.8 ^{ab}	22.0 ^b	33.8 ^{ab}	32.5 ^{ab}	45.4 ^a	34.8 ^{ab}	46.6 ^a						
	SEM	4.67	4.67	4.67	4.67	4.67	4.67	4.67						
Lag time	Coat	0.00	0.17	0.00	0.33	0.00	0.10	0.00	0.09 ^b	0.27	**	ns	*	
	Peel	0.00	0.63	0.00	0.00	0.53	0.00	0.07	0.18 ^b	0.27	**	ns	*	
	Pulp	0.03	0.00	0.00	0.70	0.80	0.00	4.97	0.93 ^{ab}	0.27	**	ns	*	
	Seed	2.20	1.67	1.50	1.30	1.07	0.80	1.13	1.38 ^a	0.27	**	ns	*	
	Mean	0.56	0.62	0.38	0.58	0.60	0.23	1.54						
	SEM	0.34	0.34	0.34	0.34	0.34	0.34	0.34						

SEM = Standard error of mean a = Gas production from the immediately soluble fraction (mL), b = gas production from the insoluble but degradable fraction (ml), a + b = potential gas production (mL), C = the rate constant of gas production (fraction/h), L = lag time; ^{a,b,c,d} Means with one or more similar superscripts within a row or within a column are not significantly different ($P>0.05$). ns: not significant. ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Table 5. Estimated Metabolizable energy, organic matter digestibility, and short-chain fatty acids of morphological fractions for seven avocado varieties in Wondo Genet.

Parameters	Fractions	Variety							Mean	SEM	P-value		
		Bacon	Ettinger	Fuerte	Hass	Local	Nabal	Pinkerton			F	V	F×V
ME (MJ/kg DM)	Coat	7.6	5.8	7.6	7.6	7.8	7.9	8.5	7.5 ^c	0.21	****	****	****
	Peel	5.4	8.1	7.0	5.6	10.8	5.2	7.3	7.1 ^c	0.21	****	****	****
	Pulp	15.3	15.3	17.7	14.6	19.1	13.7	15.2	15.8 ^a	0.21	****	****	****
	Seed	8.7	7.8	11.4	11.2	11.8	10.3	11.5	10.4 ^b	0.21	****	****	****
	Mean	9.3 ^c	9.2 ^c	10.9 ^b	9.7 ^{bc}	12.4 ^a	9.3 ^c	10.6 ^b					
	SEM	0.28	0.28	0.28	0.28	0.28	0.28	0.28					
OMD (%)	Coat	56.6	47.3	54.3	53.2	49.6	57.1	53.6	53.1 ^b	1.18	****	****	****
	Peel	34.6	33.5	39.2	34.5 ⁱ	56.2	43.8	50.0	41.7 ^d	1.18	****	****	****
	Pulp	40.9	40.4	49.2	44.1	66.9	50.6	47.2	48.5 ^c	1.18	****	****	****
	Seed	61.9	59.3	79.9	78.4	82.9	72.9	79.5	73.6 ^a	1.18	****	****	****
	Mean	48.5 ^{cd}	45.2 ^d	55.6 ^b	52.5 ^{bc}	63.9 ^a	56.1 ^b	57.6 ^{ab}					
	SEM	1.56	1.56	1.56	1.56	1.56	1.56	1.56					
SCFA (mmol/g DM)	Coat	0.84	0.57	0.77	0.73	0.52	0.87	0.75	0.72 ^b	0.03	****	****	****
	Peel	0.08	0.09	0.22	0.17	0.66	0.43	0.46	0.30 ^c	0.03	****	****	****
	Pulp	0.06	0.09	0.30	0.11	0.82	0.23	0.04	0.24 ^c	0.03	****	****	****
	Seed	0.97	0.84	1.40	1.37	1.45	1.18	1.39	1.23 ^a	0.03	****	****	****
	Mean	0.49 ^{cd}	0.39 ^d	0.67 ^b	0.59 ^{bc}	0.86 ^a	0.68 ^{ab}	0.66 ^{bc}					
	SEM	0.04	0.04	0.04	0.04	0.04	0.04	0.04					

ME= metabolizable energy; OMD= organic matter digestibility; SCFA= short-chain fatty acid; SEM= Standard error of mean; ^{a,b,c,d} Means with one or more similar superscripts within a row or a column are not significantly different ($P>0.05$). F=morphological fraction effect; V= Avocado variety effect; ns: not significant. ** $P<0.01$, *** $P<0.001$, **** $P=0.001$, **** $P<0.0001$

Chemical composition of Avocado fruits

The pulp DM content across the seven varieties was lower than previously reported ranges (Requejo-Tapia, 1999, Ly *et al.*, 2021), and similar reductions were observed in the peel and seed fractions compared with earlier studies (Okoruwa *et al.*, 2015, Egbonu *et al.*, 2018, Ly *et al.*, 2021). These differences likely reflect maturity at harvest, as DM increases with oil accumulation during ripening (Ozdemir and Topuz, 2004), as well as temperature variations during the ripening period (Tzatzani *et al.*, 2023). From a feeding perspective, lower DM indicates higher moisture content, which can reduce storage stability and increase susceptibility to spoilage (Park, 2008). However, when blended with dry feedstuffs such as dry concentrates, hay or crop residues, moisture-rich pulp and peel can improve palatability, reduce dustiness, and help complement the low nutrient density of typical basal diets (Eliyahu *et al.*, 2015). When mixed with drier ingredients, avocado by-products therefore could serve as useful balancing components, enhancing both physical characteristics and nutrient distribution in the overall ration (Wadhwa and Bakshi, 2013). Understanding DM variation in relation to maturity and oil content is therefore essential when evaluating how avocado by-products can complement and improve conventional feed formulations.

The avocado pulp showed relatively high ash content, indicating a notable mineral presence. Although ash values were lower than those reported for avocados from Gembu, Nigeria (Maitera *et al.*, 2014), but

higher than the range recorded by Nnaji and Okereke (2016), likely reflecting differences in soil mineral composition, climate, and varietal nutrient uptake. Ash content reflects the overall mineral fraction of avocado by-products and contributes to their nutritional characterization. Although avocado fractions contain minerals such as Ca, K, Na, Fe, and Mg are moderate and provide only limited support to overall dietary mineral requirements (Okibe *et al.*, 2024). Thus, while these by-products can contribute to mineral balance, they should be regarded as complementary ingredients rather than replacements for targeted mineral supplementation.

The avocado pulp contained relatively high crude protein (CP), supporting its value as a nutritional component in livestock feed. These CP levels were higher than those reported for mixed peel-pulp waste (Duguma *et al.*, 2024) but consistent with findings from other genotypes (Luitel, 2022), likely reflecting differences in sample composition, variety, and maturity. From a nutritional perspective, the CP content of fresh, non-oil-extracted pulp can support rumen microbial growth and fiber digestion when included as part of a balanced ration (Firkins, 2021), making it a useful complementary feed ingredient rather than a primary protein source. Its contribution to total dietary CP, however, depends on the feeds it replaces within the ration. Oil-extracted pomace typically contains even higher CP because lipid removal concentrates protein. Evidence from seed-based studies supports this mechanism Emelike *et al.* (2020) reported that defatted avocado seed flour had markedly higher CP (11.90%) compared to

undefatted seed flour (7.24%), demonstrating how oil extraction improves relative protein concentration in avocado by-products. While the fresh pulp used in this study contains moderate CP, its nutritional contribution is best realized when blended with drier, more fibrous ingredients to balance moisture and optimize rumen fermentation. The seed fraction showed CP values comparable to earlier reports (Talabi *et al.*, 2016, Morais *et al.*, 2017), but lower than the value reported by Nwaokobia *et al.* (2018). In addition, in avocado trees, nitrogen is mobilized from leaves before leaf abscission and moved into other parts of the plant, such as stems, roots, or developing fruits, and it also significantly affects postharvest quality (Bower and Cutting, 1988). Since nitrogen is essential for protein synthesis, this redistribution may contribute to the increased crude protein content in the pulp or seed. Overall, the CP characteristics of avocado by-products highlight their potential as complementary feed components within sustainable livestock nutrition systems rather than standalone protein sources.

The avocado peel exhibited high NDF contents, consistent with Jalal *et al.* (2024), but significantly exceeding the values from Negesse *et al.* (2009) and Marcos *et al.* (2020). These variations may reflect genetic differences, root stock selection, cultivation practices, and climatic effects in determining the fiber profile of avocado fruits, as stated by Álvarez *et al.* (2020). The peel also contained the highest acid detergent lignin (ADL) content among all fruit fractions, indicating limited digestibility due to its high lignin content and this restricts fiber digestibility and consequently reduces gas production, as lignified tissues are less accessible to rumen microbes (Wang and McAllister, 2002), although structural fiber still contributes to chewing and rumen function (Schulze *et al.*, 2014). In contrast, the pulp showed moderate NDF and lower ADL, suggesting better fermentability and energy availability, despite having the highest ADF among fractions, though still within reported ranges (Pazla *et al.*, 2023). From a nutritional standpoint, moderate NDF levels contribute to maintaining rumen motility and chewing activity (Beauchemin and Yang, 2005), whereas excessive NDF resulted in reduced dry matter intake (Shi *et al.*, 2023). The seed fraction had the lowest NDF and ADF, but its nutritional role is shaped more by its bioactive compounds such as phenolics, flavonoids, and tannins than by fermentable carbohydrates (Salazar-López *et al.*, 2020), which may influence microbial populations and fermentation pathways in the gastrointestinal tract. Inclusion levels of seed meal have been shown to reduce performance in non-ruminants (García-Contreras *et al.*, 2025), underscoring the need for careful interpretation of its nutritional role. Overall, differences in NDF, ADF, and ADL among avocado

fractions highlight the importance of evaluating each component separately when formulating diets using avocado by-products.

The ether extract (EE) content of Hass pulp was comparable to values reported by Nasri *et al.* (2021), but higher than those found by Alkhalaf *et al.* (2019), likely reflecting seasonal or locational effects (Ferreira da Vinha *et al.*, 2020). Peel EE values were lower than those of reported by Marcos *et al.* (2020), but higher than those reported by Eman *et al.* (2024). The high EE content of pulp has important nutritional implications elevated lipid levels can reduce gas and CH₄ production because fats ferment poorly and can inhibit rumen microbes, resulting in lower degradability and digestibility Marcos *et al.* (2020). Pastor *et al.* (2020) similarly reported reduced ruminal NH₃-N in goats fed high-fat pulp diets, noting that dietary EE above 6 % of DM can depress fermentation. High lipid intake may also reduce feed consumption due to effects on gut hormones, fatty-acid oxidation in the liver, and feed acceptability (Fiorentini *et al.*, 2013). These findings indicate that negative effects that could arise mainly when fresh pulp is fed alone or at high inclusion levels; when used as part of a mixed ration, the total dietary EE, rather than the pulp itself, determines fermentation outcomes. By contrast, oil-extracted avocado pomace contains much lower EE and can usually be incorporated without adverse effects (García-Vargas *et al.*, 2020). Seed EE values in this study were lower than those reported by several authors (Ifesan *et al.*, 2015, Talabi *et al.*, 2016, Ejiofor *et al.*, 2018, Nwaokobia *et al.*, 2018), but higher than the result reported by Okoruwa *et al.* (2015), (Morais *et al.*, 2017).

The seed fraction showed the highest IVDMD among all avocado components, exceeding values reported by Ly *et al.* (2021), likely due to differences in variety, maturity, or pre-treatment conditions. Its lower fiber and lignin contents enhanced microbial access, contributing to the higher digestibility. Processing techniques such as roasting, blanching, or soaking in ascorbic acid have been shown to modify seed structure, reduce anti-nutritional factors, and improve enzymatic accessibility (Fufa *et al.*, 2025), which may further enhance digestibility. However, IVDMD alone does not fully reflect feeding value. Factors such as palatability, odor, texture, and secondary metabolites can influence intake, nutrient absorption, and overall animal response (Ebrahim and Negussie, 2020, Besharati *et al.*, 2022). Although the avocado seed shows favorable digestibility, it also contains secondary metabolites that may affect its nutritional value and utilization (Okorie *et al.*, 2024) and there is still limited information on palatability and animal response. Therefore, the high IVDMD of the avocado seed observed in this study suggests

biochemical suitability for ruminant feeding however, its practical use should consider feed acceptability, processing needs, and inclusion level to optimize utilization in livestock diets. In conclusion, the relatively high IVDMD of avocado seed indicates its potential, suggesting that it can be a valuable component in livestock diets.

Mineral Composition of Different Varieties of Avocado Fruits

Macro minerals

Calcium. The calcium (Ca) content of avocado pulp in this study was substantially higher than values reported in earlier studies (Arukwe *et al.*, 2012, Tamayo *et al.*, 2018). For example, Dreher and Davenport (2013) reported 0.013% Ca in Hass pulp, whereas the present value (0.1953%) is roughly fifteen times greater, likely reflecting varietal, maturity, environmental, or methodological differences that require further verification as variety and environment related shifts in Ca concentration have also been demonstrated by Wanjiru *et al.* (2025). Peel and seed Ca levels is lower than the value reported by Morais *et al.* (2017) but greater than other values (Nwaokobia *et al.*, 2018, Tamayo *et al.*, 2018). The coat Ca content is higher than the value reported by Tamayo *et al.* (2018). Ca is essential for skeletal development and neuromuscular function (Soetan *et al.*, 2010). These results indicate considerable variability in Ca distribution among avocado morphological fractions, which contributes to their overall nutritional characterization.

Phosphorus. In this study, the phosphorus (P) content of the avocado pulp was higher compared to earlier findings (Hardisson *et al.*, 2001, Nwaokobia *et al.*, 2018). It is within the range reported by Van Rooyen and Bower (2005), though lower than the value found by Tamayo *et al.* (2018). Peel and coat P levels were comparable to previous findings, while the seed fraction exceeded earlier reports (Nwaokobia *et al.*, 2018, Tamayo *et al.*, 2018). Phosphorus is essential for skeletal development, energy metabolism, and rumen microbial function, and deficiencies often reduce feed intake and growth under grazing systems (McDowell, 1996). However, the efficiency of P utilization depends on adequate energy and protein supply in the diet. Therefore, the P content of avocado pulp and seed contributes to their nutritional value and is relevant when evaluating these by-products for inclusion in balanced rations, particularly in low-input feeding systems.

Potassium. In this study, the potassium (K) content of the avocado pulp was higher than values reported in previous studies (Morais *et al.*, 2017, Tamayo *et*

al., 2018), though lower than the value reported by Hofman *et al.* (2002). The peel showed higher K levels relative to earlier findings (Morais *et al.*, 2017, Tamayo *et al.*, 2018). The seed fraction K concentration is lower than the value reported by Morais *et al.* (2017), but higher than the value reported by Tamayo *et al.* (2018). The coat was lower than the value compared to Tamayo *et al.* (2018). These results suggests that pulp and peel can be valuable sources for livestock feed or soil enrichment. Additionally, k plays a vital role in regulating osmotic pressure and is important in maintaining acid–base balance (Saha and Pathak, 2021). Avocado pulp and peel contain relatively high K concentrations, and plays a key role in maintaining electrolyte balance (Bhuyan *et al.*, 2019), its nutritional implications depend not the nutritional implications depend on the overall dietary electrolyte balance and the mineral profile of other feed ingredients included in the ration. Excess dietary K can interfere with magnesium absorption and can present metabolic and physiologic challenges to cattle, potentially increasing the excretion of K into the environment (NRC, 2001). Therefore, while avocado by-products are promising K-rich feed ingredients, their inclusion must be balanced carefully in ration formulation.

Sodium. The sodium (Na) content of avocado pulp of the varieties Bacon, Hass and Nabal was higher than values reported (Hardisson *et al.*, 2001, Nwaokobia *et al.*, 2018) for Hass variety. The peel showed lower Na levels compared to the value reported by Morais *et al.* (2017). The seed Na content in this study was between higher values (Morais *et al.*, 2017, Nwaokobia *et al.*, 2018), and lower ones (Arukwe *et al.*, 2012, Ifesan *et al.*, 2015). These variations suggest that genotype and growing conditions influence Na accumulation across avocado fractions. Among the fractions, avocado pulp offers the most relevant Na contribution which helps in maintaining acid–base balance and hydration in animals and under heat stress, electrolyte losses can lower feed intake and growth, but Na supplementation helps to restore balance and improve performance (Ahmad and Sarwar, 2006). This observation is supported by the findings of Masters *et al.* (2005), who reported that both Na deficiency and excess can significantly impact feed intake and production in sheep, highlighting the need to monitor Na levels when incorporating avocado by-products into livestock rations.

Magnesium. The magnesium (Mg) content in the pulp fraction was higher than values reported for avocado varieties (Van Rooyen and Bower, 2005, Morais *et al.*, 2017). Similarly, the peel and seed fractions showed higher Mg levels compared to earlier findings (Morais *et al.*, 2017, Nwaokobia *et*

al., 2018, Tamayo *et al.*, 2018). The coat's Mg content was comparable to that reported by Tamayo *et al.* (2018). Mg is vital for cellular metabolism, enzyme activation and bone development in livestock (Pinotti *et al.*, 2021), making these findings valuable for improving mineral balance in feed formulations.

Trace minerals

Iron. The iron (Fe) content in the pulp fraction was higher than values reported by Nwaokobia *et al.* (2018) and Tamayo *et al.* (2018). Similarly, the peel and coat showed higher Fe concentrations compared to Tamayo *et al.* (2018). Fe levels in the seed were comparable to those in some studies (Nwaokobia *et al.*, 2018, Tamayo *et al.*, 2018), but exceeded the value reported by other researchers (Arukwe *et al.*, 2012, Morais *et al.*, 2017). Fe plays a vital role in metabolism, particularly in the formation of hemoglobin and the transport of oxygen in the blood (Soetan *et al.*, 2010) in livestock. Although Fe concentrations were relatively high, their contribution depends on bioavailability and interactions with dietary inhibitors such as Ca, phytates, and polyphenols (Piskin *et al.*, 2022). Therefore, avocado by-products may supply supplemental Fe, but should be incorporated with attention to overall mineral balance.

Zinc. In this study, the zinc (Zn) content of the pulp fraction exceeded previously reported values (Morais *et al.*, 2017, Tamayo *et al.*, 2018, Kämper *et al.*, 2020). The peel Zn content was higher than the value reported Morais *et al.* (2017), but lower than the value reported by Tamayo *et al.* (2018). The seed Zn content was comparable to Morais *et al.* (2017), higher than other reports (Arukwe *et al.*, 2012, Ifesan *et al.*, 2015, Tamayo *et al.*, 2018), yet lower than that of Nwaokobia *et al.* (2018). The coat had a lower Zn content than that reported by Tamayo *et al.* (2018). Zn is essential for all classes of livestock and poultry due to its involvement in protein synthesis, metabolic enzyme function, and insulin regulation, which affects carbohydrate metabolism (Berger and Cunha, 1993). Although avocado fractions contain measurable Zn, their nutritional value depends on inclusion level, bioavailability, and mineral–mineral interactions that can reduce Zn absorption (Gresakova *et al.*, 2021).

Copper. The copper (Cu) content of the pulp fraction was close to that reported by Morais *et al.* (2017), and higher than the values mentioned in other studies (Tamayo *et al.*, 2018, Kämper *et al.*, 2020). Peel Cu concentration was lower than that reported by Morais *et al.* (2017), and below the value reported by Tamayo *et al.* (2018). The seed contained comparable levels with other studies (Arukwe *et al.*, 2012, Nwaokobia *et al.*, 2018, Tamayo *et al.*, 2018),

but considerably lower than the value reported by Morais *et al.* (2017). The coat also had less Cu than reported the value reported by Tamayo *et al.* (2018). Cu plays a critical role in the growth and formation of bone, helps in the incorporation of Fe in hemoglobin, assists in the absorption of Fe from the gastrointestinal tract (GIT), and the transfer of Fe from tissues to the plasma (Murray *et al.*, 2000). Although avocado by-products contain measurable Cu levels, their contribution to dietary Cu supply must be evaluated within the context of overall ration composition, given the susceptibility of Cu absorption to antagonistic interactions with other minerals and the homeostatic regulation of Cu metabolism (Jansen van Ryssen and Bath, 2025).

Manganese. The manganese (Mn) content in the pulp fraction was comparable to values reported by Tamayo *et al.* (2018) and Morais *et al.* (2017), but higher than that reported by Kämper *et al.* (2020). The peel Mn content exceeded the value reported by Tamayo *et al.* (2018). The seed fraction had Mn levels similar to those reported by Arukwe *et al.* (2012) and Morais *et al.* (2017), but lower than Tamayo *et al.* (2018). The coat showed a value similar to that reported by Tamayo *et al.* (2018). The Mn content of feedstuffs is quite variable and is influenced by soil types, soil pH, fertilization, and plant species (NRC, 2001). Mn is an essential nutrient in animals that plays a role in bone mineralization, regulation of protein and energy metabolism, cellular protection (EFSA, 2013). Although Mn levels in some fractions are relatively high, their nutritional relevance depends on bioavailability and should be considered within the context of a balanced ration.

Summary of Mineral requirement in Avocado Morphological Fractions

Overall, the mineral composition of the avocado morphological fractions exhibited distinct and fraction-specific patterns when expressed relative to general ruminant mineral requirement ranges (Table 6), consistent with previous reports on fruit by-products used in ruminant feeding (Morais *et al.*, 2017, Tamayo *et al.*, 2018). Calcium concentrations were predominantly within the reported requirement ranges across all fractions, Phosphorus levels were adequate in the pulp, peel, and seed, whereas the coat consistently fell below requirements. Potassium concentrations were frequently above the general requirement range, particularly in pulp and peel fractions, reflecting the naturally high potassium content typical of fruit-derived feed materials (Czech *et al.*, 2020). Sodium concentrations were consistently low across all fractions. Magnesium concentrations were largely within the general requirement range, although a proportion of observations, particularly in seed fractions, fell below

Table 6. Content of macro and trace minerals in Avocado fractions relative to recommended mineral requirements for ruminants (%).

Fraction	Level	Macro minerals					Trace minerals			
		Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn
Coat	Higher	0	0	42.9	14.3	28.6	100	42.9	14.3	85.7
	within	100	14.3	42.9	0	71.4	0	57.1	28.6	14.3
	Less	0	85.7	14.3	85.7	0	0	0	57.1	0
Peel	Higher	0	0	85.7	0	28.6	85.7	14.3	0	71.4
	within	100	57.1	14.3	0	71.4	14.3	28.6	14.3	14.3
	Less	0	42.9	0	100	0	0	57.1	85.7	14.3
Pulp	Higher	0	0	100	0	0	100	14.3	28.6	0
	within	100	71.4	0	42.9	85.7	0	42.9	14.3	42.9
	Less	0	28.6	0	57.1	14.3	0	42.9	57.1	57.1
Seed	Higher	0	0	42.9	0	0	85.7	0	0	0
	within	100	85.7	57.1	0	57.1	14.3	28.6	28.6	14.3
	Less	0	14.3	0	100	42.9	0	71.4	71.4	85.7
Requirement		190-820	120-480	500-1000	60-180	100-250	3-5	2-4	0.7-1.1	2-4

Recommended mineral requirement for all classes of ruminants (summarized by McDowell (1983)) for macro and trace (mg/100g DM)

the reference range. Among trace minerals, iron concentrations were predominantly above the general requirement range across all fractions, while zinc and copper showed greater variability, with a higher proportion of observations falling below the reference ranges, especially in pulp and seed fractions. Manganese distribution was uneven among fractions, with peel and coat showing a higher proportion of values above the reference range compared with pulp and seed. Overall, Table 6 highlights substantial variability in mineral distribution among avocado morphological fractions. These comparisons are intended to describe compositional patterns relative to commonly cited requirement ranges rather than to indicate dietary adequacy or supplementation needs, which are influenced by regional conditions, production systems, and specific feeding objectives (McDowell, 1983).

***In vitro* gas production**

The seed fraction produced the highest gas volume, likely due to its low cell wall content (NDF, ADF, ADL) and high levels of soluble carbohydrates (Table 2). Higher gas output is commonly linked to greater carbohydrate fermentation (Ribeiro *et al.*, 2022) and enhanced microbial activity (Pastorelli *et al.*, 2023). The low ADF content observed in the seed fraction aligns with findings by Karabulut *et al.* (2007), who reported a negative correlation between ADF levels and gas production. Likewise, the high IVDMD of the seed fraction supports earlier findings that digestibility is positively correlated with gas yield (Getachew *et al.*, 2004). Together, these characteristics indicate that avocado seed has high fermentability and digestibility, making it a strong

candidate as an energy-rich feed component for ruminants.

The pulp fraction produced the lowest 24-h gas volume, reflecting reduced short-chain fatty acid (SCFA) production. Although pulp and peel showed rapid early fermentation, gas production slowed afterward, likely due to their relatively high CP and fat contents and limited fermentable carbohydrates (Table 2). Protein-rich feeds typically yield less gas (Mlambo *et al.*, 2008), and fat contributes minimally to gas production (Getachew *et al.*, 1998). Protein fermentation also releases ammonia, which buffers hydrogen ions during VFA formation and without generating CO₂ output results in a reduced total gas output (Cone and van Gelder, 1999). Similar negative correlations between CP and gas production have been reported by Abdulrazak *et al.* (2000), Bezabih *et al.* (2014), and Getachew *et al.* (2004). Overall, the pulp's compositional profile high CP and EE with limited soluble carbohydrates constrains microbial activity, leading to lower gas and SCFA production.

Gas production characteristics

In this study, the coat fraction exhibited the highest soluble fraction (a-value), indicating greater availability of rapidly fermentable substrates and rapid initial microbial activity although its practical relevance as a feed component is limited by its very low proportional contribution to fruit weight (< 1.5%; Table 1) and due to its minimal inclusion potential in practical feeding scenarios, the nutritional significance of this fraction remains limited. In contrast, the seed fraction, which represents a substantially larger proportion of the fruit (> 11.7%; Table 1), exhibited negative a-values, likely reflecting

limited soluble substrates and the presence of anti-nutritional compounds such as tannins, alkaloids, and phytic acid (Talabi *et al.*, 2016). Despite this, the seed fraction recorded the highest values for the slowly fermentable fraction (b-value) and total gas production (a + b), likely due to its rich starch and sugar content which becomes available once inhibitory effects diminish. This aligns with Getachew *et al.* (2004), who reported strong positive associations between fermentation kinetics and digestibility. In contrast, the pulp and peel fractions exhibited lower b-values, which may be attributed to their relatively high fat content (Melesse *et al.*, 2018) and elevated CP levels. Similar negative links between CP and b-values Abdulrazak *et al.* (2000) and between CP and total gas production reported by Getachew *et al.* (2004) strengthen these patterns. Overall, elevated fat and protein in pulp and peel suppress fermentative activity and limit potential gas yield.

The peel fraction showed a much lower fermentation rate constant (c) than the 5.18 h⁻¹ reported by Marcos *et al.* (2020), indicating slower microbial breakdown. The seed fraction, in contrast, exhibited the fastest fermentation rate and highest total gas production, consistent with its low fiber content and greater microbial accessibility. However, it also had the longest lag time (L), reflecting initial microbial inhibition associated with its negative a-value. The peel likewise presented an extended lag time compared with by Marcos *et al.* (2020), further supporting the influence of substrate composition on fermentation onset. Lag time represents the period needed for microbial colonization and enzymatic adaptation; long values typically result from limited soluble substrates or inhibitory compounds (Pastorelli *et al.*, 2023). Similarly, the extended lag time observed for the peel fraction supports the influence of fiber levels and phenolic compounds in delaying fermentation onset. Overall, these results demonstrate that while coat fraction kinetics provide insight into substrate fermentability, the seed fraction has greater practical importance due to its substantially higher mass contribution and fermentative potential. Nevertheless, the presence of inhibitory compounds suggests that seed utilization may benefit from processing or controlled inclusion levels, whereas pulp and peel fractions exhibit more moderate fermentation responses driven by their lipid and protein contents.

Metabolizable energy, organic matter digestibility, and short-chain fatty acids

The peel fraction showed lower estimated metabolizable energy (ME) than the 13.6 MJ/kg DM reported by Negesse *et al.* (2009), whereas the pulp exhibited comparatively high estimated ME together

with high CP and fat. Although Karabulut *et al.* (2007) reported positive associations among estimated ME, CP, and fat, other studies (Bezabih *et al.*, 2014, Abraham *et al.*, 2023), found negative correlations. In the present study, the pulp's high estimated ME agreed with low IVDMD, consistent with Abraham *et al.* (2023). This discrepancy likely reflects the lipid-depressing effect on fermentation: high fat increases calculated ME in gas-based prediction equations, yet simultaneously suppresses microbial activity, reduces carbohydrate degradation, and limits gas production. Thus, estimated ME values in lipid-rich fractions may appear high even when actual fermentative energy release is limited. This highlights the need for cautious interpretation of estimated ME in high-fat feedstuffs such as avocado pulp.

The peel fraction showed higher estimated organic matter digestibility (OMD) than the 36.5 % reported by Negesse *et al.* (2009), yet overall estimated OMD values for both peel and pulp remained low. Their elevated CP and fat contents likely contributed to reduced microbial degradation, consistent with the negative correlations reported by Bezabih *et al.* (2014) and Abraham *et al.* (2023). Although peel exhibited distinct digestibility characteristics, its practical contribution as a feed component is limited by its relatively smaller proportional contribution to total fruit mass compared with pulp (Table 1). Therefore, its digestibility characteristics are of greater than nutritional significance. Despite containing valuable nutrients, pulp and peel ferment inefficiently under in vitro conditions. Practically, these by-products are best used in combination with highly digestible forages to improve rumen degradability and maintain adequate energy supply.

The pulp fraction produced low short-chain fatty acid (SCFA) concentrations, likely due to its high CP and fat contents. Protein generates less SCFA than carbohydrate fermentation Getachew *et al.* (2004), and similar negative associations with SCFA have been reported by Hamid *et al.* (2007), Maghsoud *et al.* (2008), and Abraham *et al.* (2023). Because SCFA formation is closely linked to gas output and digestibility (Getachew *et al.*, 2004, Maheri-Sis *et al.*, 2008), reduced SCFA production reflects limited fermentable substrate availability in pulp. Although gas volume generally parallels SCFA production (Trei *et al.*, 1970), it may not always represent microbial biomass yield (Liu *et al.*, 2002). These findings highlight the difficulty of using gas and SCFA as sole indicators of fermentation efficiency, particularly in protein- and fat-rich feedstuffs like avocado pulp. These results indicate that avocado pulp contributes less to fermentative energy supply than its estimated ME value suggests. Consequently,

its inclusion level in ruminant diets should remain moderate, and it should be combined with carbohydrate-rich, rapidly fermentable feeds to improve fermentation efficiency.

CONCLUSION

Avocado varieties and their morphological fractions differed significantly in chemical composition, mineral content, and *in vitro* fermentation characteristics. Among the fractions, pulp and seed showed greater nutritional relevance due to higher lipid, protein, or fermentable carbohydrate contents, whereas peel and coat were more fibrous and less digestible. Mineral composition varied across fractions, emphasizing heterogeneous distribution rather than uniform nutritional adequacy. Fermentation results indicated high degradability of the seed fraction, while the high lipid content of pulp reduced microbial fermentation efficiency, underscoring the need for cautious inclusion. Overall, avocado by-products particularly pulp and seed can serve as complementary feed resources for ruminants when incorporated within balanced rations. Further *in vivo* studies are required to evaluate intake, optimal inclusion levels, and processing effects on nutrient utilization and animal performance.

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Compliance with ethical standards. The authors confirm that the research was carried out and managed in accordance with ethical standards. Rumen fluid was collected from an abattoir, and therefore the procedure did not require approval by an ethical committee.

Data availability. The data supporting the conclusions of this article can be obtained with the

aid of the corresponding author's email: s.terefe@yahoo.com

Author contribution statement (CRediT). **S. Terefe** – Conceptualization, Data curation, Formal analysis, Investigation, and Writing-original draft. **A. Nurfeta** – Conceptualization, Investigation, Methodology, supervision, Validation, Writing-review, and editing. **A. Tolera** – Conceptualization, Investigation, Methodology, Supervision, Validation, Writing-review, and editing.

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