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# Relationships between chemical composition and in vitro gas production parameters of maize leaves and stems

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## Abstract

This study investigated the chemical composition (proximate and Van Soest analysis) and in vitro gas production parameters of maize leaves and stems separately, and related the in vitro gas production parameters with the chemical composition, of thirteen maize cultivars. After harvest in September 2016, all plants were separated into two morphological fractions: leaves and stems. The crude protein (CP) content was greater, and the ratio of acid detergent lignin (ADL) to potentially rumen degradable fibre (calculated as the difference between neutral detergent fibre and ADL; ADL:pRDF) was lower in the leaves than in the stems in all 13 cultivars. For the leaves, the cumulative gas production between 3 and 20 hr (A2), representing cell wall fermentation in the rumen fluid, and the cumulative 72-hr gas production (GP72), representing total organic matter (OM) degradation, were moderately to weakly correlated with the chemical composition, including hemicellulose, cellulose, ADL and CP content ($R^2 < 0.40$), whilst the best relationship between the half-time value (B2), representing the rate of cell wall degradation, and chemical composition had an $R^2$ of 0.63. For the stems, the best relationship between A2, B2 and GP72 with chemical composition was greater ($R^2 \geq 0.74$) and the best relationship included hemicellulose (A2 only), cellulose and ADL (GP72 and A2 only) contents. In conclusion, maize leaves and stems differed in chemical composition, in particular CP content and ADL:pRDF. The A2 and GP72 of the stems, but not of the leaves, were highly correlated with the chemical composition, indicating that the cell wall and OM degradation of maize stems can be better predicted by its chemical composition.

**KEYWORDS**
cell wall degradation, in vitro gas production, maize leaves, maize stems

## 1 | INTRODUCTION

Crop residues are important roughage sources for ruminant animals, in particular where grassland is limited. Maize stover, the residue after harvesting the maize grains, is abundant in many countries, including China. Efficient utilization of maize stover by ruminants may decrease feed costs of the farmers due to its low price and alleviate the environmental burden caused by burning these residues on the field. In China, no more than 30% of the total maize stover was reported to be fed to ruminant animals mainly due to its low rumen degradability (Lv, Qin, Bai, & Xu, 2013). Maize stover consists of leaves and stems, and the dry matter

(DM) degradability of the stems, measured by incubation in nylon bags for 48 hr in the rumen of cattle or sheep, has been shown to be lower than that of the leaves (Harika & Sharma, 1994; Verbic, Stekar, & Resnik-Cepon, 1995). This may enable more efficient use of maize stover by separating leaves and stems, which can be fed to high-producing and low-producing or dry cows, respectively, if efficient and cost-effective separation methods are available. However, in most previous studies, the leaves and stems were separated into different fractions including leaf sheath and leaf blade, and stem rind and stem pith (Li, Xu, Liu, Fang, & Wang, 2014; Tang et al., 2006, 2008, 2009; Tolera, Berg, & Sundstol, 1999; Tolera & Sundstol, 1999), which may not be feasible and applicable on farm. Quantitative data on degradation rates of stems and leaves separately are largely lacking, but such quantitative knowledge might help to improve resource use efficiency of maize stover for cattle. Considering the impact of variation in chemical composition (protein, cellulose, hemicellulose and lignin contents) and rumen degradation on the nutritive value of ruminant feeds (Getachew, Robinson, DePeters, & Taylor, 2004), research needs to be conducted on the chemical composition and rumen degradation of the leaves and the stems of maize stover.

Cell wall degradation is critical to evaluate forage quality. Due to the difficulties in quantifying the exact cell wall content, the neutral detergent fibre (NDF) content is considered to be a rapid way to estimate the cell wall content (Theander & Westerlund, 1993). The NDF degradation, therefore, represents the cell wall degradation of the leaves and the stems. It is suggested that NDF degradability of forages is positively correlated with voluntary DM intake and fat-corrected milk yield of dairy cows (Oba & Allen, 1999a). Oba and Allen (1999b) proposed that the NDF fraction with greater degradability will leave the rumen at a greater rate, thus facilitating a greater DM intake, which stimulates milk yield (Kendall, Leonard, Hoffman, & Combs, 2009). However, Warner, Dijkstra, Hendriks, and Pellikaan (2013) did not observe a relationship between in situ fractional degradation rates of DM or acid detergent fibre (ADF) and their fractional rumen passage rates using intrinsically $^{13}$C-labelled maize silage. To our knowledge, there are no reports on the cell wall degradability of the leaves and the stems of maize stover. Although both NDF and ADF content in the leaves and the stems of maize stover were reported to be significantly related to the effective DM degradability of the leaves and stems (Verbic et al., 1995), it is unknown whether NDF and ADF content are related to the cell wall degradability of the leaves and the stems of maize stover. More information on these relationships may help to improve prediction of the cell wall degradability based on the fibre components of the leaves and the stems of maize stover, resulting in a better utilization of maize stover as a ruminant forage.

The objectives of the present study were to determine the cell wall degradability, which was evaluated by the in vitro gas production technique of the leaves and the stems of maize stover and to describe the relationships between chemical composition including hemicellulose, cellulose, ADL and CP, and the in vitro gas production parameters of the leaves and the stems of maize stover of several maize cultivars.

## 2 | MATERIALS AND METHODS

### 2.1 | Maize plants

Whole maize plants of 13 cultivars were collected on 20 September 2016, from a trial field in Wouw, the Netherlands, of Limagrain (Rilland, the Netherlands). Seeds were sown on 2 May 2016, with a plant sowing density of 95,000/ha. The fields, which had a sandy soil with pH 5.7, 40 g/kg organic matter (OM) and adequate levels of macro- and micronutrients, were fertilized with 35 m³/ha of cattle manure, 31 kg/ha of nitrogen, 10 kg/ha of phosphorus and 0.6 kg/ha of boron. The length and width of the fields to grow the 13 cultivars were approximately 50 m and 6 m respectively. The plots were next to each other, so most likely the cultivation area had no influence on the in vitro gas production parameters. The effect of the cultivation area, therefore, was not taken into consideration in the statistical evaluation. From each cultivar, 30 plants were harvested from the middle of each plot, due to inconsistent height and size of the plants at the edge of each plot. Four of the 30 plants were randomly selected and allocated to one of two duplicated samples and each sample contained all the leaves or stems from 2 plants. The ears, including the husks, grains and cobs, were removed. After separating the leaves from the stems, both the leaves and the stems were chopped into 1 cm and each duplicate weighed before being stored separately at −20°C pending freeze-drying. After drying, all the leaves and stems per cultivar duplicate were ground separately to pass a 1-mm sieve using a Peppink 100 AN cross beater mill (Peppink) and stored at room temperature until chemical analysis and in vitro gas production.

### 2.2 | Chemical analysis

Dry matter content was determined after 4 hr at 103°C in an oven (ISO 6496). The weight of total DM (TDM) of leaves and stem per plant was calculated as the product of the weight of leaves and stem per duplicate and the DM content of the leaves and stem, respectively, and then divided by two. Ash content was determined after combustion for 3 hr at 550°C in a muffle furnace (ISO 5984). The weight of total OM (TOM) of leaves and stem per plant was calculated as the product of the TDM of leaves and stem per plant and the OM content of leaves and stem on a DM basis respectively. The NDF content was determined with a heat-resistant amylase according to Van Soest, Robertson, and Lewis (1991) and expressed on ash-free basis (aNDFom). The ADF and acid detergent lignin (ADL) contents were determined according to Van Soest and McQueen (1973). The ADF content was expressed on ash-free basis (ADFom). The hemicellulose content was calculated as the difference between aNDFom and ADFom. The cellulose content was calculated as the difference between ADFom and ADL. The difference between aNDFom and ADL (i.e., hemicellulose and cellulose) was defined as potentially rumen degradable fibre (pRDF) since cellulose and hemicellulose potentially can be fully degraded by the rumen micro-organisms (Dehority, 1965; Weimer, 1992). Nitrogen (N) was determined by the Kjeldahl method, and crude protein (CP) was calculated as

**TABLE 1** Chemical composition and in vitro gas production parameters of the leaves and stems of 13 different maize cultivars[a]

| Morphological fraction | Cultivar | Ash, g/kg DM | CP, g/kg OM | aNDFom, g/kg OM | ADFom, g/kg OM | ADL, g/kg OM | Rest fraction, g/kg OM | ADL:pRDF | A1, ml/g OM | A2, ml/g OM | GP72, ml/g OM | B2, h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leaves | Ambrosini | 77* | 69* | 760* | 414* | 27* | 171 | 3.7* | 15 | 148* | 256 | 11.0* |
| | Asgaard | 79* | 59* | 803* | 436* | 24* | 139* | 3.1* | 18 | 171 | 285 | 11.3* |
| | Claudini | 87* | 96* | 709* | 364* | 14* | 195* | 2.0* | 31 | 158* | 271* | 9.8* |
| | Grosso | 89* | 91* | 725 | 388* | 21* | 184 | 3.0* | 28 | 142* | 250 | 9.8 |
| | Lg30217 | 92* | 85* | 704 | 385 | 21* | 211 | 3.0* | 29 | 138 | 251 | 10.2* |
| | Lg30218bm | 77 | 62* | 788* | 431* | 12* | 150* | 1.6* | 22 | 197* | 303* | 10.3* |
| | Lg30248 | 77* | 75* | 702* | 381 | 21 | 223* | 3.1* | 39* | 169* | 297 | 9.7* |
| | Lg31211 | 77* | 72* | 772* | 422* | 22* | 157 | 3.0* | 21* | 157* | 266 | 10.9* |
| | Lg31269 | 76* | 67* | 748* | 396 | 16* | 185* | 2.2* | 29* | 164 | 284 | 10.1* |
| | Lg3216 | 85* | 70* | 752* | 409* | 18* | 178 | 2.4* | 22 | 156* | 271* | 10.9* |
| | Palmer | 82* | 60* | 759 | 420* | 19* | 182 | 2.5* | 28 | 147* | 259 | 9.8 |
| | Pauleen | 86* | 88* | 695* | 347 | 14* | 217* | 2.0* | 37* | 163* | 280 | 9.6* |
| | Perley | 78* | 75* | 724* | 378 | 17* | 201* | 2.5* | 32* | 163 | 284 | 10.0* |
| Stems | Ambrosini | 58 | 17 | 830 | 519 | 44 | 153 | 5.6 | 27 | 112 | 224 | 8.8 |
| | Asgaard | 58 | 22 | 880 | 536 | 36 | 98 | 4.2 | 19 | 146 | 269 | 10.0 |
| | Claudini | 47 | 19 | 738 | 483 | 44 | 243 | 6.4 | 42 | 99 | 240 | 9.0 |
| | Grosso | 60 | 21 | 773 | 500 | 51 | 206 | 7.1 | 28 | 97 | 214 | 9.2 |
| | Lg30217 | 56 | 25 | 742 | 440 | 40 | 234 | 5.7 | 53 | 129 | 271 | 8.0 |
| | Lg30218bm | 73 | 30 | 843 | 512 | 29 | 127 | 3.6 | 25 | 155 | 259 | 9.0 |
| | Lg30248 | 40 | 18 | 564 | 347 | 30 | 419 | 5.5 | 76 | 140 | 306 | 7.6 |
| | Lg31211 | 61 | 23 | 803 | 498 | 39 | 175 | 5.1 | 32 | 133 | 259 | 9.2 |
| | Lg31269 | 45 | 15 | 661 | 412 | 31 | 325 | 4.9 | 59 | 134 | 288 | 8.4 |
| | Lg3216 | 53 | 16 | 813 | 526 | 48 | 171 | 6.3 | 30 | 104 | 230 | 8.9 |
| | Palmer | 52 | 17 | 819 | 521 | 57 | 164 | 7.5 | 28 | 104 | 222 | 8.9 |
| | Pauleen | 32 | 14 | 615 | 385 | 35 | 371 | 6.0 | 69 | 116 | 272 | 8.1 |
| | Perley | 30 | 18 | 636 | 372 | 35 | 347 | 5.7 | 73 | 138 | 297 | 7.4 |
| SEM | | 3.8 | 2.7 | 12.3 | 10.9 | 2.1 | 12.8 | 0.34 | 4.4 | 4.4 | 5.8 | 0.26 |
| Significance P | Cultivar (C) | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| | Fractions (F) | <.001 | <.001 | .244 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| | C × F | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | .017 |

*Note:* Values of leaves with * are significantly different from the values of the stem within cultivar ($p \leq .05$).

Abbreviations: A1, cumulative gas production within 3 hr; A2, cumulative gas production between 3 and 20 hr; ADFom, acid detergent fibre; ADL, acid detergent lignin; ADL:pRDF, the ratio of ADL to potentially rumen degradable fibre (pRDF; calculated as the difference between aNDFom and ADL) × 100; aNDFom, neutral detergent fibre; B2, time needed to reach half of A2; CP, crude protein; DM, dry matter; GP72, cumulative gas production within 72 hr; OM, organic matter; rest fraction: the non-fibre, non-protein components in OM (calculated as 1000–aNDFom–CP); *SEM*, standard error of mean.

[a]The repetition was two for the leaves and stems of each cultivar when calculating the mean of chemical composition and in vitro gas production parameters.

N × 6.25. The rest fraction consisted of the non-fibre and non-protein components in OM and calculated as 1000-aNDFom-CP.

## 2.3 | In vitro gas production

All experimental procedures with fistulated cows were conducted under the Dutch law (Experiments on Animals Act), in accordance with the European Directive 2010/63/EU. The in vitro gas production technique was performed according to the procedure described by Cone, Van Gelder, Visscher, and Oudshoorn (1996). Rumen fluid was collected 2 hr after the morning feeding from three non-lactating rumen fistulated cows (bodyweight 750 kg) fed 1 kg concentrate and grass silage ad libitum daily. The fluid was pooled, stored in a warm insulated flask, pre-filled with $CO_2$, filtered through cheesecloth with a maximum pore size of 2 mm and mixed with an anaerobic buffer/mineral solution as described by Cone et al. (1996) under continuous flushing with $CO_2$. A carefully weighed amount of OM (~0.5 g, calculated based on the DM content and the ash content) of the freeze-dried samples was incubated with 60 ml buffered rumen fluid (one part of rumen fluid and two parts of buffer) in 250-ml bottles at 39°C in a shaking water bath. Each sample was run in one bottle each time, and each sample was run twice totally during separate weeks. Gas production was recorded for 72 hr with an automated system and values expressed on an OM basis.

Cumulative gas production data were fitted to a three phasic mathematical model as described by Groot, Cone, Williams, Debersaques, and Lantinga (1996) using the NLIN procedure in SAS 9.3. The gas production curves were divided into three different sub-curves, each with an asymptote (A), a half-time value (B) and a shape parameter (C). Sub-curve 1 corresponds to the gas production between 0 and 3 hr incubation, caused by fermentation of the water-soluble components. Sub-curve 2 corresponds to the gas production between 3 and 20 hr caused by fermentation of the non-soluble components. Sub-curve 3 corresponds to the gas production between 20 and 72 hr, caused by the microbial turnover (Cone, Van Gelder, & Driehuis, 1997). The half-time value B2 is the incubation time (hr) needed to reach half of A2, representing a measure for the rate of cell wall degradation (Cone et al., 1997). To enable robust curve fitting, A1 was set as the cumulative gas production at 3 hr, and A2 was set as the cumulative gas production at 20 hr, minus that at 3 hr (Van Gelder et al., 2005).

## 2.4 | Statistical analysis

The data were analysed using the PROC GLM procedure of SAS/STAT® 9.3 (Statistical Analysis System). The model included maize cultivar ($n$ = 13), morphological fractions (leaves and stems) and their interactions as fixed effects, and a week effect. The latter was not significant and removed from the model. The in vitro gas production parameters of one sample were taken as the mean of the parameters obtained from the two runs of each sample. The repetition of the in vitro gas production parameters and chemical composition was two, as there were two samples for each cultivar (described in Section 2.3 In vitro gas production). Differences among main effects were analysed using the Tukey–Kramer's multiple comparison procedure. Regression equations were derived to predict A2, B2 and cumulative gas production at 72 hr (GP72) from each chemical component. Furthermore, variables were selected using the stepwise selection method (PROC REG procedure of SAS 9.3, 2011) with $p \leq .05$ as the significance level for the variables to enter or stay in the model. Variables considered for addition or subtraction in the stepwise approach included cellulose, hemicellulose, ADL, CP and rest fraction content.

## 3 | RESULTS

The content of ash, CP, aNDFom, ADFom, ADL and the rest fraction, as well as the ratio of ADL to pRDF (ADL:pRDF) of the leaves and the stems of the 13 maize cultivars, are shown in Table 1. The ash content in the leaves was greater than that in the stems for all the cultivars, except Lg30218bm. The leaves of all the cultivars contained more CP and had a lower ADL:pRDF than the stems. The aNDFom content in the leaves of Lg30248, Lg31269, Pauleen and Perley was greater than in the stems, whereas for Asgaard, Claudini, Lg30218bm, Lg31211 and Lg3216, the aNDFom content was lower in the leaves than in the stems. There were five cultivars, viz. Lg30217, Lg30248, Lg31269, Pauleen and Perley, containing similar amounts of ADFom in the leaves and in the stems, with the other cultivars having more ADFom content in the stems. The ADL content was significantly lower in the leaves than in the stems for all the cultivars, except Lg30248.

The in vitro gas production parameters of the leaves and the stems are also shown in Table 1. There were five cultivars (Lg30248, Lg31211, Lg31269, Pauleen and Perley) with a greater A1 of the stems than of the leaves. The leaves showed a greater A2 than the stems, except for cultivars Asgaard, Lg30217, Lg31269 and Perley, where A2 of leaves did not differ from that of stems. Leaves had a similar GP72 as stems, except for Claudini, Lg30218bm and Lg3216, where GP72 was greater for leaves than for stems. The B2 of the leaves was greater than that of the stems for 11 cultivars, with no significant difference between the leaves and the stems of Grosso and Palmer.

The total DM and OM weight and the total volumes of A2 and GP72 of the leaves and the stems per plant of the 13 cultivars are shown in Table 2. Pauleen produced less leaves, whilst Grosso and Lg30218bm produced more leaves than stem on a DM or OM basis. The total A2 (the product of A2 [ml/g OM] and the OM production [g] per plant), indicating the total amount of cell walls of the leaves and the stem per plant that can be degraded in the rumen, was significantly greater for the leaves than for the stems of 5 cultivars. The total GP72 (the product of GP72 [ml/g OM] and the OM production [g] per plant), which represented the total OM that can be degraded in the rumen, of the leaves of 3 cultivars were greater than that of the stems.

Regression equations, which describe the relationships between the chemical composition and the in vitro gas production parameters

**TABLE 2** Total dry matter (TDM) and organic matter (TOM) weight (g/plant) of a stover plant and the total volumes of A2 (A2 × TOM, L/plant) as well as total volumes of GP72 (GP72 × TOM, L/plant) of a stover plant for 13 different maize cultivars[a]

| Morphological fraction | Cultivar | TDM | TOM | Total volume of A2[b] | Total volume of GP72[c] |
|---|---|---|---|---|---|
| Leaves | Ambrosini | 33.3 | 30.7 | 4.6 | 7.9 |
| | Asgaard | 36.4 | 33.5 | 5.7 | 9.5 |
| | Claudini | 48.7 | 44.5 | 7.0 | 12.1 |
| | Grosso | 45.8* | 41.7* | 6.0* | 10.5* |
| | Lg30217 | 45.2 | 41.1 | 5.7 | 10.4 |
| | Lg30218bm | 39.2* | 36.2* | 7.2* | 11.0* |
| | Lg30248 | 53.1 | 49.1 | 8.3 | 14.6 |
| | Lg31211 | 32.5 | 30.0 | 4.7* | 8.0* |
| | Lg31269 | 44.0 | 40.6 | 6.7 | 11.6 |
| | Lg3216 | 38.1 | 34.8 | 5.5* | 9.5 |
| | Palmer | 46.7 | 42.9 | 6.3* | 11.2 |
| | Pauleen | 53.2* | 48.6* | 8.0 | 13.6 |
| | Perley | 43.5 | 40.2 | 6.6 | 11.4 |
| Stems | Ambrosini | 28.8 | 27.2 | 3.1 | 6.1 |
| | Asgaard | 29.1 | 27.4 | 4.0 | 7.4 |
| | Claudini | 62.5 | 59.7 | 5.9 | 14.3 |
| | Grosso | 39.9 | 37.5 | 3.7 | 8.0 |
| | Lg30217 | 49.3 | 46.5 | 6.0 | 12.7 |
| | Lg30218bm | 24.4 | 22.6 | 3.5 | 5.9 |
| | Lg30248 | 54.3 | 52.2 | 7.3 | 16.0 |
| | Lg31211 | 24.7 | 23.2 | 3.1 | 6.1 |
| | Lg31269 | 41.8 | 39.9 | 5.4 | 11.5 |
| | Lg3216 | 40.4 | 38.3 | 4.0 | 8.8 |
| | Palmer | 49.7 | 47.2 | 5.0 | 10.5 |
| | Pauleen | 69.4 | 67.2 | 7.8 | 18.3 |
| | Perley | 43.0 | 41.7 | 5.8 | 12.4 |
| *SEM* | | 2.15 | 2.16 | 0.30 | 0.60 |
| Significance *P* | Cultivar (C) | <.001 | <.001 | <.001 | <.001 |
| | Fractions (F) | .835 | .140 | <.001 | .299 |
| | C × F | <.001 | <.001 | <.001 | <.001 |

*Note:* Values of leaves with * are significantly different from values of the stems within cultivar (*p* ≤ .05).

Abbreviations: A2, cumulative gas production between 3 and 20 hr (for values see Table 1); GP72, cumulative gas production within 72 hr (for values see Table 1); *SEM* standard error of mean.

[a]The repetition was two for the leaves and stems of each cultivar when calculating the mean of TDM, TOM and total volumes of A2 and GP72.

[b]Indication of the total cell wall degradability.

[c]Indication of the total organic matter degradability.

of the leaves and the stems, are shown in Tables 3 and 4 respectively. The A2 of the leaves was significantly positively related to the hemicellulose content and negatively related to the ADL and CP contents, whereas the GP72 of the leaves was negatively affected by the ADL content. The B2 of the leaves was positively related to cellulose and ADL contents and negatively related to CP and rest fraction contents of the leaves. By using stepwise selection, both the ADL and CP contents were selected to predict A2 and GP72 of the leaves, whereas the rest fraction and ADL contents were selected to predict B2 of the leaves. Significant relationships were observed

between the ADL (negative relationship) and the CP (positive relationship) contents and A2 of the stems. The GP72 of the stems was negatively related to the cellulose and ADL contents and positively related to the rest fraction content. There were significant positive relationships between the hemicellulose and the cellulose contents and the B2 of the stems and a negative relationship between the rest fraction content and the B2 of the stems. Stepwise regression indicated that A2 of the stems was best predicted by inclusion of the hemicellulose (positive relationship) and the cellulose and ADL (negative relationship) contents. The best prediction of GP72 included

**TABLE 3** Relationships between in vitro gas production between 3 and 20 hr incubation (parameter A2, ml/g OM) and within 72 hr incubation (GP72, ml/g OM) and chemical composition (g/kg OM) of the leaves

| Regression equation | Adjusted $R^2$ | P | RMSE |
|---|---|---|---|
| A2 = −21 (±66.5) + 0.53 (±0.193) × hemicellulose | 0.20 | .012 | 13.54 |
| A2 = 83 (±43.6) + 0.20 (±0.115) × cellulose | 0.08 | .090 | 14.58 |
| A2 = 188 (±12.2) − 1.50 (±0.631) × ADL | 0.16 | .026 | 13.95 |
| A2 = 196 (±17.3) − 0.49 (±0.229) × CP | 0.13 | .041 | 14.19 |
| A2 = 194 (±20.8) − 0.19 (±0.112) × rest fraction | 0.07 | .103 | 14.65 |
| A2[a] = 243 (±19.7) − 1.89 (±0.545) × ADL − 0.64 (±0.194) × CP | 0.40 | .001 | 11.75 |
| GP72 = 135 (±81.4) + 0.40 (±0.237) × hemicellulose | 0.07 | .102 | 16.58 |
| GP72 = 247 (±52.2) + 0.07 (±0.137) × cellulose | −0.03 | .609 | 17.46 |
| GP72 = 305 (±13.9) − 1.66 (±0.719) × ADL | 0.15 | .030 | 15.89 |
| GP72 = 309 (±20.1) − 0.48 (±0.266) × CP | 0.08 | .085 | 16.48 |
| GP72 = 279 (±25.0) − 0.03 (±0.134) × rest fraction | −0.04 | .834 | 17.54 |
| GP72[a] = 360 (±23.6) − 2.05 (±0.654) × ADL − 0.64 (±0.233) × CP | 0.33 | .004 | 14.10 |
| B2 = 3.4 (±2.569) + 0.02 (±0.007) × hemicellulose | 0.20 | .202 | 0.52 |
| B2 = 4.7 (±1.37) + 0.01 (±0.004) × cellulose | 0.38 | <.001 | 0.46 |
| B2 = 8.9 (±0.44) + 0.07 (±0.023) × ADL | 0.26 | .004 | 0.50 |
| B2 = 12.1 (±0.62) − 0.03 (±0.008) × CP | 0.25 | .005 | 0.51 |
| B2 = 13.2 (±0.59) − 0.02 (±0.003) × rest fraction | 0.50 | <.001 | 0.41 |
| B2[a] = 11.9 (±0.67) − 0.01 (±0.003) × rest fraction + 0.05 (±0.016) × ADL | 0.63 | <.001 | 0.35 |

Abbreviations: A2, gas production between 3 and 20 hr; ADL, acid detergent lignin; B2, incubation time needed to reach half of A2; CP, crude protein; GP72, gas production within 72 hr; OM, organic matter; RMSE, root mean square error.
[a]Variables (chemical composition) were selected into the model by stepwise procedure with 0.05 as the significance level to enter or stay in the model.

the cellulose and ADL contents (both negative relationship). Only cellulose content of the stems was selected to best predict the B2 of the stems.

## 4 | DISCUSSION

In previous studies, either the DM degradability of leaves, stems, cobs, and other parts of the maize plant, or the aNDFom degradability of the leaf fractions (sheath and blade) and stem fractions (rind, pitch and node) were investigated (Harika & Sharma, 1994; Li et al., 2014; Tang et al., 2006, 2008, 2009; Tolera et al., 1999; Tolera & Sundstol, 1999; Verbic et al., 1995). However, the aNDFom degradability of the leaves (not different leaf fractions) and stems (not different stem fractions) were not investigated as such. In the present study, the aNDFom degradability of the leaves and stems was evaluated by the in vitro gas production technique, and the relationship between the in vitro gas production parameters and the chemical composition of the leaves and stems was investigated. The gas production between 3 and 20 hr is regarded as caused by fermentation of the non-soluble fraction (Cone et al., 1997), which in case of maize leaves and stems is the cell wall fraction.

On average, the total DM weight of the leaves and the stem per plant was 43.1 and 42.9 g, respectively, and the total OM weight of the leaves and the stem per plant was 39.5 and 40.8 g respectively.

The results from our study provide a reference for farmers to select the most suitable leaves and stems for their cattle based on the biomass yield and the cell wall degradation. For high-producing dairy cows, the forage with the highest cell wall degradation may be the best choice because a greater cell wall degradation is associated with a greater DM intake and milk yield (Oba & Allen, 1999b). When milk production is low and requirements for nutrients to sustain these production levels are low, the total biomass production of the leaves and the stems may become the first consideration in view of the lower feed intake level of low-producing cows. In the current trial, the plant density of all the maize cultivars is the same. In such a situation, the cultivars with greater biomass production will alleviate the need of the forages by low-producing animals. Economic models should be developed to consider both the cell wall degradation and biomass production of maize silage when fed to cows with different production levels. It should be noted that although the effect of cultivar on gas production and other characteristics was evaluated, we did not have the possibility to obtain maize cultivar plants from more than one parcel, and a limited number of plants per cultivar were obtained. Therefore, the differences reported between cultivars should be interpreted with great care, and a fully valid evaluation of cultivar effect is only possible upon obtaining multiple plant samples from multiple parcels.

In our study, the cell wall degradation of the leaves and the stems, which was evaluated by the in vitro gas production

**TABLE 4** Relationships between in vitro gas production between 3 and 20 hr incubation (parameter A2, ml/g OM) and within 72 hr incubation (GP72, ml/g OM) and chemical composition (g/kg OM) of the stems

| Regression equation | Adjusted $R^2$ | P | RMSE |
|---|---|---|---|
| A2 = 85 (±28.6) + 0.14 (±0.101) × hemicellulose | 0.07 | .188 | 19.13 |
| A2 = 146 (±27.2) – 0.05 (±0.063) × cellulose | −0.01 | .413 | 19.57 |
| A2 = 194 (±10.4) – 1.77 (±0.256) × ADL | 0.65 | <.001 | 11.47 |
| A2 = 89 (±15.4) + 1.79 (±0.768) × CP | 0.15 | .029 | 17.93 |
| A2 = 121 (±10.0) + 0.01 (±0.039) × rest fraction | −0.04 | .789 | 19.82 |
| A2[a] = 142 (±13.5) + 0.34(±0.077) × hemicellulose – 0.12 (±0.052 × cellulose – 1.61 (±0.219) × ADL | 0.82 | <.001 | 8.18 |
| GP72 = 332 (±43.3) – 0.26 (±0.152) × hemicellulose | 0.07 | .095 | 28.95 |
| GP72 = 403 (±30.4) – 0.34 (±0.071) × cellulose | 0.47 | <.001 | 21.91 |
| GP72 = 369 (±15.6) – 2.79 (±0.381) × ADL | 0.68 | <.001 | 17.09 |
| GP72 = 260 (±26.3) – 0.12 (±1.316) × CP | −0.04 | .927 | 30.71 |
| GP72 = 214 (±12.0) + 0.19 (±0.047) × rest fraction | 0.37 | <.001 | 23.80 |
| GP72[a] = 432 (±18.2) – 0.20 (±0.046) × cellulose – 2.18 (±0.315) × ADL | 0.82 | <.001 | 12.76 |
| B2 = 5.2 (±0.96) + 0.01 (±0.003) × hemicellulose | 0.33 | .001 | 0.64 |
| B2[a,b] = 4.0 (±0.55) + 0.03 (±0.001) × cellulose | 0.74 | <.001 | 0.39 |
| B2 = 7.5 (±0.69) + 0.03 (±0.017) × ADL | 0.07 | .106 | 0.75 |
| B2 = 7.6 (±0.65) + 0.05 (±0.032) × CP | 0.07 | .109 | 0.75 |
| B2 = 10.1 (±0.24) – 0.01 (±0.001) × rest fraction | 0.63 | <.001 | 0.47 |

Abbreviations: A2, gas production between 3 and 20 hr; ADL, acid detergent lignin; B2, incubation time needed to reach half of A2; CP, crude protein; GP72, gas production within 72 hr; OM, organic matter; RMSE, root mean square error.
[a]Variables (chemical composition) were selected into the model by stepwise procedure with 0.05 as the significance level to enter or stay in the model.
[b]Cellulose was the only variable that was selected into the model by stepwise procedure with 0.05 as the significance level to enter or stay in the model.

technique, varied among the cultivars, which necessitates the development of regression equations to predict the cell wall degradation based on the chemical composition. The regression equations, therefore, were developed to predict the cell wall degradability, OM degradability and the rate of cell wall degradation of the leaves and stems. The ADL content of both, the leaves and the stems, was found to be negatively related to A2 and GP72. These relationships were stronger for stems (adjusted $R^2$ 0.65-0.68) than for leaves (adjusted $R^2$ 0.15-0.16). The weaker relationship for leaves than for stems may be ascribed to the direct (covalent) or indirect (ester or ether) linkages between lignin and cellulose and hemicellulose (Ding et al., 2012; Jalc, 2002; Jeffries, 1994; Susmel & Stefanon, 1993; Vanholme, Demedts, Morreel,

Ralph, & Boerjan, 2010). This observation was consistent with previous studies (Arora & Sharma, 2009; Boon, Engels, Struik, & Cone, 2005; He et al., 2018; Tuyen, Cone, Baars, Sonnenberg, & Hendriks, 2012). The CP content of the leaves was negatively related to both A2 and GP72 (tendency only) which is in line with Cone and Van Gelder (1999) who showed that the gas production was negatively related to the protein content in the substrate. During protein fermentation, ammonia is produced which binds with $H^+$ in the buffer solutions, and as a result, the equilibrium in the buffer will shift towards $HCO_3^-$ releasing less $CO_2$ (Cone & Van Gelder, 1999). Furthermore, CP content in the leaves was negatively correlated with aNDFom content ($r = −0.78$; Table 5). Since A2 is the result of the aNDFom fermentation in rumen fluid,

| Items | aNDFom | Cellulose | Hemicellulose | CP | ADL |
|---|---|---|---|---|---|
| Cellulose | 0.93[**] | | | | |
| Hemicellulose | 0.70[**] | 0.42[*] | | | |
| CP | −0.78[**] | −0.81[**] | −0.39[*] | | |
| ADL | 0.26 | 0.24 | −0.10 | −0.22 | |
| Rest fraction | −0.96[**] | −0.85[**] | −0.74[*] | 0.56[**] | −0.24 |

**TABLE 5** Pearson's correlation coefficient between chemical composition of the leaves

Abbreviations: ADL, acid detergent lignin; aNDFom, neutral detergent fibre; rest fraction: the non-fibre, non-protein components in OM (calculated as 1000-aNDFom-CP); CP, crude protein.
[*]$p < .05$.
[**]$p < .01$.

**TABLE 6** Pearson's correlation coefficient between chemical composition of the stems

| Items | aNDFom | Cellulose | Hemicellulose | CP | ADL |
|---|---|---|---|---|---|
| Cellulose | 0.98[**] | | | | |
| Hemicellulose | 0.90[**] | 0.80[**] | | | |
| CP | 0.48[*] | 0.42[*] | 0.62[**] | | |
| ADL | 0.41[*] | 0.43[*] | 0.13 | −0.24 | |
| Rest fraction | −1.00[**] | −0.97[**] | −0.91[**] | −0.52[**] | −0.39[*] |

Abbreviations: ADL, acid detergent lignin; aNDFom, neutral detergent fibre; rest fraction: the non-fibre, non-protein components in OM (calculated as 1000-aNDFom-CP); CP, crude protein.
[*]$p < .05$.
[**]$p < .01$.

the lower aNDFom content may lead to a lower A2, which helps to explain the negative relationship between CP content and A2. To a lesser extent, the CP content influenced GP72 of the leaves. Nevertheless, A2 of the stems was positively related to CP content and GP72 of the stems was not influenced by the CP content, which may be partly attributed to the low CP content and low variation in CP content in the stems. The positive correlation ($r = 0.48$) between CP content and aNDFom content in the stems (Table 6), indicating more aNDFom can be fermented in rumen fluid when the CP content of stems was greater, may explain the positive relationship between CP content and A2 of the stems. The hemicellulose content was found to be positively related to the A2 of the stems in the best regression equation. The positive relationship between A2 and CP content of the stem, therefore, is also probably explained by the positive correlation ($r = 0.62$) between CP and hemicellulose contents. The negative relationship between cellulose content and A2 of the stems may result from the fact that the cellulose content is correlated with the ADL content ($r = 0.43$), or that more cellulose is linked with lignin in the stems. In addition, the major difference between cellulose and hemicellulose is that hemicellulose has branches with short chains and cellulose can appear in crystalline form, which render hemicellulose more degradable than cellulose (Perez, Munoz-Dorado, De la Rubia, & Martinez, 2002). Susmel, Stefanon, Mills, and Spanghero (1990) reported a greater degradability and degradation rate of hemicellulose than cellulose of forages, suggesting that cellulose and hemicellulose may have adverse effects on the cell wall degradability and OM degradability. In the best regression equations, the B2 of the leaves, representing rate of cell wall degradation, was positively related to ADL and rest fraction contents; for stems, the best regression equation to predict B2 included cellulose content only. Even though the rest fraction content was not selected by the stepwise procedure to predict the B2 of the stems, the rest fraction content still explained a large part of the variation of the B2 of the stems (adjusted $R^2 = 0.63$). The positive relationships between the fibre fractions and the B2 of the leaves and stems and the negative relationship between the rest fraction content and the B2 of the leaves and stems indicate that the rest fraction is more easily degraded than the fibre fractions in the leaves and stems. Based on the results of the 13 maize cultivars evaluated, the A2 and GP72 of the stems can be more accurately predicted by the chemical composition (adjusted $R^2$ being 0.82) than that of the leaves (adjusted $R^2$ between 0.33 and 0.40); for B2, the difference between stems and leaves in predictive capability was smaller (stems, adjusted $R^2 = 0.74$; leaves, adjusted $R^2 = 0.63$).

## 5 | CONCLUSION

Chemical composition, in particular CP content and ADL:pRDF, differed between maize leaves and stems. For most cultivars investigated, the A2 of the leaves, representing cell wall degradability, and the B2 of leaves, representing rate of cell wall degradation, were greater than that of the stems. Both A2 and GP72 of the stems were highly correlated with its chemical composition, indicating that the cell wall and OM degradation of maize stems can be better predicted by its chemical composition. For the leaves, A2 and GP72 only showed moderate relationships with the chemical composition.

### ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All experimental procedures with fistulated cows were conducted under Dutch law (Experiments on Animals Act) and approved by the relevant ethical review committees, in accordance with the European Directive 2010/63/EU.

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