

# Potential of Thermal Analysis To Estimate Chemical Composition and in Vitro Fermentation Characteristics of Maize

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Organic matter and cell wall degradation (OMD and CWD) are important quality traits of maize which may be estimated by in vitro assays using rumen fluid. However, these assays have some large disadvantages which stimulate research for alternative methods. The objective of this study was to evaluate the potential of the thermal analysis techniques, differential scanning calorimetry (DSC) and thermogravimetry (TG), to estimate OMD, CWD, and related forage quality traits of maize. For all DSC and TG properties distinguishable in DSC and TG thermograms, large variations existed among 25 maize samples. Multiple linear regression models including both DSC and TG variates could explain a major part of the variation of most forage quality traits. The relatively short analysis time, the high reproducibility of measurements, and the high predictive potential as shown by the low standard error of prediction indicate that DSC and TG may be used to predict forage quality characteristics of maize.

**Keywords:** *Degradability; cell wall; maize; differential scanning calorimetry; thermogravimetry; multiple linear regression*

## INTRODUCTION

Organic matter and cell wall degradation (OMD and CWD) are important forage quality traits of maize. In vivo degradability is accurately predicted by in vitro bioassays with rumen fluid (Tilly and Terry, 1963; Van Soest et al., 1966). The need of fistulated animals to provide rumen liquor, the variation in liquor quality, and the time needed for these assays have stimulated research for alternative ways to estimate forage degradation.

Degradation of forages depends on their chemical composition and the interactions among the components (Jung, 1989; Hatfield, 1993). Degradation of cell wall carbohydrates has been reported to be inhibited by lignin, phenolic acid ester, and *p*-coumaric acid from the cell wall (Burritt et al., 1984; Buxton and Russell, 1988; Jung, 1989; Marvin et al., 1995).

Correlation studies have shown that only part of the variation in CWD and OMD can be explained by concentrations of cell wall constituents (Marvin et al., 1995). Since ionic, hydrogen, and covalent interactions among cell wall components also affect degradability (Hatfield, 1993), accurate methods aiming to predict forage degradation characteristics (i.e., OMD, CWD) should account for effects related to variations in contents of cell wall constituents and for effects of physicochemical interactions. To overcome the disadvantages of rumen fluid-based in vitro assays, alternative methods should also be rapid and highly reproducible.

Near-infrared reflectance spectroscopy (NIRS) meets these requirements and has consequently been exploited

to predict forage degradation and related traits (Norris et al., 1976; Barton II, 1985; Bughara et al., 1992; Shenk et al., 1992; Garcia-Ciudad et al., 1993; Dardenne, 1994). Contents of protein, various cell wall constituents, fiber, and OMD are estimated accurately by NIRS. However, NIRS can not yet accurately estimate genotypic differences in CWD of whole-crop maize and maize stover (Deinum and Struik, 1989). Thermal analysis measurements depend as NIRS on the chemical composition and physicochemical properties of a sample and are reproducible and relatively rapid (Nguyen et al., 1981; Bergner and Albano, 1993). Thermal analysis methods may therefore have potential for the prediction of properties related to forage quality.

Two thermal analysis techniques, differential scanning calorimetry (DSC) and thermogravimetry (TG), are frequently used to study thermal degradation of wood, cellulose, and related materials (Beall and Eickner, 1970; Nguyen et al., 1981; Barl et al., 1986; Bergner and Albano, 1993; Krigstin et al., 1993; Cone et al., 1996). These techniques probe energy flows (i.e., DSC) or weight losses (i.e., TG) due to processes occurring within the sample as a consequence of an imposed temperature regimen. Bergner and Albano (1993) compared the potential of NIRS and thermal analysis (i.e., DSC and TG) to predict the contents of many chemical constituents including carbohydrates, ash, and lignin in peat. They concluded that a simultaneous TG/DSC technique outperformed NIRS in predicting ash and lignin (Bergner and Albano, 1993). Thus, lignin which has been shown to play an important role in forage degradation (Jung, 1989; Hatfield, 1993; Marvin et al., 1995) can be predicted by thermal analysis.

Thermal degradation studies of wood in the presence of oxygen have indicated that its thermal behavior may be regarded as a summation of the individual thermal behavior of its carbohydrate and lignin components. Nguyen et al. (1981) expected a predominant influence

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of cellulose on thermal degradation because of a large weight proportion and a high reactivity. However, Curie-point pyrolysis gas chromatography mass spectrometry showed that thermal degradation of wood is more complex (Pouwels, 1989). Structural information such as crystallinity of cellulose and perhaps also the degree of cross-linking within hemicellulose influence the nature of the thermogram.

Since DSC and TG thermograms of cell walls will contain information of its individual chemical components and about structural features and these features are known to affect microbial degradation of cell walls, we expected that thermal analysis may have potential to predict forage quality traits. In this study we investigated thermal degradation of powder samples of maize stalks by means of DSC and TG and related these properties to *in vitro* fermentation characteristics and contents of several chemical constituents of the samples. The aim of this study was to assess the potential of thermal analysis for the prediction of forage quality traits of maize.

## MATERIALS AND METHODS

**Plant Material and Sample Preparation.** For this experiment stalk samples of 25 different recombinant inbred lines of maize (RILs) were available. RILs were produced from a cross between two homozygous inbred lines contrasting for degradability, Co125 (poorly degradable) and W401 (highly degradable), followed by five generations of inbreeding.

On September 13, 1990, stalk fractions were collected shortly before silage stage from 10 plants/RIL. The stalk material was subsequently chopped with a laboratory chopper, dried at 70 °C, and ground with a beater cross mill (Retsch, type SK 1) using a 1 mm sieve. This 1 mm powder was used for all analyses except for DSC. For DSC measurements the samples were further ground by a ball grinding mill (Retsch, type MM 2) under liquid nitrogen and subsequently dried at 40 °C for 1–2 days.

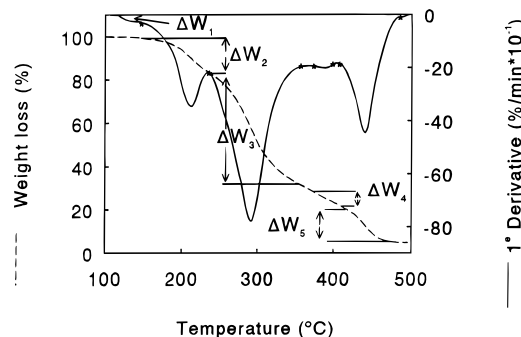
**In Vitro Fermentation.** Ground stalk samples were incubated at 39 °C in a buffered inoculum for 48 h as described before (Marvin et al., 1996). The inoculum contained rumen fluid collected from a cannulated ox maintained on a hay diet.

After the incubation, the undigested plant material was removed by filtration through fritted glass filter crucibles (pore size: 40–100  $\mu\text{m}$ ) and washed several times with warm water. The filtrate was collected and centrifuged (10 min, 4 °C, 10000g). The resulting supernatant was adjusted to pH 9 by adding NaOH and stored at -20 °C. At a convenient time frozen samples were thawed and acidified to pH 3 by adding  $\text{H}_3\text{PO}_4$ . Volatile fatty acids (among others acetate and propionate) were extracted with ethyl acetate and quantified by GLC using a WCOT fused silica gel column (25 m  $\times$  0.32 mm; Chrompack) with FFAB-CB coating. Prior to the extraction the suspension was saturated with NaCl, and hexanoic acid was added as internal standard. All *in vitro* incubations were carried out two times in triplicate.

OMD and CWD were determined for all maize samples as described before (Marvin et al., 1995) using identical *in vitro* incubations as were used in this study for determination of the production of volatile fatty acids upon incubation with rumen fluid.

**Chemical Analysis.** The amounts of neutral and acid detergent fiber (NDF and ADF) and permanganate lignin (pLIG) in the stalk samples were determined by the method of Goering and Van Soest (1970). Cellulose (CEL) concentration was calculated as the difference between ADF and pLIG, and hemicellulose (HEM) concentration was calculated as the difference between NDF and ADF.

Total phenolic content was estimated according to Morrison (1972) using ferulic acid as standard, and the contents of phenolic acid esters *p*-coumaric and ferulic acid were determined by HPLC as described before (Marvin et al., 1995). All measurements were carried out in duplicate.

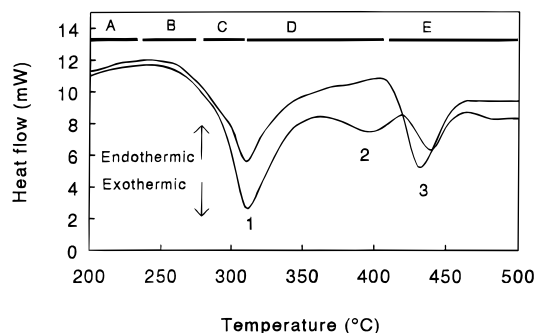


**Figure 1.** TG thermogram showing weight loss curve (---) and its derivative (—). The five weight loss processes ( $\Delta W_1$ – $\Delta W_5$ ) are indicated.

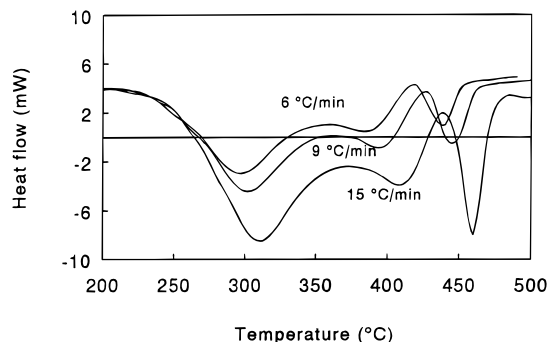
**Thermal Analysis. Differential Scanning Calorimetry (DSC).** DSC measurements were performed on a Perkin-Elmer DSC7 (Perkin-Elmer, Norwalk, CT) using closed aluminum pans with two pinholes. Heat flow was recorded from 100 to 500 °C with various linear heating rates (e.g., 6, 9, 10, 15, and 30 °C $\cdot$ min $^{-1}$ ) and 26, 30, or 35 mL $\cdot$ min $^{-1}$  air flow. About 0.6–0.7 mg of powder was used for a measurement. The area of a peak in the thermogram provides an estimate of the enthalpy ( $\Delta H$ ). Since in our experiments base-line separation of peaks did not occur, the positions where the first derivative equals zero were taken as the boundaries of a peak of which the area was determined. All graphs were normalized on sample weight basis before enthalpy was calculated (Perkin-Elmer software, 7 series/UNIX, version 4.0). Samples contained 4–6% water. The initial weight of the sample at the start of the experiment was used for the normalization procedure. The onset temperature of a peak ( $T_{\text{onset}}$ ) was determined using Perkin-Elmer software (7 series/UNIX, version 4.0). The DSC was calibrated after every 10–15 measurements according to Perkin-Elmer guidelines using zinc ( $\Delta H = 108$  J/g,  $T_{\text{onset}} = 419$  °C) and indium ( $\Delta H = 29$  J/g,  $T_{\text{onset}} = 157$  °C). Every measurement was carried out with new (disposable) pans of identical mass.

**Thermogravimetry (TG).** TG measurement was performed on a Perkin-Elmer TGA7 (Perkin-Elmer, Norwalk, CT) using an air flow of 23 mL $\cdot$ min $^{-1}$ . Weight loss was recorded as percentage loss occurring in the temperature region from 100 to 500 °C with a linear heating rate of 10 °C $\cdot$ min $^{-1}$ . The sample consisted of 1 mm powder, and the sample weight varied between 2 and 4 mg. A weight loss process was defined as the amount of material lost from a sample between two zero points around a peak in the first derivative curve as indicated in Figure 1. Five weight loss processes could be distinguished in the TG thermogram (Figure 1). Repeated analyses of the same maize sample using this method showed that the weight loss in all five weight loss processes less than 2.5% variation and for the corresponding midtemperatures less than 1.5 °C variation. Due to the high repeatability and large variation between the maize samples, it was not necessary to carry out duplicate measurements in order to obtain reliable values. The TG was calibrated with Curie-point standard (Allumel and Perkaloy).

**Statistical Analysis.** All laboratory analyses were carried out on the 25 samples. Correlation and multiple linear regression (MLR) analysis were performed using common statistical procedures (Snedecor and Cochran, 1980). The multiple linear regression model,  $y_i = b_0 + \sum b_i x_i$ , included DSC and TG variates as explanatory variates ( $x_i$ ) and chemical components and fermentation characteristics as response variates ( $y_i$ ). Selection of the best prediction model from all possible prediction models was carried out on the basis of the minimum Mallows  $C_p$  (Mallows, 1973), using data of all 25 samples. The predictive potential of selected MLR models was assessed using the leave-one-out procedure as described by Bergner and Albano (1993). With this method, for each sample, a residual after prediction is calculated of the prediction based on a calibration of the best model using the set of data on all samples except the one for which a prediction (and



**Figure 2.** DSC thermograms of two maize samples extremely differing in thermal behavior. The five distinguishable thermal regions (A–E) are indicated.



**Figure 3.** DSC thermogram of maize analyzed at different heating rates. Only heating rates 6, 9, and 15 °C·min<sup>-1</sup> are shown.

a residual after prediction) should be obtained. The standard error of predictions was calculated from all 25 residuals after predictions.

## RESULTS

**Differential Scanning Calorimetry (DSC).** *Optimization of Measurement.* In this study DSC thermograms of 1 mm powder samples were poorly reproducible. This problem was due to poor homogeneity of the samples and low amounts of material needed for DSC measurements. This problem was solved by milling the samples to a finer powder.

Three distinct exothermic peaks (1–3) were recognized in DSC thermograms of extra milled maize samples in the temperature range of 250–500 °C (Figure 2). These exothermic peaks were preceded by an endothermic region occurring from 100 to 250 °C. The characteristic of the exothermic peaks depended on the heating rate. Peak areas increase and peak temperatures shift toward higher values when heating rate is elevated (Figure 3). However, the onset temperature of a peak was not influenced by the heating rate. This effect of heating rate is accompanied by a decrease of reproducibility, probably due to limited heat transfer within samples. The air flow also affected the nature of the DSC thermogram and reproducibility of the measurement (not shown).

Since, the aim of this study was to assess the potential of DSC and/or TG to predict forage quality traits, not only the reproducibility of the measurement should be taken into account but also the accuracy of data extraction from the thermogram should be maximal. A better peak separation and absence of heat conductivity problems within the sample favor slow heating rates, but smaller peak areas will cause larger errors when extracting the data from the thermograms.

For maize samples the optimal reproducibility of the measurements and the optimal accuracy of data extrac-

**Table 1.** Variation in DSC Properties of Stalk Samples of Maize<sup>a</sup>

	$\Delta H_1$ (J·(g of dry matter) <sup>-1</sup> )	$\Delta H_2$ (J·(g of dry matter) <sup>-1</sup> )	$\Delta H_3$ (J·(g of dry matter) <sup>-1</sup> )	$T_{\text{onset}1}$ (°C)	$T_{\text{onset}2}$ (°C)	$T_{\text{onset}3}$ (°C)
min	1240	0 <sup>b</sup>	355	268	0 <sup>b</sup>	414
max	2248	674	2063	312	385	446
mean	1575	287	948	289	345	429

<sup>a</sup> Abbreviations:  $\Delta H_1$ ,  $\Delta H_2$ , and  $\Delta H_3$ , enthalpy release of peaks 1, 2, and 3, respectively;  $T_{\text{onset}1}$ ,  $T_{\text{onset}2}$ , and  $T_{\text{onset}3}$ , onset temperature of peaks 1, 2 and 3, respectively. <sup>b</sup> Peak 2 was absent.

**Table 2.** Variation in TG Properties of Stalk Samples of Maize<sup>a</sup>

	$\Delta W_1$ (%)	$\Delta W_2$ (%)	$\Delta W_3$ (%)	$\Delta W_4$ (%)	$\Delta W_5$ (%)	$T_{\Delta W_1}$ (°C)	$T_{\Delta W_2}$ (°C)	$T_{\Delta W_3}$ (°C)	$T_{\Delta W_4}$ (°C)	$T_{\Delta W_5}$ (°C)
min	0.4	10.5	41.2	3.7	12.1	120	199	284	365	422
max	3.7	23.6	56.4	14.4	24.1	157	216	306	412	454
mean	1.5	16.8	49.2	9.5	17.9	136	210	294	380	439

<sup>a</sup> Abbreviations:  $\Delta W_1$ ,  $\Delta W_2$ ,  $\Delta W_3$ ,  $\Delta W_4$ , and  $\Delta W_5$ , extent of weight loss of processes 1, 2, 3, 4, and 5, respectively;  $T_{\Delta W_1}$ ,  $T_{\Delta W_2}$ ,  $T_{\Delta W_3}$ ,  $T_{\Delta W_4}$ ,  $T_{\Delta W_5}$ , midtemperature of processes 1, 2, 3, 4, and 5, respectively.

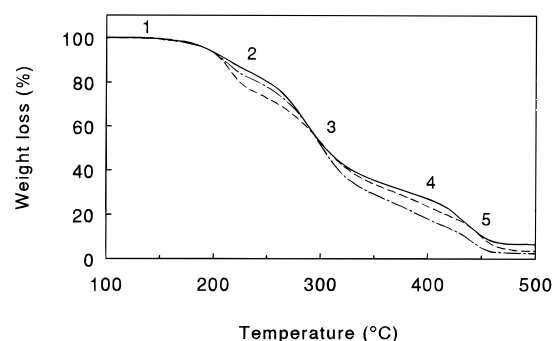
tion were found at a heating rate of 10 °C·min<sup>-1</sup> and an air flow of 30 mL·min<sup>-1</sup>, using samples of 0.6–0.7 mg. At these conditions repeated analysis of the same sample only showed small differences for peak area (enthalpy) and onset temperature. For enthalpy ( $\Delta H$ ) and onset temperature ( $T_{\text{onset}}$ ) this variation was 55 J·(g of dry matter)<sup>-1</sup> and 2.1 °C for peak 1, 7.4 J·(g of dry matter)<sup>-1</sup> and 1.4 °C for peak 2, and 33 J·(g of dry matter)<sup>-1</sup> and 0.4 °C for peak 3. Heating rates up to 10 °C·min<sup>-1</sup> are often used when thermal degradation of plant material is studied (Beall and Eickner, 1970; Barl et al., 1986; Bergner and Albano, 1993; Krigstin et al., 1993; Cone et al., 1996), and this heating rate has been used before to estimate contents of various chemical components in plant material (Bergner and Albano, 1993).

*Variation between Maize Samples.* Large variation was observed among the samples for all three peaks in the DSC thermogram with regard to the extent of  $\Delta H$  and  $T_{\text{onset}}$ . Figure 2 shows the thermogram of two maize samples having extreme differences in thermal behavior. The variation among the samples for  $\Delta H$  and  $T_{\text{onset}}$  of all three peaks is summarized in Table 1. The onset temperature of peak 1 was located at temperatures around 289 °C, peak 2 around 345 °C, and peak 3 around 429 °C. Generally,  $\Delta H$  was largest for peak 1 and lowest for peak 2. For some maize samples peak 2 was even absent (Figure 2).

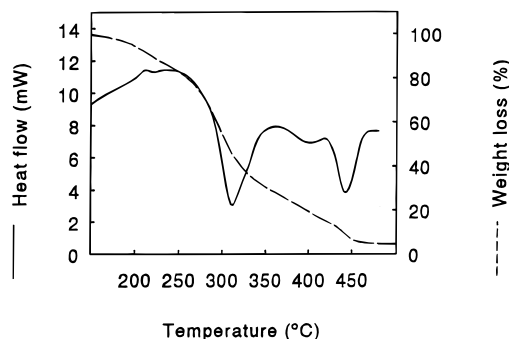
**Thermogravimetry (TG).** *Optimization of Measurement.* TG thermograms of maize samples showed weight loss from the samples with increasing temperature. Between 100 and 500 °C five distinct weight loss processes ( $\Delta W_1$ – $\Delta W_5$ ) were observed (Figure 1).

In contrast to DSC, TG gave no reproducibility problems with 1 mm maize powder when a heating rate of 10 °C·min<sup>-1</sup> was used. Instead, duplicate measurements of 1 mm powder were nearly identical showing variations less than 2.5% for weight loss and less than 1.5 °C for midtemperatures of the weight loss processes. Midtemperature is the temperature at maximum weight loss rate.

*Variation between Maize Samples.* Among the maize samples large variation was observed in the extent of weight loss as well as in accompanying midtemperature (Table 2 and Figure 4). Five distinct weight loss processes were observed as follows:  $\Delta W_1$  (near 136 °C),  $\Delta W_2$  (near 210 °C),  $\Delta W_3$  (near 294 °C),  $\Delta W_4$  (near 380



**Figure 4.** TG thermograms of three maize samples showing different weight loss curves. The weight loss processes are indicated as 1–5. In this figure the variation in the processes 1 and 5 is not clearly visible.



**Figure 5.** Combined DSC (—) and TG (---) thermograms of a single maize sample.

**Table 3.** Variation in *In Vitro* Fermentation Properties and Chemical Composition of Stalk Samples of Maize<sup>a</sup>

	min	max	mean
Fermentation Characteristic			
OMD (%)	64	82	73
CWD (%)	46	66	55
ratio Ac:Prop	1.87	2.59	2.23
Chemical Constituent (g·(kg of dry matter) <sup>-1</sup> )			
NDF	435	685	567
ADF	253	411	330
HEM	182	273	237
CEL	199	333	265
pLIG	43.9	73.0	56.9
tPHEN	91.1	115.7	98.5
PCA	13.8	23.0	19.1
FA	4.0	6.1	4.7

<sup>a</sup> OMD, organic matter degradation; CWD, cell wall degradation; ratio Ac:Prop, ratio of acetate and propionate; NDF, neutral detergent fiber; ADF, acid detergent fiber; HEM, hemicellulose; CEL, cellulose; pLIG, permanganate lignin; tPHEN, total phenolics; PCA, *p*-coumaric acid ester; FA, ferulic acid ester.

°C), and  $\Delta W_5$  (near 439 °C). The amount of mass lost from the sample was largest for process 3 ( $\Delta W_3$ , 40–60% weight loss). Weight losses in processes 2 and 5 were of the same order (10–24% weight loss), and only minor amounts were lost in processes 1 and 4 (<14%). For each maize sample, processes 3 (i.e.,  $\Delta W_3$ ), 4 (i.e.,  $\Delta W_4$ ), and 5 (i.e.,  $\Delta W_5$ ) of the TG thermogram coincided with peaks 1, 2, and 3 of the DSC thermogram, respectively. This phenomenon is shown in Figure 5 for one maize sample.

**Fermentation and Cell Wall Composition.** *In vitro* fermentation and chemical characteristics of the maize stalk samples differed greatly (Table 3). The concentration ranges observed for all chemical components and degradability (OMD and CWD) of the stalk fraction of these maize samples are in agreement with earlier observations (Hunt et al., 1992; Wolf et al., 1993; Marvin et al., 1995). In addition, the maize samples

**Table 4.** Coefficients of Correlation (*r*) between Fermentation and Chemical Characteristics of Maize Stalk Samples and DSC Variates<sup>a</sup>

	DSC variates					
	$\Delta H_1$ (J·(g of dry matter) <sup>-1</sup> )	$\Delta H_2$ (J·(g of dry matter) <sup>-1</sup> )	$\Delta H_3$ (J·(g of dry matter) <sup>-1</sup> )	$T_{onset1}$ (°C)	$T_{onset2}$ (°C)	$T_{onset3}$ (°C)
OMD	0.39	-0.47	ns	0.52	ns	0.60
CWD	ns	-0.35	ns	0.52	ns	0.36
ratio	-0.53	0.56	ns	-0.44	ns	-0.59
Ac:prop						
NDF	-0.57	0.38	ns	ns	ns	-0.63
ADF	-0.63	ns	ns	ns	ns	-0.64
HEM	ns	0.37	ns	ns	ns	-0.46
CEL	-0.64	ns	ns	ns	ns	-0.65
pLIG	-0.55	ns	ns	ns	ns	-0.51
tPHEN	ns	ns	ns	-0.37	ns	ns
PCA	-0.48	ns	ns	ns	ns	-0.59
FA	ns	ns	ns	ns	ns	ns

<sup>a</sup>Key to abbreviations is given in the footnote to Table 3; ns, not significant;  $|r| > 0.34$ ,  $P < 0.05$ .

differed in the ratio of acetate and propionate production as measured after *in vitro* rumen fermentation.

**Relationships between Thermal Behavior and Cell Wall Characteristics.** *Correlation Analysis.* A correlation study was carried out using thermal, chemical, and fermentation characteristics in order to obtain a better understanding of their interrelationships. Tables 4 and 5 show the coefficients of correlation between cell wall characteristics and DSC or TG variates, respectively. Constituent concentrations or fermentation properties were poorly or moderately correlated to any DSC variable ( $|r| < 0.65$ , Table 4). The enthalpy of peak 3 and the onset temperature of peak 2 showed no significant single correlations with any variate. On the other hand, TG variates showed close relationships with contents of various chemical components. Especially, weight loss of process 3 exhibited strong correlations with contents of neutral and acid detergent fiber (NDF and ADF;  $r = 0.90$  and  $0.89$ , respectively), cellulose (CEL;  $r = 0.89$ ), hemicellulose (HEM;  $r = 0.81$ ), permanganate lignin (pLIG;  $r = 0.78$ ), organic matter degradation (OMD;  $r = -0.70$ ), and ratio of the volatile fatty acids acetate and propionate (ratio Ac:Prop;  $r = 0.82$ ). Weight loss of processes 4 and 5 did not correlate significantly with any variate.

*Multiple Linear Regression Analysis.* To investigate the possibility that a combination of DSC and/or TG variates could improve prediction/estimation of constituent contents and fermentation characteristics, we performed multiple linear regression (MLR) analysis. The best fitting regression models for the various traits are summarized in Tables 6 and 7.

MLR models based on DSC variates alone could explain 76 and 74% of the variation in contents of ADF and CEL, respectively, and 70% of that of NDF. The variation in contents of HEM, total phenolics (tPHEN), and ferulic acid ester (FA) was poorly fitted by models with only DSC variates (<29%). The fermentation properties OMD and ratio Ac:Prop were explained moderately well (52–65%) and CWD poorly (34%).

For all maize traits TG-prediction models were more powerful in comparison to DSC-prediction models, except for the trait *p*-coumaric acid ester (PCA; see Table 6). TG-based models explained 94, 87, 87, and 77% of the variation in contents of NDF, ADF, CEL, and pLIG, respectively. Variation in contents of tPHEN and PCA were poorly explained by the TG models showing  $R^2_{adj}$  below 40% (Table 6). In comparison to DSC models,  $R^2_{adj}$  of the TG models for OMD, CWD,

**Table 5. Coefficients of Correlation (*r*) between Fermentation and Chemical Characteristics of Maize Stalk Samples and TG Variates<sup>a</sup>**

	TG variates									
	$\Delta W_1$ (%)	$\Delta W_2$ (%)	$\Delta W_3$ (%)	$\Delta W_4$ (%)	$\Delta W_5$ (%)	$T_{\Delta W_1}$ (°C)	$T_{\Delta W_2}$ (°C)	$T_{\Delta W_3}$ (°C)	$T_{\Delta W_4}$ (°C)	$T_{\Delta W_5}$ (°C)
OMD	0.54	0.67	-0.70	ns	ns	0.36	ns	0.52	0.52	ns
CWD	ns	0.39	ns	ns	ns	ns	ns	0.37	0.42	ns
ratio Ac:Prop	-0.44	-0.86	0.82	ns	ns	ns	-0.50	-0.60	-0.49	-0.40
NDF	-0.65	-0.74	0.93	ns	ns	-0.51	-0.40	-0.46	-0.44	-0.48
ADF	-0.64	-0.70	0.89	ns	ns	-0.51	-0.39	-0.49	-0.41	-0.42
HEM	-0.53	-0.66	0.81	ns	ns	-0.39	ns	ns	-0.40	-0.50
CEL	-0.65	-0.68	0.89	ns	ns	-0.53	-0.44	-0.48	-0.40	-0.48
pLIG	-0.51	-0.66	0.78	ns	ns	-0.39	ns	-0.43	-0.37	ns
tPHEN	-0.39	ns	ns	ns	ns	ns	ns	ns	ns	ns
PCA	-0.42	ns	0.45	ns	ns	-0.37	-0.55	-0.53	ns	-0.49
FA	ns	-0.40	0.62	ns	ns	ns	ns	ns	ns	-0.47

<sup>a</sup> Key to other abbreviations is given in the footnote to Table 3; ns, not significant;  $|r| > 0.34$ ,  $P < 0.05$ .

**Table 6. Statistics of MLR Models for Fermentation Characteristics and Contents of Several Chemical Constituents in Maize Stalks Based on DSC and/or TG Variates<sup>a</sup>**

variate	MLR model									
	DSC			TG			DSC/TG			
	$R^2_{adj}$ (%)	SEC	<i>N</i>	$R^2_{adj}$ (%)	SEC	<i>N</i>	$R^2_{adj}$ (%)	SEC	SEP	<i>N</i>
Fermentation Characteristic										
OMD (%)	64.5	2.96	3	78.8	2.29	4	93.5	1.27	1.49	6
CWD (%)	33.9	4.10	3	58.5	3.25	4	88.1	1.74	2.13	8
ratio Ac:Prop	52.2	0.13	3	93.4	0.05	3	93.4	0.05	0.05	3
Chemical Constituent (g·(kg of dry matter) <sup>-1</sup> )										
NDF	69.6	32.6	3	93.9	14.6	5	95.5	12.5	14.7	7
ADF	75.8	20.9	3	86.8	15.4	4	91.7	12.2	14.1	6
HEM	28.9	18.1	3	75.5	10.6	5	90.7	6.5	9.1	10
CEL	73.5	17.6	4	86.7	12.4	4	90.4	10.6	12.2	5
pLIG	58.9	5.4	3	77.2	4.1	5	80.5	3.7	4.6	6
tPHEN	17.8	5.8	2	34.4	5.2	3	61.6	4.0	5.16	7
PCA	57.5	1.7	3	40.3	1.9	4	88.6	0.9	1.2	11
FA	8.8	0.5	2	70.0	0.2	7	84.0	0.2	0.3	8

<sup>a</sup> Key to other abbreviations is given in the footnote to Table 3; SEC, standard error of calibration; SEP, standard error of prediction; *N*, number of variates in the model.

**Table 7. Best Fitting Regression Models for Fermentation Characteristics and Contents of Several Chemical Constituents Based on DSC and/or TG Variates**

	equation <sup>a</sup>	$R^2_{adj}$
OMD	$0.142x_4 + 0.659x_6 + 1.028x_9 - 0.285x_{14} - 0.390x_{17} + 0.241x_{18} - 48.6$	93.5
CWD	$0.224x_4 + 0.920x_6 + 0.870x_9 - 0.509x_{12} - 0.176x_{13} - 0.519x_{14} - 0.632x_{17} + 0.458x_{18} - 21.6^{ns}$	88.1
ratio Ac:Prop	$-0.014x_9 + 0.026x_{10} + 0.008x_{18} + 0.839$	93.4
NDF	$-0.101x_2 - 3.793x_6 - 80x_7 + 11.69x_{10} + 8.95x_{11} + 6.38x_{12} + 3.51x_{15} + 400^{ns}$	95.5
ADF	$-0.478x_4^{ns} - 3.195x_6 - 12.27x_8 - 9.72x_9 + 1.302x_{17} - 2.24x_{18} + 1553$	91.7
HEM	$0.045x_1 + 0.053x_2 - 0.023x_3 + 0.351x_4 - 5.529x_9 - 4.383x_{12} - 0.910x_{13} - 1.175x_{14} + 2.077x_{15} - 1.047x_{17} + 589$	90.7
CEL	$-3.606x_6 - 7.827x_9 + 2.170x_{15} - 0.515x_{16} - 1.675x_{18} + 1581$	90.4
pLIG	$-0.135x_4^{ns} - 1.103x_6 - 1.963x_9 + 0.839x_{14} + 0.433x_{17} - 0.505x_{18} + 259.6$	80.5
tPHEN	$-0.013x_2^{ns} - 0.356x_4 - 0.868x_6 + 0.433x_{10}^{ns} + 0.844x_{12} + 0.808x_{17} - 0.545x_{18} + 204.0$	61.6
PCA	$-0.002x_1^{ns} - 0.023x_2 - 0.068x_4 + 0.006x_5 - 0.477x_6 - 0.439x_9 - 1.057x_{11} + 0.079x_{13} + 0.121x_{17} - 0.109x_{18} + 189.7$	88.6
FA	$-0.0005x_1 + 0.0003x_3 + 1.00x_7 + 0.175x_{10} + 0.077x_{12} + 0.092x_{14} + 0.014x_{16} - 0.025x_{17} - 19.96$	84.0

<sup>a</sup>  $x_1 = \Delta H_1$ ;  $x_2 = \Delta H_2$ ;  $x_3 = \Delta H_3$ ;  $x_4 = T_{onset1}$ ;  $x_5 = T_{onset2}$ ;  $x_6 = T_{onset3}$ ;  $x_7 = \text{ratio } \Delta H_2:\Delta H_3$ ;  $x_8 = \Delta W_1$ ;  $x_9 = \Delta W_2$ ;  $x_{10} = \Delta W_3$ ;  $x_{11} = \Delta W_4$ ;  $x_{12} = \Delta W_5$ ;  $x_{13} = T_{\Delta W_1}$ ;  $x_{14} = T_{\Delta W_2}$ ;  $x_{15} = T_{\Delta W_3}$ ;  $x_{16} = T_{\Delta W_4}$ ;  $x_{17} = T_{\Delta W_5}$ ;  $x_{18} = \text{ash (g·(kg of dry matter)}^{-1})$ ; ns, not significant ( $\alpha > 0.1$ ).

and ratio Ac:Prop were all considerably higher and accompanied by lower standard error of calibrations (SECs). Especially the extreme high  $R^2_{adj}$  (i.e., 93%) and extreme low SEC (i.e., 0.05) of TG models for ratio Ac:Prop were striking.

MLR models based on both DSC and TG variates showed considerably higher explanatory/predictive potential than models based on DSC or TG variates alone (Table 6). The best DSC/TG-based models explained more than 90% of the variation in OMD, ratio Ac:Prop, NDF, ADF, HEM, and CEL and more than 84% of the variation in CWD, PCA, and FA. The explanation of the variation in contents of pLIG and tPHEN was somewhat lower (i.e., 81 and 62%, respectively). The best fitting DSC/TG models for the various fermentation and chemical characteristics all had low SEC and

contained 3–11 explanatory variates (Table 6). The DSC/TG model for HEM and PCA contained the most variates (10 and 11, respectively) and the DSC/TG model for ratio Ac:Prop and CEL the least (3 and 5 variates, respectively). The best DSC/TG models for the various response variates included both DSC and TG variates, except for ratio Ac:Prop. For this property adding of DSC variates to the TG model gave no improvement (Table 6). The best DSC/TG models for OMD and CWD both contained  $\Delta W_2$ , midtemperature of  $\Delta W_2$  and  $\Delta W_5$  of the TG variates, and  $T_{onset}$  of peaks 1 and 3 ( $T_{onset1}$  and  $T_{onset3}$ ) of the DSC variates (Table 7). The explanatory variates  $\Delta W_2$ ,  $T_{onset3}$  and  $T_{\Delta W_5}$  are frequently present in DSC/TG models for cell wall constituents (Table 7). To validate the predictive potential of the best MLR models for the various chemical

constituents and fermentation characteristics of maize stalks (i.e., DSC/TG models), standard errors of prediction (SEP) were calculated for these models (Table 6). The SEP values of the various DSC/TG models were close to the SEC values but always somewhat (i.e., 15–30%) higher.

## DISCUSSION

Thermal degradation processes of powder samples of maize stalks were studied by differential scanning calorimetry and thermogravimetry, and thermal degradation characteristics were related to *in vitro* fermentation characteristics and concentrations of various chemical components.

DSC thermograms showed two main and one minor exothermic peak. The peak area and associated peak temperature appeared to be a function of the heating rate. Similar dependency has been observed in studies investigating thermal degradation of woody samples (Beall and Eickner, 1970; Nguyen et al., 1981). Furthermore, DSC thermograms of maize stalk samples very much resemble thermograms obtained by DSC or related techniques at similar conditions for alfalfa (Varju, 1974), wood, or related plant material (Nguyen et al., 1981; Krigstin et al., 1993; Cone et al., 1996). Such a similarity is likely since stalk samples of maize mainly consist of cell walls with similar composition as cell walls of alfalfa and wood (i.e., cellulose, hemicellulose, lignin, proteins, and ash).

Interpretation of thermal degradation curves of wood and related plant materials in the presence of oxygen as monitored by DSC or TG is very complicated because of the complex mixture of high and low molecular weight polymers in these samples, simultaneously occurring thermal reactions (both endothermic and exothermic), oxidation reactions, and simultaneously decomposing constituents. Krigstin et al. (1993) distinguished five regions (A–E) in DSC thermograms of willow. For maize these regions are located between 30 and 230 °C (A), 230 and 280 °C (B), 280 and 315 °C (C), 315 and 420 °C (D), and 420 and 500 °C (E) and are indicated in Figure 2. Region A is a net endothermic region and is ascribed to energy absorption due to evaporation and drive off of some of the initial gasses which are produced by early pyrolysis (Krigstin et al., 1993). The sum of the thermal reactions becomes exothermic in region B. At these temperatures the initial degradation of HEM (starting at ca. 225 °C) and LIG (starting at ca. 250 °C) dominates. LIG decomposition gradually progresses from this point and extends throughout the whole temperature range, while HEM decomposes somewhat faster showing a maximum decomposition rate near 273 °C (Nguyen et al., 1981). The exothermic B region is dominated by slow oxidation, depolymerization, and dehydration reactions. The sum of the reactions become even more exothermic between 280 and 315 °C (region C). Levoglucosans are produced and decomposition of CEL starts in this temperature region (Varju, 1974; Nguyen et al., 1981). Decomposition of CEL may show main exothermic peaks near 330 and 420 °C (Varju, 1974).

The exotherm regions B and C are followed by a region in which endothermic reactions dominate (region D). Many maize samples showed a small exothermic peak after the initial net endothermic reactions in region D. Between 420 and 500 °C (region E) secondary reactions of pyrolysis products dominate which yield highly reactive char. This char is further oxidized resulting in highly exothermic reactions (Krigstin et al., 1993).

In Figure 5 it is apparent that the large exothermic peak between 414 and 446 °C is accompanied by a relatively small weight loss from the sample. High positive correlation ( $r = 0.84$ , not shown) between enthalpy and weight loss in this temperature region (i.e.,  $\Delta H_3$  and  $\Delta W_3$ , respectively) further suggests a relationship. Susott (1982) has suggested that the enthalpy release in this region may be due to cross-linking and crystallization of remaining carbonaceous matter in the char.

At high temperatures and in the presence of oxygen, oxidative reactions will dominate decomposition and pyrolysis reactions. This was confirmed by the absence of weight loss in TG thermograms above 380 °C and by the absence of exothermic peaks in DSC thermograms when thermal analysis was performed in an oxygen free atmosphere.

Temperature ranges of thermal processes of individual chemical components as demonstrated for wood are overlapping (Krigstin et al., 1993). This suggests that, generally, no single DSC peak or TG process can be exclusively linked to a single chemical component of a sample. The lack of exclusive simple correlation between individual chemical components and thermal characteristics as distinguished in this paper indicates that this holds for maize too. Nevertheless, contents of several cell wall components (NDF, ADF, CEL, and HEM) showed high simple positive correlations with  $\Delta W_3$ . Due to strong (negative) correlations between NDF, CEL, HEM, and OMD ( $|r| > 0.8$ , not shown),  $\Delta W_3$  also showed a good relationship with OMD ( $r = -0.70$ ).

No satisfactory information about fermentation characteristics and/or contents of various chemical components of the samples was obtained from the DSC and/or TG thermograms when using single correlations. Therefore, to assess the suitability of DSC and/or TG to predict forage quality traits better, MLR analyses were performed. The MLR models for these traits which were based on variates extracted from the DSC curves alone fitted poorly, whereas models based on TG variates were considerable more successful (Table 6). However, the best explanation of the variation for the traits was obtained with models combining DSC and TG variates, except for total phenolics. These DSC/TG models had low standard errors of prediction indicating that they may have potential for predicting forage quality properties, in particular for prediction of CWD. A superior predictive performance of models which use both DSC and TG variates, as compared to models based on either of the two, has been observed before by Berger and Albano (1993) when studying thermal degradation of peat.

The accuracy of the predictions of contents of chemical components and OMD in maize stalks by the use of DSC in combination with TG, as was observed in this study, is at least similar to those obtained for forages by NIRS (Norris et al., 1976; Barton II, 1985; Bughrara et al., 1992; Shenk et al., 1992; García-Ciudad et al., 1993; Dardenne, 1994). Bergner and Albano (1993) compared NIRS and DSC/TG for prediction of contents of various chemical components in peat. These authors concluded that the prediction of contents of most chemical constituents was similar for NIRS and DSC/TG. However, for LIG and ash, DSC/TG outperformed NIRS. In this study the number of maize samples used to show the predictive potential of thermal analysis is too small to enable a fair comparison between NIRS and thermal analysis. The outcome of the DSC/TG regression studies, however, is promising and indicates that in the

future this technique may become useful for analyzing forage quality traits.

In conclusion thermal degradation properties as monitored by DSC and TG vary greatly among stalk samples of maize differing in chemical composition. The results described in this paper suggest that thermal degradation properties are related to the chemical composition and may be useful as a tool to predict various forage quality traits.

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