

Communication

# Chemical Composition and In Vitro Nutritive Evaluation of Pomegranate and Artichoke Fractions as Ruminant Feed

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**Abstract:** The aim of this work was to assess the chemical composition and in vitro ruminal fermentation of samples ( $n = 3$ ) of pomegranate (peels (PPs) and seeds (PSs)) and artichoke (hearts (AHs) and stems (ASs)) wastes. Dried orange pulp (DOP) and tomato pomace (TP) were used as reference feeds. All wastes had low dry matter (DM; lower than 33.0 and 12.0% for pomegranate and artichoke, respectively). The DM of pomegranate fractions was rich in sugars (>42.0%) and contained low protein (<8.0%) and neutral detergent fiber (NDF; <27.0%), whereas that of both artichoke fractions had high protein (>18.0%) and NDF (>36.0%) and low sugars content (<9.2%). Pomegranate seeds were more rapidly and extensively fermented in vitro than PPs, but both were less degradable and contained less metabolizable energy (ME) than DOP (7.43, 11.0 and 12.5 MJ ME/kg DM, respectively). Although AHs were more rapidly fermented and produced more volatile fatty acids (VFAs) than ASs, both had lower ME content than TP (9.50, 7.25 and 12.5 MJ ME/kg DM). The analyzed wastes had lower ME content than other by-products, but they were extensively fermented by ruminal microorganisms and could be used as ruminant feeds.

**Keywords:** pomegranate wastes; artichoke wastes; in vitro; rumen fermentation; energy content; gas production



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## 1. Introduction

Food waste has a substantial environmental and economic impact and represents an inefficient use of global resources, and thus it is essential to reduce the amount of wasted food. Fruits and vegetables produce the largest share of food waste, considering the waste generated during cultivation, harvesting, processing, distribution, and consumption. Current estimates indicate that in the EU, as much as 41.4 and 45.7% of fruit and vegetable production, respectively, is lost or wasted throughout the entire food supply chain, and these figures can rise to over 55% in other regions worldwide [1]. Furthermore, fruits and vegetables account for 76% of the total food waste generated during primary production, especially at the postharvest stage, when between 37 and 55% of the total harvest can be discarded [2] due to the high-quality standards imposed by consumers. Moreover, food waste can also be extremely polluting and its handling is usually challenging [3].

Pomegranate (*Punica granatum* L.) fruits are consumed worldwide, and its production is estimated to be greater than  $3.8 \times 10^6$  t per year [4]. Pomegranate processing into juice or other products generates a large quantity of waste products, mainly peels, which can represent as much as 50% of the fruit [5]. Previous studies have evaluated the effect of including pomegranate waste products in ruminant diets on animal performance and product quality [5–8], and although no effect has been observed on milk production or growth performance, the antioxidant status of cow's and ewes' milk and kid's meat has been increased, with a healthier fatty acid profile. However, information on their nutritive value and ruminal fermentation is more limited [8].

Artichoke (*Cynara scolymus* L.) is another crop widely consumed worldwide, with a production of approximately  $0.7 \times 10^6$  t per year [9]. However, only a small proportion of

the crop is edible and waste products (leaves, external bracts, and stems) may represent approximately 80% of the harvested biomass [10]. Previous studies have evaluated the characteristics of ensiled artichoke waste [11,12] and its effect on milk quality in both dairy sheep, observing a reduction in fat and total free fatty acids and an increase in total free amino acids in cheese [13], and dairy goats, observing a healthier lipid and mineral profile for human consumption in milk [14]. However, information on characteristics and ruminal fermentation of artichoke wastes is still scarce. Therefore, the objective of this study was to evaluate the chemical composition, *in vitro* ruminal fermentation, and energy content of samples of pomegranate and artichoke fruits and their fractions.

## 2. Materials and Methods

All the experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of the Comunidad Autónoma de Madrid (Approval number PROEX 212.2/22). Animal care and ruminal sampling followed the Spanish regulations for experimental animal protection.

### 2.1. Pomegranate and Artichoke Samples

Three pomegranate (*Punica granatum* L.) and artichoke (*Cynara scolymus* L.) samples were obtained from local markets in 3 different weeks during the autumn season. Each sample of pomegranate consisted of 10 fruits that were weighed and manually separated into peels (albedo, rind, and membrane) and seeds (aril and seeds). Each artichoke sample consisted of 15 pieces, which were weighed and divided into hearts and external parts (outer leaves and stems). Both fractions of each sample (3 samples for both pomegranate and artichoke) were independently weighed and dried at 40 °C in an air-forced oven until constant weight to determine the dry matter (DM) content. In addition, one sample of each dried orange pulp (DOP) and tomato pomace was evaluated in the study to be used as reference feeds for pomegranate and artichoke samples, respectively, as both by-products are widely used in ruminant feeding in practice and have similarities in the chemical composition with the samples studied. The DOP sample was commercially available, whereas tomato pulp was obtained from a tomato processing industry. After drying, all samples were ground to pass a 1 mm screen using an automatic centrifugal mill (Retsch ZM 200, Haan, Germany) to carry out chemical composition analysis and *in vitro* ruminal incubations.

### 2.2. Animals, Feeding and Ruminal Fluid

Four adult Lacaune sheep ( $64.3 \pm 2.11$  kg body weight; 3 years old), with a permanent rumen cannula, were used as rumen fluid donors for *in vitro* incubations. The sheep were fed a diet based on grass hay and concentrate in a 2:1 proportion, which contained 114 g of crude protein (CP), 365 g of neutral detergent fiber (NDF), and 160 g of acid detergent fiber (ADF) per kg DM. The diet was administered daily in two equal portions at 9:00 and 18:00 at a restricted level (45 g dry matter (DM)/kg body weight<sup>0.75</sup>) and animals had free access to freshwater and were individually housed in floor pens.

About 400 g of ruminal content was manually obtained from each sheep through the rumen cannula before the morning feeding (9:00 h) using barbecue tongs with shovels. The ruminal content was filtered through four layers of cheesecloth and the obtained fluid was immediately transported to the laboratory (less than 15 min after collection) into thermal flasks (one for the fluid from each donor sheep) for conducting the *in vitro* incubations.

### 2.3. *In Vitro* Incubations: Experimental Design and Sampling

Two similar *in vitro* trials were carried out to determine gas production kinetics and fermentation parameters of the fractions of pomegranate and artichoke and of the reference feeds. The incubations were conducted as described by De Evan et al. [15] in two consecutive weeks—gas production kinetics first and fermentation parameters second. Briefly, 200 mg of DM of each sample (3 samples per each studied fraction and the two

reference feeds) was carefully weighed into 60mL glass vials. The ruminal fluid of each sheep was independently mixed with a pre-warmed (39 °C) culture medium [16] in a 1:4 ratio which was modified by excluding the trypticase and replacing the  $(\text{NH}_4)\text{HCO}_3$  with  $\text{NaHCO}_3$  to obtain a N-free medium. Each vial was filled with 20 mL of the mixture using a Watson-Marlow 520UIP31 peristaltic pump (Watson-Marlow Fluid Technology Group, Cornwall, UK) under  $\text{CO}_2$  flushing. The vials were sealed with rubber stoppers and incubated at 39 °C. In addition, vials without substrates (blanks; two per inoculum) were included to correct for the endogenous gas production. This procedure was followed to obtain 4 different replicates (one vial per sheep inoculum) per incubated sample.

In the first in vitro trial the vials were incubated for 120 h and the amount of gas produced in each vial was measured at different time intervals (3, 6, 9, 12, 15, 22, 26, 31, 36, 48, 58, 72, 96, 120 and 144 h after incubation) using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona, Spain) and a plastic syringe. The second in vitro trial was performed in a different week as described before and lasted for 24 h. After this time, the gas production was measured, the content of the vials was homogenized by handshaking, and the pH was determined with a pHmeter Crison GPL 21 (Crison Instruments, Barcelona, Spain). Finally, 3 mL of each vial content was mixed with 3 mL of 0.5 M HCl and frozen at  $-20$  °C until the volatile fatty acid (VFA) and  $\text{NH}_3\text{-N}$  concentrations analyses.

In addition, the potential DM degradability (PDMD) was estimated using an Ankom Daisy<sup>II</sup> incubator (Ankom Technology Corp., Fairport, NY, USA), at the same time as the incubation was performed to measure the gas production kinetics. Three hundred mg of each feed was weighed into filter bags (Ankom Corp #57; 25  $\mu\text{m}$  pore size; Ankom Technology Corp., Fairport, NY, USA) in triplicate, and the bags were incubated at 39 °C in a 1:4 mixture of ruminal fluid (mixture of all sheep) and the culture medium described previously [16]. After 144 h, the bags were washed with cold water, dried at 60 °C for 48 h, and weighed to calculate the PDDM. This value was used to estimate the DM effective degradability (DMED) as described later.

#### 2.4. Analyses of Chemical Composition

All chemical fractions were analyzed in duplicate. The DM (ID 934.01), ash (ID 942.05), and ether extract (EE; ID 920.39) were analyzed following the procedures of AOAC [17]. Other chemical analysis and analysis of  $\text{NH}_3\text{-N}$  and volatile fatty acids (VFA) concentrations in the vial's content were performed as described by De Evan et al. [15].

#### 2.5. Calculations and Statistical Analysis

Gas production data were fitted to the model  $\text{Gas} = A (1 - e^{(-c(t - \text{lag}))})$  using the Proc NLIN of the SAS [18]. In this model, A is the asymptotic or potential gas production, c is the fractional gas production rate, lag is the time until gas production begins, and t is the gas measurement time. In addition, the average gas production rate (AGPR) was defined as the gas production rate in the period from the incubation start to the time taken to reach half of the A value, and it was calculated as:  $\text{AGPR} = A c / [2 (\ln 2 + c \text{lag})]$ . The DMED was estimated as:  $\text{DMED} = [(\text{PDMD} \times c) / (c + kp)] e^{(-kp \times \text{lag})}$  for a kp (rumen passage rate) of 0.042 per h. The metabolizable energy (ME) content of the samples was estimated from the amount of gas produced at 24 h of incubation (G24; mL per 300 mg of DM incubated) and the content in CP and EE (expressed as g/kg DM) using the following equation [19]:  $\text{ME} = 2.43 + 0.1206 \times \text{G24} + 0.0069 \times \text{CP} + 0.0187 \times \text{EE}$ .

All statistical analyses were performed with the SAS package [18]. Data on chemical composition of pomegranate and artichoke fractions were analyzed independently for each by-product as a one-way analysis of variance, with the by-product fraction being the main effect. Gas production and fermentation parameters were analyzed using the PROC MIXED of SAS using the following statistical model, in which the effect of the by-product fraction was considered fixed and that of the inoculum was considered random:  $Y_{ij} = \mu + T_i + Y_j + e_{ij}$ , where  $Y_{ij}$  = observation;  $\mu$  = overall mean for each parameter;  $T_i$  = effect of

by-product fraction;  $Y_j$  = effect of inoculum; and  $e_{ij}$  = random error. Values of  $p < 0.05$  were considered statistically significant, and those  $< 0.10$  were considered trends.

### 3. Results and Discussion

#### 3.1. Chemical Composition of Pomegranate and Artichoke Wastes

The chemical composition of the pomegranate and artichoke fractions and of the reference feeds is shown in Table 1. On average, the pomegranate fruits contained 36.5 and 63.5% of peels and seeds (fresh matter basis). The DM content of the pomegranate was greater ( $p = 0.027$ ) in peels than in seeds, although it was low in both fractions. Both pomegranate fractions were characterized by low CP and EE content ( $< 7.2\%$  and  $1.6\%$  of DM, respectively), but had high total sugars levels ( $> 46\%$  of DM). Peels were more fibrous than seeds, having more than twice the amount of NDF, ADF and lignin than seeds ( $p \leq 0.003$ ), whereas CP and sugars content were significantly lower ( $p \leq 0.008$ ) in peels than seeds. The NDICP was a low proportion of total CP in seeds, but it was greater ( $p < 0.001$ ) in peels, reaching an average 28.1% of total CP. A sample of DOP was selected as a reference feed due to its similar low CP and high sugars content. The chemical composition of the DOP sample was in the range previously reported in the feed tables [20–22].

**Table 1.** Chemical composition (g/100 g dry matter unless otherwise stated) of pomegranate and artichoke fractions ( $n = 3$ ) and of a sample of each dried orange pulp (DOP) and tomato pomace used as reference feeds <sup>1</sup>.

| Item                          | Pomegranate |       |                  |           | Artichoke |        |       |                  | Tomato Pomace |       |
|-------------------------------|-------------|-------|------------------|-----------|-----------|--------|-------|------------------|---------------|-------|
|                               | Peels       | Seeds | SEM <sup>1</sup> | $p =$     | DOP       | Hearts | Stems | SEM <sup>1</sup> |               | $p =$ |
| Dry matter (g/100 g)          | 32.4        | 20.0  | 2.57             | 0.027     | 91.1      | 11.9   | 7.02  | 0.386            | 0.001         | 26.0  |
| Ash                           | 3.59        | 2.47  | 0.152            | 0.007     | 3.11      | 9.70   | 9.05  | 0.252            | 0.140         | 3.70  |
| Crude protein (CP)            | 3.80        | 7.17  | 0.474            | 0.008     | 5.84      | 24.0   | 18.6  | 0.65             | 0.004         | 17.3  |
| Ether extract                 | 1.60        | 1.55  | 0.186            | 0.872     | 4.90      | 2.31   | 1.55  | 0.231            | 0.083         | 10.7  |
| Total sugars                  | 42.6        | 75.3  | 1.4              | $< 0.001$ | 46.5      | 9.09   | 6.90  | 0.858            | 0.145         | 12.3  |
| Neutral detergent fiber (NDF) | 26.7        | 12.8  | 0.90             | $< 0.001$ | 16.3      | 36.2   | 51.2  | 2.05             | 0.007         | 54.1  |
| Acid detergent fiber          | 18.6        | 8.64  | 1.10             | 0.003     | 9.73      | 23.7   | 34.8  | 1.52             | 0.007         | 40.8  |
| Lignin                        | 6.80        | 3.90  | 1.255            | 0.178     | 0.81      | 7.45   | 6.57  | 0.872            | 0.514         | 21.7  |
| NDICP (% CP) <sup>2</sup>     | 28.1        | 4.97  | 1.49             | $< 0.001$ | 5.11      | 26.5   | 24.2  | 3.18             | 0.629         | 12.8  |
| Lignin (% NDF)                | 25.1        | 30.2  | 4.25             | 0.444     | 4.97      | 20.4   | 12.9  | 2.01             | 0.056         | 40.1  |

<sup>1</sup> SEM: standard error of the mean ( $n = 3$ ); <sup>2</sup> NDICP: neutral detergent insoluble crude protein expressed as g/100 g crude protein.

The type of pomegranate by-product analyzed in different studies varies highly, and therefore some differences in the chemical composition can be expected. In agreement with our results, others [23,24] found greater CP content in seeds than in peels, but the content of NDF and ADF was greater in the seeds, which contrasts with our results. In previous studies [23–26], CP content of pomegranate peels varied from 2.5 to 8.4% (DM basis), whereas NDF, ADF and EE content ranged from 20.6 to 31.6, from 11.7 to 21.2, and from 0.40 to 5.25% of DM, respectively, and our samples were within the ranges previously reported for CP, NDF and ADF, although greater EE content was observed.

The nutrient content (DM basis) of pomegranate seeds after extracting the juice reported by others [23,24] was variable for CP (11–15%), NDF (43–68%), ADF (31–49%), ash (0.7–2.8%) and EE (0.6–10%). We observed lower content of CP, NDF and ADF in the seeds compared to these studies, but ash and EE contents were in the range described. These differences could be explained by the high concentration of sugars in the seeds of our study (75% of DM), which would cause a reduction in the concentration of other nutrients. In general, differences in chemical composition of pomegranate by-products can be justified by variations in production and growing conditions and in pomegranate varieties, but also by differences in processing.

Artichoke hearts and stems represented an average 46.5 and 53.6% (fresh matter basis) of the whole vegetable, respectively, and both fractions contained low DM (Table 1). The DM of both artichoke fractions were characterized by high CP and NDF content, although about 25% of the total CP was linked to NDF, thus reducing its availability for ruminants. In addition, both fractions had low EE and total sugars content. Artichoke hearts had greater CP and lower NDF and ADF than stems ( $p \leq 0.007$ ), but there were no differences between fractions in ash, total sugars and lignin content. As previously reported [11,12,15,27–29], the CP, NDF, ADF, lignin and EE content of artichoke stems (DM basis) can range from 10 to 18%, 43 to 57%, 30 to 43%, 4.3 to 10%, and 0.8 to 5.5%, respectively, and the composition of the stems analyzed in our study is generally within the range of values reported. The variations observed in the chemical composition of artichoke by-products among studies may be largely due to the proportion of each fraction.

Tomato pomace was selected as a reference feed for comparison with artichoke samples due to its similar CP and NDF content, although tomato pomace contained more EE and total sugars than both artichoke fractions. The chemical composition of the tomato pomace sample used in our study agrees well with the values reported previously [20,21].

### 3.2. In Vitro Fermentation of Pomegranate Wastes

Both pomegranate fractions and the DOP used as a reference were fermented in vitro with ruminal fluid from sheep to assess the gas production kinetics (Table 2). Compared to peels, pomegranate seeds had greater ( $p < 0.001$ ) values of *A*, *c*, *Lag* and AGPR, indicating greater fermentation of pomegranate seeds compared to peels. Both DMED and ME content were greater ( $p < 0.001$ ) for the seeds, which agrees well with the greater content in sugars and lower NDF content of this fraction compared with peels (Table 1).

**Table 2.** Parameters of gas production kinetics of pomegranate (n = 3) and artichoke fractions (n = 3) and of a sample of each dried orange pulp and tomato pomace used as reference feeds <sup>1</sup>.

| Sample            | Gas Production Parameters |                |                |             |          |                            |
|-------------------|---------------------------|----------------|----------------|-------------|----------|----------------------------|
|                   | A (mL/g)                  | <i>c</i> (%/h) | <i>Lag</i> (h) | AGPR (mL/h) | DMED (%) | ME <sup>2</sup> (MJ/kg DM) |
| Pomegranate peels | 147                       | 4.23           | 0.053          | 4.49        | 36.0     | 7.43                       |
| Pomegranate seeds | 244                       | 7.83           | 1.788          | 11.4        | 53.2     | 11.0                       |
| SEM <sup>3</sup>  | 1.6                       | 0.171          | 0.1422         | 0.127       | 0.58     | 0.044                      |
| <i>p</i> =        | <0.001                    | <0.001         | <0.001         | <0.001      | <0.001   | <0.001                     |
| Reference feed    |                           |                |                |             |          |                            |
| Dried orange pulp | 360                       | 8.61           | 0.306          | 20.7        | 61.7     | 12.5                       |
| Sample            |                           |                |                |             |          |                            |
| Artichoke hearts  | 188                       | 3.71           | 2.63           | 4.40        | 33.2     | 9.50                       |
| Artichoke stems   | 206                       | 2.78           | 3.37           | 3.61        | 23.1     | 7.25                       |
| SEM               | 1.9                       | 0.061          | 0.193          | 0.071       | 0.64     | 0.119                      |
| <i>p</i> =        | <0.001                    | <0.001         | 0.014          | <0.001      | <0.001   | <0.001                     |
| Reference feed    |                           |                |                |             |          |                            |
| Tomato pomace     | 199                       | 7.28           | 2.33           | 8.28        | 31.5     | 11.1                       |

<sup>1</sup> A: potential gas production; *c*: fractional rate of gas production; *Lag*: is the time needed to start gas production; AGPR: average gas production rate; DMED: dry matter effective degradability estimated for a rumen particulate outflow of 0.042 per h; <sup>2</sup> ME: metabolizable energy was calculated from gas production at 24 h as well as chemical composition as proposed by Menke and Steingass [19]; <sup>3</sup> SEM: standard error of the mean (n = 12; 3 samples × 4 replicates).

Mirzaei-Aghsaghali et al. [23] observed greater in vitro gas production for pomegranate peels compared with seeds in 96 h in vitro incubations with ruminal fluid from steers, but the seeds were obtained after extracting the juice and had lower content in non-structural carbohydrates than the peels. In contrast, Delavar et al. [24] reported similar gas production patterns for both peels and seeds. The gas production values observed in the current study for both pomegranate fractions were greater than others reported previously [24,30,31],

which can be partly due to the fact that our samples had not previously been used for juice extraction. In addition, the source of ruminal fluid and the experimental methodology can also influence in vitro gas production.

Both pomegranate fractions were less fermented than DOP despite all samples having high total sugars and low NDF content. However, pomegranate NDF was more lignified than the NDF of DOP (Table 1) and lignin is one of the main factors that reduce ruminal fiber degradation. The ME content of the DOP used as a reference (12.5 MJ ME/kg DM) was similar to the values previously reported in the feed tables (12.2, 11.5 and 12.1 MJ ME/kg DM) for [20–22], respectively, indicating that this sample could be considered representative of this by-product. The ME of pomegranate peels and seeds was 61.9 and 91.7% of that of DOP, respectively. As DOP can replace cereals in the diet of ruminants [32], pomegranate by-products, and especially those including whole seeds, can be used as an energy source for ruminants.

Compared with peels, pomegranate seeds showed greater ( $p < 0.001$ ) gas and total VFA production after 24 h of incubation (Table 3), and consequently lower pH ( $p < 0.001$ ) as the total VFA production and ruminal pH are usually negatively correlated. The molar proportion of acetate was greater ( $p < 0.001$ ) in pomegranate peels than in the seeds, whereas the propionate proportion was lower ( $p < 0.001$ ), resulting in a greater ( $p < 0.001$ ) acetate/propionate ratio for the peels fraction. The observed differences in the VFA profile are consistent with the lower sugars and greater NDF content of peels compared to seeds and is in accordance with the results of Kara [25], who studied the in vitro fermentation of pomegranate peels using ruminal fluid from goats as the inoculum.

**Table 3.** Fermentation parameters of pomegranate ( $n = 3$ ) and artichoke fractions ( $n = 3$ ) and of a sample of each dried orange pulp and tomato pomace used as reference feeds after 24 h of incubation with ruminal fluid from sheep <sup>1</sup>.

| Sample            | Molar Proportions (mol/100 mol) |        |                       |              |                 |               |           |                 |                           |
|-------------------|---------------------------------|--------|-----------------------|--------------|-----------------|---------------|-----------|-----------------|---------------------------|
|                   | Gas (mL/g DM)                   | pH     | Total VFA (μmol/g DM) | Acetate (Ac) | Propionate (Pr) | Butyrate (Bt) | Minor VFA | Ac/Pr (mol/mol) | NH <sub>3</sub> -N (mg/L) |
| Pomegranate peels | 96.3                            | 6.76   | 4.48                  | 57.5         | 28.6            | 11.8          | 2.13      | 2.02            | 61.6                      |
| Pomegranate seeds | 210                             | 6.46   | 8.46                  | 52.3         | 34.7            | 10.4          | 2.61      | 1.52            | 79.2                      |
| SEM <sup>2</sup>  | 1.60                            | 0.010  | 0.092                 | 0.43         | 0.49            | 0.56          | 0.160     | 0.040           | 1.95                      |
| $p =$             | <0.001                          | <0.001 | <0.001                | <0.001       | <0.001          | 0.105         | 0.048     | <0.001          | <0.001                    |
| Reference feed    |                                 |        |                       |              |                 |               |           |                 |                           |
| Dried orange pulp | 235                             | 6.44   | 8.90                  | 64.8         | 21.0            | 10.9          | 3.30      | 3.09            | 87.8                      |
| Sample            |                                 |        |                       |              |                 |               |           |                 |                           |
| Artichoke hearts  | 109                             | 6.72   | 6.57                  | 62.0         | 25.7            | 7.57          | 4.64      | 2.42            | 182                       |
| Artichoke stems   | 97.7                            | 6.79   | 6.15                  | 64.5         | 24.1            | 6.88          | 4.57      | 2.68            | 162                       |
| SEM <sup>2</sup>  | 1.70                            | 0.037  | 0.810                 | 0.27         | 0.30            | 0.103         | 0.066     | 0.039           | 2.5                       |
| $p =$             | 0.025                           | 0.217  | 0.003                 | <0.001       | <0.001          | <0.001        | 0.482     | <0.001          | <0.001                    |
| Reference feed    |                                 |        |                       |              |                 |               |           |                 |                           |
| Tomato pomace     | 135                             | 6.80   | 6.28                  | 66.0         | 23.2            | 7.32          | 3.48      | 2.84            | 162                       |

<sup>1</sup> VFA: volatile fatty acids; minor VFA: calculated as the sum of isobutyrate, isovalerate, and valerate; and <sup>2</sup> SEM: standard error of the mean ( $n = 12$ ; 3 samples  $\times$  4 replicates).

The greater NH<sub>3</sub>-N concentrations ( $p < 0.001$ ) observed for the seeds agrees well with the greater CP content and the lower NDICP proportion of this fraction compared with the peels (Table 1), which would result in a greater amount of N available to be used by rumen microorganisms. The fermentation pattern of the DOP used as a reference (Table 3) was quite similar to that of pomegranate seeds, although fermentation of DOP resulted in a greater acetate/propionate ratio, probably due to differences in the chemical composition. In addition, DOP showed greater NH<sub>3</sub>-N content than both pomegranate fractions despite having intermediate CP content, which could be due to greater CP degradability in the DOP as indicated by the lower amount of NDICP (Table 1).

### 3.3. In Vitro Fermentation of Artichoke Wastes

The parameters of gas production of artichoke fractions and the sample of tomato pomace used as a reference are shown in Table 2. Potential gas production (A) of artichoke stems was 9.6% greater ( $p < 0.001$ ) than that of artichoke hearts. Although the NDF content of the stems was significantly greater (51.2 vs. 36.2% of DM), the lower lignification of the stems' NDF (12.9% of lignin in the NDF) compared with the hearts' (20.4%) can help to explain these results. Despite the lower potential gas production, the hearts fermented more rapidly than the stems, resulting in significantly greater ( $p < 0.001$ ) values of  $c$ , AGPR, DMED and ME, and lower ( $p = 0.014$ ) Lag values. Others [29,30] reported greater gas production than in our study for artichoke stems and bracts, respectively, after in vitro incubation with ruminal fluid from sheep for 96 h. Similarly, greater in vitro DM degradability for ensiled artichoke waste (69%) [12], for fresh artichoke (50.6%) [33], and for artichoke stems (65.6%) [30] and bracts (63.4%) [29] have been observed in other studies. These results reflect the great variability in the values that can be found in the literature, which can be attributed to the variability in the composition of by-products, but also to the variable methodologies used to determine DM degradability.

Both artichoke fractions were less rapid and extensively fermented than the tomato pomace used as a reference feed, which was likely due to the greater content in easily fermented fractions in the tomato pomace [3]. Tomato pomace has been classified as a medium-quality fibrous ingredient [3], and its ME content reported in the feed tables was between 9.5 and 11.2 MJ /kg DM [20,21] which is in good agreement with the value observed in our study (11.1 MJ/kg DM). The ME content of artichoke hearts and stems was 85.6 and 65.3% of that of tomato pomace, respectively, indicating that they have lower nutritive value for ruminants than this by-product.

Artichoke hearts produced greater ( $p \leq 0.025$ ) gas and total VFA than artichoke stems in 24 h of in vitro ruminal incubation (Table 3), which is in agreement with the greater gas production rates ( $c$  and AGPR) and DMED values previously observed for hearts. The greater ( $p < 0.001$ ) acetate proportions and lower ( $p < 0.001$ ) proportions of propionate observed for stems are consistent with the greater NDF content of this fraction compared with artichoke hearts (Table 1), which is also reflected in the greater acetate/propionate ratio ( $p < 0.001$ ) observed for the stems. Madrid et al. [33] analyzed the in vitro fermentation of both fresh and boiled whole artichoke using ruminal fluid from goats and observed greater proportions of acetate and lower proportions of propionate than in the present study, but it should be taken into account that VFA production was measured after 72 h incubation and acetate proportion usually increases and that of propionate decreases as incubation time progresses [34]. Compared with artichoke hearts, the stems had lower ( $p < 0.001$ )  $\text{NH}_3\text{-N}$  concentration, which could be attributed to their lower CP content. Compared with the sample of tomato pomace used as a reference, both artichoke fractions produced lower amounts of gas, but similar amounts of total VFA. However, the estimated ME content of tomato pomace was greater than that for artichoke stems, which could be due to the greater EE concentration of tomato pomace.

## 4. Conclusions

Discarded pomegranates had low dry matter content, but the dry matter was rich in sugars and contained low protein and fiber. Pomegranate peels were more fibrous than seeds and were less degraded in the rumen. The metabolizable energy content of pomegranate seeds was similar to that of dried orange pulp and therefore it could be a high-energy feed for ruminants. However, pomegranate peels had lower energy content and therefore the energy content of discarded pomegranates would be influenced by the proportion of each fraction. Discarded artichokes had very low dry matter content, but the dry matter was rich in protein and medium-lignified fiber. The estimated metabolizable energy content of artichoke hearts and stems indicates that they have lower nutritive value for ruminants than tomato pomace. Both wastes could be used in ruminant feeding, but

given their low dry matter content, effective low-cost storage methods are needed for their preservation.

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