



Relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm

II. Ruminant and post-ruminant degradabilities

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Abstract

Correlations between kernel vitreousness and ruminant *in situ* (RDMD) and total tract dry matter (TDMD; sum of ruminant *in situ* and post-ruminant *in vitro* measurements) degradabilities were determined for 33 diverse corn germplasm sources. These included a wide range of endosperm characteristics from opaque 2 (o2) types to densely packed flint types, and a number of intermediates. Harvests were done at two growth stages; 1/2 milk-line (ML) and black-layer (BL). Kernels from middle portion of ears were oven dried at 40 °C for 72 h and ground through a Wiley mill (6 mm screen) for measurement of *in situ* RDMD after 0 and 14 h of incubation using two steers (1.5 g/bag × 8 replicates per time point per steer in 5 cm × 5 cm bags of 50 µm pore size). Residue from the 14 h bags proceeded to an 8 h *in vitro* enzymatic post-ruminant digestion after which the residue was oven dried at 62 °C for 48 h and dry matter content determined. Inbred by harvest-stage interactions were observed for 0-h disappearance and TDMD. Vitreousness had strong negative correlations with degradability

Abbreviations: A-fraction, zero-hour disappearance or bag wash loss; BL, black-layer harvesting stage; DT, drying technique; GEM, Germplasm Enhancement of Maize project; HS, harvest stage; ML, 1/2 milk-line harvesting stage; NIRS, near infrared reflectance spectroscopy; pRDMD, post-ruminant dry matter degradability; RDMD, ruminant dry matter degradability; TDMD, total dry matter degradability; TSTARCD, total tract starch degradability; WQS C2, Wisconsin quality synthetic cycle 2.

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measurements, particularly for more mature (BL) samples (-0.728 , -0.770 and -0.603) versus ML (-0.569 , -0.541 and -0.338) for 0 h disappearance, RDMD and TDMD, respectively. Vitreousness was highly correlated with corn degradability, especially at a black-layer stage of harvest, in this diverse corn germplasm.

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1. Introduction

Recent research has evaluated corn germplasm for differences in starch degradability (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002; Johnson et al., 2002; Taylor and Allen, 2005) to improve corn grain and silage utilization by ruminants. Rumen degraded starch supplies energy to the animal through volatile fatty acid production and metabolism, and also contributes to protein metabolism through microbial mass, whereas post-ruminal starch is degraded to glucose (Hall, 2002). Corn germplasm differences in total tract starch degradation (TSTARCHD) are often less than for ruminal starch degradation (RSTARCHD), because of post-ruminal compensation (Huntington, 1997). However, variation still exists among germplasm of different genetic background for TSTARCHD (Ngonyamo-Majee et al., 2004; Taylor and Allen, 2005). Taylor and Allen (2005) evaluated floury and vitreous commercial corn hybrids and reported 615 g/kg versus 290 g/kg and 969 g/kg versus 921 g/kg for proportion of RSTARCHD and TSTARCHD, respectively. We (Ngonyamo-Majee et al., 2004) found consistent mean differences, across four harvest stages, between *o2* (Oh43) and normal (Oh43) corn grain (698 g/kg vs. 468 g/kg, 870 g/kg vs. 789 g/kg and 940 g/kg vs. 831 g/kg for proportion of ruminal dry matter degradability (RDMD), and total tract dry matter degradability (TDMD) and TSTARCHD, respectively). Taylor and Allen's (2005) work was done *in vivo*, whereas our work involved *in situ* rumen degradability followed by an *in vitro* post-ruminal digestion procedure by Calsamiglia and Stern (1995). The *opaque2* (*o2*) genes elevate concentrations of lysine and tryptophan (Mertz et al., 1964). Earlier studies by Dado and Briggs (1996) reported that 6 and 12 h *in vitro* starch degradability of seven high-lysine varieties averaged 17% and 7% units higher than the normal check hybrid, respectively. Other mutations introduced to improve starch digestibility include the *sugary* (*su*) allele that accumulates higher levels of sucrose compared to normal corn (Perera et al., 2001), and the *waxy* (*wx*) gene, characterized by the complete replacement of amylose by amylopectin (Watson, 2003). Although there has been success in the introduction of these genes to improve starch digestibility, they have not had impact in animal feed industry because of poor agronomic qualities. Hence, current efforts from industry on identifying corn germplasm with high starch digestibility have focused on selection from within the high yielding germplasm pool.

In a previous study (Ngonyamo-Majee, 2005) a wide range in the endosperm properties (kernel vitreousness, density, and hardness) of 33 germplasm sources was found. These inbreds allowed studying the relationships between their endosperm properties and ruminal and post-ruminal starch degradabilities from material of pure genetic background, which was not allowed from the use of hybrids in the other studies (Philippeau and Michalet-

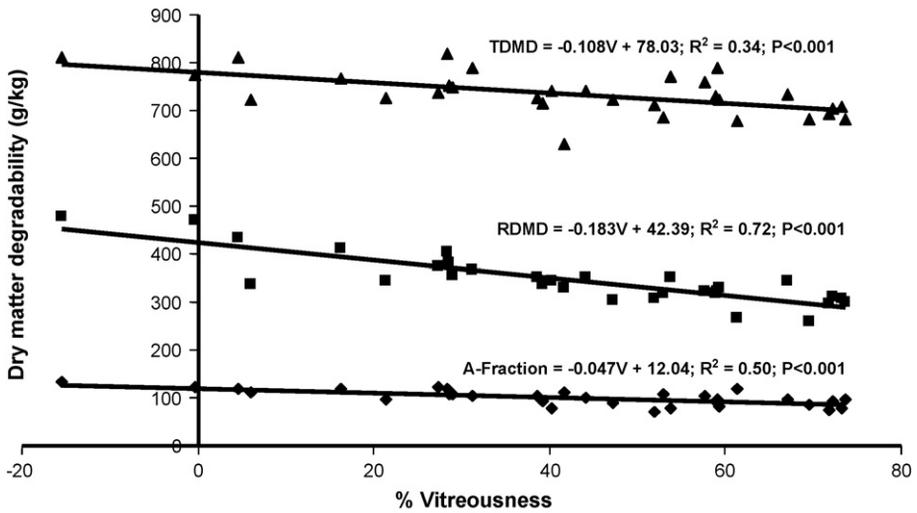


Fig. 1. Regression of vitreousness (V) with zero-hour bag loss (A-fraction), ruminal dry matter degradability (RDMD) and total dry matter degradability (TDMD) of the 31 (excluding the two outliers *su2*(Oh43 and *wx1su2*(Oh43) from the original 33 germplasm sources) germplasm sources at 1/2 milk-line stage.

Doreau, 1997; Correa et al., 2002; Taylor and Allen, 2005). The objectives of this experiment were to: (1) evaluate the degradabilities of 33 corn germplines of diverse genetic background, and (2) determine the correlations between corn endosperm properties measured manually and predicted by near-infrared reflectance spectroscopy (NIRS) versus degradability

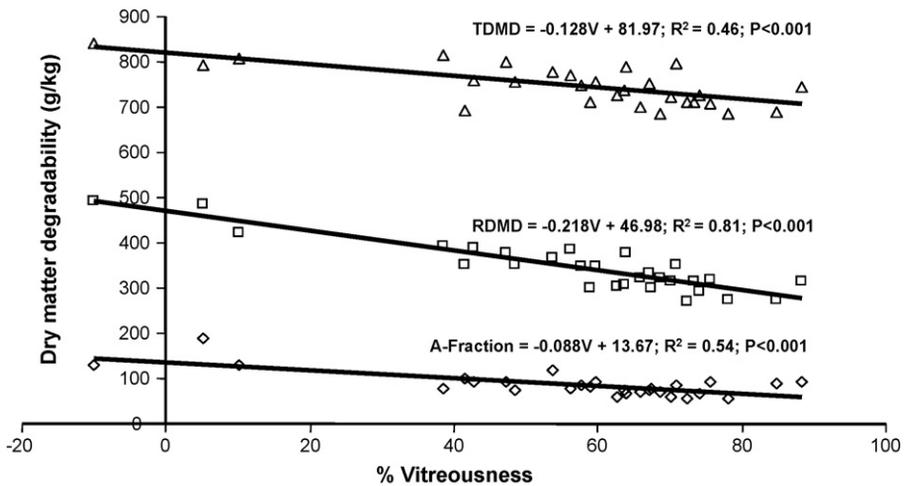


Fig. 2. Regression of vitreousness (V) with zero-hour bag loss (A-fraction), ruminal dry matter degradability (RDMD) and total tract dry matter degradability (TDMD) of the 31 (excluding the two outliers *su2*(Oh43 and *wx1su2*(Oh43) from the original 33 germplasm sources) germplasm sources at black-layer stage.

measurements. It is believed that a combination of NIRS technology and correlations data for physical traits with starch digestibility will provide faster and cheaper selection tools that may be used to support corn hybrid breeding programs and selection for improved starch digestibility.

2. Materials and methods

This is the second in a series from one study that evaluated the relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm. This article discusses the evaluation of ruminal and post-ruminal dry matter degradabilities of the 33 germplasm sources and their correlation with endosperm properties. The companion report covers the development of NIRS calibrations as a rapid and non-destructive procedure to determine corn vitreousness, density, and other hardness measures (Nkonyamo-Majee et al., 2008).

2.1. Field production

Thirty-three inbred lines were selected for starch and endosperm characteristics likely related to starch granule packing (Jennings et al., 2002a,b) and ruminal starch degradation (Philippeau and Michalet-Doreau, 1997). The range of endosperm characteristics extended from the *opaque 2* (*o2*) types with very loosely packed starch granules to densely packed flint types (Jennings et al., 2002a,b), and a number of intermediates. These included 17 lines from the germplasm enhancement of maize (GEM) project at Iowa State University; six flint lines; six near-isogenic variants of Oh43 carrying *opaque-2* (*o2*), *floury-2* (*fl2*), *sugary-2* (*su2*), *amylose-extender-1* (*ae1*), *soft endosperm* (*h1*), and *waxy-1 sugary-2* (*wx1su2*) alleles that affect endosperm composition; an experimental breeding population from University of Wisconsin-Agronomy department (WQS C2); and three conventional U.S. inbreds as check lines; B73 (Iowa Stiff Stalk Synthetic); Oh43 (Ohio State University); and W64A (University of Wisconsin). Germplasm selection for check lines carrying the allelic mutations (*o2*, *fl2*, *su2*, *wx1*, and *ae1*) was limited to largely the older material that had these mutant genes successfully introduced. Hence, the current popular inbreds from Lines of Holden germplasm background (e.g. LH 185 and LH198) were not included, as they have not had any of these mutations introduced (Coors, 2001, personal communication).

The inbred lines were grown at University of Wisconsin West Madison Research Station during summer of 2002 in 3.04 m × 0.76 m rows in a randomized complete block design with three replications. Planting was done on 6 May 2002 and plots were thinned to leave about 10 plants per row before the sixth leaf stage giving about 60,000 plants/ha density. At flowering, each plant was self-pollinated by hand to preserve the endosperm characteristics specific to each inbred. Each inbred row was split for harvest at two maturity stages; 1/2 milk-line (ML) and black-layer (BL) reflective of corn silage and high-moisture corn harvests, respectively. The harvest stages were identified by assessing the movement and position of the milk-line on the backside of kernels. Hence, this resulted in different harvesting dates for the inbreds. After harvest by hand, ears were immediately frozen

within 15 min in the field using liquid nitrogen and then stored at -75°C in a freezer until shelling.

2.2. Laboratory procedures

Kernels from middle portions of ears were shelled when frozen (-80°C), thawed and oven dried at 40°C to avoid disruption of kernel cell structure during processing (Philippeau and Michalet-Doreau, 1997; Ngonyamo-Majee et al., 2004). Total starch and protein were predicted using near infrared transmittance (NIT) techniques using regression equations for corn grain developed by Pioneer, a Dupont company. Kernel vitreousness and density were determined by NIRS using calibration equations that we developed in a previous study using the same germplasm (Ngonyamo-Majee et al., 2008). These calibrations produced good prediction models with high R^2 values (0.90 and 0.92) and residual predictive values (RDP) (3.73 and 2.50) for vitreousness and density, respectively. The NIRS procedure used dried samples ground to pass a 1 mm screen size using the Stenvert micro hammer-cutter mill and put in glass covered cuvettes sealed with paper stoppers. These were scanned in duplicate using NIR spectrophotometer (FOSS NIRSystems model 6500, Silver Springs, MD, USA) with a range of 400–2498 nm.

2.3. DM degradability

Two steers fitted with ruminal cannulae were fed *ad libitum* a diet composed of a concentrate (corn grain) to forage (alfalfa hay) ratio of 40:60. Steers were adapted to their diets for a 2-week period prior to the start of ruminal *in situ* incubations. Corn kernels dried as previously described were ground through a Wiley mill (6 mm screen, Arthur H. Thomas, Philadelphia, PA) and approximately 1.5 g of the ground material weighed into 5 cm \times 5 cm dacron bags with 53 ± 10 μm pore size (Ankom Co., Fairport, NY) with heat-sealed seams. The *in situ* study was designed to ensure that all treatments were balanced across steers and time. Immediately before being placed in the rumen, bags containing feed were placed in a large mesh bag (36 cm \times 42 cm, with a nylon zipper) that was soaked in water (39°C) for 15 min before insertion into the rumen *via* the ruminal cannula. Ruminal incubation time was 14 h with eight replicates. The choices on rumen incubation time point and sample grind size were based on Sapienza (2002) who reported increased sensitivity of *in situ* rumen assays (at 12–16 h incubation times) to detect differences in corn grain degradation with values similar to *in vivo* data from several studies conducted at the Livestock Nutrition Center of Pioneer Hi-bred Int. (a Du Pont company). Immediately after removal from the rumen, mesh bags were repeatedly dunked in a 20 l bucket of cold water to remove debris from the outside of the bag and to stop enzymatic activity. Water in the buckets was replaced for the second steer. Mesh bags then were placed in an automatic washing machine filled with water and allowed to agitate on a gentle cycle with the lid open to prevent spinning (Cherney et al., 1990). Then, bags were dried at 62°C for 48 h to determine dry matter (DM) content. Zero-hour bags also were immersed in water (39°C) for 15 min before washing. This was done to determine DM that is soluble or fine-particles that escapes through the bag pores. After DM content was determined, 14 h bags were subjected to an enzymatic incubation to simulate post-ruminal digestion (Calsamiglia and Stern, 1995). First, bags

were 2 h incubated in pepsin and after rinsing, directly incubated in pancreatin buffer for 6 h. The final residue was rinsed and oven dried at 62 °C for 48 h and DM content of the residue was determined and used to calculate TDMD. The following formulas were used to calculate proportion of 0 h DM disappearance (A-Fraction), RDMD, and TDMD:

$$\text{A-fraction (g/kg DM)} = \left(\frac{\text{initial dry weight} - 0 \text{ h wash residue dry weight}}{\text{initial dry weight}} \right) \times 1000$$

$$\text{RDMD (g/kg DM)} = \left(\frac{\text{initial dry weight} - \text{ruminal 14 h } in \text{ situ residue dry weight}}{\text{initial dry weight}} \right) \times 1000$$

$$\text{TDMD (g/kg DM)} = \left(\frac{\text{initial dry weight} - \text{enzyme residue dry weight}}{\text{initial dry weight}} \right) \times 1000$$

2.4. Data analysis

The SAS (2001) Proc Mixed procedure was used to analyze for all parameters with replicate and replicate \times inbred effects included in the model as random factors. Means were compared by the least significant difference test, in the case of a significant ($P < 0.05$) treatment effect. Correlation coefficients between endosperm physico-chemical properties (vitreousness, density, and protein) with the A-fraction, RDMD and TDMD were determined for the two maturity stages.

3. Results

3.1. Kernel density, vitreousness and nutrient composition

Kernel density, vitreousness and composition data for the 33 germplasm sources are presented in Table 1. There were wide ranges among the inbreds for all physical and chemical composition parameters. The GEM material was higher in CP content than check lines or mutants. Comparisons of physical characteristics showed *o2*(Oh43) and *fl2*(Oh43) with lowest densities and vitreousness, while NC flints (NC398 and NC414) and two GEMlines (AR16035:S02-666-1-B-B and AR16035:S02-611-1-B-B) were the most dense inbreds. Starch values were within the normal range for yellow dent corn grain in U.S. (680–740 g/kg DM) (Watson, 2003). Contrary to what we expected, there was a decline in kernel density and starch ($P < 0.0001$) and an increase ($P < 0.05$) in CP, with advanced maturity. Vitreousness increased with maturity ($P < 0.001$).

Table 1
Kernel physico-chemical properties

Inbred	Vitreousness (%)	Density (g/cm ³)	Starch (g/kg DM)	Protein (g/kg DM)
su2(Oh43)	72.3	1.225	704	127
o2(Oh43)	-12.8	1.065	700	96
ae1(Oh43)	41.5	1.252	686	134
fl2(Oh43)	2.5	1.092	698	108
hl1(Oh43)	7.4	1.128	703	120
wx1su2(Oh43)	53.1	1.200	744	127
CML176*	16.2	1.197	685	100
AR16035:S02-447-1-B	74.7	1.215	698	159
AR16035:S02-611-1-B-B	80.4	1.245	724	140
AR16035:S02-666-1-B-B	73.1	1.247	724	163
CHIS775:N1912-254-1-B	41.1	1.240	720	122
CHIS775:N1912-347-1-B	35.0	1.183	745	105
CHO5015:N12-387-1-B	61.6	1.230	721	142
CHO5015:N15-143-1-B	63.2	1.238	702	142
CHO5015:N15-182-1-B	62.2	1.222	712	128
CHO5015:N15-8-1-B	45.1	1.162	763	101
CUBA164:2008a-23-1-B	51.4	1.200	684	126
CUBA164:S15-184-1-B	71.9	1.240	683	169
CUBA164:S15-192-2-B	34.9	1.212	687	126
CUBA164:S15-435-1-B	38.1	1.220	718	138
CUBA164:S2008a-3-1-B	65.0	1.253	728	106
DREP150:N2011d-13-1-B	40.1	1.245	696	173
FS8B(T):N1802-35-1-B	47.7	1.203	710	103
UR13085:N0215-3-1-B	64.0	1.173	713	122
NC398	72.6	1.287	710	130
NC410	69.1	1.235	723	118
NC412	53.5	1.238	716	109
NC414	62.4	1.257	696	102
NC416	39.9	1.237	680	117
WQS C2	66.5	1.232	710	114
B73	33.4	1.198	704	104
Oh43	58.8	1.208	718	106
W64A	75.7	1.230	697	136
Mean	50.8	1.213	708	123
SE	1.8	0.006	6.0	2.9
LSD	9.1	0.019	20.5	9.9
P-value	<0.001	<0.001	<0.001	<0.001
<u>Maturity:</u>				
ML	44.2	1.229	722	123
BL	57.6	1.197	689	124
SE	0.78	0.002	5.0	1.1
P<	<0.001	<0.001	<0.001	<0.05
<u>Interaction</u>				
Inbred x Maturity	<0.001	<0.001	<0.2000	<0.05

Table 2
Ruminal and post-ruminal dry matter degradabilities

Inbred	A-fraction	RDMD	pRDMD	TDMD
su2(Oh43)	103	452	698	836
o2(Oh43)	130	484	658	826
ae1(Oh43)	106	342	489	661
f12(Oh43)	156	480	584	784
h1(Oh43)	125	427	671	810
wx1su2(Oh43)	105	459	693	834
CML176	118	411	520	768
AR16035:S02-447-1-B	94	309	558	694
AR16035:S02-611-1-B-B	93	314	599	724
AR16035:S02-666-1-B-B	103	270	565	682
CHIS775:N1912-254-1-B	113	374	626	765
CHIS775:N1912-347-1-B	107	383	593	749
CHO5015:N12-387-1-B	82	315	571	705
CHO5015:N15-143-1-B	86	310	615	734
CHO5015:N15-182-1-B	63	290	597	712
CHO5015:N15-8-1-B	102	353	578	727
CUBA164:2008a-23-1-B	69	324	606	734
CUBA164:S15-184-1-B	78	278	591	704
CUBA164:S15-192-2-B	92	323	603	730
CUBA164:S15-435-1-B	101	366	650	775
CUBA164:S2008a-3-1-B	90	339	689	793
DREP150:N2011d-13-1-B	90	322	586	719
FS8B(T):N1802-35-1-B	86	363	599	743
UR13085:N0215-3-1-B	77	325	562	704
NC398	74	305	570	702
NC410*	-	-	-	-
NC412	90	326	611	739
NC414	88	328	636	755
NC416	90	360	645	772
WQS C2	85	335	577	718
B73	99	399	698	817
Oh43	73	365	656	780
W64A	68	292	570	696
Mean	95	356	612	749
SE	8.3	5.0	4.3	9.2
LSD	17.3	61.7	39.6	41.1
P-value	<0.001	<0.001	<0.001	<0.001
Maturity				
ML	101	365	602	741
BL	88	347	619	721
SE	1.1	2.0	2.7	2.7
P-value	<0.001	<0.001	<0.001	<0.001
Interaction				
Inbred x Maturity	<0.001	<0.001	<0.01	<0.01

*NC410 had limited sample size harvested, hence was not included for degradability studies.

A-fraction: 0-h disappearance; RDMD: ruminal dry matter degradability; pRDMD: post-rumen dry matter degradability; TDMD: total dry matter degradability; SE: standard error; LSD: least significant difference at $P < 0.05$; ML: 1/2 milk-line harvest stage; BL: black-layer harvest stage. Check lines are highlighted.

3.2. Degradability

Degradability parameter data are presented in Table 2. Because of the strong ($P < 0.0001$) positive correlations for RDMD versus RSTARCD ($r = 0.98$) (Correa et al., 2002) and for TDMD versus TSTARCD ($r = 0.90$) (Ngonyamo-Majee et al., 2004), DM degradability was used as a predictor of starch degradability. Black-layer samples had lower ($P < 0.001$) A-fraction DM and TDMD, but similar RDMD compared to ML samples. Most GEM material had higher degradability than WQS C2 population. Kernel TDMD for WQS C2 was also lower ($P < 0.05$) than values from the flint check lines. We found inbred by harvest stage interactions for A-fraction ($P < 0.0001$), RDMD ($P < 0.0001$) and TDMD ($P < 0.01$).

3.3. Correlation coefficients

Correlations between physico-chemical properties of the germplasm combined for two maturities are shown in Table 3. Kernel vitreousness was positively ($P < 0.0001$) correlated to density and kernel CP, but not starch ($P = 0.4801$) and oil ($P = 0.0512$), when both harvest stages were included. However, treating the harvest stages separately improved ($P < 0.001$) the correlations of vitreousness with density and kernel CP for ML and BL stages, respectively (Tables 4 and 5). Kernel density had a similar poor correlation with kernel CP ($P < 0.0001$). Corn starch had a weak positive correlation ($P = 0.0526$) with density, relatively strong negative correlation with oil ($P < 0.0001$) and DM content at harvest ($P < 0.0001$), and poor negative correlation ($P = 0.0133$) with kernel CP.

The correlations between endosperm physico-chemical properties and degradability are summarized in Tables 3–5. Vitreousness had a strong negative correlation with all degradability parameters ($P < 0.001$), particularly for more mature (BL) samples versus ML. Other kernel properties that showed strong ($P < 0.01$) negative correlations with degradability were density and kernel CP content.

Table 3

Correlations (r -value) for kernel endosperm physico-chemical properties and degradability parameters combined for the two harvesting stages

	0-h	RDMD	pRDMD	TDMD	Protein	Starch	Density	Vitreousness
DM	-0.30***	-0.07 NS	0.08 NS	0.02 NS	0.02 NS	-0.45***	-0.30***	0.28***
0-h		0.62***	0.08 NS	0.36***	-0.32***	0.19*	-0.42***	-0.65***
RDMD			0.48***	0.80***	-0.51***	0.07 NS	-0.61***	-0.62***
pRDMD				0.91***	-0.28**	0.05 NS	-0.19*	-0.14 NS
TDMD					-0.45***	0.07 NS	-0.43***	-0.40***
Protein						-0.20*	0.43***	0.41***
Starch							0.15 NS	-0.06 NS
Density								0.58***

DM: dry matter concentration (g/kg); 0-h: bag wash DM loss (g/kg); RDMD: ruminal dry matter digestibility (g/kg); pRDMD: post-ruminal dry matter digestibility (g/kg); TDMD: total dry matter digestibility (g/kg); NS: non-significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 4
Correlations (*r*-value) for kernel endosperm physico-chemical properties and degradability parameters at 1/2 milk-line stage

	0-h	RDMD	pRDMD	TDMD	Oil	Protein	Starch	Density	Vitreousness
DM	−0.24*	−0.12 NS	−0.02 NS	−0.04 NS	−0.12 NS	0.07 NS	−0.00 NS	0.04 NS	0.21*
0-h		0.54***	0.12 NS	0.34**	0.29**	−0.29**	−0.02 NS	−0.44 (−0.45)***	−0.57 (−0.61)***
RDMD			0.50***	0.80***	0.29**	−0.49***	0.07 NS	−0.59 (−0.72)***	−0.54 (−0.74)***
pRDMD				0.91***	0.18 NS	−0.35**	0.25*	−0.14 NS	−0.10 NS
TDMD					0.27*	−0.49***	0.20 NS	−0.39 (−0.50)***	−0.34 (−0.54)***
Oil						−0.36***	−0.62***	−0.15 NS	−0.34***
Protein							−0.28**	0.54***	0.37***
Starch								0.03 NS	0.22*
Density									0.74***

Values in parantheses are for correlations after removal of *su2*(Oh43) and *wx1su2*(Oh43) which were highly influencing the relationships (outliers). DM: dry matter concentration (g/kg); 0-h: bag wash DM loss (g/kg); RDMD: ruminal dry matter degradability (g/kg); pRDMD: post-ruminal dry matter degradability (g/kg); TDMD: total tract dry matter degradability (g/kg); NS: non-significant.

* P<0.05.

** P<0.01.

*** P<0.001.

Table 5
Correlations (*r*-value) for kernel endosperm physico-chemical properties and degradability parameters at black-layer stage

	0-h	RDMD	pRDMD	TDMD	Protein	Starch	Density	Vitreousness
DM	−0.09 NS	−0.17 NS	−0.25*	−0.26*	0.10 NS	0.08 NS	−0.15 NS	0.03 NS
0-h		0.77***	0.16 NS	0.49***	−0.39**	0.10 NS	−0.68(−0.68)***	−0.73(−0.75)***
RDMD			0.48***	0.81***	−0.53***	0.14 NS	−0.69(−0.73)***	−0.77(−0.86)***
pRDMD				0.90***	−0.21 NS	0.09 NS	−0.15 NS	−0.33**
TDMD					−0.42***	0.13 NS	−0.44(−0.47)***	−0.60(−0.70)***
Protein						−0.26*	0.36**	0.51***
Starch							−0.14 NS	−0.07 NS
Density								0.74***

Values in parantheses are for correlations after removal of *su2*(Oh43) and *wx1su2*(Oh43) which were highly influencing the relationships (outliers). DM: dry matter concentration (g/kg); 0-h: bag wash DM loss (g/kg); RDMD: ruminal dry matter degradability (g/kg); pRDMD: post-ruminal dry matter degradability (g/kg); TDMD: total tract dry matter degradability (g/kg); NS: non-significant.

* P<0.05.
** P<0.01.
*** P<0.001.

4. Discussion

4.1. Kernel density, vitreousness and nutrient composition

The relatively high CP values from the GEM material; AR16035:S02-611-1-B-B; CHO5015:N12-387-1-B; AR16035:S02-447-1-B, AR16035:S02-666-1-B-B CUBA164:S15-184-1-B; and DREP150:N2011d-13-1-B, are similar to those reported by Pollak (2003). Kernel density is the sum of the densities of its nutrient components (*i.e.* starch = 1.5; protein = 1.1; oil = 0.9; water = 1.0 g/cm³) and internal voids filled with air (density near zero) (Paulsen et al., 2003). Thus, differences in chemical composition may account for variation in density. The negative vitreousness values observed from o2(Oh43) (–12.8%) is a result of the fixed NIR calibration equation (intercept was not forced to pass through zero), otherwise its value is 0%. Since kernel structural integrity and resistance to deformation is thought to result from sub-cellular associations based primarily on the interaction of matrix protein with imbedded starch (Shandera and Jackson, 2002), we would expect inbreds with relatively soft endosperm to have lower protein. Singh et al. (2001) found the GEM accessions containing slightly less starch (659–691 g/kg DM) than two public Corn Belt inbreds (B73 and Mo17) with 680–697 g starch/kg DM in the grain. In this study, we found a wider range of starch values for the GEM accessions and the Corn Belt inbreds (B73, Mo17 and W64A). Studies by Pollak (2003) involving the same GEM material analyzed by NIR, had starch values of 731, 717, 703 and 656 g/kg DM for CHO5015:N15-8-1-B, FS8B(T):N1802-35-1-B, B73, and DREP150:N2011d-13-1-B, respectively. These compare well with our starch values for the same material. Starches isolated from the GEM accessions (Singh et al., 2001) possessed thermal, paste and gel properties statistically different ($P < 0.05$) from commercial hybrids and common Corn Belt hybrids.

Contrary to what we expected, there was a decline in kernel density and starch and an increase in CP, with advanced maturity. Vitreousness increased with maturity and this agrees with previous findings (Correa et al., 2002). Kernels tend to accumulate storage nutrients with maturity, but their proportion may decline as other components increase. Interactions found between maturity and inbred type on density and CP could be caused by the genetic differences in the way the inbreds respond to environmental stresses of temperature and moisture during the growing season (Westgate, 1994).

4.2. Degradability

Because of the strong ($P < 0.0001$) positive correlations for RDMD *versus* RSTARCD ($r = 0.98$) (Correa et al., 2002) and for TDMD *versus* TSTARCD ($r = 0.90$) (Ngonyamo-Majee et al., 2004), DM degradability was used as a predictor of starch degradability. Inbred comparisons showed most GEM material and flint check lines had higher degradability than WQS C2 population developed for improved silage quality with high stover digestibility (Frey et al., 2004). This shows potential for GEM lines that achieved high degradability values; *i.e.* CUBA164:S15-435-1-B; CUBA164:S2008a-3-1-B and CHIS775:N1912-254-

1-B and were also found to contain higher starch and protein contents (Table 1). Because of the compensatory digestion that occurs in the intestines from material escaping the rumen, as shown by data for post-ruminal DM degradability (pRDMD), there is a reduction of the variation in TDMD among the inbreds. The same effect was observed in a previous study (Ngonyamo-Majee et al., 2004), and similar findings were reported in other studies (Huntington, 1997; Hall, 2002; Offner and Sauvant, 2004). Nevertheless, still lower TDMD values were observed from the flints compared to check lines and the OH43 mutant carrying inbreds.

Interactions found for inbred by harvest stage on the A-fraction, RDMD and TDMD may be explained by the lack of a significant reduction of degradability with maturity by particularly the soft endosperm mutants, *i.e.* o2(OH43) and fl2(OH43). These inbreds actually had an increase in degradability parameters with maturity. The magnitude of interactions on TDMD was reduced probably as a result of the compensatory degradation that occurred post-ruminally, as discussed earlier. Westgate (1994) and Paulsen et al. (2003) reported that corn plants of different genetic background modify their inherent physiological and biochemical processes differently in response to environmental stresses of temperature and moisture. This will affect the final kernel endosperm hardness properties, and hence degradability.

4.3. Correlation coefficients

4.3.1. Endosperm physico-chemical properties

Correlations between physico-chemical properties of the germplasm show interesting relationships that may be exploited in hybrid selection trials. The negative correlation between starch content and most corn traits, especially CP content has also been reported by Se'ne et al. (2001). Further, they also found vitreousness to be poorly correlated to CP ($r=0.3$), which agrees with our data. Several authors have reported similar poor correlations between protein content and kernel physical characteristics (Abdelrahman and Hoseney, 1984; Dorsey-Redding et al., 1991; Mestres et al., 1991). Philippeau et al. (2000) reported α , β , and δ -zeins to be positively correlated ($P<0.01$) with vitreousness, whereas true glutelins were negatively correlated ($P<0.01$) with vitreousness. Zeins are the predominant protein fraction, averaging 680 g/kg of total protein and glutelins 20 g/kg (Philippeau et al., 2000). We hypothesize that the opposing directions of the relationship between zeins (positive) and true glutelins (negative), could have reduced the correlations of protein content with vitreousness and density, since total protein was analysed. This shows the need to conduct more detailed characterization of endosperm proteins in the 33 germplasm lines. Identification of protein type could allow corn breeders to select or genetically modify corn germplasm for low vitreousness and improved starch degradability. Nonetheless, inbreds which had lower density, lower protein content, and lower vitreousness ratings included o2(OH43), fl2(OH43), h1(OH43), and CHO5015:N15-8-1-B. The higher protein content of the more vitreous endosperm allows the cysteine-rich prolamins to be spatially closer to each other and be prone to intermolecular disulphide-bonding that produce tighter protein networks. This gives the hard endosperm characteristic and restricts accessibility of starch granules to microbial and enzymatic attack.

4.3.2. Endosperm characteristics and degradability

The strong negative correlation between vitreousness and all degradability parameters agrees with other findings (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002; Ngonyamo-Majee et al., 2004). After taking note of the influence that *su2* and *wx1su2* mutations had on the correlation, we removed this data which improved ($P < 0.01$) *r*-value for both harvest stages (Figs. 1 and 2). The inbred *su2*(Oh43) and its double mutant *wx1su2*(Oh43) showed high vitreousness as well as high degradability values for the two harvest stages. This suggests that still other factors affect degradability. This could be associated with the *su2* mutation, common in both inbreds, which gives a hard- and glassy-translucent endosperm with a wrinkled pericarp. The sugary mutant causes an accumulation of water-soluble polysaccharide (WSP or phytoglycogen; Marshall and Tracy, 2003). Normal corn contains about 2 g WSP/kg DM, whereas an earlier mutant of the sugary allele (*su1*) contains 25–35 g WSP/kg DM (Marshall and Tracy, 2003). The WSP is a highly branched polysaccharide consisting of $\alpha(1-4)$ glucan with $\alpha(1-6)$ branch points, giving the sugary endosperm its smooth texture (Boyer and Shannon, 1984) and hard, vitreous endosperm. This texture creates endosperm characteristics of hard, vitreous endosperm, conflicting with the expected negative relationship between vitreousness and degradability. The ability of *su2* mutation to accumulate higher levels of sucrose compared to normal corn (Perera et al., 2001) results in higher degradability values (Willcox et al., 1994). The *wx1* mutation on the double mutant *wx1su2*, alters starch composition (*i.e.* the proportion of amylose to amylopectin) and accumulates slightly less total starch in the mature kernels than non-mutant types (Boyer and Shannon, 1984). This double mutant *wx1su2* was developed by corn breeders searching for high quality cultivars with excellent seed quality. However, this endosperm mutant combination may not result in the high quality, if the appropriate background genes are not present. This agrees with our findings as the double mutant failed to show any improvement on degradability compared to the single mutant *su2*(Oh43).

The other kernel properties of density and CP content also showed strong negative correlations with degradability, which is not surprising, as kernel density and CP were positively correlated with vitreousness. Nonetheless, vitreousness accounted for more variation in digestibility than kernel density or CP content.

5. Conclusions

Although effects of both maturity stage and corn germplasm type were observed for vitreousness and degradability, germplasm type had the strongest influence. This was particularly true for zero-hour disappearance and the ruminally degraded fraction. The results also suggest that endosperm carbohydrate properties other than vitreousness affect corn degradability. This was shown by the opposing relationship of kernel hardness properties (vitreousness and density) for *su2* and *wx1su2* mutants with degradability. The existence of inbreds with combinations of positive physico-chemical properties (*i.e.* high starch and protein contents and low to medium vitreousness and density) and high degradability found among some of the GEM material make them attractive as future germplasm to be exploited for developing highly nutritive (for both energy and protein) corn hybrids for silage and grain production.

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