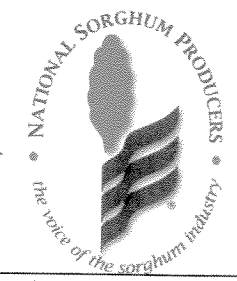
Table 1. Sorghum Samples Grown, Harvested, Processed and Shipped to NREL for Research Purposes.

Code	Entry Type	Sorghum Type	Maturity Harvest	Maturity Entry	Brown Midrib	Male Sterile	Produced In
07CMP001	Hybrid	Forage	Vegetative	PS	N	N	Bushland Tx
07CMP002	Hybrid	Forage	Vegetative	PS	N	N	Bushland Tx
07CMP003	Hybrid	Forage	Vegetative	PS	bmr12	N	Bushland Tx
07CMP004	Hybrid	Forage	Vegetative	PS	N	N	Bushland Tx
07CMP005	Hybrid	Forage	Vegetative	PS	N	N	Bushland Tx
07CMP006	Hybrid	Forage	Hard Dough	ML	N	N	Bushland Tx
07CMP007	Hybrid	Forage	Hard Dough	M	bmr18?	N	Bushland Tx
07CMP008	Hybrid	Forage	Hard Dough	ML	N	N	Bushland Tx
07CMP009	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP010	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP011	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP012	Hybrid	Forage	Hard Dough	ML	N	N	Bushland Tx
07CMP013	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP014	Hybrid	Forage	Hard Dough	ML	bmr12	N	Bushland Tx
07CMP015	Hybrid	Forage	Hard Dough	M	bmr12	N	Bushland Tx
07CMP016	Hybrid	Forage	Hard Dough	ML	N	N	Bushland Tx
07CMP017	Hybrid	Forage	Hard Dough	ML	bmr?	N	Bushland Tx
07CMP018	Hybrid	Forage	Hard Dough	ME	bmr?	N	Bushland Tx
07CMP019	Hybrid	Forage	Hard Dough	M	bmr?	Y	Bushland Tx
07CMP020	Hybrid	Forage	Hard Dough	E	N	N	Bushland Tx
07CMP021	Hybrid	Forage	Hard Dough	L	N	N	Bushland Tx
07CMP022	Hybrid	Forage	Hard Dough	L	N	N	Bushland Tx
07CMP023	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP024	Cultivar	Sweet	Soft Dough	L	N	N	Bushland Tx
07CMP025	Cultivar	Sweet	Soft Dough	L	N	N	Bushland Tx
07CMP026	Cultivar	Sweet	Soft Dough	L	N	N	Bushland Tx
07CMP027	Cultivar	Sweet	Soft Dough	L	N	N	Bushland Tx
07CMP028	Cultivar	Sweet	Soft Dough	L	N	N	Bushland Tx
07CMP029	Hybrid	Forage	Hard Dough	M	N	N	Bushland Tx
07CMP030	Hybrid	Forage	Hard Dough		N	N	Bushland Tx
07CMP031	Hybrid	Forage	Hard Dough	M	bmr6?	N	Bushland Tx
07CMP032	Hybrid	Forage	Hard Dough	ML	bmr6?	N	Bushland Tx
07CMP033	Hybrid	Grain	Mature	M	N	N	Bushland Tx
07CMP034	Hybrid	Grain	Mature	M	N	N	Bushland Tx
07CMP055	Hybrid	Forage	Vegetative	PS	N	N	College Station Tx
07CMP099	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP106	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP107	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP108	Hybrid	Forage	Silage	PS	N	N	College Station Tx
07CMP109	Hybrid	Forage	Silage	PS	N	N	College Station Tx
07CMP112	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP113	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP114	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP115	Cultivar	Sweet	Silage	L	N	N	College Station Tx
07CMP100	Hybrid	Forage	Silage	PS	bmr12	N	College Station Tx



Code	Entry Type	Sorghum Type	Maturity Harvest	Maturity Entry	Brown Midrib	Male Sterile	Produced In
07CMP110	Hybrid	Forage	Silage	PS	N	N	College Station Tx
07CMP111	Hybrid	Forage	Silage	PS	N	N	College Station Tx
07CMP101	Hybrid	Forage	Silage	ML	bmr?	N	College Station Tx
07CMP102	Hybrid	Forage	Silage	PS	N	N	College Station Tx
07CMP103	Cultivar	Sweet	Silage	ML	N	N	College Station Tx
07CMP104	Hybrid	Forage	Silage	PI	N	Y	College Station Tx
07CMP105	Hybrid	Forage	Silage	PI	N	N	College Station Tx
07CMP035	Hybrid	Forage	Hard Dough	M	bmr6	N	College Station Tx
07CMP036	Hybrid	Forage	Hard Dough	M	N	N	College Station Tx
07CMP037	Hybrid	Forage	Hard Dough	M	bmr6	N	College Station Tx
07CMP038	Hybrid	Forage	Hard Dough	M	N	N	College Station Tx
07CMP039	Hybrid	Forage	Vegetative	PS	bmr6	?	College Station Tx
07CMP040	Hybrid	Forage	Hard Dough	ML	bmr18	N	College Station Tx
07CMP041	Hybrid	Forage	Vegetative	PS	N	?	College Station Tx
07CMP042	Hybrid	Grain	Mature	ML	bmr12	N	College Station Tx
07CMP043	Parental Line	Grain	Mature	ML	N	N	College Station Tx
07CMP044	Parental Line	Grain	Mature	ML	bmr12	N	College Station Tx
07CMP045	Hybrid	Forage	Vegetative	PS	N	N	College Station Tx
07CMP046	Hybrid	Forage	Vegetative	PS	N	N	College Station Tx
07CMP047	Hybrid	Forage	Vegetative	PS	N	N	College Station Tx
07CMP048	Hybrid	Forage	Vegetative	PS	N	N	College Station Tx
07CMP049	Hybrid	Forage	Soft Dough	M	N	PI	College Station Tx
07CMP054	Hybrid	Grain	Mature	ML	N	N	College Station Tx
07CMP062	Seed Parent	Grain	Mature	ML	N	N	College Station Tx
07CMP137	Seed Parent	Grain	Mature	ML	N	N	Halfway Tx
07CMP082	Cultivar	Sweet	Hard Dough	ML	N	N	College Station Tx
07CMP125	Cultivar	Sweet	Bagasse	ML	N	N	College Station Tx
07CMP157	Cultivar	Sweet	Bagasse	ML	N	N	College Station Tx
07CMP172	Cultivar	Sweet	Hard Dough	ML	N	N	College Station Tx
07CMP083	Cultivar	Sweet	Hard Dough	ML	N	N	College Station Tx
07CMP126	Cultivar	Sweet	Bagasse	ML	N	N	College Station Tx
07CMP158	Cultivar	Sweet	Bagasse	ML	N	N	College Station Tx
07CMP173	Cultivar	Sweet	Hard Dough	ML	N	N	College Station Tx
07CMP084	Cultivar	Sweet	Hard Dough	L	N	N	College Station Tx
07CMP127	Cultivar	Sweet	Bagasse	L	N	N	College Station Tx
07CMP159	Cultivar	Sweet	Bagasse	L	N	N	College Station Tx
07CMP174	Cultivar	Sweet	Hard Dough	L	N	N	College Station Tx
07CMP085	Cultivar	Sweet	Hard Dough	M	N	N	College Station Tx
07CMP128	Cultivar	Sweet	Bagasse	M	N	N	College Station Tx
07CMP160	Cultivar	Sweet	Bagasse	M	N	N	College Station Tx
07CMP175	Cultivar	Sweet	Hard Dough	M	N	N	College Station Tx
07CMP086	Cultivar	Sweet	Hard Dough	M	N	N	College Station Tx
07CMP129	Cultivar	Sweet	Bagasse	M	N	N	College Station Tx
07CMP161	Cultivar	Sweet	Bagasse	M	N	N	College Station Tx
07CMP176	Cultivar	Sweet	Hard Dough	M	N	N	College Station Tx
07CMP087	Cultivar	Sweet	Hard Dough	L	N	N	College Station Tx
07CMP130	Cultivar	Sweet	Bagasse	L	N	N	College Station Tx
07CMP162	Cultivar	Sweet	Bagasse	L	N	N	College Station Tx

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Code	Entry Type	Sorghum Type	Maturity Harvest	Maturity Entry	Brown Midrib	Male Sterile	Produced In
07CMP177	Cultivar	Sweet	Hard Dough	L	N	N	College Station Tx
SB2223G	Hybrid	Forage					Plainview, TX

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MEMO

To: Dr. Jeff Dahlberg
National Sorghum Producers
From: Ed Wolfrum
Date: June 17, 2008
Subject: Results of NIR study of Sorghum Samples

We have collected NIR spectra of the sorghum samples provided to us by the National Sorghum Producers (NSP). We have also performed wet chemical analysis of four sorghum samples. This memo summarizes the results to date, and makes recommendations for samples chosen for subsequent wet chemical analysis based on the NIR spectroscopy results. We recommend a discussion of these results among the interested parties prior to selecting subsequent samples for compositional analysis.

Wet Chemistry

All wet chemical analysis was performed by Mr. Justin Sluiter. Complete wet chemical analysis was completed on samples CMP07002, 005, 101, and 102 (for the balance of this memo, the first 5 characters of the sample names will be deleted). Compositional analysis is underway for sample 015, but it is not yet complete. This sample did not extract well with our Dionex Automated Solvent Extractor (ASE-200), and we had to perform a traditional Sohxlet extraction. We will be providing these results very shortly.

The results of the compositional analysis of the first 4 samples were provided to the NSP previously. The summary results are shown below. The column "all nonstruct" is the sum of "% non-structural inorganics", "%sucrose", "%water extractives other", and %ethanol extractives". The column "S+E+W" is the sum of "%sucrose", "water extractives other", and "%ethanol extractives".

The glucan composition varies quite a bit, as does the extractives content. Note the inverse correlation between these two components. Since the mass closure must equal 100%, higher structural carbohydrate values will typically yield lower extractives content. There is little variation in lignin content among the four samples analyzed.

Sample	%Structural Inorganics	%Non-structural inorganics	%Structural Protein	% Sucrose	%Water Extractable Others	%Ethanol Extractives	% Lignin	% Glucan	% Xylan	Total %	All nonstruct	S+E+W
002	5.50	4.40	0.00	3.85	13.52	3.20	14.44	32.44	17.36	99.84	24.96	20.56
005	5.28	3.77	0.00	4.94	14.74	3.31	14.20	31.51	17.18	100.19	26.77	23.00
101	5.60	3.75	0.00	0.52	12.12	2.88	13.92	38.01	21.60	101.83	19.27	15.52
102	4.11	3.49	0.00	1.23	7.90	3.11	14.18	40.53	22.50	101.71	15.73	12.24

NIR Spectroscopy

All NIR spectroscopy was performed by Mr. Ryan Ness. A FOSS 6500 NIR spectrometer was used to collect spectra in reflectance mode. The samples were scanned in random order of the course of three days. There were 11 sample names for which we had two samples: 82, 83, 84,125, 126, 130, 157, 158, 162, 172, 173, and 182. We arbitrarily appended the letter "B" to the second replicate sample scanned. We understand from Prof. Rooney that these are "replicates and regrowth samples".

The spectra were exported in ASCII format and combined with the sample information file provided by the NSP. The merged dataset was imported into the multivariate statistical analysis package Unscrambler 9.7 (Camo USA) for principal component analysis (PCA). The main outputs of a PCA analysis are the so-called "scores plots", which show the relationship among samples in "principal component space". Samples that are close together in a PCA scores plot have similar spectra; samples far apart in a PCA scores plot have dissimilar spectra.

Figures 1-4 show the results of a principal component analysis (PCA) on the NIR spectral data. The only difference between these plots is the manner in which the points are labeled.

In Figure 1, duplicate NIR scans are shown in RED, while NIR scans of the "replicates and regrowth samples" are shown in GREEN. The variability in the duplicate NIR scans is slightly higher than typical, but preprocessing of the spectra prior to PCA analysis removes the majority of the variance (data not shown). Note that some of the replicates do not lie near each other. While sample 157 lies at the extreme right-hand side of the plot, its replicate (157B) is at the extreme top of the plot. This is also true of samples 158 and 158B, 84 and 84B, and 173 and 173B. It will be useful to learn the history of these samples to see how similar these "regrowth/replicates" are.

In Figure 2, the samples for which we have wet chemistry data are colored RED. The two samples with the lower glucan composition (002 and 005) are in the lower part of the plot, while the two samples with the higher glucan composition (101 and 102) are in the upper part of the plot. This may indicate that glucan composition is somehow captured in the 2nd rather than the 1st principal component (PC2 rather than PC1). These four samples are very close to the y-axis, which corresponds to little variability along the first principal component (PC1), which represents over 50% of the variability in the spectral data.

In Figures 3 and 4, the PCA results are grouped by sorghum type and growth location, respectively. We see some segregation of the results by both of these variables. Figure 3 shows that no forage sorghum samples have PC1 values greater than 15, while sweet sorghum samples have PC1 values up to approximately 50. Figure 4 shows that samples from College Station more variation along PC1 than for the Bushland samples. We do not know whether this is an environmental effect or whether the College Station samples were simply more genetically diverse.

We have found that variation in NIR spectra often corresponds to variation in composition. Thus, we recommend performing compositional analysis on a group of samples that span principal component space. That is, we recommend samples at the extremes of the x- and y-axes, along with some samples centrally located. Looking at Figure 1, we can see that samples 46-49, 84, and 173B are on the extreme left-hand side of Figure 1, while samples 172-177, 86, and 157 all are on the extreme right-hand side of the plot. Analyzing one or more samples from each of these groups would be beneficial. It would be sensible to consider genetic and environmental factors as well when deciding which samples should be subject to wet chemical analysis, so a discussion of the causes of the segregation seen in Figures 3 and 4 would be useful

Conclusions & Recommendations

The preliminary NIR and compositional analysis work show compositional variability among the sorghum samples. Additional compositional analysis work should be focused on those samples showing the greatest variability in NIR spectral signature. However, an informed choice of samples will require consideration of the environmental and genetic nature of the samples, so it would be very useful to hold a conversation with all interested parties prior to selecting the next set of samples for compositional analysis.

***Figure 1**. PCA scores plot of the NIR spectral data. The samples in RED are duplicate NIR scans of the same sample. The samples in GREEN are the identically-labeled replicate samples. The second sampled scanned was arbitrarily labeld with a B.*

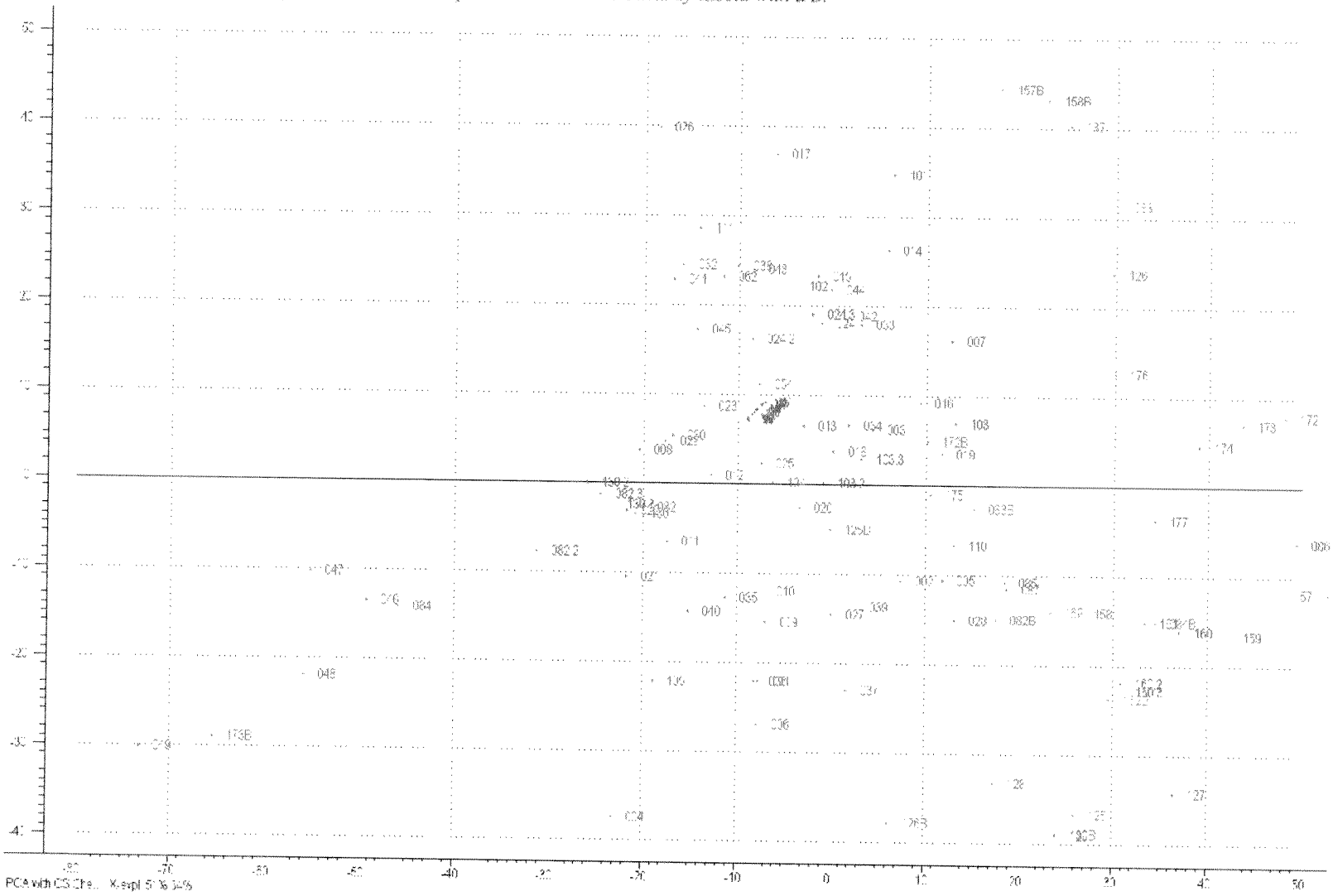

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***Figure 2**. PCA scores plot of the NIR spectral data. The samples in RED have wet chemistry data. The glucan compositions are superimposed on the plot.*

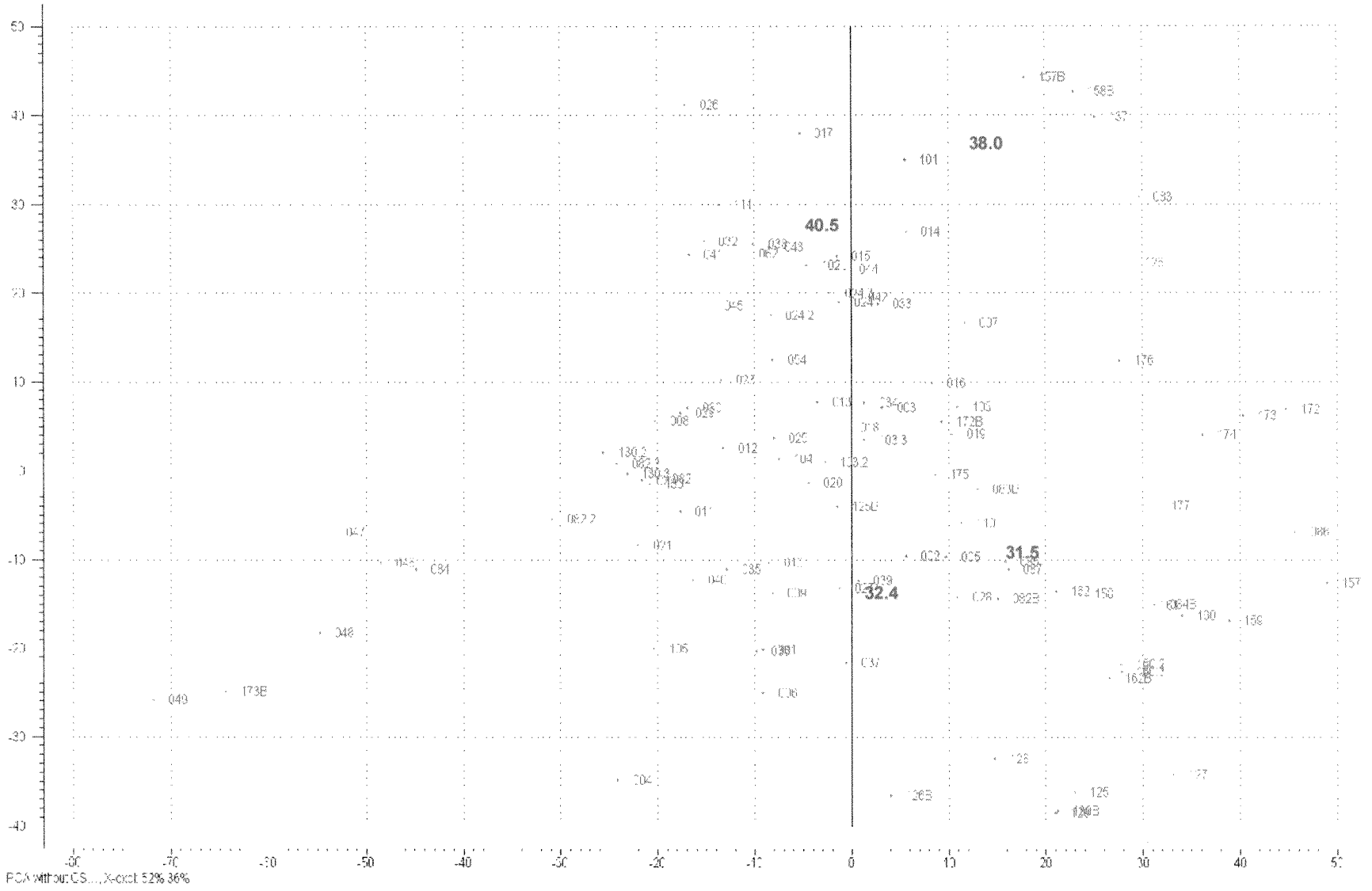


***Figure 3**. PCA scores plot of the NIR spectral data. These are the same data as Figure 1, grouped by sorghum type. RED=FORAGE, PURPLE=SWEET, GREEN=GRAIN. There appears to be some segregation along PC1 between forage and sweet sorghum types, with no forage sorghums having PC1>20. This suggests more spectral variation in this direction for the sweet sorghums relative to the forage sorghums.*

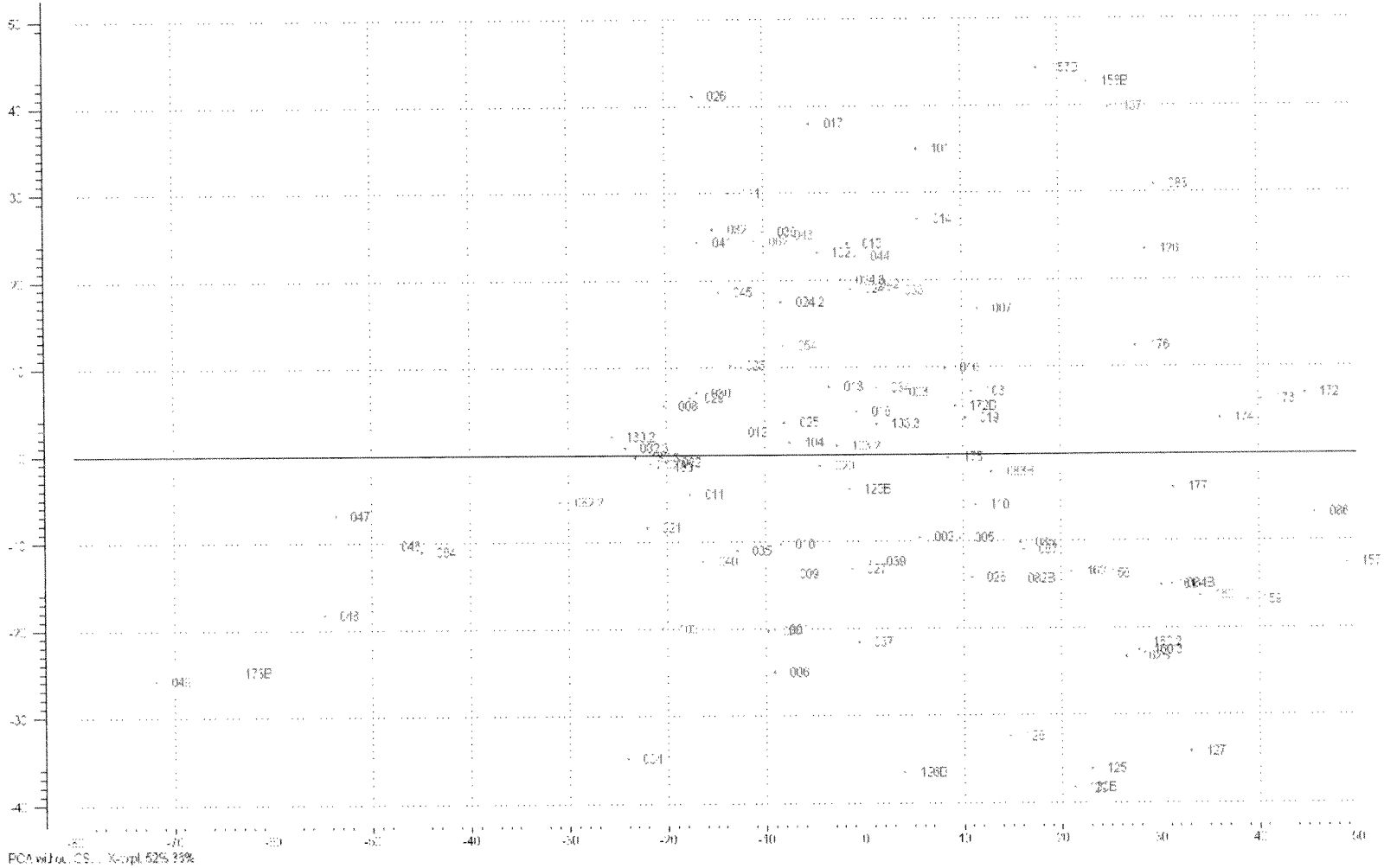
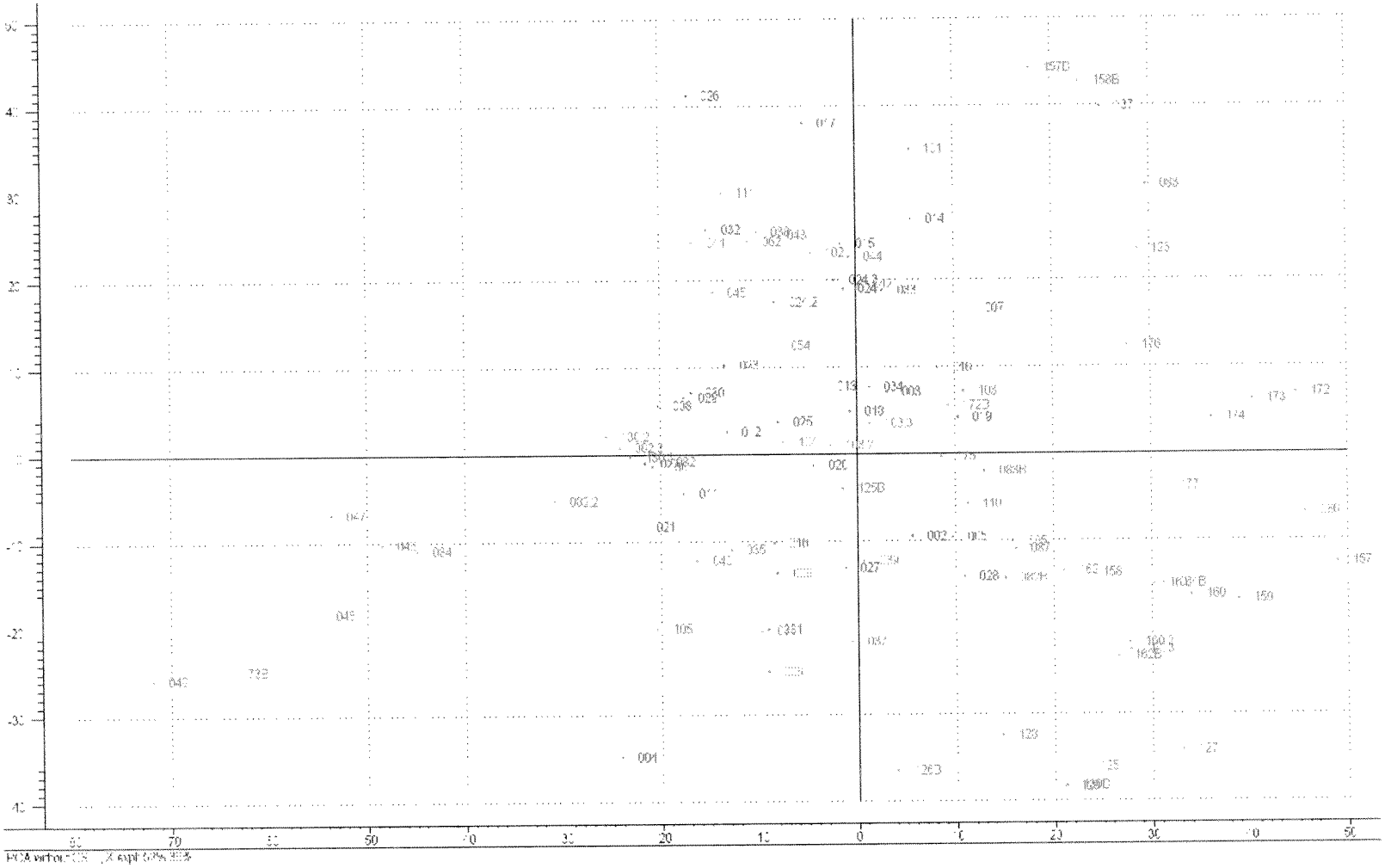


Figure 4. *PCA scores plot of the NIR spectral data. These are the same data as Figure 1, grouped by location, RED=BUSHLAND, GREEN=COLLEGE STATION, PURPLE=HALFWAY. There appears to be more spectral variation along PC1 in the College Station sample relative to the Bushland samples.*



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National Renewable Energy Laboratory

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MEMO

To: Dr. Jeff Dahlberg
National Sorghum Producers
From: Ed Wolfrum
Date: July 18, 2008
Subject: Results of Compositional Analysis of Sorghum Samples

This memo summarizes the results of the compositional analysis of sorghum samples provided by the NSP to NREL. Mr. Justin Sluiter, Mr. Ryan Ness and Ms. Courtney Payne performed the compositional analyses. Mr. Justin Sluiter and I reviewed the data for consistency and accuracy.

The results are summarized in Table 1. Each entry in this table is the average of 2 duplicate analyses. Five samples, 07CMP002, 07CMP005, 07CMP015, 07CMP101, and 07CMP102 were analyzed for starch. Only one of these, 07CMP015, contained starch. This sample did not extract well on our Dionex ASE 200 automated solvent extractor. We have seen in the past that high-starch samples extract very poorly on the ASE 200. Instead of using the ASE, we performed a 24-hour sequential Soxhlet extraction on this sample, and then analyzed for starch. Results indicated the sample had 22.2% starch by mass. This is apportioned in the Table below as glucan. Thus, the actual structural glucan in this sample is 44.2%-22.2%=20%.

As part of our internal quality assurance procedures, we analyzed sample 07CMP043 three times (once by one analyst, twice by another). The results are shown highlighted in the table. This is a fair representation of the precision of our methods.

Protein analyses are not yet complete; we send samples out to a commercial analytical laboratory for nitrogen analysis, and then use a protein-nitrogen conversion factor to estimate total protein. These data should be available within 2 weeks.

We are currently investigation how these results correlate with the NIR spectral data we previously collected.

Table 1. Compositional Analysis Results.

NOTES:

1. Sample 07CMP015 was analyzed for starch. Results indicated the sample had 22.2% starch by mass. This is apportioned in the table as glucan. Thus, the actual structural glucan in this sample is 44.2%-22.2%=20%. No other samples contained starch.

Sample	%Structural Inorganics	%Non-structural inorganics	% Sucrose	%Water Extractable Others	%Ethanol Extractives	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Fructose	Acetyl	Total %	% Solubles	% Structurals
07CMP002	5.50	4.40	3.85	13.52	3.20	14.44	32.44	17.36	0.53	2.36	0.75	1.50	99.84	24.96	74.88
07CMP004	4.18	4.64	4.24	14.06	3.11	13.29	29.08	17.33	0.71	1.64	0.00	2.18	94.47	26.05	68.42
07CMP005	5.28	3.77	4.94	14.74	3.31	14.20	31.51	17.18	0.62	2.27	0.44	1.91	100.19	26.77	73.42
07CMP015	4.39	4.46	1.56	10.93	2.90	9.56	44.22	13.30	0.71	1.54	0.50	1.28	95.36	19.84	75.51
07CMP026	4.70	1.95	3.53	15.23	3.58	13.91	32.54	14.54	0.84	1.63	0.00	2.09	94.53	24.29	70.25
07CMP039	3.14	3.31	1.84	8.23	3.51	16.88	35.62	18.82	0.61	1.31	0.00	2.36	95.64	16.90	78.74
07CMP043	4.86	2.69	0.10	8.59	3.32	15.14	36.55	21.13	0.75	1.87	0.00	2.08	97.07	14.69	82.38
07CMP043	4.61	2.86	0.76	7.64	3.02	15.16	35.99	20.90	0.62	1.31	0.00	2.79	95.67	14.29	81.39
07CMP043	4.56	3.08	2.11	6.31	2.81	15.41	36.89	21.26	0.73	2.01	0.00	2.10	97.26	14.31	82.95
07CMP045	5.19	3.21	0.27	10.96	3.38	13.44	36.21	19.70	0.87	2.09	0.00	1.28	96.61	17.83	78.78
07CMP047	3.69	3.17	0.30	15.11	4.37	15.85	32.68	17.11	0.94	1.89	0.00	1.75	96.87	22.95	73.92
07CMP049	3.22	4.17	0.22	11.16	3.66	15.85	33.58	18.01	0.87	1.86	0.00	1.56	94.16	19.21	74.95
07CMP083	2.41	2.26	0.36	16.83	3.51	16.90	31.70	17.41	0.89	1.75	0.00	2.36	96.38	22.96	73.42
07CMP101	5.60	3.75	0.52	12.12	2.88	13.92	38.01	21.60	0.65	1.93	0.18	0.67	101.83	19.27	82.56
07CMP102	4.11	3.49	1.23	7.90	3.11	14.18	40.53	22.50	0.59	1.96	0.31	1.81	101.71	15.73	85.97
07CMP105	4.92	2.37	0.66	21.66	4.47	12.81	29.72	15.42	0.69	1.79	0.00	1.78	96.29	29.15	67.14
07CMP110	4.03	4.17	1.24	6.97	3.14	17.41	36.65	19.02	0.67	1.88	0.00	1.30	96.46	15.51	80.95
07CMP127	2.05	0.95	17.21	13.59	1.85	11.50	31.51	14.74	0.56	1.34	0.00	2.10	97.38	33.59	63.79
07CMP157	2.41	0.59	21.27	18.83	2.26	9.52	24.90	12.63	0.70	1.67	0.00	1.86	96.64	42.95	53.69
07CMP172	4.59	-0.06	21.19	20.63	3.00	9.58	21.61	11.89	0.67	1.77	0.00	1.63	96.49	44.76	51.73
SB2223G	5.89	1.05	14.40	16.56	3.37	10.75	23.17	15.48	0.86	2.42	0.00	2.16	96.12	35.37	60.75
min	*2.05*	*-0.06*	*0.10*	*6.31*	*1.85*	*9.52*	*21.61*	*11.89*	*0.53*	*1.31*	*0.00*	*0.67*	*94.16*	*14.29*	*51.73*
mean	*4.25*	*2.87*	*4.85*	*12.93*	*3.23*	*13.80*	*33.10*	*17.49*	*0.72*	*1.82*	*0.10*	*1.84*	*97.00*	*23.87*	*73.12*
max	*5.89*	*4.64*	*21.27*	*21.66*	*4.47*	*17.41*	*44.22*	*22.50*	*0.94*	*2.42*	*0.50*	*2.79*	*101.83*	*44.76*	*85.97*