National Corn-to-Ethanol Research Center

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Laboratory Research Division

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Investigation of the Impact of Particle Size on DDGS Proximate Analysis

Purpose of Study

This study investigates the impact of particle size on the analysis of crude protein and crude fat in Distiller's Dried Grains with Solubles (DDGS). AOAC recommends that animal feed samples be ground to pass a 2 mm screen if they contain heat-sensitive analytes (AOAC method 950.02), but commercial laboratories may routinely grind samples to pass a smaller screen. Particle size is important because it may affect the calibration of indirect measurement instruments (such as NIR), and there is preliminary evidence that some direct analytical methodologies such as the determination of fat, fiber or starch are sensitive to it.

Experimental Procedure

A DDGS sample with medium range crude protein level was selected from the DDGS Library at NCERC. The protein level chosen as intermediate was based on AFIA sample ranges to be between 27 and 28%. The sample was mechanically stirred to render it homogeneous and a particle size analysis was performed on an unground 100 g subsample portion (Table 1). Then, the entire DDGS sample was subdivided into five portions and each ground through one of the following screen sizes: 2.0 mm, 1.4 mm, 1.0 mm and 0.425 mm, corresponding to sieve sizes 10, 14, 18 and 40 respectively. The 1.4 mm (14) screen size was selected instead of 1.5 because 1.5 mm mesh sieves are not readily available commercially.

Crude protein tests were performed on each particle size portion including the original unground sample. The test method was the AFIA recommended method AOAC 990.03, and ten replicates from each particle size portion were tested: 50 samples in total + 10 QC samples. Similarly, crude fat tests were performed on each particle size portion including the original unground sample. The test method was the AFIA recommended method AOAC 945.16, and ten replicates from each particle size portion were tested: 50 samples in total + 10 QC samples.

Data Analysis

The data collected is reported on Table 1, Table 2, Table 3, Figure 1 and Appendix 1 for the ANOVA and F-test reports.

Sieve Size #	Opening Diameter (mm)	Mass left on sieve (%)
10	2.00	1.3
14	1.40	10.2
18	1.00	21.1
40	0.425	54.3
bottom	--	13.4

Table 1: Particle Size Distribution

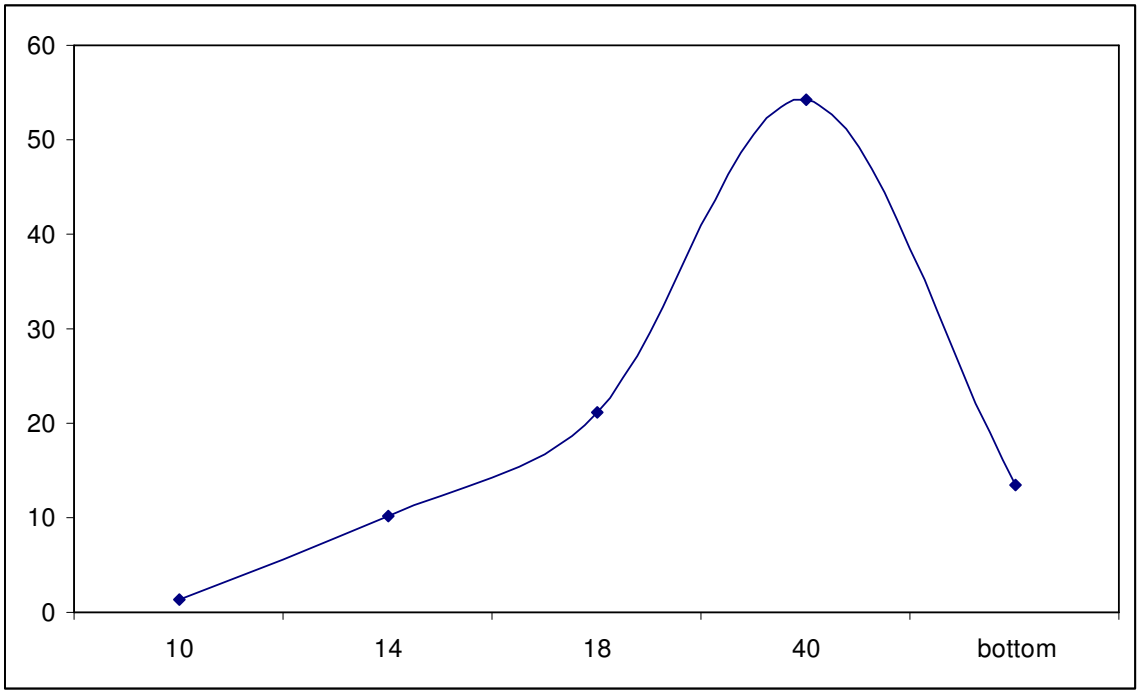

Figure 1: Cumulative Particle-size distribution

The particle size distribution of the unground sample (Table 1, Fig. 1) shows the majority of the sample falling between particle sizes of 1.4 mm to 0.425 mm. It is important to note that particle size reflects an upper boundary, and does not indicate that all the particles going through a particular size will be the same size. There is very little sample greater than 2 mm, so we can consider for this sample the unground portion to be similar enough to the 2 mm portion. We also see this reflected in the proximate data.

In this study, we wanted to check how tightly the given particle sizes are clustered together. The data shows that the lowest %RSD for both analyses corresponds to the smallest particle size, namely 0.425 mm. For the protein analyses we see a slight increase

in the average compositions with decreasing particle size, while in the fat analyses we don't see a similar trend for sizes up to 1 mm, although we see variations. We do observe a dramatic increase in overall fat *content* when particle size decreases from 1 mm to 0.425 mm.

We compared the highest RSD (1-mm) to the lowest (0.425-mm) to see if the difference is significant. A rough estimate suggests that the RSD for 0.425-mm fat is significantly smaller than the RSD for 1-mm fat, but the RSD for 0.425-mm protein is not different from the RSD for the 2-mm protein.

To test whether the differences in analysis according to particle size were statistically significant on the whole, we performed analysis of variance (ANOVA) at the 0.05 level (see Appendix 1). The data shows that particle size is a statistically significant parameter in both fat and protein analysis. However, an F-test (P= 0.05) between different particle sizes show no clear trend; for example, the difference is most pronounced between 0.425-mm and 1-mm but no difference is noted with the F-test between unground and 0.425-mm samples (Appendix 1). This is an indication that particle size is not the only factor affecting the differences noticed. Method sensitivity is another possible cause for the variability of the data, since fat extraction is improved by an increase in overall surface area.

	Unground		**2.00**		**1.40**		**1.00**		**0.425**	
	Protein	Fat	Protein	Fat	Protein	Fat	Protein	Fat	Protein	Fat
1	28.11	9.15	27.74	9.24	28.46	8.91	29.11	9.20	28.86	9.95
2	27.52	9.15	28.35	9.26	27.48	9.00	27.69	8.08	28.17	10.14
3	26.98	9.01	26.92	9.53	28.31	9.14	26.16	8.37	28.58	10.09
4	27.44	8.79	26.77	8.51	27.97	8.78	28.55	8.95	28.55	10.06
5	27.93	9.33	27.48	9.31	28.32	9.20	28.02	8.44	28.38	10.12
6	27.55	9.33	26.96	8.96	28.14	9.26	26.31	9.96	28.23	10.24
7	28.08	9.61	28.17	9.04	27.68	9.25	25.11	9.25	28.42	10.08
8	27.00	9.09	26.74	9.65	28.12	9.41	27.40	8.37	28.26	10.32
9	26.74	9.45	27.69	8.20	27.25	9.09	27.75	8.24	27.87	10.43
10	26.94	9.37	28.60	9.50	25.74	9.23	26.69	9.67	28.82	10.01
AVG	27.43	9.23	27.54	9.12	27.75	9.13	27.28	8.85	28.41	10.14
RSD	**1.83**	**2.57**	**2.48**	**5.06**	**2.91**	**2.05**	**4.43**	**7.33**	**1.06**	**1.44**

Table 2: Protein and fat analyses, with averages and RSD for all particle sizes

To test method sensitivity we used the cumulative RSD obtained from the averages for protein analyses of all particle sizes to compare to the cumulative RSD for the averages of fat analyses (Table 3). The cumulative RSD from the average of the fat analysis across particle sizes is much higher than that of the protein analysis, indicating a sensitivity to method.

Table 3: Sensitivity to method

	Ave. Protein	Ave. Fat
UG	27.43	9.23
2	27.54	9.12
1.4	27.75	9.13
1	27.28	8.85
0.425	28.41	10.14
AVG	**27.68**	**9.29**
RSD	**1.60**	**5.32**

Conclusions and Recommendations

In this study, the particle size determination showed the unground DDGS sample to be similar to the 2 mm grind size (98.7% of our sample had particle size equal to or smaller than 2 mm).

There is a statistically significant difference between the analyses of unground, 2 mm, 1.4 mm 1 mm and 0.425 mm samples, indicating that particle size affects analysis, but the trend is not clear. The variation of the analytical results also appears to be related to method sensitivity to changes in surface area of the sample. Further study is required to understand whether there are other factors affecting the observed variations and how they may be related.

Based on these findings we would recommend different strategies when analyzing for protein or fat:

- If a sufficient fraction of the DDGS particles are smaller than 2 mm, for protein analysis then there seems to be no advantage in grinding the sample, as the results show a slightly better RSD in the unground sample than the other sizes. Leaving the sample unground will also minimize the loss of moisture and preserve the integrity of volatile components. If the particle size is larger than that, a grind to pass 2 mm size would be the recommended alternative, to minimize moisture and volatiles loss.
- For crude fat analysis, the method will yield higher fat values for the smaller particle size, due to higher availability of fats with decreased size, so it should be recommended that the sample be ground to pass through a mesh smaller than 1 mm. If there is a concern about the loss of moisture and volatiles, the mesh size recommended by AOAC, 0.75 mm, would be appropriate for a more precise value of crude fat.

Appendix 1: Statistical Data

Anova: Single Factor (Protein)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
UG	10	274.29	27.43	0.25
2	10	275.42	27.54	0.47
1.4	10	277.47	27.75	0.65
1	10	272.79	27.28	1.46
0.425	10	284.14	28.41	0.09

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.86	4.00	1.97	3.36	0.02	2.58
Within Groups	26.29	45.00	0.58			
Total	34.16	49.00				

Anova: Single Factor
(Fat)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
UG	10	92.28	9.23	0.06
2	10	91.20	9.12	0.21
1.4	10	91.27	9.13	0.03
1	10	88.53	8.85	0.42
0.425	10	101.44	10.14	0.02

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.80	4.00	2.45	16.40	2.34E-08	2.58
Within Groups	6.72	45.00	0.15			
Total	16.51	49.00				

F-Test Protein

	1-mm	*0.425-mm*
Mean	27.279	28.414
Variance	1.462788	0.09156
Observations	10	10
df	9	9
F	**15.97628**	
P(F<=f) one-tail	0.000163	
F Critical one-tail	3.178893	

F-Test Fat

	1-mm	*0.425-mm*
Mean	8.853	10.144
Variance	0.421201	0.02136
Observations	10	10
df	9	9
F	**19.71915**	
P(F<=f) one-tail	6.86E-05	
F Critical one-tail	3.178893	

	UG	*0.425-mm*
Mean	27.429	28.414
Variance	0.251232	0.09156
Observations	10	10
df	9	9
F	**2.743908**	
P(F<=f) one-tail	0.074376	
F Critical one-tail	3.178893	

	UG	*0.425-mm*
Mean	9.228	10.144
Variance	0.056307	0.02136
Observations	10	10
df	9	9
F	**2.63608**	
P(F<=f) one-tail	0.082471	
F Critical one-tail	3.178893	