March, 2010

Predicting Stability of Distiller's Wet Grains (DWG) with Color Analysis

R. M. Lehman, *United States Department of Agriculture*
Kurt A. Rosentrater, *United States Department of Agriculture*

Food Bioprocess Technol (2010) 3:204–212
DOI 10.1007/s11947-008-0090-x

ORIGINAL PAPER

Predicting Stability of Distiller's Wet Grains (DWG) with Color Analysis

K. A. Rosentrater · R. M. Lehman

Received: 26 November 2007 / Accepted: 28 April 2008 / Published online: 28 May 2008

Abstract Distiller's wet grain (DWG) is one of the coproducts from the fuel ethanol industry. Although many studies have investigated the nutritional properties of DWG, little work has investigated the storability and shelf life for these feed products or how to measure these quantities. The objectives of this research were to measure the development of microorganisms and their respiration over time in freshly produced DWG and to determine if there was a quantitative relationship between these microbiological parameters and a more easily measured physical property, DWG color. The numbers of aerobic heterotrophic bacteria, molds and yeasts, and carbon dioxide generated by microbial respiration were measured at t=0, 1, 2, 4, and 7 days as were Hunter color (L, a, b) values. All of the microbial parameters increased significantly over time ($p<0.05$). Hunter L and a values appeared to change over time as well, but these differences became significant only at t=7 days; at this time period, Hunter b changed significantly also. Hunter a and b values were negatively correlated with aerobic heterotroph numbers (r=−0.74 for Hunter a; r=−0.77 for Hunter b), yeast and mold counts (r=−0.78 for Hunter a; r=−0.81 for Hunter b), and CO_2 production (r=−0.89 for Hunter a; r=−0.87 for Hunter b). Hunter L values had moderate positive correlations with the microbial parameters (r values ranged from 0.42 to 0.57). Using Hunter a and b color parameters as predictor variables, multiple linear and nonlinear regressions produced R^2 values of 0.751, 0.665, and 0.816 for aerobic heterotrophs, molds and yeasts, and CO_2 generation, respectively. Additional research should quantify spoilage criteria, the relationship to palatability, and determine how best to use color changes as indicators of each.

Keywords Biofuels · Coproducts · Degradability · Distiller's wet grains · DWG · Ethanol · Properties

K. A. Rosentrater (✉) · R. M. Lehman
USDA-ARS, North Central Agricultural Research Laboratory,
2923 Medary Ave.,
Brookings, SD 57006, USA
e-mail: krosentr@ngirl.ars.usda.gov

Introduction

The fuel ethanol industry has been growing tremendously over the last decade, and it is poised to expand even more over the next several years. As of March 2008, 143 manufacturing plants in the US had an aggregate production capacity of nearly 31.0 billion liters per year (8.2 billion gallons per year), with another 64 plants under construction or renovation, with an additional 19.7 billion liters per year (5.2 billion gallons per year) (BBI 2008; RFA 2008). Ethanol production, which is primarily corn-based in the US, results in three main product streams in roughly equal proportions: (1) fuel ethanol; (2) nonfermentable constituents; and (3) carbon dioxide. The process of converting corn to ethanol consists of several steps, including grinding, cooking, liquefying, saccharifying, fermenting, and distilling the corn grain. In-depth information can be found in Tibelius (1996), Weigel et al. (1997), Dien et al. (2003), and Jaques et al. (2003), but will not be discussed further here.

The nonfermentable components (i.e., protein fiber, lipids, and ash) are removed after fermentation, before distillation, as whole stillage. This stillage is centrifuged to remove water, which is then evaporated to produce a stream known as condensed distiller's solubles (CDS), which is then recombined with the centrifuge solids, dried to ensure a stable shelf life, and then this combined product is sold as distiller's dried grains with solubles (DDGS). This com-

posite coproduct is used for feed rations, either locally or shipped via truck or rail to reach distant customers. Sometimes these composite streams are not dried, but are instead sold locally as distiller's wet grains (DWG). The sale of both types of distiller's grains contributes substantially to an ethanol plant's incoming revenue stream and are thus vital to each plant's operations.

The use of DWG will continue to play a key role as the ethanol industry grows. Increased use of DWG (versus DDGS) in the marketplace can decrease the overall energy budget for fuel ethanol manufacturing, as it decreases the need for drying, and thus reduces subsequent environmental impacts such as greenhouse gas emissions by these operations. DWG is a feed material with highly digestible nutrients (Garcia and Kalscheur 2006; Schingoethe et al. 2002); it has been the topic of many research studies over the years, including use in beef (Ham et al. 1994; Klopfenstein 1996; Larson et al. 1993; Lodge et al. 1997; Mustafa et al. 2000; Ojowi et al. 1997; Shand et al. 1998) and dairy (Al-Suwaiegh et al. 2002; Birkelo et al. 2004; Chiou et al. 1999; Schingoethe et al. 1999) rations. However, there is also a growing interest in monogastric (especially swine) diets as well (Pedersen et al. 2005).

Several studies have examined the nutritional properties of these byproduct feeds (Belyea et al. 1998; Spiehs et al. 2002; Belyea et al. 2004; and Shurson et al. 2004). Much of the available chemical, nutritional, and physical property research on ethanol coproduct streams has been reviewed by Rosentrater and Muthukumarappan (2006).

There has been very little work aimed at quantifying storability and allowable shelf life for these feed products. As DWG remains wet until use, spoilage by microorganisms may occur rapidly, depending on ambient weather conditions. Lehman and Rosentrater (2007) investigated microbial growth in DWG samples at a commercial fuel ethanol plant. Rosentrater and Lehman (2008) measured the physical and chemical properties of these samples, and found strong correlations between microbial activity and color of DWG. Rosentrater (2006) discussed the need to develop rapid sensing techniques for ethanol coproducts, as these tools could be useful to both ethanol processing plants and livestock feeding operations. Color measurement is one such rapid measurement technique.

Because our previous work (Lehman and Rosentrater 2007; Rosentrater and Lehman 2008) indicated a linkage between DWG color and microbial growth, the goal of this study was to quantitatively test the relationships between DWG color parameters, microbial numbers, and respiration (produced by microbial activity) over time. It was our contention that microbial development in DWG could be quantified in a simple, rapid manner, so that livestock and ethanol producers will have a means to determine DWG spoilage vis-à-vis acceptability.

Materials and Methods

Experimental Setup and Sampling

The DWG for this study exited the ethanol processing plant, which was a relatively new plant in the upper Midwest, via a screw conveyer, which dropped this material onto one of several piles in an uncovered holding bunker. DWG was then transferred by a payloader into semi-trucks, which subsequently transported the DWG to local feedlots and dairies. We collected the freshly produced DWG into 2-gal Ziplocs bags. DWG was homogenized and subsamples were collected for basic physical/chemical characterization analyses. Homogenized, fresh DWG was distributed (aseptically) into sterile, plastic (100×15 mm) Petri dishes (40). The DWG in each dish had the same depth (15 mm), and the surface was uniformly flat. Dishes with DWG were arranged, uncovered, on trays on cart outside the lab building, to simulate storage conditions at nearby ethanol plants and livestock farms. Trays of dishes were protected from birds, varmints, and large insects with netting (1.6 mm mesh), but otherwise exposed to the elements. The dishes of DWG were numbered and four randomly selected dishes were removed for analysis at each sampling interval. Four dishes were randomly selected at the outset of the experiment (t=0 day) for analysis. At subsequent time points (1, 2, 4, and 7 days), four randomly selected dishes were retrieved for analyses. Ambient temperature and precipitation were recorded via an automated weather station located 1 km from the experimental site.

Initial Characterization of DWG

Bulk samples of the DWG were physically characterized at the outset of the experiment (t=0 day), and as such, were as close to sterile as possible for these types of materials. For all physical properties, each was studied using 16 replicates. Moisture content was determined following Standard Method S352.2 (ASAE 2004), using a forced-convection laboratory oven (Thelco Precision, Jovan Inc., Winchester, VA) at 103 °C for 72 h. Thermal conductivity, resistivity, and diffusivity were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, WA), that utilized the line heat-source probe technique (Baghe-Khandan et al. 1981). Bulk density was measured by filling a standard ½-l bushel tester (Seedburo Equipment Co., Chicago, IL). Color was measured using a spectrophotocolorimeter (LabScan XE, Hunter Associates Laboratory, Reston, VA) using the L-a-b opposable color scales (Hunter Associates Laboratory 2002) per manufacturer guidelines. To measure color, each Petri dish containing DWG was placed under the machine's sample observation port (which was a ½-in. diameter opening), and reflectance spectra was measured. pH was

measured following Standard Method 02–52 (AACC 2000). Chemical properties included crude protein, crude fiber, and crude fat, which were determined using Official Methods 990.03, 978.10, and 920.39, respectively (AOAC 2003), and ash content, which was measured following Standard Method 08–03 (AACC 2000). Each chemical constituent was determined using four replicates.

After all initial (i.e., at t=0) physical and chemical properties were determined, additional color and moisture measurements, in conjunction with microbial properties, were subsequently recorded at t=1, 2, 4, and 7 days.

Characterization of DWG Stability over Time

One quadrant from each plate was aseptically collected for dilution spread plating on solid microbiological growth media using standard procedures (Koch 1994). Our previous work on these same DWG (Lehman and Rosentrater 2007) and the work of others using different distiller's grain products (Nofsinger et al. 1983; Pedersen et al. 2004) indicated that yeasts and molds would be the dominant microorganisms populating DWG. Serial dilutions of the DWG were made in sterile phosphate-buffered saline (PBS: 1.18 g Na_2HPO_4, 0.223 g $NaH_2PO_4{\cdot}H_20$, and 8.5 g NaCl per liter; pH 7.5) and triplicate plates were inoculated at 10^{-1} to 10^{-6} dilutions of sample for each media. Yeasts and molds were quantified on Dichloran Rose Bengal choramphenicol agar (DRBC, Oxoid), using 100 mg/l chloramphenicol supplement (Tournas et al. 2001) and incubated in the dark at 23 °C for 7 days. Aerobic heterotrophs (bacteria plus fungi) were enumerated on plate count media (PCA, Oxoid) incubated in the dark at 23 °C for 7 days. Total numbers of colony-forming units (CFU) (average of three plates) were expressed on a gram dry weight basis by accounting for the moisture content of the DWG. CO_2 production (carbon mineralization or respiration) was used to estimate aerobic stability in a manner analogous to silage (Ashbell et al. 2002). The second quadrant was aseptically collected and 5 g (wet weight) was placed in a 250-ml serum vial and sealed. After 24 h incubation in the dark at 23 °C, a 2.5-ml headspace sample was collected via the septum and headspace CO_2 concentrations were quantified by gas chromatography (Shimadzu 14b, 2-ml injection loop, a 1/8" stainless steel Porapack Q (80/100 mesh) column operated at 60 °C, and an electron capture detector at 260 °C). The remaining two quadrants for each plate were analyzed for color (following methods previously described).

Data Analysis

Colony-forming units (CFU) for aerobic heterotrophs and yeast and molds, as well as headspace CO_2 (ppm) concentrations after 24 h of incubation, color, and moisture content were averaged for the four Petri plate samples collected at each time point and were reported along with one standard deviation (1 SD). Formal statistical analyses on all collected data were performed via Microsoft Excel v. 2003 (Microsoft Corporation, Redmond, WA), Minitab v. 14.11 (Minitab Inc., State College, PA), and SAS (SAS Institute, Cary, NC) software, using a Type I error rate (α) of 0.05, and included summary statistics, which are given as mean ± 1 SD; ANOVA and General Linear Models (to test for differences over time); Pearson linear correlation analysis; linear regression; and multiple linear and nonlinear regression.

Results and Discussion

Initial Characteristics of DWG

Physical and chemical properties of the initial DWG (i.e., at t=0) are shown in Table 1. The DWG samples studied had fairly high moisture contents, with an average of 61.74% wet basis (wb), or 161.44% dry basis (db), and were therefore highly susceptible to rapid spoilage, according to other published recommendations (Beauchat 1981) for feed materials. Because DWG moisture content is so high, storage recommendations for this coproduct stream typically range between 4 and 7 days (Tjardes and Wright 2002), depending on storage conditions (i.e., temperature, humid-

Table 1 Physical and chemical properties of distillers wet grains (DWG) at t=0[a]

Property	Mean	SD[b]
Physical		
Moisture content (% db)	161.44	4.27
Thermal		
Conductivity (W/m °C)	0.18	0.06
Resistivity (m °C/W)	5.31	0.95
Diffusivity (mm^2/s)	0.10	0.01
Bulk density (kg/m^3)	888.30	33.14
Color		
Hunter L (−)	49.69	1.21
Hunter a (−)	8.43	0.23
Hunter b (−)	24.06	0.45
Chemical		
pH	4.53	0.02
Protein (% db)	27.85	0.45
Fiber (% db)	5.90	0.48
Fat (% db)	13.03	0.10
Ash (% db)	5.20	0.08

[a] n=16 for all properties studied, except for protein, fiber, fat, and ash, which utilized n=4
[b] SD is standard deviation

Table 2 Microbial properties of distillers wet grains (DWG) over time[a]

Time (d)	Aerobic Heterotrophs (CFU/g dry mass)		Molds & Yeasts (CFU/g dry mass)		CO_2 Production (ppm)	
	Mean	SD[b]	Mean	SD[b]	Mean	SD[b]
0	4.26×10^{1} A	4.92×10^{1}	BD[c]	–	470 A	106
1	1.42×10^{4} B	2.98×10^{3}	1.88×10^{4} A	4.84×10^{3}	2230 B	175
2	4.95×10^{5} C	2.13×10^{5}	4.62×10^{5} B	7.14×10^{4}	6266 C	637
4	3.12×10^{8} D	2.24×10^{8}	4.38×10^{8} C	3.59×10^{8}	8118 D	977
7	7.22×10^{8} D	2.57×10^{8}	9.55×10^{8} D	3.15×10^{8}	17564 E	1,094

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of time was significant at the α=0.05 level for that specific dependent variable; n=4 for all microbial properties studied
[b] *SD* is standard deviation
[c] BD = below detection limit of 3 CFU/g dry mass for average of n = 4 independent replicates

ity, etc.). The other physical and chemical property results for the DWG in this study were similar to values published elsewhere (Lehman and Rosentrater 2007; Rosentrater and Lehman 2008). It is well-known that production practices vary over time at a single fuel ethanol plant, as well as between plants; this has resulted in DWG with chemical and physical properties slightly different than those that we have studied before. All initial color parameters, however, were somewhat higher: Hunter *L* of 49.69 (versus 31.81 from our other studies); Hunter *a* of 8.43 (versus 5.04); Hunter *b* of 24.06 (versus 15.24). These results indicate that the DWG for this study was initially brighter, redder, and more yellow compared to the DWG in our previous studies. All measured properties generally exhibited relatively low standard deviations, except for bulk density, which had some variation. On further examination, however, the standard deviation for bulk density (33.14 kg/m^3) corresponded to a coefficient of variation (CV) of only 3.73%, which was actually quite low.

Characteristics of DWG Stability over Time

All microbial stability properties (Table 2), as well as color values (Table 3), changed over time. In addition, all of the microbial activity data exhibited an increase in variance as their respective mean levels increased. Because of the heteroscedastic nature of these data (which is common for biological data), the data were transformed using a logarithmic transformation (i.e., Y=log (Y + 1)), according to the procedures discussed by Sokal and Rohlf (1995) and Zar (1996) before testing for mean separations. It appears that the populations of aerobic heterotrophs as well as molds and yeasts increased over time, and these differences were statistically significant (p<0.05). CO_2 production, which is an indicator of microbial activity and thus stability, significantly increased for each subsequent time point as well. This indicated that microbial growth and activity, which limit material storability, were indeed occurring in the DWG over time. In this study, the growth of microorganisms was stimulated by the relatively high daytime temperatures (> 25 °C) and several precipitation events (Fig. 1) that provided moist conditions conducive to fungal growth.

There were six rainfalls during the experiment one of which resulted in approximately 0.35 in. of rain; one resulted in 0.22 in.; three of which were between approximately 0.04 and 0.12 in, and one had approximately 0.01 in. This directly affected the moisture content of the samples over time. But, these excess moisture levels had little impact on microbial development in the samples over

Table 3 Physical properties of distillers wet grains (DWG) over time[a]

Time (d)	Hunter L		Hunter a		Hunter b		Moisture (% db)	
	Mean	SD[b]	Mean	SD[b]	Mean	SD[b]	Mean	SD[b]
0	49.69 AB	1.21	8.43 A	0.23	24.06 A	0.45	161.44 A	4.27
1	48.26 A	1.08	7.15 B	0.40	22.19 A	0.18	290.71 B	21.66
2	53.6 BC	1.13	7.4 AB	0.22	24.1 A	0.35	252.68 C	4.15
4	48.42 A	2.44	7.26 B	1.12	22.47 A	1.93	449.95 D	14.84
7	56.21 C	6.19	1.64 C	1.10	8.25 B	2.42	116.42 E	17.70

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of time was significant at the α=0.05 level for that specific dependent variable; n=4 for all properties studied, except for color properties (Hunter *L*, *a*, *b*) at t=0, which utilized n=16
[b] SD is standard deviation

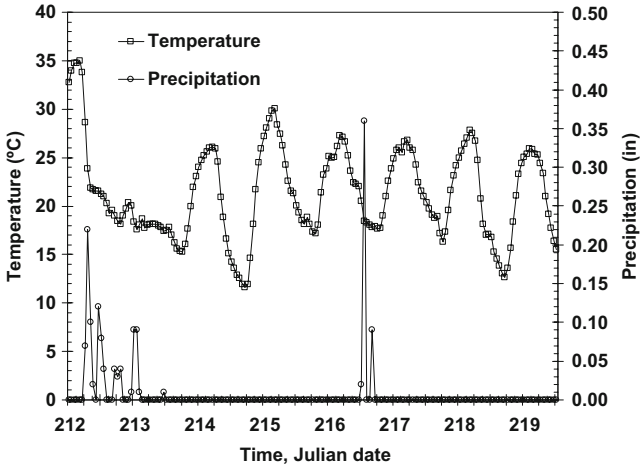

Fig. 1 Temperature and precipitation record during the experiment. Time is the Julian date (212 is July 31, t=0 day, for the initiation of the study) and is indicated on the x-axis at noon of each day

time, as the DWG already had very high levels of free water to begin with, which has been shown by our previous work (Rosentrater and Lehman 2008).

Color appeared to change over time as well, although these changes were not as straightforward as the microbial activity data. Although some differences were significant, Hunter L did not clearly change as a result of time until t=7 days. Hunter a significantly decreased at t=1 days, and then again at t=7 days. Hunter b, however, did not significantly change until t=7 days. It definitely appears that something occurred between t=4 and t=7 days. Visual inspection of the samples during this time frame indicated that these differences were caused by the visual color changes of the DWG as it aged, as well as the presence of visible mold on the surface of the DWG.

The relationships between microbial activity, color parameters, and moisture content over time were further investigated using linear correlation analysis. The 36 resulting Pearson product-moment correlations (Speigel 1994) are provided in Table 4. Twenty-two of these were significant (p<0.05); the remainder were not. The correlation coefficient (r) quantifies the strength of the linear relationship between two variables. As expected based on our previous work (Lehman and Rosentrater 2007; Rosentrater and Lehman 2008), all color values and microbial counts had strong correlations with time, as evidenced by the resulting correlation coefficients, which ranged from 0.50 to 0.98. Moreover, it appeared that the color evolution of DWG was indeed related to microbial measures, especially Hunter a and b values, which were negatively correlated with aerobic heterotroph numbers (r=−0.74 for Hunter a; r=−0.77 for Hunter b), yeast and mold counts (r=−0.78 for Hunter a; r=−0.81 for Hunter b), and CO_2 production (r=−0.89 for Hunter a; r=−0.87 for Hunter b). Hunter L values, on the other hand, did not exhibit high correlations with any of the microbial parameters (r values ranged from 0.42 to 0.57). Molds and aerobic heterotrophs are suspected to catalyze spoilage of DWG (Lehman and Rosentrater 2007). Microbial activity, as measured by CO_2 production, generally had stronger relationships with color parameters than the microbial numbers. Despite general expectations that microbial numbers and activities should be tightly linked, this is often not the case. The relationship between microbial population densities and activities of those populations has been shown to vary from non-existent to very strong (Mills and Bell 1986). As expected, moisture content over time had very low correlations with microbial development.

Table 4 Pearson linear correlation coefficients (r) between stability and color properties of DWG over time (and associated p values; with a significance level of 0.05)

	Day	Aerobic Heterotrophs	Molds & Yeasts	CO_2 Production	L	a	b	Moisture
Day	1							
Aerobic heterotrophs	0.8753 (0.0001)	1						
Molds and yeasts	0.8682 (0.0001)	0.8898 (0.0001)	1					
CO_2 Production	0.9807 (0.0001)	0.8746 (0.0001)	0.8606 (0.0001)	1				
L	0.4976 (0.0257)	0.5621 (0.01)	0.4181 (0.067)	0.5735 (0.0083)	1			
a	−0.8661 (0.0001)	−0.7381 (0.0002)	−0.7835 (0.0001)	−0.8880 (0.0001)	−0.4623 (0.0404)	1		
b	−0.8506 (0.0001)	−0.7689 (0.0001)	−0.8092 (0.0001)	−0.8686 (0.0001)	−0.4489 (0.0474)	0.9875 (0.0001)	1	
Moisture	−0.1030 (0.6666)	−0.1897 (0.4020)	−0.1730 (0.4650)	−0.2260 (0.3380)	−0.5110 (0.0210)	0.4660 (0.0380)	0.5090 (0.0220)	1

Table 5 Linear regression results for stability properties of distillers wet grains (DWG) as a function of color parameters

Response	Predictor	Intercept		Slope		R^2
		Estimate	p value	Estimate	p value	
Aerobic Heterotrophs	Hunter L	-1.99×10^{9}	0.018	4.29×10^{7}	0.010	0.277
	Hunter a	7.99×10^{8}	0.0001	-9.28×10^{7}	0.0001	0.520
	Hunter b	9.98×10^{8}	0.0001	-3.91×10^{7}	0.0001	0.569
Yeasts & Molds	Hunter L	-1.91×10^{9}	0.107	4.28×10^{7}	0.067	0.128
	Hunter a	1.12×10^{9}	0.0001	-1.32×10^{8}	0.0001	0.593
	Hunter b	1.40×10^{9}	0.0001	-5.53×10^{7}	0.0001	0.636
CO_2 Production	Hunter L	−36048	0.023	839	0.008	0.291
	Hunter a	20593	0.0001	−2140	0.0001	0.777
	Hunter b	24067	0.0001	−847	0.0001	0.741

Because correlations involving color parameters hold potential for developing predictive relationships between product color and microbial development over time, which could lead to low-cost visual sensing strategies for quality control, these potential relationships were further investigated using linear regression. As these results indicate, both the Hunter a and the Hunter b color parameters seem to fit the data fairly well. Results for the Hunter L, on the other hand, appear to be strongly influenced by a single data point (which occurred because of a single L value, measured at t=7 days). Table 5 provides parameter estimates, associated p values for each estimate, and the resulting coefficient of determination (R^2) for each of these regression lines. Linear regression results show that, overall, Hunter L did not predict any of the microbial stability parameters very well (R^2 ranged from 0.128 to 0.291), although the regression parameters were significant for aerobic heterotrophs. Hunter a, on the other hand, did predict all microbial stability parameters fairly well (R^2 ranged from 0.520 to 0.777; all regression parameters were significant). Likewise, linear regression using Hunter b predicted all microbial properties well (R^2 ranged from 0.569 to 0.741).

Multiple linear and nonlinear regressions were then pursued to investigate the possibility of increasing the predictive power of the color parameters. Statistical results for these regressions are provided in Table 6. Figure 2A displays the best fit regression surface for aerobic hetero-

Table 6 Multiple linear regression results for stability properties of distillers wet grains (DWG) as a function of color parameters

Response	Predictor	Parameter Estimate	Standard Error	t value	90% Confidence Limits		p value
Aerobic Heterotrophs	Intercept	1.35×10^{9}	1.69×10^{8}	8.014	1.06×10^{9}	1.65×10^{9}	0.0000
	Hunter a	3.41×10^{8}	1.03×10^{8}	3.304	1.62×10^{8}	5.21×10^{8}	0.0042
	Hunter b	-8.44×10^{7}	1.50×10^{7}	−5.617	-1.11×10^{8}	-5.83×10^{7}	0.0000
	Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	p value	
	Regression	1.48×10^{18}	2	7.38×10^{17}	25.64	0.0000	
	Error	4.89×10^{17}	17	2.88×10^{16}			
	Total	1.97×10^{18}	19				
Yeasts & Molds	Intercept	1.58×10^{9}	3.27×10^{8}	4.828	1.01×10^{9}	2.14×10^{9}	0.0002
	Hunter a	1.06×10^{8}	1.50×10^{8}	0.702	-1.56×10^{8}	3.68×10^{8}	0.4919
	Hunter b	-9.76×10^{7}	6.09×10^{7}	−1.601	-2.04×10^{8}	8.44×10^{6}	0.1278
	Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	p value	
	Regression	2.36×10^{18}	2	1.18×10^{18}	16.84	0.0001	
	Error	1.19×10^{18}	17	7.00×10^{16}			
	Total	3.55×10^{18}	19				
CO_2 Production	Intercept	3.14×10^{4}	7.04×10^{3}	4.458	1.91×10^{4}	4.36×10^{4}	0.0004
	Hunter a	-3.17×10^{3}	6.98×10^{2}	−4.542	-4.38×10^{3}	-1.96×10^{3}	0.0003
	Hunter b	-6.94×10^{4}	4.39×10^{4}	−1.581	-1.46×10^{5}	6.98×10^{3}	0.1324
	Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	p value	
	Regression	5.90×10^{8}	2	2.95×10^{8}	37.59	0.0000	
	Error	1.33×10^{8}	17	7.84×10^{6}			
	Total	7.23×10^{8}	19				

trophs (CFU/g dry mass) as a function of both Hunter *a* and Hunter *b*. The resulting surface equation (F=25.64) was:

$$\text{Aerobic heterotrophs} = 1.35 \times 10^9 + 3.41 \times 10^8 1\text{n}(a) - 8.44 \times 10^7 b \quad (1)$$

where *a* is Hunter *a* value (−), and *b* is Hunter *b* value (−). This equation had a resulting R^2 of 0.751, which was substantially higher than any of the color parameters individually (0.277, 0.520, 0.569 for Hunter *L*, *a*, and *b*, respectively; Table 5), and thus provides a fairly comprehensive approach to predicting aerobic heterotrophs in DWG using color analysis. Figure 2B shows the best fit surface for molds and yeasts (CFU/g dry mass) as a function of Hunter *a* and *b*. This equation (F = 16.84) was:

$$\text{Molds \& yeasts} = 1.58 \times 10^9 + 1.06 \times 10^8 a - 9.76 \times 10^7 b \quad (2)$$

where *a* is Hunter *a* value (−), and *b* is Hunter *b* value (−). This equation had an R^2 of 0.665, which was only slightly higher than those predicted by either Hunter *a* (0.593) or Hunter *b* (0.636) separately (Table 5). As the individual regression coefficients were determined not be significant, it appears that prediction of yeasts and molds in DWG would probably be predicted better using linear regression of the individual Hunter *a* and *b* color parameters. Figure 2C displays the best fit regression surface for CO_2 production (ppm) as a function of both Hunter *a* and *b*. The resulting surface (F=37.59) was:

$$CO_2 = 3.14 \times 10^4 - 3.17 \times 10^3 a - 6.94 \times 10^4 / b \quad (3)$$

where *a* is Hunter *a* value (−), and *b* is Hunter *b* value (−). This equation had an R^2 of 0.816, which was only slightly greater than either Hunter *a* (0.777) or Hunter *b* (0.741) individually (Table 5), and the coefficient estimate for Hunter *b* was determined to not be significant. Thus, it appears that CO_2 production is better predicted using the individual Hunter *a* and *b* color parameters.

Consequently, it appears that Hunter *a* and Hunter *b* can be successfully used to predict the microbial stability of DWG. Of course, each batch of DWG will vary somewhat in color, and the baseline (i.e., at t=0 day) will necessarily need to be quantified. Hunter *a* and *b* appear to individually predict microbial stability better than Hunter *L*, but multiple linear and nonlinear regression appear to have an even greater ability to predict microbial development, particularly activities, over time.

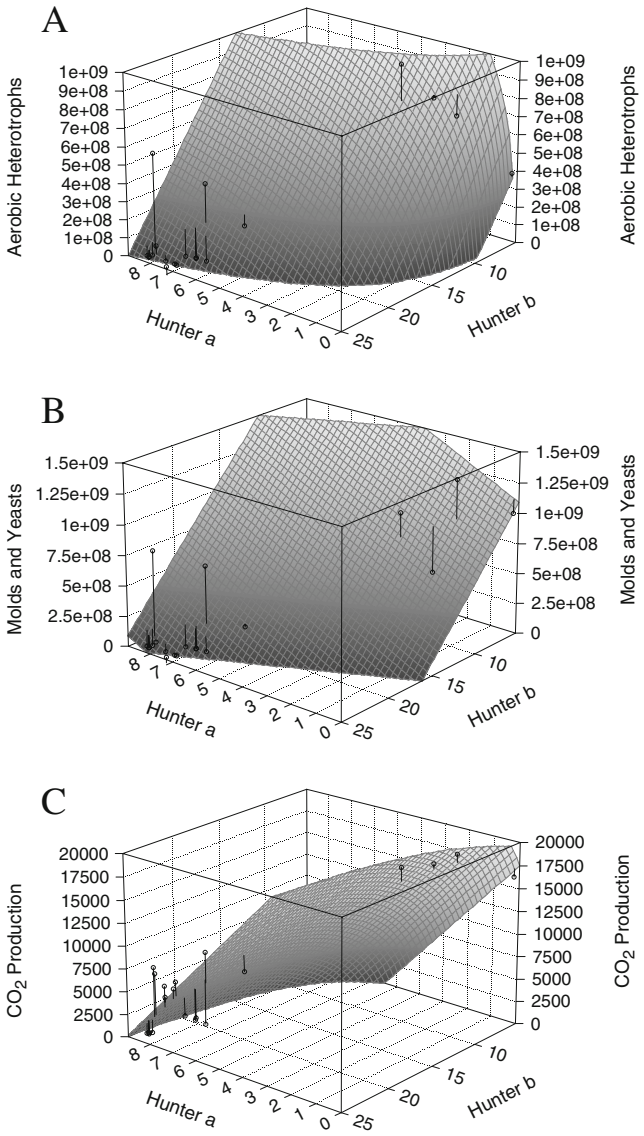

Fig. 2 Color parameters can be simultaneously used as an indicator of DWG storage stability. (A) Aerobic heterotrophs (CFU/g dry mass) as a function of Hunter *a* and *b* values. (B) Molds and yeasts (CFU/g dry mass) as a function of Hunter *a* and *b* values. (C) CO_2 production (ppm) as a function of Hunter *a* and *b* values

Conclusions

Many studies have investigated the use of distiller's wet grains (DWG) in livestock diets, but little work has been pursued outside this domain. We have quantified the increase in microbial numbers and activity over time in DWG. Concurrently, color changed as well, and this may be related to the microbial changes that occurred. We measured both simultaneously over a 7-day period, and we found that Hunter *a* and *b* values were negatively correlated with aerobic heterotroph numbers, yeast and mold counts, and CO_2 production, and thus these color parameters may be viable indicators of microbial activity in DWG. Hunter *L* values, on the other hand, did not exhibit high correlations with any of the microbial properties that we measured. Using color parameters as predictor variables with both

linear regression as well as multiple linear and nonlinear regression, it appears that Hunter *a* and Hunter *b* can be successfully used to predict the microbial stability of DWG. Of course, each batch of DWG will vary in terms of color and initial microbial populations, as well as physical and chemical properties, thus baseline data for each DWG sample will need to be quantified. Although with this study progress has been made toward developing spoilage criteria for DWG (as none has yet been established), further studies on allowable shelf life for DWG under various environmental conditions, as well as palatability, and ultimate safety for livestock consumption, are warranted.

Acknowledgements We thank the collaborating ethanol processing plant for access to their facility and for samples for our experiment. We gratefully acknowledge technical assistance from Jenna Carsrud, Vanessa Cheesbrough, Amy Christie, and Kendra Jensen. And, we thank Theron Cooper for many insightful discussions regarding distiller's grains.

Disclaimer Mention of a trade name, propriety product, or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply approval of a product to the exclusion of others that may be suitable.

References

AACC (2000). *Approved methods of the American association of cereal chemists* (10th ed.). St. Paul, MN: American Association of Cereal Chemists.

Al-Suwaiegh, S., Fanning, K. C., Grant, R. J., Milton, C. T., & Klopfenstein, T. J. (2002). Utilization of distillers grains from the fermentation of sorghum or corn in diets for finishing beef and lactating dairy cattle. *Journal of Animal Science*, *80*, 1105–1111.

AOAC (2003). *Official methods of analysis of AOAC international* (17th ed.). Gaithersburg, MA: AOAC International.

ASAE (2004). *S352.2: Moisture measurement—grains and seeds. ASAE standards* (51th ed.). St. Joseph, MI: ASAE.

Ashbell, G., Weinberg, Z. G., Hen, Y., & Filya, I. (2002). The effects of temperature on the aerobic stability of wheat and corn silages. *Journal of Industrial Microbiology and Biotechnology*, *28*, 261–263.

Baghe-Khandan, M., Choi, S. Y., & Okos, M. R. (1981). Improved line heat source thermal conductivity probe. *Journal of Food Science*, *46*(5), 1430–1432.

BBI (2008). *Plant list*. Grand Forks, ND: BBI International Available at: http://www.ethanolproducer.com/plant-list.jsp. Accessed 04 March 2008.

Beauchat, L. R. (1981). Microbial stability as affected by water activity. *Cereal Foods World*, *26*, 345–349.

Belyea, R. L., Eckhoff, S., Wallig, M., & Tumbleson, M. E. (1998). Variability in the nutritional quality of distillers solubles. *Bioresource Technology*, *66*, 207–212.

Belyea, R. L., Rausch, K. D., & Tumbleson, M. E. (2004). Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. *Bioresource Technology*, *94*, 293–298.

Birkelo, C. P., Brouk, M. J., & Schingoethe, D. J. (2004). The energy content of wet corn distillers grains for lactating dairy cows. *Journal of Dairy Science*, *87*, 1815–1819.

Chiou, P. W. S., Chang, S. H., Chiang, J. K., Yu, B., & Chen, C. R. (1999). Studies on the use of wet sorghum distiller's grains in lactating cows. *Asian-Australasian Journal of Animal Sciences*, *12*(6), 895–900.

Dien, B. S., Bothast, R. J., Nichols, N. N., & Cotta, M. A. (2003). The U.S. corn ethanol industry: An overview of current technology and future prospects. In *The third international starch technology conference-coproducts program proceedings* (pp. 10–21). University of Illinois.

Garcia, A. D., & Kalscheur, K. F. (2006). *Ensiling wet distillers grains with other feeds. Extension extra 4029*. Brookings, SD: South Dakota State University.

Ham, G. A., Stock, R. A., Klopfenstein, T. J., Larson, E. M., Shain, D. H., & Huffman, R. P. (1994). Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. *Journal of Animal Science*, *72*, 3246–3257.

Hunter Associates Laboratory (2002). *Universal software user's manual (Version 2.5)*. Reston, VA: Hunter Associates Laboratory.

Jaques, K. A., Lyons, T. P., & Kelsall, D. R. (2003). *The alcohol textbook*. Nottingham, UK: Nottingham University Press.

Klopfenstein, T. (1996). Distillers grains as an energy source and effect of drying on protein quality. *Animal Feed Science and Technology*, *60*, 201–207.

Koch, A. L. (1994). Growth measurement. In P. Gerhardt, R. Murray, W. Wood, & N. Krieg (Eds.), *Methods for general and molecular bacteriology* (pp. 248–277). Washington, D.C.: American Society for Microbiology.

Larson, E. M., Stock, R. A., Klopfenstein, T. J., Sindt, M. H., & Huffman, R. P. (1993). Feeding value of wet distillers byproducts for finishing ruminants. *Journal of Animal Science*, *71*, 2228–2236.

Lehman, R. M., & Rosentrater, K. A. (2007). Microbial development in distillers wet grains produced during fuel ethanol production from corn (Zea mays). *Canadian Journal of Microbiology*, *53*, 1046–1052.

Lodge, S. L., Stock, R. A., Klopfenstein, T. J., Shain, D. H., & Herold, D. W. (1997). Evaluation of corn and sorghum distillers byproducts. *Journal of Animal Science*, *75*, 37–43.

Mills, A. L., & Bell, P. (1986). Determination of individual organisms and their activities in situ. In R. Tate (Ed.), *Microbial autecology: A method for environmental studies* (pp. 27–60). New York: John Wiley & Sons, Inc.

Mustafa, A. F., McKinnon, J. J., & Christensen, D. A. (2000). The nutritive value of thin stillage and wet distillers' grains for ruminants: review. *Asian-Australasian Journal of Animal Sciences*, *13*(11), 1609–1618.

Nofsinger, G. W., VanCauwenberge, J. E., Bothast, R. J., & Kwolek, W. F. (1983). An evaluation of chemical methods to extend the allowable storage time of wet distillers' grains. *Journal of Agricultural Food Chemistry*, *31*, 276–279.

Ojowi, M., McKinnon, J. J., Mustafa, A. F., & Christensen, D. A. (1997). Evaluation of wheat-based wet distillers' grains for feedlot cattle. *Canadian Journal of Animal Science*, *77*(3), 447–454.

Pedersen, C., Jonsson, H., Lindberg, J. E., & Roos, S. (2004). Microbiological characterization of wet wheat distillers' grain, with focus on isolation of Lactobacilli with potential as probiotics. *Applied Environmental Microbiology*, *70*, 1522–1527.

Pedersen, C., Roos, S., Jonsson, H., & Lindberg, J. E. (2005). Performance, feeding behavior and microbial diversity in weaned piglets fed liquid diets based on water or wet wheat-distillers grain. *Archives in Animal Nutrition*, *59*(3), 165–179.

RFA (2008). *Ethanol biorefinery locations—U.S. fuel ethanol industry biorefineries and production capacity.* Washington, D.C.: Renewable Fuels Association. Available at: http://www.ethanolrfa.org/. Accessed 04 March 2008.

Rosentrater, K. A. (2006). Some physical properties of distillers dried grains with solubles (DDGS). *Applied Engineering in Agriculture*, *22*(4), 589–595.

Rosentrater, K. A., & Lehman, R. M. (2008). Physical and chemical properties of corn distillers wet grains. *Applied Engineering in Agriculture*, *24*(1), 57–62.

Rosentrater, K. A., & Muthukumarappan, K. (2006). Corn ethanol coproducts: generation, properties, and future prospects. *International Sugar Journal*, *108*(1295), 648–657.

Schingoethe, D. J., Brouk, M. J., & Birkelo, C. P. (1999). Milk production and composition from cows fed wet corn distillers grains. *Journal of Dairy Science*, *82*, 574–580.

Schingoethe, D. J., Kalscheur, K. F., & Garcia, A. D. (2002). *Distillers grains for dairy cattle. Extension extra 4022*. Brookings, SD: South Dakota State University.

Shand, P. J., McKinnon, J. J., & Christensen, D. A. (1998). Eating quality of beef from animals fed wet brewers' grains and wheat-based wet distillers' grains. *Canadian Journal of Animal Science*, *78*(1), 143–146.

Shurson, G. C., Spiehs, M. J., & Whitney, M. (2004). The use of maize distiller's dried grains with solubles in pig diets. *Pig News and Information*, *25*(2), 75N–83N.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry—the principles and practice of statistics in biological research* (3rd ed.). New York, NY: WH Freemand & Company.

Speigel, M. R. (1994). *Statistics*. New York, NY: McGraw-Hill Inc.

Spiehs, M. J., Whitney, M. H., & Shurson, G. C. (2002). Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *Journal of Animal Science*, *80*, 2639–2645.

Tibelius, C. (1996). *Coproducts and near coproducts of fuel ethanol fermentation from grain*. Agriculture and Agri-Food Canada–Canadian Green Plan Ethanol Program: Starchy Waste Streams Evaluation Project. Available at: http://res2.agr.ca/publications/cfar/index_e.htm. Accessed 10 January 2007.

Tjardes, K., & Wright, C. (2002). *Feeding corn distiller's co-products to beef cattle. Extension extra 2036*. Brookings, SD: South Dakota State University.

Tournas, V., Stack, M. E., Mislivec, P. B., Koch, H. A., & Bandler, R. (2001). Yeasts, molds, and mycotoxins. In *Bacteriological analytical manual* (8th ed., Revision A, pp. 14–17). Washington, D.C.: U.S. Food and Drug Administration.

Weigel, J. C., Loy, D., & Kilmer, L. (1997). *Feed co-products of the dry corn milling process*. Iowa State University, Iowa Corn Promotion Board, Iowa Department of Agriculture, Renewable Fuels Association, National Corn Growers Association. Available at: http://www.iowacorn.org/ethanol/ethanol_17.html. Accessed 10 January 2007.

Zar, J. H. (1996). *Biostatistical analysis* (3rd ed.). Upper Saddle River, NJ: Prentice Hall.