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Rumen fermentation profile and methane mitigation potential of mango and avocado byproducts as feed ingredients and supplements

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Fruit byproducts represent a sustainable alternative to conventional feed for ruminants, addressing food-feed competition and environmental concerns, particularly through their potential to reduce enteric methane emissions via bioactive compounds. This study explored the use of mango and avocado byproducts as feed ingredients and supplements. In experiment 1, mango peel (MP), mango seed kernel (MSK), mango seed coat (MSC), avocado peel (AP), and avocado seed (AS) were independently tested to determine their chemical composition, in vitro digestibility, and rumen fermentation parameters, including gas production and methane emissions. In experiment 2, rumen fermentation parameters were evaluated across five treatment groups: The control group received 200 mg of alfalfa hay alone, without any supplementation. The remaining four groups each received 200 mg of alfalfa hay as the basal diet, supplemented with 15 mg of one of the following microencapsulated extracts: mango peel extract (MPE), avocado peel extract (APE), mango seed kernel extract (MSKE), or avocado seed extract (ASE). Both experiments were conducted over three runs, with each run including three replicates per treatment group, resulting in a total of nine replicates per group. Data were analyzed using linear mixed models with Bonferroni-adjusted pairwise comparisons ($p < 0.05$). MSK had the highest crude protein content, whereas AP had the highest ether content. MSC and AP presented the highest fiber fractions. AP and MP showed higher total phenolic content and antioxidant capacity. In experiment 1, AS, MP and MSK resulted in greater in vitro dry matter digestibility, and cumulative gas production compared to MSC and AP. Acetate to propionate ratios were higher in AS, MSC, and MSK. Methane production (ml/g dry matter incubated) was highest in MSK (43.7), while AP (19.8) and MSC (18.7) produced the lowest, representing almost 55% reduction compared to MSK ($P < 0.001$). MP (40.9) and AS (42.2) had intermediate methane values. Ammonia nitrogen was highest in AP and lowest in MSC. In experiment 2, MSKE, ASE and the control had the highest cumulative gas production, whereas APE reduced methane production by 16% compared to the control and lowered the acetate-to-propionate ratio. Compared with the control, all the encapsulated extracts lowered the ammonia nitrogen concentration. Overall, MP, MSK, and AS have emerged as the most promising ingredients because of their relatively high digestibility, and fermentation efficiency, whereas APE and MPE have potential as feed supplements for reducing in vitro methane production.

Keywords Fruit byproducts, Phenolic compounds, Encapsulated extracts, Digestibility, Methane, Ruminal fermentation

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The increasing demand for food production on a global scale has led to a substantial quantity of organic waste being generated by the agro-industrial sector, which presents considerable economic and environmental challenges. Each year, approximately 1.6 billion tons of food valued at about $1.2 trillion are lost or wasted globally, which represents roughly one-third of the total food produced[1,2]. Landfilling, incineration, and dumping are the predominant techniques employed for the disposal of these agro-industrial byproducts, all of which lead to environmental contamination[3,4]. Moreover, ruminant livestock production plays a crucial role in ensuring global food security and meeting nutrition requirements[5]. Nevertheless, the livestock sector accounts for 14% of total anthropogenic greenhouse gas emissions worldwide, with ruminants accounting for more than 81% of enteric methane (CH_4) emissions[6]. Additionally, CH_4 is responsible for 2–12% of the energy loss in ruminants[7], trapping heat much more efficiently than carbon dioxide (CO_2)[8]. Therefore, investigating sustainable feeding strategies that can effectively reduce CH_4 emissions while simultaneously fulfilling the nutritional requirements of the increasing ruminant population is important. The use of agro-industrial byproducts as alternative feed resources presents a potential solution for addressing the issues outlined above because of their good nutritional profile and the presence of bioactive compounds[9]. For instance, byproducts such as dried citrus pulp, corn distiller's dried grains with solubles, exhausted olive cake, grape marc, tomato pomace, pomegranate peel and seed have been successfully incorporated into ruminant diets, demonstrating benefits like reduced feeding costs and decreased environmental impact associated with byproduct disposal[10–12]. However, their efficacy varies due to differences in composition, processing, and bioactive compound bioavailability.

Mangoes (*Mangifera caesia* Jack) and avocados (*Persea americana* Mill), both extensively cultivated tropical fruits, produce significant amounts of waste throughout their cultivation and processing stages. The annual production of avocados reaches approximately 6 million metric tonnes[13]. The waste generated from avocado production consists primarily of peels and seeds, which make up 13–18% of the fruit's fresh weight[14]. Mango, one of the world's most important tropical fruits, produces approximately 46 million tonnes annually, with processing resulting in 35–60% waste, mostly peels and kernels[15]. These byproducts are rich in a wide variety of bioactive components, such as polyphenols, flavonoids and tannins[16]. These bioactive compounds have a significant effect on the ruminal microbiota, fermentation, and digestion, ultimately aiding in the reduction of CH_4. However, their susceptibility to degradation under rumen conditions (e.g., pH fluctuations, enzymatic activity) limits their practical application[17]. To overcome this challenge, encapsulation technologies, such as beta-cyclodextrin (β-CD), offer a novel strategy to enhance bioactive stability and controlled release. β-CD, a cyclic oligosaccharide with a hydrophilic exterior and hydrophobic cavity, forms inclusion complexes with phenolic compounds[18], shielding them from degradation and improving solubility[17,19]. This ensures bioactive integrity during digestion, enabling sustained interaction with rumen microbes to suppress CH_4 production[19]. While prior studies have explored mango and avocado byproducts incorporation in ruminants diet in various forms such as silage multimineral blocks, and predominantly their peels or pomace[20,21], inconsistencies in the results of previous studies have led us to investigate these byproducts further, as their conclusive effects on rumen fermentation and CH_4 mitigation are not yet well established. Furthermore, their use as encapsulated bioactive sources for targeted CH_4 mitigation remains underexplored. This study addresses these gaps through a two-phase approach. First, the nutritional composition, in vitro dry matter digestibility (IVDMD), and fermentation characteristics of mango and avocado byproducts were evaluated to establish their baseline suitability as ruminant feed resources. Second, in vitro fermentation dynamics of alfalfa hay supplemented with β-CD-encapsulated phenolic extracts from these byproducts were investigated to assess their potential to optimize rumen function and reduce CH_4 emissions. Through this dual approach, the study aimed to contribute to the development of sustainable dietary interventions in ruminant nutrition.

Materials and methods

Fruit byproducts and processing

Fruit materials were procured from local markets and restaurants in Saltillo, Coahuila, Mexico. Twenty-five kilograms of commercially ripened mangoes were obtained from the local market, and the pulp, peel, and seeds were separated. The outer layer of the mango seeds (endocarp or seed coat) was detached from the remaining seed material (seed kernel). Additionally, approximately 15 kg of avocado peels and seeds were collected as waste from local restaurants. All mango byproducts were cut into small pieces (approximately 1.0 to 2.0 cm) and subjected to drying at 42 °C for 12 h[22], while avocado byproducts were dried at 60 °C for 8 h in a hot air oven (Excalibur 3926 TB). After drying, the byproducts were ground to a particle size of 1 mm, sieved, and stored in closed, light-protected containers until further analysis.

Chemical composition analysis

The chemical composition of Mango and Avocado fruit byproducts and alfalfa hay (used as the basal diet in Experiment 2) were determined in triplicate for each sample. Dry matter (DM) was determined using a hot air oven, where samples were weighed before and after drying, and DM was calculated by subtracting the moisture loss from the initial weight. Except stated otherwise, all chemical analyses were done using AOAC method[23]. Crude protein (CP) content was measured via a Kjeltec 8100 (P. R. China) (method 990.03), and ether extract (EE) was determined via Soxtec Avanti (FOSS, Höganäs Sweden) (method 920.39) and the ash content was measured via a muffle furnace (Analitica s.a.s., Pesaro, Italy) (method 942.05). The ash-corrected neutral detergent fiber (NDF) contents were analyzed by adding heat-resistant α-amylase, after which acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed via FT 122 Fibertec (FOSS Analytical Co., Ltd. China 215121), following Van Soest et al.[24]. Non-fibrous carbohydrates (NFC) were calculated using the formula: $NFC = 100 - (CP + EE + NDF + Ash)$[25].

Preparation of mango and avocado byproducts extracts

Ultrasonic extractions for both mango and avocado fruit byproducts, such as mango peel, mango seed kernel, mango seed coat, avocado peel, and avocado seed were performed using ethanol as a solvent[26]. A sample weighing 1 g was extracted with 50 mL of solvent for 120 min at 45 °C. After extraction, the samples were filtered with filter paper. All extracts were stored at 4 °C until analysis.

Total phenolic content analysis

The total phenolic content (TPC) of mango and avocado byproducts was determined via the Folin–Ciocalteu method[26]. Briefly, 0.1 mL of the extract from the byproduct samples was added to a test tube, followed by the addition of 1.9 mL of distilled water. After that, 2.5 mL of Lowry C solution and 0.25 mL of Folin reagent were added, and the mixture was vortexed. After mixing, the tubes were kept in the dark for 30 min, after which UV–Vis absorption was measured at 750 nm (Cary Win UV). A calibration curve prepared with gallic acid was used to quantify the TPC (mg GAE/g DM).

Total antioxidant capacity analysis

The antioxidant capacities of the mango and avocado byproducts were assessed using the ABTS methods[26]. The antioxidant capacity was quantified via a calibration curve prepared with Trolox. To perform the ABTS method, a solution of ABTS• radical was produced by combining 2.45 mM $K_2S_2O_8$ with a 7 mM ABTS solution and distilled water. In test tubes, 0.1 mL of byproduct extract, 3.9 mL of ethanol, and 1 mL of ABTS• radical solution (diluted at a 1:10 ratio with distilled water) were combined and mixed. The absorbance of the samples was measured at 734 nm using a UV–Vis spectrophotometer (Cary Win UV) after a duration of 6 min.

Experiment 1

This study evaluated the fermentation characteristics of five distinct fruit byproducts: mango peel (MP), avocado peel (AP), mango seed kernel (MSK), avocado seed (AS), and mango seed coat (MSC). Each byproduct was treated as a separate experimental treatment, without combining them with any other feed ingredients or additives to independently assess its fermentation profile, gas production, and methane emissions. The experiment was conducted over three runs, with each run including three replicates per treatment group, resulting in a total of nine replicates per group.

Ruminal inoculum preparation

Two healthy cows were selected and fed ad libitum a total mixed ration (TMR) formulated to meet or exceed the predicted requirements of the NRC[27] for energy, protein, minerals, and vitamins. The diet primarily consisted of corn silage and a concentrate mixture at a 47:53 forage-to-concentrate ratio. Rumen fluid used in this study was obtained post-slaughter from a local slaughterhouse, where digesta was collected from the rumen and manually squeezed, allowing the liquid portion to be extracted into a glass bottle. The collected fluid (approximately 1000 ml) was immediately placed into a pre-warmed thermos maintained at 39 °C. The rumen fluid was immediately transferred to the laboratory. The rumen fluid was sieved through four layers of muslin cloth and placed in an Erlenmeyer flask at 39 °C under continuous CO_2 flushing. The buffer mixture for in vitro rumen fermentation was prepared using the method described by Menke and Steingass[28]. The buffer contained macro-minerals, micro-minerals, a reducing agent, and resazurin as a reduction indicator. The buffered ruminal inoculum mixture was continuously gassed with CO_2 and maintained at 39 °C, following the standard procedure described by Menke and Steingass[28]. The mixture was prepared using a 1:2 ratio of rumen fluid to buffer solution, with 10 ml of rumen fluid and 20 ml of buffer solution used in each syringe.

In vitro incubation

A precise amount of each byproduct (200 mg) was weighed into 100 mL calibrated glass syringes (Fortuna®, Häberle Labortechnik, Germany). The syringes were prepared in triplicate for each byproduct, resulting in a total of 15 treatment syringes per run (5 fruit byproducts × 3 replicates). Additionally, three blank syringes containing no substrate were included in each run to correct the gas production results by subtracting the gas production in these blanks from those observed in the treatment groups. The experiment was repeated in three independent runs, and each syringe resulted in 9 replications for each fruit byproduct ($n = 9$). Syringes were incubated with 30 mL of buffered ruminal inoculum for 24 h in a water bath maintained at 39 °C. The syringes were manually swirled every 2 h throughout the incubation period to ensure thorough mixing of the substrate with the ruminal fluid. Gas production was measured at intervals of 3, 6, 12, and 24 h. The CH_4 production of the byproducts was assessed separately via the AMPTS II and Gas Endeavour systems (Bioprocess Control, Lund, Sweden) following a modified methodology based on Nell[29]. For CH_4 quantification, a distinct set of incubation bottles ($n = 9$ per byproduct) was connected to the Gas Endeavour system's CO_2 absorption unit, which was filled with 3 M NaOH to selectively remove CO_2 and hydrogen sulfide (H_2S). The absorption unit was linked to a Flow Cell Array and Data Acquisition (DAQ) unit, operating on the principles of liquid displacement and buoyancy to quantify CH_4 production (mL per gram of incubated feed). All CH_4 flow rates were normalized to standard conditions (1 atm, 0 °C, dry gas).

Analysis of fermentation parameters

After 24 h of incubation, the buffered rumen fluid samples were transferred to a 50 mL centrifugation tube and analyzed for pH (Sartorius PB-20, Göttingen, Germany). After pH evaluation, the buffered rumen fluid was centrifuged at 150,000 × g at 4 °C for 15 min. After that, volatile fatty acid (VFA) analysis was performed via an Acclaim 4 × 250 mm organic acid column with HPLC (ICS 3000, Dionex Corporation, San Francisco, CA)

following the methodology of Sucu[30]. Ammonia nitrogen (NH_3-N) was determined according to the AOAC[23] using a Kjeltech autoanalyzer (Gerhardt, Bonn, Germany).

In vitro dry matter digestibility

The IVDMD of fruit byproducts (MP, AP, MSK, AS, and MSC) was investigated using the Daisy II Incubator (Ankom Technology, USA) following the method described by Goering and Van Soest[24]. Fresh rumen liquor was collected, blended, and purged with CO_2 at 39 °C as described in the previous section. A 250 mg sample of each fruit byproduct was weighed and added to ANKOM F57 filter bags, which were heat sealed and introduced into 5-L incubation jars of the Daisy II Incubator with 24 bags per jar. The filtered rumen fluid was diluted in the buffered medium at a 1:4 (v/v) ratio. Two litres of buffered rumen fluid were anaerobically transferred into each incubation jar and closed with a plastic lid with a single-way valve to prevent the accumulation of fermentation gases. Each fruit byproduct was incubated in triplicate within a single jar, and three separate jars were used for the digestibility experiment, resulting in a total of 9 replications per byproduct ($n = 9$). In addition to the fruit byproducts, each jar contained two standard feed ingredients (alfalfa hay and maize) for reference and three blank bags for correction purposes. The jars were placed in a revolving incubator (ANKOM Daisy) where they were maintained at 39 °C with continuous rotation to ensure the correct immersion of the bags in buffered rumen fluid. After 48 h of incubation, the bags were rinsed gently with cold water. The percentage of IVDMD was then calculated on a dry matter basis via the following equation:

$$\mathrm{IVDMD} = (100 - (\mathrm{W3} - (\mathrm{W1} \times \mathrm{C1})) / (\mathrm{W2} \times \mathrm{DM})) \times 100$$

where W1 = bag weight, W2 = sample weight, W3 = final bag weight after in vitro treatment, and C1 = blank bag correction (final oven-dried weight/original blank bag weight).

Experiment 2

The study included five treatment groups. The control group received 200 mg of alfalfa hay alone (AA), without any supplementation. The remaining four groups each received 200 mg of alfalfa hay as the basal diet, supplemented with 15 mg of one of the following microencapsulated extracts: mango peel extract (MPE), avocado peel extract (APE), mango seed kernel extract (MSKE), or avocado seed extract (ASE). The experiment was conducted over three runs, with each run including three replicates per treatment group, resulting in a total of nine replicates per group. Alfalfa hay served as the control and basal diet in all treatment groups, following methodology from previous literature[31–33]. This design aimed to assess the effects of the β-CD microencapsulated extracts of these fruits byproducts, when added to alfalfa hay on rumen fermentation characteristics.

Microencapsulation of mango and avocado byproduct extracts

The extracts were microencapsulated in β-CD via inclusion complex formation, following a modified method adapted from Li et al.[34]. Briefly, 0.75 g of β-CD was dissolved in 25 mL of distilled water to prepare a saturated aqueous solution. The previously prepared mango and avocado byproduct extracts were slowly added to this mixture at room temperature, and the mixture was stirred magnetically for 2 h to allow proper interaction between β-CD and the bioactive compounds in the extracts. Following stirring, the solvents were evaporated by incubating the mixture at 30 °C in the incubator (Nuve S500) until dry capsules were obtained. The microcapsules were subsequently stored in a desiccator to protect them from moisture and degradation until further use.

Rumen fermentation characteristics

Fermentation characteristics (gas production, CH_4, pH, VFA, and NH_3-N) of the microencapsulated extracts of mango and avocado byproducts were determined according to the methodology described in experiment 1. The organic matter digestibility (OMD) were estimated using the following equations provided by Menke and Steingass[28].

OMD (g/kg DM) = 8.89 × gas production (ml/200 mg DM per 24 h) + 0.448 × crude protein (g/kg DM) + 0.651 × crude ash (g/kg DM) + 149.

Statistical analysis

Statistical analysis was carried out via SPSS Version 29.0.1.0 (171). Data normality was assessed using the Shapiro Wilk test before statistical analysis. The fermentation parameters of experiment 1 were analyzed via a linear mixed model. Fruit byproducts (MP, AP, MSK, AS, and MSC) were treated as fixed factors, whereas experimental runs were treated as random factors. Pairwise comparisons for the mixed model analysis were conducted using Bonferroni correction. The IVDMD of fruit byproducts was evaluated via one-way ANOVA, followed by Tukey's post hoc test to determine significant differences among treatments.

Experiment 2 employed a mixed model analysis to evaluate the effects of microencapsulated extracts of these fruits' byproducts. Treatments (AA, MPE, APE, MSKE, and ASE) were considered fixed factors, whereas experimental runs were treated as random factors. Pairwise comparisons between treatments were conducted via Bonferroni correction. For both experiments, statistical significance was set at $P < 0.05$. All results are presented as group means with pooled standard errors of the mean.

Results

Experiment 1

Chemical composition

The DM content in these fruit byproducts ranged from 211 g/kg to 514 g/kg on a fresh basis, indicating varying moisture levels among the byproducts (Table 1). The CP levels were highest in MSK (76 g/kg), followed by AP

[1]Ingredients	Group					
	Alfalfa hay	Mango peel	Avocado peel	Mango seed kernel	Avocado seed	Mango seed coat
DM (fresh matter)	888	211	224	308	514	425
CP	140	41	60	76	51	16
EE	21	20	170	64	13	5
NDF	554	148	491	122	169	789
ADF	446	136	403	87	113	632
ADL	101	60	201	20	60	202
Ash	74	31	28	21	15	8
[2]NFCs	212	759	252	718	752	182

Table 1. Chemical composition of avocado and Mango by products on a DM basis (values expressed as g/kg DM). [1]*DM* dry matter, *CP* crude protein, *EE* ether extract, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *ADL* acid detergent lignin, *NFCs* non fiber carbohydrates. [2]NFCs = 100−(CP + EE + NDF + Ash)[25].

[1]Parameters	Group					[2]SEM	*P*-VALUE
	Mango peel	Avocado peel	Mango seed kernel	Avocado seed	Mango seed coat		
In vitro gas production (mL/0.2 g DM)							
3 h	11.6[a]	3.0[d]	9.3[b]	6.4[c]	2.9[d]	0.17	< 0.001
6 h	22.2[a]	8.2[d]	18.0[b]	12.5[c]	5.2[e]	0.39	< 0.001
12 h	33.1[a]	13.8[c]	29.8[b]	29.3[b]	9.7[d]	0.49	< 0.001
24 h	42.6[c]	22.0[d]	50.0[a]	48.0[b]	14.9[e]	0.34	< 0.001
IVDMD %	78.7[a]	27.6[c]	74.3[b]	79.4[a]	16.5[d]	4.14	< 0.001
CH_4 (ml/g DM incubated)	40.9[b]	19.8[c]	43.7[a]	42.2[ab]	18.7[c]	0.46	< 0.001

Table 2. Effects of Mango and avocado byproducts on gas volume, in vitro dry matter digestibility and methane production. [1]3–24 h: gas production (mL/0.2 g DM) measured at 3, 6, 12, and 24 h; IVDMD: in vitro dry matter digestibility measured over 48 h of incubation; CH_4: methane. [2]SEM: pooled standard error of the mean. [a–e]Means with different superscripts in a row differ significantly ($P < 0.05$).

(60 g/kg), AS (51 g/kg), MP (41 g/kg), and MSC (16 g/kg). The EE content, an indicator of lipid or fat content, was high in AP (170 g/kg) compared to other fruit byproducts. MSC and AP have the highest NDF, ADF and ADL values. The ash content was relatively low across all the byproducts, with MP having the highest ash content at 31 g/kg and MSC having the lowest ash content at 8 g/kg. NFC content varied significantly, with MP (759 g/kg), AS (752 g/kg), and MSK (718 g/kg) exhibiting the highest values, whereas AP (252 g/kg) and MSC (182 g/kg) had the lowest.

Gas production, in vitro dry matter digestibility and methane production

In vitro total gas production was measured at 3, 6, 12, and 24 h for various fruit byproducts, and the results revealed significant differences among the fruit byproducts at each time point ($P < 0.001$, Table 2). The gas production at the 3rd hour was highest ($P < 0.001$) for MP, followed by MSK, AS AP and MSC. A similar pattern of gas production was observed at 6 and 12 h. The cumulative 24 h gas production was highest for MSK, followed closely by AS ($P < 0.001$) (Table 2). MP had moderate gas production, whereas AP and MSC had lower gas production ($P < 0.001$). The IVDMD was significantly greater ($P < 0.001$, Table 2) in the AS, MP and MSK groups than in the AP and MSC groups. MSK produced the highest CH_4 ($P < 0.001$), while MP and AS had intermediate levels, and both AP and MSC presented lower and similar levels (Table 2).

Volatile fatty acids, pH and ammonia nitrogen

The molar proportions of VFA and total VFA (TVFA) production differed significantly ($P < 0.001$) among by products (Table 3). The TVFA production was highest in MSK (58.2 mmol/L) and lowest in the MSC (33.2 mmol/L). Regarding molar proportions, the seed byproducts (MSK and AS) exhibited lower acetate (64.9–66.1%) and propionate (18.4–19.0%) percentages but higher butyrate levels (14.8–16.6%) compared to the peel byproducts, which had acetate around 66.3–67.2% and propionate at 21.7%. Consequently, the acetate to propionate ratio was significantly higher in the seeds of both fruits (3.5–3.6) relative to the peel byproducts (3.0–3.1). MSC presented the highest pH, whereas MSK and AS presented the lowest pH ($P < 0.001$). The NH_3-N concentration was highest in AP ($P < 0.001$), followed by MSK, whereas AS, MP and MSC presented significantly lower and similar values.

[1]Parameters	Group					[2]SEM	*P*-Value
	Mango peel	Avocado peel	Mango seed kernel	Avocado seed	Mango seed coat		
Acetate %	66.3^{ab}	67.2^{a}	64.9^{c}	66.1^{b}	67.4^{a}	0.17	< 0.001
Propionate %	21.7^{a}	21.7^{a}	18.4^{b}	19.0^{b}	18.9^{b}	0.17	< 0.001
Butyrate %	11.9^{d}	11.1^{e}	16.6^{a}	14.8^{b}	13.7^{c}	0.14	< 0.001
TVFA (mmol/L)	54.8^{b}	42.4^{c}	58.2^{a}	55.8^{b}	33.2^{d}	0.37	< 0.001
Acetate: Propionate	3.0^{b}	3.1^{b}	3.5^{a}	3.5^{a}	3.6^{a}	0.03	< 0.001
pH	6.7^{c}	6.8^{b}	6.7^{d}	6.7^{d}	6.9^{a}	0.02	< 0.001
NH_3-N (mg/dl)	3.6^{c}	5.1^{a}	4.0^{b}	3.3^{c}	3.3^{c}	0.10	< 0.001

Table 3. Effect of Mango and avocado byproducts on in vitro rumen fermentation parameters. [1]*TVFA* total volatile fatty acid; *NH_3–N* ammonia nitrogen. [2]*SEM* pooled standard error of the mean. [a–d]Means with different superscripts within a row differ significantly ($P < 0.05$).

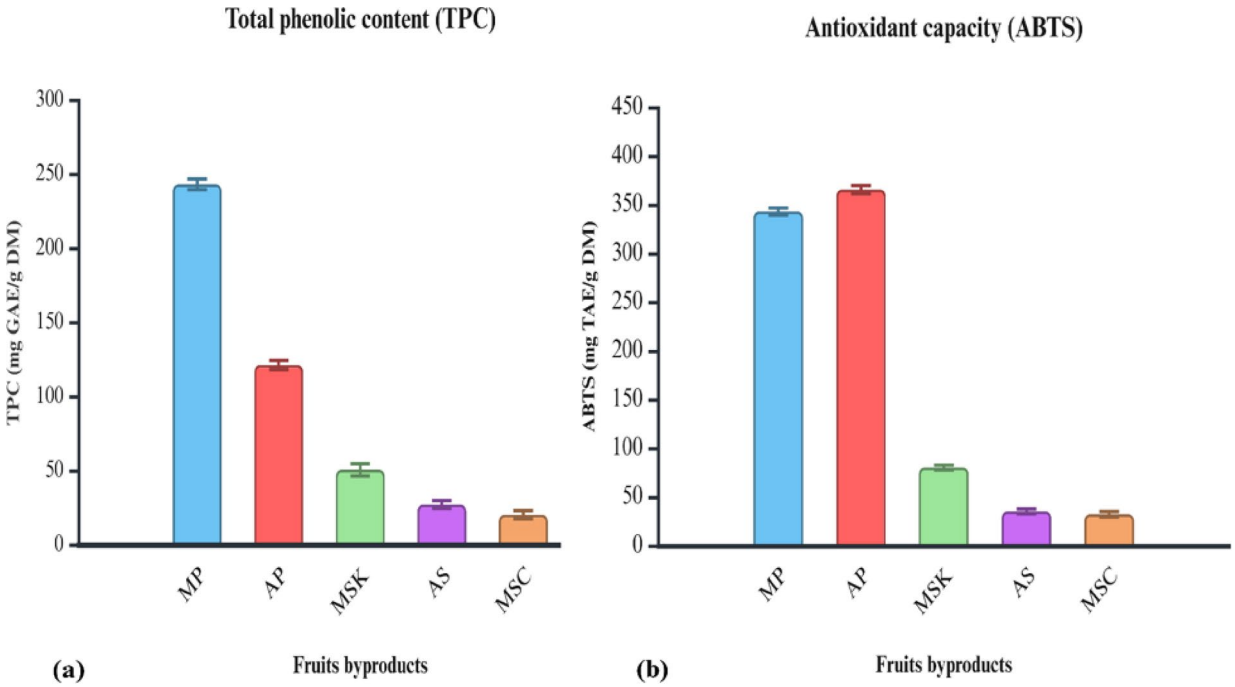

Fig. 1. Total phenolic content (TPC) (**a**) and antioxidant capacity (ABTS) (**b**) of mango and avocado fruit byproducts. The TPC was determined via the Folin–Ciocalteu method, and the results are expressed as mg gallic acid equivalents (GAE) per g DM. The antioxidant capacity was measured via the ABTS radical scavenging assay and is expressed as mg Trolox equivalents (TAE) per g DM. Each value represents the mean of three replicates. Abbreviations: *MP* mango peel, *AP* avocado peel, *MSK* mango seed kernel, *AS* avocado seed, *MSC* mango seed coat.

Experiment 2

Total phenolic content and antioxidant capacity

The total phenolic content and antioxidant capacity of the byproducts (MP, AP, MSK, AS, and MSC) are shown in (Fig. 1a, b), respectively. The highest TPC values were observed for MP and AP, with values of 243.8 mg GAE/g and 121.6 mg GAE/g, respectively. MSK and AS had moderate TPC values of 50.9 mg GAE/g and 27.5 mg GAE/g, respectively, whereas MSC had the lowest value of 20.7 mg GAE/g.

However, in terms of antioxidant capacity, AP had the highest concentration (366.3 mg TAE/g), with MP slightly lower at 343.7 TAE/g. The seed byproducts of both fruits had lower antioxidant capacities, with MSK at 80.9 mg TAE/g, AS at 35.9 mg TAE/g, and MSC at 32.9 mg TAE/g.

Gas production

Gas production for three hour was not significantly different among the treatments ($P = 0.071$, Table 4). However, gas production at the sixth hour, twelfth hour and twenty-fourth hour intervals was significantly different among the treatments ($P < 0.05$). At 24 h, the MPE and APE treatments resulted in significantly lower mean gas production compared to the AA, ASE, and MSKE ($P < 0.001$).

[2]Parameters	[1]Group					[3]SEM	*P*-Value
	AA	MPE	APE	MSKE	ASE		
Gas production (ml/0.2 g DM)							
3 h	10.4	9.8	10.2	10.6	10.4	0.19	< 0.071
6 h	19.6^{ab}	19.7^{ab}	18.8^{b}	19.7^{ab}	20.4^{a}	0.28	< 0.04
12 h	30.8^{ab}	29.5^{bc}	29.0^{c}	30.6^{ab}	31.4^{a}	0.29	< 0.001
24 h	42.1^{a}	40.1^{b}	40.1^{b}	42.4^{a}	42.2^{a}	0.33	< 0.001

Table 4. Effect of microencapsulated extracts supplementation of Mango and avocado byproducts on in vitro total gas volume during different time intervals of incubation. [1]*AA* alfalfa hay, *MPE* mango peel encapsulated extract, *APE* avocado peel encapsulated extract, *MSKE* mango seed kernel encapsulated extract, *ASE* avocado seed encapsulated extract. [2]3–24 h: gas production (mL/0.2 g DM) measured at 3, 6, 12, and 24 h. [3]SEM: pooled standard error of the mean. [a–d]Means with different superscripts in a row differ significantly ($P < 0.05$).

[2]Parameters	[1]Group					[3]SEM	*P*-VALUE
	AA	MPE	APE	MSKE	ASE		
OMD %	63.6^{a}	61.8^{b}	61.8^{b}	63.9^{a}	63.7^{a}	0.29	< 0.001
CH_4 (ml/g DM incubated)	41.1^{b}	38.5^{c}	36.4^{d}	43.3^{a}	38.5^{c}	0.28	< 0.001
pH	6.7	6.7	6.7	6.6	6.7	0.02	0.189
NH_3-N (mg/dl)	5.6^{a}	3.7^{b}	3.3^{c}	3.8^{b}	3.6^{bc}	0.06	< 0.001

Table 5. Effect of microencapsulated extracts of Mango and avocado byproducts on organic matter digestibility, methane, pH, and ammonia nitrogen after 24 h of in vitro fermentation. [1]*AA* alfalfa hay, *MPE* mango peel encapsulated extract, *APE* avocado peel encapsulated extract, *MSKE* mango seed kernel encapsulated extract, *ASE* avocado seed encapsulated extract. [2]*OMD* organic matter digestibility, CH_4 methane, NH_3–*N* ammonia nitrogen. [3]SEM: pooled standard error of the mean. [a–d]Means with different superscripts in a row differ significantly ($P < 0.05$).

[2]VFA	[1]Group					[3]SEM	*P*-VALUE
	AA	MPE	APE	MSKE	ASE		
Acetate %	65.6^{a}	63.0^{b}	59.2^{c}	65.6^{a}	65.8^{a}	0.19	< 0.001
Propionate %	20.2^{c}	22.5^{b}	26.4^{a}	21.0^{c}	22.1^{b}	0.20	< 0.001
Butyrate %	14.2^{a}	14.5^{a}	14.4^{a}	13.5^{a}	12.2^{b}	0.26	< 0.001
TVFA	53.3^{c}	57.1^{b}	58.0^{b}	68.1^{a}	53.0^{c}	0.42	< 0.001
Acetate: propionate	3.3^{a}	2.8^{c}	2.2^{d}	3.1^{a}	3.0^{b}	0.03	< 0.001

Table 6. Effect of microencapsulated extracts of Mango and avocado byproducts on volatile fatty acids produced during in vitro rumen fermentation. [1]*AA* alfalfa hay, *MPE* mango peel encapsulated extract, *APE* avocado peel encapsulated extract, *MSKE* mango seed kernel encapsulated extract, *ASE* avocado seed encapsulated extract. [2]*VFA* volatile fatty acid, *TVFA* total volatile fatty acid. [3]*SEM* pooled standard error of the mean. [a–d]Means with different superscripts in a row differ significantly ($P < 0.05$).

Organic matter digestibility, methane, pH and ammonia nitrogen

The OMD was significantly ($P < 0.001$) greater in MSKE, ASE and AA than in MPE and APE (Table 5). CH_4 analysis revealed that APE produced significantly ($P < 0.01$) less methane, followed by MPE and ASE. While MSKE produced significantly more ($P < 0.01$) methane as compared to all treatments (Table 5). The rumen pH was not influenced by encapsulated mango or avocado byproducts ($P = 0.189$). The NH_3-N concentration was significantly greater in the AA group than in the encapsulated fruit byproduct extract groups.

Volatile fatty acids

The molar proportion of VFAs varied significantly among the treatments ($P < 0.001$, Table 6). APE exhibited the highest propionate proportion, whereas AA and ASE had the greatest acetate proportion. Butyrate proportions were similar across most treatments but lowest in ASE (12.15%) ($P < 0.001$). TVFA concentration also varied significantly ($P < 0.001$), with MSKE presenting the highest levels, followed by APE and MPE, while AA and ASE showed lower values. The acetate to propionate ratio was significantly lower in the APE than in the other treatments, while the highest values were observed in the AA and MSKE treatments ($P < 0.001$).

Discussion

Experiment 1

A notable variation was observed in the chemical composition, IVDMD, and rumen fermentation characteristics of mango and avocado byproducts, which can be attributed to differences in their structural components. The chemical composition of the mango and avocado byproducts in this study aligns with previous findings, with similar DM concentrations on fresh basis[20,35], comparable CP values in MSK reported by Ashoush et al.[36] and Mutua et al.[37], and similar EE contents in AP reported in earlier studies[20,35]. Likewise, The NDF and ADF concentrations in MSC and AP resemble those reported by previous authors[20,32,35] indicating a relatively high fiber content that could influence ruminal digestibility.

The TPC and antioxidant capacity differed considerably across fruit types and byproduct fractions. These values align with the wide TPC range of 10.84 to 281.4 mg GAE/g values reported for mango and avocado byproducts, with variations attributable to extraction methodologies[35,38,39]. The peel byproducts of both fruits are richer sources of phenolic compounds and antioxidants than their respective seed byproducts. This disparity can be attributed to the differential phytochemical profiles across fruit portions, with peels generally containing higher concentrations of polyphenols and antioxidants than seeds do[40]. This distribution likely results from the peel's role as a protective barrier, facing greater exposure to environmental stressors such as UV radiation and pathogens, which stimulates increased production of these defensive compounds[41]. In terms of gas production, AP and MSC resulted in lower gas production. The reduced gas production in MSC can be attributed to its substantially higher lignin content (201 g/kg) compared to AS (60 g/kg and MSK (20 g/kg). Moreover, the high lignification of MSC and AP further limits the accessibility and degradation of fiber polysaccharides by ruminal microbes[42]. Our findings are consistent with those of García-Rodríguez et al.[43], who correlated high lignin content with lower gas volumes for grape seeds and pepper skins. As gas production correlates positively with organic matter fermentation[28], the higher values of gas production for the seeds and peels of mango suggest a greater extent of fermentation, likely due to their richness in readily fermentable carbohydrates (NFCs: 718–759 g/kg)[44]. The high lignin content in AP and MSC likely contributes to lower IVDMD, as lignin limits the accessibility and degradability of NDF polysaccharides by ruminal microbes[42]. Moreover, high fat levels in AP can form a coating around feed particles, further limiting microbial degradation. These factors collectively impede the breakdown of nutrients in the rumen, leading to decreased IVDMD. The high IVDMD values obtained for mango byproducts, specifically MP and MSK, are in accordance with previously reported values[20,45] underscoring their potential as fermentable feed ingredients. Based on the nutritional and fermentation characteristics, MSC appears to have a limited nutritive value, primarily due to its high lignin content, which can restrict digestibility. Given its composition, MSC could be utilized in ruminant diets to enhance rumen fill, similar to low-quality roughages such as wheat and rice straw. However, its high lignin content may limit its effectiveness as a primary feed ingredient.

The VFA profiles varied significantly among the fruit byproducts, reflecting differences in their chemical composition and fermentation characteristics, attributable to varying carbohydrate compositions and structures[20]. TVFA was highest in MSK and AS, likely due to the fermentability of their fiber fractions or NFC, despite their lower NDF content. Conversely, MSC and AP exhibited the lowest TVFA, aligning with their high lignin content (Table 1), which likely impeded microbial degradation of fiber[46]. Propionate production was highest in MP and AP, and this higher propionate level in peel byproducts indicates improved fermentation efficiency, as propionate acts as a hydrogen sink, potentially reducing methanogenesis[42]. In contrast, the high CH_4 production observed in MSK and AS aligns with their higher TVFA production, particularly due to a greater acetate to propionate ratio, which is linked to increased hydrogen availability in the rumen, indirectly supporting methanogenesis[47]. Notably, while AS had high CH_4 production, AP did not, despite both being from avocado. This difference can be attributed to their distinct chemical compositions, particularly the higher fiber content and EE in AP, which reduces digestibility and microbial fermentation efficiency, compared to AS, which has lower fiber (ADL: 60 g/kg) and greater digestibility, promoting increased CH_4 production.

NH_3-N concentrations varied significantly among the fruit byproducts, reflecting differences in their protein content and fermentation characteristics. The low NH_3-N concentration observed with MP suggests efficient nitrogen utilization, likely due to increased assimilation of degraded nitrogen into microbial proteins. This finding aligns with the favorable VFA profile of MP, particularly its high propionate production, indicating active microbial fermentation. This may also be influenced by phenolic compounds that inhibit proteolytic activity[48].

Conversely, the high NH_3-N level of AP aligns with its greater crude protein content, suggesting that AP more readily degrades protein in the rumen. The MSCs presented relatively low NH_3-N levels, which is consistent with their overall poor fermentability, likely because the high lignin content limits protein degradation. The supplementation of these byproducts with nitrogen sources has the potential to increase ruminal fermentation by supplying adequate ammonia for microbial protein synthesis while reducing nitrogen waste.

Experiment 2

Mango and avocado byproducts, which are rich in flavonoids and phenolic compounds, have been characterized in numerous studies[15,16,49]. β-CD based encapsulation of mango and avocado byproduct extracts was used in this study to investigate the effects on rumen fermentation characteristics and CH_4 emissions. Encapsulation enhances the stability and bioavailability of these bioactive compounds, facilitating their interaction with the rumen microbiota[19]. The dosage of 15 mg for the microencapsulated extracts was chosen on the basis of previous studies[19,50,51] investigating the effects of microencapsulated plant extracts on rumen fermentation characteristics. Alfalfa hay was chosen as basal diet due to its consistent nutrient profile, high protein and fibre content, and widespread use in ruminant nutrition. As a single ingredient, it simplifies the experimental design and allows for a clearer assessment of the effects of supplemented microencapsulated phenolic extracts on rumen fermentation characteristics.

The reduction in gas production and OMD observed in the treatments supplemented with peel extracts of both mango and avocado fruits at 24 h suggests that these extracts influence ruminal fermentation. This reduction is likely due to the presence of bioactive compounds, particularly phenolics. Phenolic compounds are known to modulate rumen fermentation by affecting microbial activity[48]. The consistency between gas production, OMD, and CH_4 concentrations indicates a potential shift in fermentation patterns. Lower gas production often correlates with reduced methanogenesis, which can increase feed efficiency and reduce greenhouse gas emissions[52]. However, the simultaneous decrease in OMD suggests that while these extracts may have CH_4 mitigating properties, they might also modulate microbial populations responsible for fiber digestion, potentially affecting overall nutrient utilization.

The CH_4 production results (expressed as ml/g DM incubated) suggest that peel extracts from both mango and avocado, as well as avocado seed extract, can reduce CH_4 production in vitro. Bioactive compounds such as phenolic acids, tannins, flavonoids, and saponins, known for their antimethanogenic properties[48,53], are present in high concentrations in these byproducts[54]. The presence of phenolic compounds in mango and avocado peel extracts, including naringin, quercetin, mangiferin, ellagic acid, catechins, glycosides, and procyanidins[15,49,55], can contribute to this reduction in CH_4 and gas production[56,57]. Moreover, it has been reported that flavonoids and tannins have the ability to reduce CH_4 production, as they have antimicrobial activities through interference with the cellular integrity of methanogenic archaea as well as protozoa[58].

The peel extract treatments resulted in decreased acetate proportion and acetate to propionate ratios, indicating a shift in fermentation pathways. The increase in propionate in the peel extracts of both fruits may have resulted from the encapsulated extracts increasing the abundance of the Prevotellaceae and Veillonellaceae families[59], which utilize H_2 to produce propionate, providing a competitive pathway to methanogenesis[60]. Moreover, tannins can inhibit the activity of methanogens and protozoa, reducing CH_4 production and redirecting hydrogen toward propionate production pathways[61]. This mechanism is likely responsible for the increased propionate proportion and reduced acetate to propionate ratio in the APE group. The rumen pH in this study was not significantly affected by the treatments and remained within the optimal range for microbial activity. In the present study, the NH_3-N concentration was lower in all the encapsulated groups than in the control, and the lower NH_3-N levels in the encapsulated phenolic extract groups could result from protein phenolic compound complexes, inhibition of predominant protein degrading or ammonia producing microbiota, and ammonia utilization for microbial protein synthesis[48,62]. The antimicrobial properties of compounds such as mangiferin, ellagic acid, catechins, glycosides, and flavonoids present in mango and avocado extracts affect the rumen microbial ecosystem, reducing amino acid deamination[63]. These compounds suppress ammonia-producing bacterial growth and reduce protozoal populations, which contribute to protein degradation and ammonia production[64]. These findings suggest that encapsulated phenolic extracts from mango and avocado byproducts can modulate rumen fermentation dynamics, including gas production, VFA profiles, and methanogenesis. However, future studies must include microbial ecology analyses to provide a comprehensive understanding of these effects and optimize their application in ruminant diets.

Conclusion

The findings of experiment 1 suggest that mango byproducts such as peels and seed kernels, along with avocado seeds, have promising potential as feed. Among these, MP emerged as the most effective treatment, as it significantly increased propionate production, reducing CH_4 production (ml/g DM incubated) in vitro, and maintaining optimal rumen pH, leading to improved rumen fermentation efficiency. Experiment 2 revealed that the extracts derived from mango and avocado peels significantly reduced CH_4 production (ml/g DM incubated, in vitro) and acetate proportion and the acetate-to-propionate ratio, indicating a shift in fermentation pathways. These findings suggest that encapsulated phenolic extracts from peels of mango and avocado fruits can modulate rumen fermentation dynamics, including gas production, VFA profiles, and CH_4 emissions. However, future research should include comprehensive in vivo and in vitro microbial analyses to fully understand and optimize the application of these byproducts in ruminants.

Data availability

The datasets generated and analysed during this study are included within the article and are also available from the corresponding author upon reasonable request.

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Author contributions

H.J., E.S., D.C., and I.F. conceived and designed the study. H.J., M.Z.A., M.Gi., (Melania Giammarco) and B.K. developed the methodology. D.C., E.S., M.Gi., and I.F. supervised the project. H.J., M.Gi., M.Z.A., and I.F. wrote the original draft. H.J., M.Z.A., P.P., M.Ga., (Min Gao) and J.E. performed data curation, formal analysis, and software implementation. H.J., E.S., L.P., J.E., and M.Ga. controlled the content and structure. All the authors reviewed and edited the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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