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# Chemical and fermentative characteristics of agricultural byproducts and their mixtures with roughages incubated with rumen fluid from slaughtered dromedaries

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**Abstract:** In pursuit of novel strategies aimed to promote animal production while exploiting agricultural residues, using the dromedary camel rumen fluid degradation capabilities combined with a gas production (GP) technique approach, we investigated the in vitro kinetic parameters and some physical and chemical characteristics of three agro-industrial byproducts (AIBP) and their mixtures with three roughages. Chemical composition, buffering capacity, apparent degradability, pH, and in vitro GP kinetic parameters were evaluated for citrus pulp, tomato peels, apple pomace, their mixtures (50:50 % DM basis) with milk thistle, crown daisy, and polyphyte hay. GP of AIBP ranged from 200 to 240 mL/g DM. Roughages produced significantly less gas whereas mixtures gave intermediate amounts of gas. Gas from AIBP evolved with significantly ($P < 0.05$) shorter lag time (0.33 h) compared with roughages and mixtures. Mixing roughages with AIBP significantly ($P < 0.05$) increased the initial and final pH. The buffering capacity significantly increased with some mixtures and decreased with some others. In conclusion, ruminal fluid from slaughtered dromedaries, like the conventional bovine inoculum, fermented the major plant polymers and can be used in in vitro degradability trials. Rapidly fermentable AIPB mixed with roughages can improve some nutritional aspects, providing dietary fiber and correcting some in vitro kinetic parameters.

**Key words:** Agro-industrial byproducts, buffering capacity, *Camelus dromedaries*, fibrous forages, in vitro gas production, ruminal inoculum.

## 1. Introduction

Limited availability of feeds is the main constraint for animal production for many countries and all additional resources to meet the animal needs can alleviate the production cost. Integration of agro-industrial byproducts may offer wide perspectives for improving the nutritional balance. Industrial processing of fruits and vegetables generates a huge amount of agro-industrial byproducts (AIBP) every year around the world and represents 30%–50% of the initial product (1). Many AIBP are converted in several applications such as metabolites productions, gas production in biodigestors, and substrates for various fermentations and are potentially convertible to feeds for animal production (2).

Depending on the industrial processes used such as juice extraction from citrus or tomato paste production, the initial structure and chemical composition of fruit or vegetable undergo various modifications or alterations, resulting in a product with new characteristics not necessarily compatible with the needs of ruminal microbiota and the animal physiology. Some nutritive constituents can disappear and others can increase, leading to an unbalanced nutritional profile. Moreover, some byproducts show a high content of rapidly fermentable total sugars, such as citrus and apple byproducts, and can be used as carbon and energy sources while others, such as olive cake, have poor protein and fiber content, and some others contain valuable bioactive compounds. Intrinsic buffering capacity and pH of byproducts are important aspects for optimal microbial activities in the rumen. Healthy animals are able to buffer ingested feeds with saliva, within physiological limits, but feeds with low buffering capacity increase saliva secretion, with consequent acidosis risk. AIBP present different buffering capacity (BC) mainly related to their salts of organic acids and protein content (3). Metabolic gas produced from fermented feeds is another important

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aspect when byproducts are used as feeds for ruminants due to the risk of bloating. Mixing some AIBP with one another or with roughage forages can offer the possibility to correct some of their nutritional and physicochemical traits, such as unbalanced nutritional composition, low buffering capacity, and excess of gas production by ruminal fermentation. At the same time, the process can valorize the forages themselves. Among AIBP, tomato peels, apple pomace, and citrus pulp are produced with the highest tonnage (1). Milk thistle and crown daisy are, on the other hand, interesting examples of highly exploitable abundant wild forages in the Mediterranean area (4, 5).

Rumen fluid from fistulated bovines has been widely used for in vitro evaluation of degradability of various feeds and byproducts. whereas rumen fluid from slaughtered dromedaries (RFSD), despite its suitability for the same purpose (6), has received little attention so far. In this study, we attempted to investigate the suitability of the RFSD in vitro fermentation with the objective to evaluate the in vitro degradation characteristics of fibrous forages and highly fermentable industrial citrus, tomato, and apple byproducts, as well as their mixtures. The forages used, besides polyphyte hay, included crown daisy and milk thistle, two spontaneous weeds of promising value which abound as wild vegetation in the semiarid Mediterranean areas (7–10).

## 2. Materials and methods

### 2.1. Plant material and pure polymers

As previously reported (4), three agro-industrial byproducts from tomatoes (*Solanum lycopersicum*), apple (*Malus communis*), and citrus or sweet orange (*Citrus sinensis*, L. Osb.), and three types of forages were tested. Crown daisy (CD; *Chrysanthemum coronarium* L.) and milk thistle (MT; *Silybum marianum* L. Gaertn.) were cropped in Northern Sardinia, Italy, respectively at the following coordinates: 40.523444°N, 8.7445370°E and 40.3271250°N, 8.9602000°E. Both CD and MT were sown in early November, and fertilization was applied with 35 kg of N $ha^{-1}$ and 100 kg of $P_2O_5$ $ha^{-1}$. No irrigation, fertilizer, or herbicides were applied after sowing. Untreated plants of CD and MT were harvested at late growth stage (June) to maximize biomass production, and immediately air-dried. Apple pomace (AP), citrus pulp (CP), and tomato peels (TP), composed by skins, pulp, seeds, damaged fruits, and vegetables not suitable for human consumption were collected from local warehouses, and one sample for each material (1 kg) was oven- dried at 60 °C until constant weight. All feed samples were ground by a hammer mill (Pulverisette 19, Fritsch GmbH, Laborgeratebau, Germany) with a screen size of 1 mm and independent samples of about 2 g were randomly collected to be used for incubation and chemical analysis. AIBP were dried at 65 °C for 72 h and milled to pass 1 mm screen and kept in plastic bottle until use. In order to compare the chemical and kinetic characteristics with a conventional feed for cows, we used polyphyte hay in these trials, as previously described (11).

### 2.2. Chemical analyses

As previously reported (4), chemical composition was determined according to AOAC procedures (12) for DM, crude protein, ether extract, and ash. Neutral detergent fiber was determined according to Mertens (2002) (13). Acid detergent fiber content was sequentially determined after NDF according to Robertson and Van Soest (1981) (14). Additionally, total sugars (TS) were determined according to the colorimetric assay (15) using glucose as standard sugar. Cellulose was computed as neutral detergent fiber (NDF)-acid detergent fiber (ADF) and hemicellulose as the difference between ADF and ADL (acid detergent lignin). Apparent degradability (AD) was estimated with a modified Tilley and Terry procedure (16), washing the nondegraded residual after fermentation of the substrate with bidistilled water and filtering it through a nylon bag with 50 μm pores and no enzymatic treatment was done. The blank contained no substrates. Residual matter was dried for 24 h at 75 °C. AD was computed as the difference between initial and filtered residual DM corrected with the blank at the end of incubation. For buffering capacity assay, a modified procedure based on the one reported by Giger-Reverdin et al. (17) was used. Briefly, 1.0 g DM of substrate was suspended in 20 mL of bidistilled water and shaken for 2 h. Initial pH was measured and then corrected to pH 7.0 by N HCl or NaOH, followed by titration with N acetic acid, using a pH meter with a temperature compensation probe. Buffering capacity was expressed in milliequivalent per liter of N acetic acid per g of DM, used to decrease the pH by one unit in the interval of pH from 7.0 to 4.0.

### 2.3. Animal donor and in vitro incubation

Ruminal fluid from two mature male Camels (*Camelus dromedarius*) weighing between 345 and 355 kg was used at each trial. The animals were transferred from the arid zone of South Biskra (Algeria) to the municipal slaughterhouse of Constantine (Algeria). In this region, dromedaries grazed freely native fibrous and sclerophyllous plants mainly belonging to Chenopodiaceae, Zygophyllaceae, Plumbaginaceae, and several other herbaceous species at minor abundance. Water was provided only every 2-3 days in this arid zone. Fresh rumen content of two slaughtered dromedaries was collected aseptically in insulated flasks immediately after evisceration, mixed and filtered through two cheese cloth layers under oxygen-free $CO_2$. The filtrate was used as inoculum. In 250-mL glass bottles, 500 mg (DM) or 250 mg of each single substrate for the mixtures were incubated with 25 mL of filtered rumen fluid diluted in 75 mL of buffered solution (18). In order

to check for the presence of the main functional bacterial groups (cellulolytic, xylanolytic, pectinolytic, amylolytic, and proteolytic species) in the inoculum from the ruminal content, 500 mg of each pure polymer: fibrous cellulose CF11 (Whatman), xylan (Sigma), pectin (Fluka), soluble starch (Merck), and casein (Sigma) were used as internal standards. Blanks were prepared in the same manner, omitting substrates. All substrates were fermented in duplicate in two different trials and four kinetics were obtained for each substrate. Incubation was conducted at 39 °C in a water bath, and cumulative gas was measured using a related time pressure transducer apparatus (19). This apparatus releases a fixed amount of the evolved gas from fermented substrate in relation with the time of reaction. The fixed amount of gas was set during the calibration procedure according to the operating manual (20).

**2.4. Data analysis**

The gas produced during in vitro fermentation was fitted to the following exponential model: (21, 22):

$$gas(t) = b/(1- \exp(-c(t-lag))),$$

where gas(t) is the evolved gas during the incubation, b the asymptotic gas production (GP) (mL gas/g DM), and c the fractional constant rate of GP (b/h). The time when the half GP is reached ($t_{½}$) was computed as (ln(0.5)/c). GP was also estimated at 6 h (b6), 12 h (b12), 24 h (b24), and 48 h (b48) of incubation. Kinetic parameters were estimated using the nonlinear model procedure of the Statistical Analysis System software (23). Means of chemical and fermentative parameters of substrates were compared using Student Newman Keuls test at the significance level of P = 0.05. The substrates were organized in four groups: byproducts, fibrous forages, pure polymers, and mixtures, and the pooled means of their kinetic parameters were compared in the same way. The effects of substrates and their mixtures and the effects of group of substrates on the kinetic parameters were evaluated with the general linear model procedure of the same software according to the equation: $Yi = \mu + Si + ei$, where $Yi$ is the dependent variable (b, c, lag, $t_{½}$, etc.), $\mu$ the overall mean, $Si$ the effect of substrate or the effect of the group of substrates, and $ei$ the residual random error of experiment.

## 3. Results

**3.1 Chemical composition**

The compositional values of the two low-quality spontaneously growing forages, i.e. milk thistle and crown daisy, along with those of the three industrial byproducts, i.e. tomato peels, citrus pulp, and apple pomace were previously reported for a parallel study with bovine rumen fluid incubations (4), and only additional data are reported here. The polyphyte hay contents were as follows: DM, 93.2; organic matter, 91.7; crude protein (CP), 3.5; ether extract (EE), 3.0; neutral detergent fiber (NDF), 69.2; acid detergent fiber (ADF), 39.8; acid detergent lignin (ADL), 2.5; acid insoluble ash (AIA), 0.25; hemicellulose, 21.0; and cellulose, 37.3. All values are in g/100g DM. In terms of total sugars, apple pomace had the highest content (77.8%, P < 0.05 DM), followed by citrus pulp 50.6, tomato peels 31.2, polyphyte hay 20.0, crown daisy 9.5, and milk thistle 7.9.

Except for tomato peels (NDF: 33.1% DM), the byproducts were significantly less fibrous than roughages and contained less ADF and cellulose, but high amounts of total sugars. For ruminant diet purposes, the tomato byproduct showed the interesting compositional profile with 12.1% CP, 33.1% NDF, and 31.2% for total sugars. Apple pomace showed a different nutritional content characterized by a large amount of total sugars (77.8% DM) and low CP content (2.3% DM). The citrus pulp showed an intermediate compositional profile with a high level of total sugars and the lowest acid lignin contents (0.02% DM). Roughages were highly fibrous with elevated contents in neutral fiber NDF (>68% DM), ADF (>39%), hemicelluloses (>16%), and cellulose (>37%), but their total sugar contents were significantly lower than those observed for byproducts: 7.9 and 9.5 g/100 g DM for milk thistle and crown daisy, respectively. Polyphyte hay showed intermediate contents of total sugars, cellulose, and ADF among byproducts and roughages.

**3.2. pH and buffering capacity**

The initial pH (pHi) of the byproducts was acidic for apple pomace, tomato peels, and citrus pulp (Table 1). Fibrous feeds showed different pHi: alkaline for milk thistle, near the neutrality for crown daisy and acidic for polyphyte hay. The pHi of all mixtures significantly increased (P < 0.05), relative to the byproduct. However, pHi of tomato peels–crown daisy, tomato peels–hay, citrus pulp–crown daisy, and citrus pulp–hay mixtures remained under pH 5.0. Mixing industrial byproducts with roughages resulted in significant changes in buffering capacity (BC), compared with buffered saliva (BS), filtered ruminal fluid (RF), and their mixture (BS+RF). The tomato byproduct showed the highest BC despite its low pHi. The BC of tomato peels–hay mixture was significantly higher than that of polyphyte hay alone. Surprisingly, this was not the case for tomato peels–milk thistle mixture despite the high BC of the milk thistle alone. For tomato peels–crown daisy mixture, BC was nearly intermediate and significantly higher than that of crown daisy alone. AIBP, roughages, and their mixtures appeared significantly less buffered than BS and RF+BS, presumably due to the minor content of carbonates and phosphates. Purified cellulose and starch polymers have no BC, while xylan, casein, and pectin showed different values. Among the substrates, crown daisy and milk thistle showed the lowest apparent degradability, while apple

**Table 1.** Initial and final pH, buffering capacity, and apparent degradability of fermented substrates, pure polymers, inoculum, and incubation medium.

| | pHi | Final pH after incubation | Buffering capacity (mEquivalents. $L^{-1}$ of N acetic acid/g DM) | Apparent degradability (g/100 g DM) |
|---|---|---|---|---|
| *Medium and inoculum* | | | | |
| Rumen fluid | $7.48^{b(1)}$ | § | $0.96^{d}$ | § |
| Buffered saliva | $6.89^{c}$ | § | $3.33^{a}$ | § |
| Rumen fluid + Buffered Saliva | $6.88^{c}$ | § | $1.49^{b}$ | § |
| *SEM* | *0.13* | § | *0.46* | § |
| *Byproducts* | | | | |
| Apple pomace | $4.39^{jk}$ | $6.60^{cd}$ | $0.24^{ij}$ | $96.0^{a}$ |
| Citrus pulp | $4.09^{k}$ | $6.56^{cd}$ | $0.49^{g}$ | $96.2^{a}$ |
| Tomato peels | $4.11^{k}$ | $6.71^{bcd}$ | $1.26^{c}$ | $86.2^{b}$ |
| *SEM* | *0.06* | *0.01* | *0.19* | *1.13* |
| *Fibrous forages* | | | | |
| Milk thistle | $8.16^{a}$ | $6.77^{bc}$ | $1.52^{b}$ | $54.6^{f}$ |
| Crown daisy | $6.19^{f}$ | $6.75^{bc}$ | $0.34^{hi}$ | $49.6^{f}$ |
| Polyphyte hay | $5.66^{g}$ | $6.71^{bc}$ | $0.54^{f}$ | $67.3^{de}$ |
| *SEM* | *0.45* | *0.02* | *0.23* | *1.53* |
| *Byproducts and fibrous forages mixtures* | | | | |
| Apple pomace: milk thistle | $6.56^{e}$ | 6.70b$^{c}$ | $0.55^{f}$ | $72.0^{de}$ |
| Apple pomace: polyphyte hay | $5.67^{h}$ | $6.77^{bc}$ | $0.34^{hi}$ | $86.8^{b}$ |
| Citrus pulp: milk thistle | $5.66^{h}$ | $6.87^{b}$ | $0.54^{f}$ | $72.1^{de}$ |
| Tomato peels: milk thistle | $5.29^{i}$ | $7.22^{a}$ | $0.70^{e}$ | $63.0^{e}$ |
| Apple pomace: crown daisy | $5.08^{j}$ | $6.73^{bc}$ | $0.28^{ij}$ | $69.1^{de}$ |
| Citrus pulp: polyphyte hay | $4.75^{k}$ | $6.63^{cd}$ | $0.44^{fgh}$ | $76.0^{cd}$ |
| Citrus pulp: crown daisy | $4.66^{l}$ | $6.48^{d}$ | $0.37^{ghi}$ | $75.4^{cd}$ |
| Tomato peels: polyphyte hay | $4.46^{m}$ | $6.78^{bc}$ | $0.82^{e}$ | $81.3^{bc}$ |
| Tomato peels: crown daisy | $4.39^{m}$ | $6.57^{cd}$ | $0.74^{e}$ | $68.0^{de}$ |
| *SEM* | *0.16* | *0.34* | *0.04* | *1.62* |
| *Polymers* | | | | |
| Casein | $5.74^{h}$ | $7.09^{a}$ | $0.34^{hi}$ | $95.84^{ab}$ |
| Cellulose | $4.52^{ij}$ | $6.54^{cd}$ | $0.004^{l}$ | $99.0^{a}$ |
| pectin | $2.73^{l}$ | $6.69^{bcd}$ | $0.20^{jk}$ | $99.75^{a}$ |
| Starch | $5.36^{h}$ | $6.59^{cd}$ | $0.01^{l}$ | $99.25^{a}$ |
| Xylan | $5.81^{g}$ | $6.72^{bcd}$ | $0.11^{kl}$ | $99.6^{a}$ |
| *SEM* | *0.36* | *0.06* | *0.01* | *0.51* |

(1)Means in the same column with different superscripted letters differ significantly ($P < 0.05$); §, not applicable; SEM, standard error of the means.

pomace and citrus pulp had the highest AD followed by tomato pulp. Polyphyte hay had comparable AD to several mixtures and seven mixtures among nine showed similar AD to polyphyte hay (Table 1).

Following the medium and inoculum group, fibrous forages showed the highest pHi (Table 2), but due to the highest BC of medium and inoculum group, all final pH were similar except the byproducts group. In terms

of apparent degradability, the mixtures group showed intermediate values between the byproducts and fibrous forages group. The pure polymers used as internal standards in the trials were almost entirely degraded.

### 3.3. In vitro degradation kinetics

Microbial degradation kinetics of byproducts showed different in vitro fermentation patterns evidenced by the gas production (Figures 1–3). AIPB and their mixtures with fibrous forages completely exhausted their metabolic gas before the end of 72 h of incubation. Apple pomace and its mixtures showed a simple parabolic pattern, while tomato peels and their mixtures had a more complex pattern. When mixed with crown daisy, tomato peels and citrus pulp produced considerably less gas than each byproduct alone: −46.5% and −38.5%, respectively (Table 3), while apple pomace gave remarkably less gas when mixed with crown daisy (−69.5%). Citrus and apple byproducts produced more gas than tomatoes but the amount of gas

**Table 2.** Pooled means of initial and final pH, buffering capacity, and apparent degradability of fermented substrates, pure polymers, inoculum and incubation medium grouped in five categories.

| | pHi | Final pH after incubation | Buffering capacity (mEquivalents. $L^{-1}$ of N acetic acid/g DM) | Apparent degradability (g/100 g DM) |
|---|---|---|---|---|
| Medium and inoculum | $6.74^{a(1)}$ | § | $1.92^{a}$ | § |
| Byproducts | $4.2^{b}$ | $6.64^{b}$ | $0.66^{bc}$ | $91.5^{b}$ |
| Mixtures | $5.17^{b}$ | $6.75^{a}$ | $0.53^{bc}$ | $73.7^{c}$ |
| Forages | $6.74^{a}$ | $6.74^{a}$ | $0.8^{ab}$ | $56.7^{d}$ |
| Polymers | $4.71^{b}$ | $6.72^{a}$ | $0.13^{c}$ | $98.7^{a}$ |
| *SEM* | *0.19* | *0.01* | *0.1* | *1.78* |

[(1)]Means in the same column with different superscripted letters differ significantly ($P < 0.05$); §, not applicable; SEM, standard error of the means.

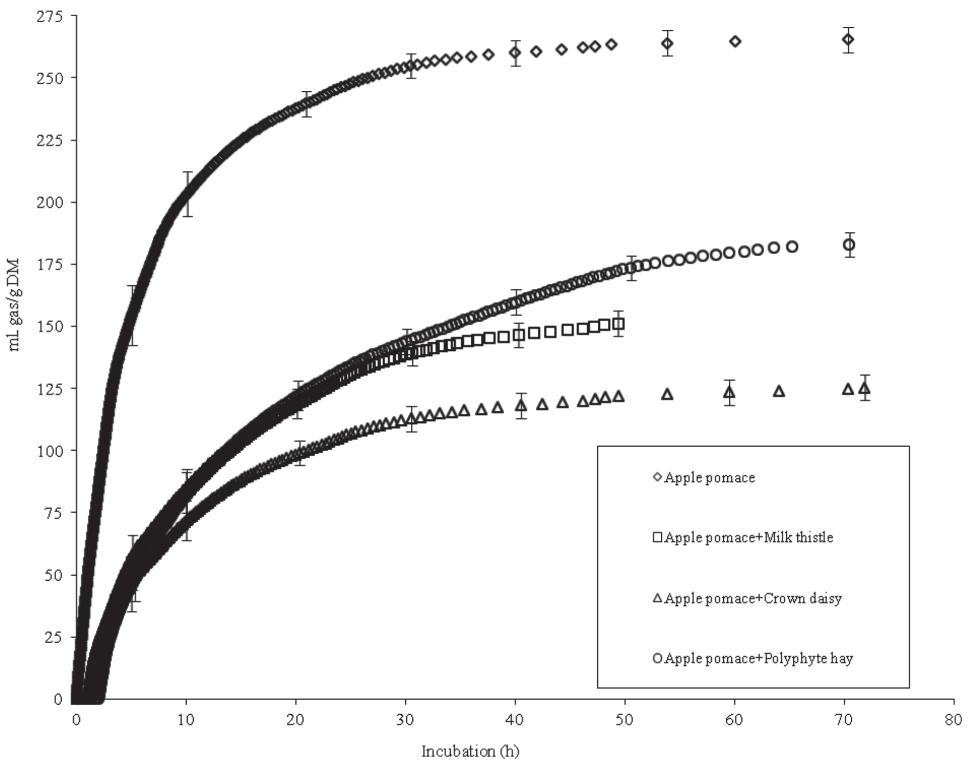


**Figure 1.** Observed gas production by 1 g DM of apple pomace and their mixtures with milk thistle, crown daisy, and polyphyte hay. The bars represent the standard error of the means.

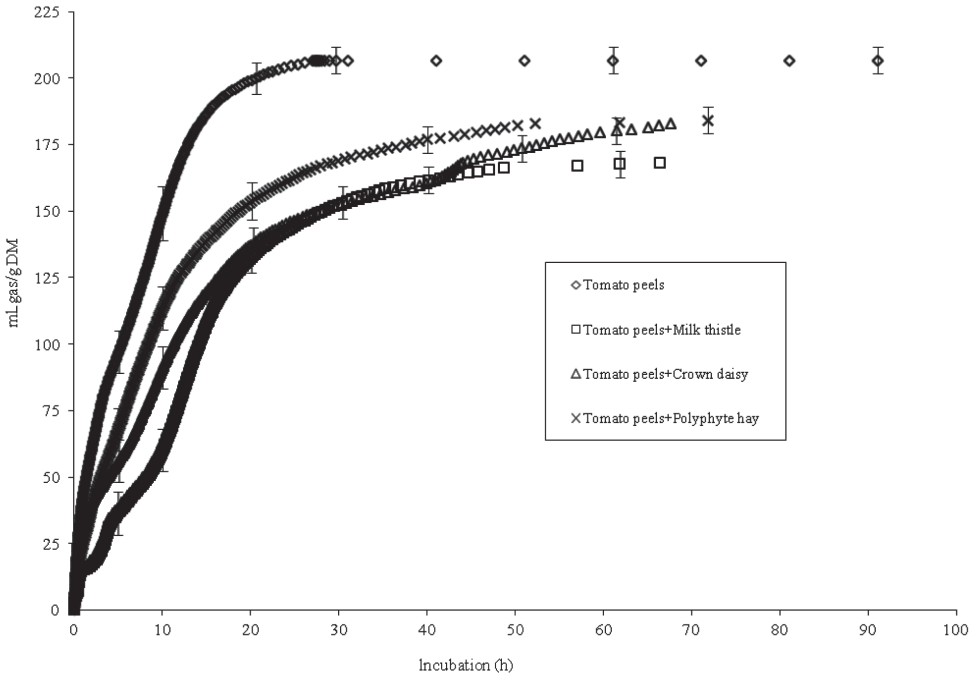


**Figure 2.** Observed gas production by 1 g DM of tomato peels and their mixtures with milk thistle, crown daisy, and polyphyte hay.

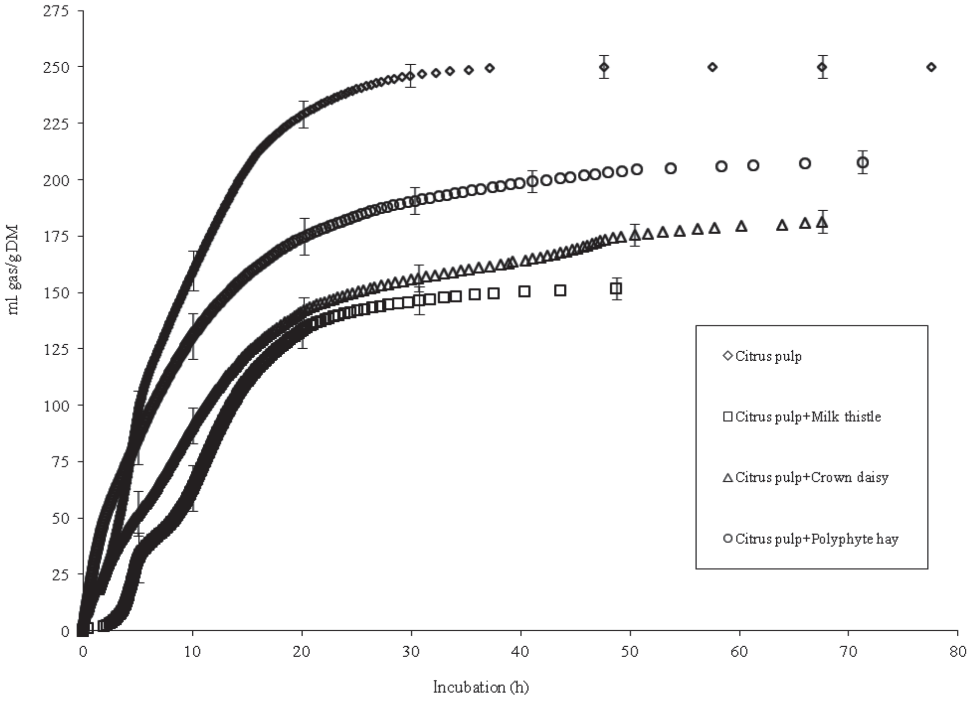


**Figure 3.** Observed gas production by 1 g DM of citrus pulp and their mixtures with milk thistle, crown daisy, and polyphyte hay.

produced after 6 h was statistically not different between byproducts. In vitro GP started after comparably short lag times: 0.31, 0.19, and 0.47 h, respectively for tomato, citrus, and apple byproducts. Half-life production values of the released gas were reached at different times but the results were statistically comparable for apple, citrus, and

**Table 3.** Estimated kinetic parameters of in vitro fermentation of 1g DM of citrus, apple and tomato byproducts and their mixtures with milk thistle, crown daisy, and polyphyte hay compared with pure polymers.

| Groups of substrates | Constant rate of GP c (b/h) | Asymptotic GP b [(1)] | Lag-time (h) | Half-life time (h) | GP at 6 h[(1)] | GP at 12 h[(1)] | GP at 24 h[(1)] | GP at 48 h[(1)] | se |
|---|---|---|---|---|---|---|---|---|---|
| *Byproducts* | | | | | | | | | |
| Citrus pulp | 0.144$^{ab(2)}$ | 239.5$^{bc}$ | 0.19$^{d}$ | 4.96$^{c}$ | 138.0$^{a}$ | 195.5$^{a}$ | 230.7$^{ab}$ | 239.0$^{b}$ | 5.3 |
| Apple pomace | 0.181$^{a}$ | 231.5$^{bc}$ | 0.48$^{d}$ | 3.89$^{c}$ | 153.5$^{a}$ | 204.7$^{a}$ | 228.2$^{ab}$ | 231.5$^{b}$ | 6.3 |
| Tomato peels | 0.108$^{bcd}$ | 203.4$^{cd}$ | 0.31$^{d}$ | 6.41$^{bc}$ | 97.2$^{bc}$ | 147.8$^{bc}$ | 188.1$^{bc}$ | 202.2$^{bc}$ | 8.4 |
| *SEM* | *0.007* | *5.19* | *0.10* | *0.24* | *6.25* | *6.74* | *5.8* | *5.24* | |
| *Fibrous forages* | | | | | | | | | |
| Milk thistle | 0.072$^{cde}$ | 90.5$^{ghi}$ | 3.64$^{cd}$ | 9.72$^{bc}$ | 31.7$^{de}$ | 52.3$^{fg}$ | 74.3$^{ef}$ | 87.5$^{gf}$ | 2.4 |
| Crown daisy | 0.055$^{de}$ | 70.3$^{i}$ | 2.12$^{d}$ | 12.16$^{b}$ | 20.5$^{e}$ | 34.9$^{g}$ | 52.1$^{f}$ | 65.2$^{g}$ | 0.7 |
| Polyphyte hay | 0.072$^{cde}$ | 105.1$^{fghi}$ | 1.92$^{d}$ | 11.66$^{bc}$ | 36.0$^{de}$ | 57.9$^{fg}$ | 81.3$^{ef}$ | 87.7$^{efg}$ | 3.5 |
| *SEM* | *0.006* | *4.28* | *0.56* | *0.92* | *3.0* | *4.1* | *4.42* | *4.15* | |
| *Mixtures* | | | | | | | | | |
| Citrus pulp– milk thistle | 0.125$^{bc}$ | 128.1$^{efgh}$ | 7.13$^{bc}$ | 5.54$^{bc}$ | 77.6$^{cd}$ | 99.6$^{def}$ | 121.7$^{de}$ | 127.8$^{def}$ | 3.7 |
| Citrus pulp– crown daisy | 0.076$^{cde}$ | 171.3$^{de}$ | 1.71$^{d}$ | 9.13$^{bc}$ | 62.7$^{cde}$ | 102.5$^{def}$ | 143.6$^{cd}$ | 166.8$^{cd}$ | 3.4 |
| Citrus pulp–polyphyte hay | 0.116$^{bcd}$ | 202.3$^{cd}$ | 0.001$^{d}$ | 6.02$^{bc}$ | 111.1$^{bc}$ | 151.5$^{bc}$ | 189.4$^{bc}$ | 201.5$^{bc}$ | 5 |
| Apple pomace–milk thistle | 0.087$^{bcd}$ | 143.8$^{ef}$ | 0.55$^{d}$ | 7.98$^{bc}$ | 58.5$^{cde}$ | 93.2$^{ef}$ | 126.0$^{de}$ | 141.6$^{de}$ | 5 |
| Apple pomace–crown daisy | 0.109$^{bcd}$ | 125.0$^{efgh}$ | 0.50$^{d}$ | 6.59$^{bc}$ | 61.1$^{cde}$ | 91.6$^{ef}$ | 115.3$^{de}$ | 123.8$^{def}$ | 3.8 |
| Apple pomace–polyphyte hay | 0.111$^{bcd}$ | 200.0$^{cd}$ | 0.25$^{d}$ | 7.09$^{bc}$ | 97.0$^{bc}$ | 144.4$^{bcd}$ | 181.5$^{bc}$ | 187.0$^{bc}$ | 10.4 |
| Tomato peels–milk thistle | 0.114$^{bcd}$ | 125.0$^{efgh}$ | 8.05$^{b}$ | 6.06$^{bc}$ | 62.0$^{cde}$ | 93.2$^{ef}$ | 116.8$^{de}$ | 124.3$^{def}$ | 2.9 |
| Tomato peels–crown daisy | 0.07$^{cde}$ | 137.0$^{efg}$ | 2.36$^{d}$ | 9.97$^{bc}$ | 47.0$^{de}$ | 77.9$^{efg}$ | 111.7$^{de}$ | 132.2$^{def}$ | 3.2 |
| Tomato peels–polyphyte hay | 0.105$^{bcd}$ | 145.3$^{ef}$ | 0.77$^{d}$ | 6.59$^{bc}$ | 67.4$^{cd}$ | 103.4$^{def}$ | 133.2$^{d}$ | 144.2$^{cd}$ | 2.5 |
| *SEM* | *0.005* | *7.7* | *0.6* | *0.4* | *5.0* | *6.8* | *7.5* | *7.64* | |
| *Polymers* | | | | | | | | | |
| Xylan | 0.17$^{ab}$ | 203.1$^{cd}$ | 1.59$^{d}$ | 4.22$^{c}$ | 129.3$^{ab}$ | 175.4$^{ab}$ | 198.7$^{b}$ | 202.9$^{bc}$ | 8.4 |
| Pectin | 0.104$^{bcd}$ | 285.6$^{a}$ | 1.12$^{d}$ | 7.06$^{bc}$ | 129.2$^{ab}$ | 198.5$^{a}$ | 257.1$^{a}$ | 282.0$^{a}$ | 7.1 |
| Starch | 0.064$^{cde}$ | 235.0$^{bc}$ | 8.22$^{b}$ | 11.28$^{bc}$ | 75.1$^{cd}$ | 125.4$^{cde}$ | 182.5$^{bc}$ | 222.0$^{b}$ | 6.1 |
| Cellulose | 0,032$^{e}$ | 265.0$^{ab}$ | 18.33$^{a}$ | 22.66$^{a}$ | 47.0$^{de}$ | 85.0$^{ef}$ | 141.6$^{cd}$ | 205.6$^{bc}$ | 6.4 |
| Casein | 0.102$^{bcd}$ | 82.2$^{hi}$ | 3.55$^{cd}$ | 7.13$^{bc}$ | 37.2$^{de}$ | 57.1$^{fg}$ | 74.0$^{f}$ | 81.1$^{gf}$ | 4 |
| *SEM* | *0.01* | *16.6* | *1.52* | *1.6* | *9.0* | *12.2* | *14.2* | *15.34* | |

[(1)] mL of gas/g DM; [(2)] means in the same column with different superscripts differ significantly ($P < 0.05$); se, residual standard error; SEM, standard error of the means; GP, gas production.

tomato byproducts. In terms of gas constant rate of GP c, the apple byproduct showed the highest constant rate: 0.181 b/h and the tomato peels the lowest value: 0.108 b/h. Fibrous feeds did not show significant differences in their kinetic parameters, but crown daisy, with the lowest gas production, was the least fermentable feed compared to polyphyte hay and milk thistle. Potential gas production b, as expected, decreased in all mixtures when compared with the byproducts alone. Gas produced by citrus and apple byproducts was comparable with that produced by some of the pure plant polymers: xylan, pectin, starch, and cellulose, denoting that ruminal microflora easily accessed to the high degradable substrates.

In terms of lag time, fibrous feeds showed comparable values with their mixture with byproducts, but byproducts alone showed a very short lag time, only 0.3 h after the incubation started. Similarly, the half-time of gas production increased significantly by more than 2 h when byproducts were mixed with fibrous feeds. With byproducts and fibrous feeds mixture, the rate constant of GP c was reduced by around 30%, when compared with byproducts alone (0.14 vs 0.099 b/h).

All three byproducts showed comparable short lag times. However, tomato peels showed the highest half-time of GP (6.41 h) and the lowest constant of fractional rate c (0.108 b/h). Forages showed comparable kinetic parameters and, among them, crown daisy was the least fermentable (70.3 mL/g DM), with the highest half-time (12.16 h). Forages produced significantly less gas than byproducts, and their mixture produced intermediate amounts of gas (156.1 mL), and showed 7.3 h half-life time and 0.1 b/h constant of fractional rate c. Forages and their mixtures with byproducts showed comparable lag times which were significantly higher than those of byproducts fermented alone (0.33 h), i.e. nearly 15 times higher. Based on their constant of fractional rate c, asymptotic GP b, and lag and half-life time, AIBP were the most fermentable group of substrates and the fibrous forages the least fermentable one, except for the lag parameter (Table 4). The mixtures group showed intermediate fermentative characteristics between the compared to the other two groups. Pure polymers used as internal standards showed a comparable c with the mixtures group but the highest asymptotic GP, lag time, and half-life time.

## 4. Discussion

A comparison with a previous work based on the use of the same feedstocks but with bovine rumen fluid (4) could be made. Overall, camel inoculum shows higher gas production than bovine inoculum at early stages (6 h) and lower cumulative gas production at the asymptotic stage for all byproducts and for milk thistle when used as single substrates. For crown daisy, the bovine values at 6 h are, on the other hand, higher and so are the values of the mixtures at all times. Using rumen fluid of fistulated sheep and fitting their data with the same exponential model, Besharati et al. (24) reported a lower GP at 6 h of incubation: 136.1 mL for AP and 84.6 mL for TP. Lower results for GP at 6 h were also reported by Tagliapietra et al. (4): 60 mL for AP and 73 mL for TP. These authors used rumen fluid from dry Holstein–Friesian cows and fitted data with a logistical model. Similar differences were also reported for GP at 24 h of incubation but not for GP at 48 h, probably due to the diversity of the ruminal microbiota of each ruminant species. The $t_{½}$ values tend to be shortened in camel for single substrates while they tend to be longer for the mixtures. All final pH values of AIBP and their mixtures with fibrous forages, fermented in buffered saliva (BS), remained in a range of pH (6.48–7.22) compatible with the metabolic activity of the ruminal microflora, despite their different pHi. Giger-Reverdin et al. (17) reported a higher pHi (5.77) for apple pomace, and Moharrery (25) reported also a higher pHi (4.73) for TP. All AIBP and their mixtures with fibrous forages were degraded at various extents, depending on their nutrients content and structure, suggesting their compatibility with the metabolic activity of the ruminal microflora.

**Table 4.** Pooled means of estimated kinetic parameters of in vitro fermentation of 1g DM of groups of substrates.

| Groups of substrates | Constant rate of GP c (b/h) | Asymptotic GP b [(1)] | Lag time (h) | Half-life time (h) | GP at 6 h[(1)] | GP at 12 h[(1)] | GP at 24 h[(1)] | GP at 48 h[(1)] | se |
|---|---|---|---|---|---|---|---|---|---|
| Byproducts | 0.140[a(2)] | 221.8[a] | 0.33[c] | 5.2[c] | 125.5[a] | 178.1[a] | 212.0[a] | 221.2[a] | 6.9 |
| Fibrous forages | 0.072[c] | 95.7[c] | 2.28[b] | 11.5[a] | 32.1[d] | 52.4[d] | 74.4[d] | 89.6[d] | 2.8 |
| Polymers | 0.092[b] | 213.7[a] | 6.7[a] | 10.7[a] | 81.9[b] | 126.3[b] | 169.0[b] | 197.5[b] | 6.3 |
| Mixtures | 0.1[b] | 156.1[b] | 2.1[b] | 7.3[b] | 70.3[c] | 107.9[c] | 140.0[c] | 153.9[c] | 4.4 |
| *SEM* | *0.005* | *7.06* | *0.48* | *0.52* | *4.67* | *6.15* | *6.76* | *6.84* | |

[(1)] mL of gas/g DM; [(2)] means in the same column with different superscripts differ significantly ($P < 0.05$); se, residual standard error; SEM, standard error of the means; GP, gas production.

The use of agro-industrial byproducts as nonconventional feeds for ruminants is a common practice with various levels of inclusion in the diet. It is very important to define this level of inclusion in order to avoid physiological or metabolic disorders in various groups of ruminants. Inclusion of 300 g/kg DM of tomato pulp increased the fat content in milk of lactating ewes, as reported by Abbeddou et al. (26). In a trial conducted with crossbred heifers, Cribbs et al. (27) reported that DM intake, feed to gain ration, and average daily gain (ADG) decreased and several metabolic parameters were altered with inclusion of dehydrated citrus pulp at the level of 20% in substitution of corn. Increasing the level of inclusion on pelleted citrus pulp, from 0 to 2.5 kg/d per animal, Villarreal et al. (28) observed a linear decrease of DM, DM intake, and organic matter digestibility. Mixing apple pomace and tomato pulp (50:50) can replace alfalfa hay up to 30% in the diet of Holstein dairy cows without negative effects on milk composition as reported by Abdollahzadeh et al. (29). Ahn et al. (30) reported that inclusion of AP in goat (*Capra hircus*) diet mixed with rice straw, rice bran or concentrates at 30% to 60% of the diet improved DM intake, nutrient digestibility and nitrogen retention, compared with diet without AP. These authors reported no negative effects even if apple pomace was included at a high level (60% of the diet). As regards differences concerning ruminal microbiology of cows vs camels, recent reports found that concerning the two most abundant bacterial phyla, the camel rumen, while keeping similar proportions of Bacteroidetes, displays considerably less Firmicutes. Moreover, the numbers of Spirochaetes, Fibrobacteres, and Verrucomicrobia were higher in camels than in cows. All these evidences are consistent with a higher content of fiber in the diet (31).

We can conclude, on the one hand, that rumen fluid extracted from slaughtered dromedaries is a valuable tool for testing the kinetics parameters of the in vitro degradation of feeds with various characteristics. On the other hand, mixed substrates fermentation kinetics showed different instances of lowering gas production when byproducts are used as ruminant feeds. Mixing byproducts of the agricultural industry with fibrous feeds can thus be envisaged as a strategy to correct many of their fermentative and physico-chemical characteristics, improving their unbalanced nutritional profile also by lowering the total sugars. The buffering capacity was also influenced by mixtures; therefore, caution can be recommended for the maintenance of an optimal ruminal pH.

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