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Impact of synthetic antioxidants on lipid peroxidation of distiller's dried grains with solubles and distiller's corn oil stored under high temperature and humidity conditions[1]

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ABSTRACT: This experiment evaluated the effect of antioxidants, oil content in distiller's dried grains with solubles (DDGS), quality of distiller's corn oil, and storage time on lipid peroxidation. A source of low-oil DDGS (LO-DDGS; 5.0% ether extract [EE], as-fed basis), high-oil DDGS (HO-DDGS; 13.0% EE, as-fed basis), and 2 sources of distiller's corn oil (DCO; 1.20, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables [MIU], respectively [DCO-1], and 1.20, 0.01, and 0.10% MIU, respectively [DCO-2]) were obtained. Each of the 4 ingredients was divided into 18 representative subsamples (approximately 908 g for DDGS or 2 kg of DCO). Six subsamples of each ingredient were mixed with either no supplemental antioxidants (CON), Rendox-CQ (REN; 1,000 mg/kg EE; Kemin, Industries, Des Moines, IA), or Santoquin-Q4T (SAN; 1,500 mg/kg EE; Novus International, St. Louis, MO). Each mixture ($n = 72$) was split into thirds, and 1 portion was immediately frozen at –20°C (d 0). Two portions were stored under hot (38.6 ± 0.1°C) and humid conditions (94.0 ± 0.3% relative humidity) for 14 or 28 d. The MIXED procedure of SAS was used to evaluate the effects of ingredient, antioxidant, storage time, and interactions, with d-0 values used as a covariate. From d 14 to 28, peroxide value (PV), *p*-anisidine value (AnV), and thiobarbituric acid reactive substances (TBARS) of DCO and DDGS increased by 3- to 4-fold ($P < 0.05$). Over the entire storage period, PV of DCO-1 and HO-DDGS (12.3 ± 0.3 and 12.6 ± 0.3 mEq O_2/kg oil, respectively) exceeded ($P < 0.05$) that of DCO-2 and LO-DDGS (9.6 ± 0.3 and 9.3 ± 0.3 mEq O_2/kg oil, respectively). Adding REN or SAN ($P < 0.05$) reduced TBARS and AnV relative to CON (TBARS = 11.0 ± 0.2 mg malondialdehyde Eq/kg oil and AnV = 6.5 ± 0.2) over the entire period (mean of d 14 and 28), but TBARS and AnV did not differ ($P > 0.05$) between antioxidants (TBARS = 6.1 ± 0.2 and 5.9 ± 0.2 mg malondialdehyde Eq/kg oil, respectively, and AnV = 1.9 ± 0.2 and 1.8 ± 0.2 for REN and SAN, respectively). The PV on d 14 and 28 and overall was less ($P < 0.05$) when either antioxidant was added relative to CON (16.0 mEq O_2/kg) and was greater for ingredients treated with SAN ($P < 0.05$) compared with REN (8.8 ± 0.2 and 8.0 ± 0.2 mEq O_2/kg oil for SAN and REN, respectively). In summary, antioxidants reduced peroxidation of DDGS and DCO by approximately 50% during 28 d of storage at 38.6°C and 94.0% relative humidity, but neither antioxidant completely stabilized the ingredients.

Key words: antioxidants, distiller's corn oil, distiller's dried grains with solubles, ingredient storage, lipid peroxidation

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INTRODUCTION

More than 85% of U.S. ethanol plants extract corn oil before manufacturing corn distiller's dried grains with solubles (**DDGS**), which results in large availability of distiller's corn oil (**DCO**; RFA, 2014). Corn oil and DCO are high in PUFA that are highly susceptible to peroxidation (NRC, 2012).

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Table 1. Initial concentration of antioxidant in high-oil distiller's dried grains with solubles (HO-DDGS), low-oil distiller's dried grains with solubles (LO-DDGS), and distiller's corn oil (DCO) before storage at 38°C and 94% relative humidity[1,2,3,4]

Item	HO-DDGS	LO-DDGS	DCO-1[5]	DCO-2[6]	PSEM[7]	*P*-value
Rendox-CQ, mg/kg crude fat	977[a]	946[b]	1,004[c]	962[ab]	4	<0.01
Santoquin-Q4T, mg/kg crude fat	1,463[a]	1,455[a]	1,486[b]	1,476[ab]	6	<0.01

[a–c]Means with different superscripts differ ($P < 0.05$).

[1]Rendox-CQ (active ingredient is *t*-butylhydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) was added at 1,000 mg/kg of lipid.

[2]Santoquin-Q4T (active ingredients are ethoxyquin and TBHQ; Novus International, St. Louis, MO) was added at 1,500 mg/kg of lipid.

[3]Control batches (without added antioxidants) were analyzed for Rendox-CQ and Santoquin-Q4T and contained undetectable levels.

[4]Means represent 6 batches per ingredient.

[5]DCO-1 = 1.20, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables, respectively.

[6]DCO-2 = 1.20, 0.01, and 0.10% moisture, insoluble impurities, and unsaponifiables, respectively.

[7]PSEM = pooled SEM.

Susceptibility to peroxidation varies depending on the fatty acid composition of the lipid source and the presence of natural antioxidants. Lipid peroxidation is accelerated by exposure to heat, air, moisture, and pro-oxidant metals, which may be introduced during processing and storage (Belitz et al., 2009). Therefore, lipid-rich ingredients may be peroxidized to varying extents depending on storage and processing conditions (Dibner et al., 2011; Song and Shurson, 2013).

Lipids extracted from corn germ oil and thin stillage were peroxidized at greater rates than oil extracted from DDGS during 14 d of storage at 40°C (Winkler-Moser and Breyer, 2011). The reduced rate of peroxidation of oil in DDGS may be due to protection provided by antioxidants indigenous to corn, which are concentrated in DDGS (Winkler-Moser and Breyer, 2011). Addition of synthetic antioxidants is an effective method to prevent peroxidation of lipids that are contained in fats and oils (Valenzuela et al., 2002; Chen et al., 2014). The most common synthetic antioxidants are *t*-butyl-4-hydroxyanisole (**BHA**), 2,6-di-*t*-butylhydroxytoluene (**BHT**), *t*-butylhydroquinone (**TBHQ**), ethoxyquin, and 2,6-di-ter-butyl1-4-hydroxymethyl-phenol (Guo et al., 2006). However, little is known about the effects of these antioxidants and their combinations on lipid peroxidation in DCO and DDGS stored under high temperature and humidity. Therefore, the objective of this experiment was to investigate the amount of lipid peroxidation that can occur in DDGS and DCO, with or without the addition of synthetic antioxidants, when stored under temperature and humidity conditions typical of international trade.

MATERIALS AND METHODS

Low-oil DDGS (**LO-DDGS**; 5.0 ± 0.15% ether extract [**EE**], as-fed basis) and DCO (Voila; 1.2, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables [**MIU**], respectively [**DCO-1**]; 99.5% EE; POET Nutrition, Sioux Falls, SD) were obtained from the same ethanol plant (POET, Lake Crystal, MN). High-oil DDGS (**HO-DDGS**; 13.0 ± 0.19% EE, as-fed basis) was obtained from CHS Inc. (Inver Grove Heights, MN) and was produced by Highwater Ethanol (Lamberton, MN). Distiller's corn oil from a second source (1.20, 0.01, and 0.10% MIU, respectively [**DCO-2**]; 99.5% EE) was obtained from POET Nutrition. The DCO-2 was manufactured using an alternative propriety method to compare with the process used to produce DCO-1. Consequently, MIU content of DCO-1 was greater than DCO-2 (MIU = 1.76 [1.20, 0.08, and 0.48% MIU, respectively] and 1.31% [1.20, 0.01, and 0.10% MIU, respectively] for DCO-1 and DCO-2, respectively).

Each of the 4 ingredients was divided into 18 representative subsamples (approximately 908 g for each DDGS source or 2 kg for each DCO source). For each ingredient, 6 subsamples were mixed with either no supplemental antioxidants (**CON**; n = 6/ingredient), Rendox-CQ (**REN**; n = 6/ingredient; TBHQ as active ingredient; Kemin Industries, Des Moines, IA), or Santoquin-Q4T (**SAN**; n = 6/ingredient; active ingredient: ethoxyquin and TBHQ; Novus International, St. Louis, MO) using a stationary food mixer (Kitchen Aid, Benton Harbor, MI) on the lowest setting for at least 3 min (n = 72 batches). Batches were formulated to contain 0 (CON) or 1,000 mg REN/kg crude fat or 1,500 mg SAN/kg crude fat according to the maximum level recommended by the manufacturer, but analyzed values varied slightly (Table 1). No mold inhibitors were added to any samples, and mold inhibitors are not added to DDGS during manufacturing, transport, or storage.

Each batch was divided into 3 equal samples (total = 216 samples) of approximately 300 g of DDGS or 600 mL of DCO to achieve similar volumes of material in each container, assuming a bulk density of 0.4896 g/mL for DDGS (Letsche et al., 2009). Within each batch, 2 samples were placed in 2 plastic containers and the third sample was placed in screw-top plastic bottles.

The headspace of each bottle was purged with N gas, and bottles were frozen (–20°C) until later analysis to determine initial (d-0) peroxidation measurements. Plastic containers (n = 144) were covered with synthetic cheesecloth material, secured by a rubber band, and placed in an environmentally controlled chamber (model z-32; Cincinnati Sub Zero, Cincinnati, OH). Samples were blocked across the chamber to account for potential environmental gradients in temperature and relative humidity (**RH**), and samples representing the same batch were placed adjacent to each other. The chamber was set to maintain 38°C and 90% RH, but actual temperature and RH varied slightly from these settings. Temperature and humidity were monitored at 5-min intervals using a ThermaData Logger (Thermoworks, Lindon, UT). Light inside the environmental chamber was turned off except during sample collection, and the window on the unit was covered to prevent exposure to external light. One randomly selected sample from each batch was removed from the chamber on d 14 (n = 6/treatment), and the remaining sample in each batch was removed at d 28 (n = 6/treatment). Upon removal, selected samples were placed in screw-top plastic bottles. The headspace of each bottle was purged with N gas, and samples were frozen (–20°C) until analysis.

Laboratory Analyses

Samples of DDGS were ground, and lipids were extracted as described by Folch et al. (1957). Distiller's corn oil samples and DDGS oil extracts from d 0 were analyzed for ethoxyquin and TBHQ via gas chromatography–mass spectroscopy as described by Guo et al. (2006). Levels of REN and SAN were then calculated based on the analyzed antioxidant concentration of these proprietary products. All samples were analyzed for fatty acid profile (methods Ce 2-66 [AOCS, 2013] and 996.06 [AOAC, 2012]), thiobarbituric acid reactive substances (**TBARS**), peroxide value (**PV**) using chloroform (method Cd 8-53; AOCS, 2013), and *p*-anisidine value (**AnV**; method Cd 18-90; AOCS, 2013). The TBARS assay was a modified version of the American Oil Chemists' Society procedure (Cd 19-90; AOCS, 2013) using malonaldehyde as a standard (Pegg, 2001). Initial samples of DCO were analyzed for moisture (method Ca 2c-25; AOCS, 2013), insolubles (method Ca 3a-46; AOCS, 2013), unsaponifiables (method Ca 6a-40; AOCS, 2013), and free fatty acids in crude and refined fats and oils (method Ca 5a-40; AOCS, 2013). All assays were conducted at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) unless noted otherwise.

Mold was observed in all containers containing DDGS and none containing DCO after 14 or 28 d of storage. Upon removal from the chamber, the moldy fraction was separated from the fraction that did not appear moldy, and each portion was weighed to calculate apparent mold content. Apparent mold content (%) was calculated using the following equation:

$$\text{apparent mold} = [\text{moldy weight}/(\text{moldy weight} + \text{nonmoldy weight})] \times 100.$$

Moldy and nonmoldy fractions were homogenized for all chemical analyses. Samples were submitted for total mold count using the plate dilution technique described by Tournas et al. (2001) at Minnesota Valley Testing Laboratories, Inc. (New Ulm, MN).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC) with repeated measures to evaluate the fixed effects of ingredient (HO-DDGS, LO-DDGS, DCO-1, and DCO-2), antioxidant addition (CON, REN, and SAN), time (d 14 or 28), and all interactions in a 4 × 3 × 2 factorial arrangement. Specifically, the model included the fixed effects of ingredient, antioxidant, time, ingredient × antioxidant, ingredient × time, antioxidant × time, and ingredient × antioxidant × time using the heterogeneous compound symmetry covariance structure. Initial (d-0) values were used as covariates for each variable, and apparent mold concentration was used as a covariate in the analysis of all variables except total mold count. Covariates were included as described, regardless of significance. Initial values (d 0) were analyzed using a similar model including the fixed effects of ingredient, antioxidant, and ingredient × antioxidant. Block was included as a random effect in all models. Batch (n = 72) was the experimental unit. Results are reported as least squares means. To facilitate mean separation, the PLM procedure of SAS (version 9.3; SAS Inst. Inc.) was used with the Tukey adjustment. Correlations between nutrient content and the concentration of PV, TBARS, and AnV among ingredients were determined using PROC CORR of SAS. Significance was declared at $P < 0.05$, whereas values of $0.05 \geq P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

The high concentration of PUFA in corn oil causes it to be more susceptible to peroxidation compared with lipid sources containing high concentrations of MUFA or SFA (Belitz et al., 2009). Linoleic and linolenic acid are peroxidized at rates 12 and 25 times faster than oleic acid (Belitz et al., 2009). Furthermore, heat, oxygen, moisture, and pro-oxidant metals may

be introduced during processing and storage of ingredients and complete feeds, which accelerate the peroxidation process. Antioxidants impede or reduce the rate of peroxidation, but antioxidants cannot reverse peroxidation once it has occurred (Belitz et al., 2009).

Researchers have reported that lipids in DDGS (Song and Shurson, 2013) and concentrated lipid sources such as corn oil (Dibner et al., 2011) are highly variable in amount of peroxidation. Recently, the ethanol industry has adopted oil extraction, which contributes to variable EE content in DDGS, ranging from 4.88 to 13.23% crude fat on a DM basis (Kerr et al., 2013). However, connections between EE content of DDGS and its susceptibility to peroxidation have not been established. Song and Shurson (2013) reported that PV and TBARS of DDGS varied from 4.2 to 84.1 mEq O_2/kg oil and 1.0 to 5.2 mg malondialdehyde Eq/kg of oil, respectively. In the current study, relatively low initial values (d 0) of PV (1.42 ± 0.01, 1.23 ± 0.01, 1.30 ± 0.01, and 1.13 ± 0.01 mEq O_2/kg), TBARS (1.99 ± 0.01, 1.97 ± 0.01, 1.98 ± 0.01, and 1.99 ± 0.01 mg malondialdehyde Eq/kg of oil), and AnV (0.17 ± 0.003, 0.17 ± 0.003, 0.17 ± 0.003, and 0.17 ± 0.003) were observed for HO-DDGS, LO-DDGS, DCO-1, and DCO-2, respectively, which suggests that the ingredient sources used herein were not excessively peroxidized before conducting the study (Table 2).

The environmentally controlled chamber used in this study provided excellent control of desired storage conditions because the actual temperature (38.6 ± 0.1°C) and RH (94.0 ± 0.3%) varied very little during the 28-d storage period. These conditions reflect a "typical" scenario for ingredients shipped internationally and stored in hot, humid climatic conditions (e.g., Southeast Asia). No other studies have been published to measure the extent of lipid peroxidation in DCO or DDGS during storage under these conditions. The initial level of peroxidation varied ($P < 0.05$) among treatments on d 0 (Table 2). Therefore, initial values were used as covariates. The PV, TBARS, and AnV of DDGS and DCO increased from d 14 to 28 ($P < 0.05$) of storage (Table 2). Similarly, other researchers have reported that PV (Naz et al., 2005; Winkler-Moser and Breyer, 2011) and AnV (Naz et al., 2005) increase in corn oil during storage at ≤40°C. Peroxide value and TBARS of canola oil increased after heating (65°C) for 17 d (Wanasundara and Shahidi, 1994). Similarly, PV, TBARS, and AnV increased in sunflower oil heated at 60°C for 21 d (Chen et al., 2014). However, peroxidation is a dynamic process resulting in the production and subsequent degradation of numerous compounds. Lipid hydroperoxides are primary products of lipid peroxidation, which subsequently react to form numerous compounds including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds, which also degrade in subsequent reactions (Belitz et al., 2009). The rate of degradation of peroxides depends on thermal conditions (Liu et al., 2014). Consequently, PV followed a bell-shaped curve of production and degradation in lipids exposed to extreme temperatures (80 to 150°C; DeRouchey et al., 2004; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). Similarly, TBARS concentration followed a bell-shaped curve in corn oil, canola oil, tallow, or poultry fat during heating at 185°C for 12 h (Liu et al., 2014). Therefore, low values of TBARS and PV may be misleading in lipids exposed to extreme (≥80°C) temperatures because they may indicate late stages of the peroxidation process. However, our results suggest that PV, TBARS, and AnV are acceptable indicators of peroxidation in DCO and DDGS exposed to storage conditions with relatively lower temperatures (≤65°C) because at 28 d, peroxidation products were still on the increasing portion of the curve. Unfortunately, historical conditions of lipid production and handling are often unknown when selecting the most appropriate peroxidation measures to use when evaluating various feed ingredients containing lipids. Furthermore, various additional procedures can be used to assess the extent of peroxidation, and these methods recently have been reviewed by others (Liu et al., 2014).

There were significant 3-way interactions among ingredients, antioxidants, and storage time. The PV, TBARS, and AnV on d 14 and 28 and overall declined ($P < 0.05$) for all ingredients treated with either REN or SAN relative to CON (Table 3). However, there were no differences ($P > 0.05$) between the antioxidants within each ingredient. The PV of DCO-1 and HO-DDGS was greater ($P < 0.05$) than DCO-2 and LO-DDGS on d 14 and 28 and overall. Similarly, the TBARS concentration of DCO-2 was less ($P < 0.05$) than DCO-1 and less ($P < 0.05$) for LO-DDGS than HO-DDGS on d 28 and overall. Despite the fact that DCO-1 and LO-DDGS were manufactured at the same ethanol plant, the PV was 25% lower ($P < 0.05$) in LO-DDGS than in DCO-1 after 28 d of storage. This finding suggests that extracted DCO is more easily peroxidized than intact oil remaining in LO-DDGS. Extracted oils are more digestible than intact oil in grain and grain coproducts by pigs, which may be due to oil remaining in grains being embedded within the fiber matrices making it less accessible to lipases (Adams and Jensen, 1984; Kil et al., 2010; Kim et al., 2013). However, whether fiber provides similar protection against lipid peroxidation is unknown. The AnV value was 25% lower ($P < 0.05$) in DCO-1 compared with the other ingredients, but the explanation for this is unclear. The decreased PV and TBARS for DCO-2 relative to DCO-1 suggest that the concentration of MIU affects the peroxidation status of DCO during storage. The MIU concentration of DCO-2

Table 2. Main effects of ingredient, antioxidant, and day of storage on characteristics of high-oil distiller's dried grains with solubles (HO-DDGS), low-oil distiller's dried grains with solubles (LO-DDGS), and distiller's corn oil (DCO) stored at 38°C and 94% relative humidity[1]

	Ingredient[2]					Antioxidant[4]				*P*-value[5]			
Item	HO-DDGS	LO-DDGS	DCO-1	DCO-2	PSEM[3]	CON	REN	SAN	PSEM	EI	EA	EI × d	EA × d
Peroxide value, mEq/kg lipid[6]													
d 0	1.42^{a}	1.23^{b}	1.30^{c}	1.13^{d}	0.01	1.24^{a}	1.28^{ab}	1.29^{b}	0.01				
d 14	4.63^{a}	3.33^{b}	4.42^{a}	3.42^{b}	0.11	5.80^{a}	2.87^{b}	3.18^{c}	0.09				
d 28	20.55^{a}	15.26^{b}	20.24^{a}	15.79^{b}	0.43	26.29^{a}	13.12^{b}	14.47^{c}	0.32				
Mean[7]	12.59^{a}	9.30^{b}	12.33^{a}	9.60^{b}	0.26	16.04^{a}	8.00^{b}	8.82^{c}	0.17	<0.01	<0.01	<0.01	<0.01
TBARS, mg MDA Eq/kg lipid[6,8]													
d 0	1.99	1.97	1.98	1.99	0.01	1.98	1.99	1.99	0.01				
d 14	3.46^{a}	2.82^{bc}	3.27^{ab}	2.77^{c}	0.14	4.45^{a}	2.49^{b}	2.30^{b}	0.13				
d 28	13.19	11.80	12.97	11.32	0.49	17.66^{a}	9.76^{b}	9.55^{b}	0.32				
Mean	8.32^{a}	7.31^{bc}	8.12^{ab}	7.05^{c}	0.27	11.05^{a}	6.12^{b}	5.92^{b}	0.16	<0.01	<0.01	0.06	<0.01
p-Anisidine value[6]													
d 0	0.17	0.17	0.17	0.17	<0.01	0.11^{a}	0.22^{b}	0.18^{b}	<0.01				
d 14	1.98^{a}	1.79^{ab}	1.46^{b}	1.71^{ab}	0.10	3.78^{a}	0.61^{b}	0.82^{b}	0.12				
d 28	5.14^{ab}	6.23^{a}	4.08^{b}	5.02^{ab}	0.37	9.30^{a}	3.24^{b}	2.81^{b}	0.24				
Mean	3.56^{a}	4.01^{a}	2.77^{b}	3.37^{a}	0.18	6.54^{a}	1.92^{b}	1.82^{b}	0.15	<0.01	<0.01	<0.01	<0.01
Linoleic acid, % of lipid[6]													
d 0	52.92^{a}	53.21^{b}	53.08^{ab}	51.96^{c}	0.06	52.78	52.73	52.88	0.05				
d 14	53.05^{a}	52.66^{b}	53.28^{a}	52.26^{b}	0.07	52.75	52.86	52.83	0.06				
d 28	53.53^{a}	52.9^{bc}	53.2^{ab}	52.33^{c}	0.11	52.98	52.94	53.04	0.06				
Mean	53.29^{a}	52.78^{b}	53.24^{a}	52.29^{b}	0.08	52.86	52.90	52.94	0.03	<0.01	0.37	<0.01	0.21
Linolenic acid, % of lipid[6]													
d 0	1.68^{a}	1.71^{b}	1.30^{c}	1.34^{d}	<0.01	1.51	1.51	1.51	<0.01				
d 14	1.65^{a}	1.71^{b}	1.33^{c}	1.37^{d}	0.03	1.51	1.52	1.52	0.01				
d 28	1.6^{a}	1.72^{b}	1.33^{c}	1.37^{d}	0.03	1.50	1.49	1.52	0.01				
Mean	1.62^{a}	1.71^{b}	1.33^{c}	1.37^{d}	0.03	1.50	1.51	1.52	<0.01	<0.01	0.16	<0.01	0.04
SFA, % of lipid[6]													
d 0	17.69^{a}	18.60^{b}	16.45^{c}	16.65^{d}	0.03	17.38^{a}	17.40^{a}	17.26^{b}	0.02				
d 14	17.44^{a}	18.85^{b}	16.29^{c}	16.38^{c}	0.10	17.27	17.22	17.23	0.03				
d 28	16.75^{a}	18.44^{b}	16.34^{a}	16.42^{a}	0.12	17.01	16.98	16.97	0.03				
Mean	17.10^{a}	18.64^{b}	16.31^{c}	16.40^{c}	0.11	17.14	17.10	17.10	0.02	<0.01	0.31	<0.01	0.89
MUFA, % of lipid[6]													
d 0	27.35^{a}	26.10^{b}	28.56^{c}	29.37^{d}	0.03	27.87	27.88	27.79	0.03				
d 14	27.65	26.41	28.47	29.27	0.12	27.96	27.93	27.95	0.03				
d 28	27.83	26.61	28.55	29.27	0.14	28.07	28.08	28.04	0.05				
Mean	27.74^{a}	26.51^{b}	28.51^{c}	29.27^{d}	0.13	28.02	28.01	28.00	0.03	<0.01	0.86	0.33	0.69
PUFA, % of lipid[6]													
d 0	54.60^{a}	54.93^{b}	54.39^{a}	53.30^{c}	0.07	54.28	54.24	54.39	0.06				
d 14	54.72^{a}	54.39^{b}	54.58^{ab}	53.61^{c}	0.08	54.26	54.37	54.34	0.06				
d 28	55.15^{a}	54.64^{bc}	54.50^{bc}	53.67^{d}	0.12	54.48	54.44	54.56	0.06				
Mean	54.93^{a}	54.52^{b}	54.54^{b}	53.64^{c}	0.09	54.37	54.41	54.45	0.04	<0.01	0.29	<0.01	0.17

$^{a–d}$Within a row, means without a common superscript differ ($P < 0.05$).

[1]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration {[apparently moldy fraction/(apparently moldy fraction + apparently fresh fraction)] × 100}.

[2]DCO-1 = 1.20, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables, respectively; DCO-2 = 1.20, 0.01, and 0.10% moisture, insoluble impurities, and unsaponifiables, respectively.

[3]PSEM = pooled SEM.

[4]CON = no supplemental antioxidants; REN = Rendox-CQ (active ingredient is *t*-butylhydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 mg/kg of lipid; SAN = Santoquin-Q4T (active ingredients are ethoxyquin and TBHQ; Novus International, St. Louis, MO) at 1,500 mg/kg of lipid.

[5]EI = effect of ingredient; EA = effect of antioxidant.

[6]Effect of time (d 14 vs. 28, $P < 0.05$).

[7]Main effect mean of ingredient or antioxidant, regardless of day.

[8]TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde.

Table 3. Interactive effects of ingredient, antioxidant, and sampling day on characteristics of high-oil distiller's dried grains with solubles (HO-DDGS), low-oil distiller's dried grains with solubles (LO-DDGS), and distiller's corn oil (DCO) stored at 38°C and 90% relative humidity[1,2,3]

	Ingredient												
	HO-DDGS			RO-DDGS			DCO-1			DCO-2			
	Antioxidant			Antioxidant			Antioxidant			Antioxidant			
Item	CON	REN	SAN	CON	REN	SAN	CON	REN	SAN	CON	REN	SAN	PSEM[4]
Peroxide value, mEq/kg lipid[5,6]													
d 14	7.13^{a}	3.12^{bc}	3.63^{b}	4.49^{d}	2.68^{c}	2.83^{c}	6.86^{a}	2.99^{bc}	3.40^{bc}	4.73^{d}	2.68^{c}	2.84^{bc}	0.37
d 28	31.37^{a}	13.93^{bc}	16.35^{b}	20.50^{d}	11.73^{bc}	13.55^{bc}	31.02^{a}	14.57^{bc}	15.14^{bc}	22.26^{d}	12.26^{c}	12.83^{c}	0.37
Mean[7]	19.25^{a}	8.52^{cde}	9.99^{c}	12.49^{b}	7.21^{e}	8.19^{de}	18.94^{a}	8.78^{cde}	9.27^{cd}	13.50^{b}	7.47^{e}	7.84^{de}	0.21
TBARS, mg MDA Eq/kg lipid[5,6,8]													
d 14	5.05^{a}	2.90^{cd}	2.41^{d}	3.75^{bc}	2.37^{d}	2.34^{d}	4.83^{a}	2.61^{cd}	2.39^{d}	4.16^{ab}	2.08^{d}	2.06^{d}	0.20
d 28	21.06^{a}	9.53^{b}	8.99^{b}	14.26^{d}	11.00^{bc}	10.13^{bc}	21.24^{a}	8.41^{b}	9.27^{b}	14.08^{cd}	10.08^{b}	9.81^{b}	0.69
Mean	13.05^{a}	6.22^{b}	5.70^{b}	9.00^{c}	6.69^{b}	6.24^{b}	13.04^{a}	5.51^{b}	5.83^{b}	9.12^{c}	6.08^{b}	5.93^{b}	0.37
p-Anisidine value[5,6]													
d 14	3.94^{a}	0.96^{b}	1.04^{b}	3.76^{a}	0.65^{b}	0.97^{b}	3.79^{a}	0.18^{b}	0.40^{b}	3.62^{a}	0.64^{b}	0.87^{b}	0.17
d 28	9.10^{a}	3.43^{bc}	2.88^{bc}	9.48^{a}	4.96^{b}	4.26^{b}	9.69^{a}	1.22^{c}	1.33^{c}	8.95^{a}	3.35^{bc}	2.77^{bc}	0.48
Mean	6.52^{a}	2.19^{b}	1.96^{bcd}	6.62^{a}	2.80^{b}	2.61^{b}	6.74^{a}	0.70^{d}	0.87^{cd}	6.29^{a}	2.00^{bc}	1.82^{bcd}	0.29
Linoleic acid, % of lipid[5]													
d 14	53.02	53.14	52.99	52.74	52.63	52.61	52.99	53.40	53.44	52.24	52.27	52.27	0.11
d 28	53.59	53.56	53.45	53.03	52.85	52.83	53.07	53.13	53.39	52.23	52.23	52.51	0.14
Mean	53.31^{a}	53.35^{a}	53.22^{ab}	52.88^{abc}	52.74^{bc}	52.72^{bc}	53.03^{ab}	53.26^{ab}	53.41^{a}	52.24^{c}	52.25^{c}	52.39^{c}	0.10
Linolenic acid, % of lipid													
d 14	1.65	1.65	1.63	1.70	1.71	1.71	1.32	1.34	1.34	1.36	1.37	1.38	0.03
d 28	1.6	1.58	1.62	1.72	1.70	1.73	1.31	1.33	1.34	1.36	1.36	1.38	0.03
Mean	1.63	1.62	1.63	1.71	1.70	1.72	1.31	1.34	1.34	1.36	1.37	1.38	0.03
SFA, % of lipid[5]													
d 14	17.47	17.4	17.47	18.79	18.87	18.88	16.42	16.22	16.22	16.39	16.4	16.35	0.13
d 28	16.76	16.71	16.78	18.4	18.4	18.53	16.38	16.36	16.29	16.5	16.47	16.29	0.14
Mean	17.12^{a}	17.05^{a}	17.12^{a}	18.59^{b}	18.63^{b}	18.70^{b}	16.4^{c}	16.29^{c}	16.26^{c}	16.45^{c}	16.44^{c}	16.32^{c}	0.11
MUFA, % of lipid													
d 14	27.57	27.65	27.72	26.44	26.39	26.41	28.52	28.46	28.43	29.32	29.24	29.25	0.13
d 28	27.77	27.88	27.84	26.62	26.56	26.66	28.65	28.57	28.44	29.26	29.31	29.24	0.16
Mean	27.67	27.76	27.78	26.53	26.47	26.54	28.58	28.52	28.43	29.29	29.27	29.25	0.13
PUFA, % of lipid[5]													
d 14	54.7	54.81	54.64	54.47	54.36	54.35	54.28	54.72	54.75	53.58	53.62	53.63	0.12
d 28	55.21	55.16	55.08	54.77	54.57	54.58	54.36	54.43	54.71	53.57	53.57	53.87	0.15
Mean	54.96^{a}	54.99^{a}	54.86^{ab}	54.62^{abc}	54.47^{bc}	54.46^{bcd}	54.32^{cd}	54.57^{abc}	54.73^{ab}	53.58^{e}	53.59^{e}	53.75^{de}	0.11

$^{a–e}$Within a row, means without a common superscript differ ($P < 0.05$).

[1]DCO-1 = 1.20, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables, respectively; DCO-2 = 1.20, 0.01, and 0.10% moisture, insoluble impurities, and unsaponifiables, respectively.

[2]CON = no supplemental antioxidants; REN = Rendox-CQ (active ingredient is *t*-butylhydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 mg/kg of lipid; SAN = Santoquin-Q4T (active ingredients are ethoxyquin and TBHQ; Novus International, St. Louis, MO) at 1,500 mg/kg of lipid.

[3]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration {[apparently moldy fraction/(apparently moldy fraction + apparently fresh fraction)] × 100}.

[4]PSEM = pooled SEM.

[5]Ingredient × antioxidant ($P < 0.05$).

[6]Ingredient × antioxidant × day ($P < 0.05$).

[7]Main effect mean of ingredient × antioxidant, regardless of day.

[8]TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde.

was 26% less than DCO-1, with the main differences being for insoluble impurities (87.5% less) and unsaponifiables (80% less). Contaminants and metals such as Fe, Cu, and Mn increase the rate of peroxidation of unsaturated fats (Flider and Orthoefer, 1981). However, peroxidation is also influenced by the degree of unsaturation of the fatty acids in the oils. Concentrations of linoleic acid and total PUFA, as a percentage of lipid, were lower ($P < 0.05$) in DCO-2 and LO-DDGS compared with DCO-1 or HO-DDGS, but these differences

Table 4. Main effects of ingredient, antioxidant, and day of storage on characteristics of high-oil distiller's dried grains with solubles (HO-DDGS) and low-oil distiller's dried grains with solubles (LO-DDGS) stored at 38°C and 94% relative humidity

	Ingredient			Antioxidant[1]				*P*-value[2]			
Item	HO-DDGS	LO-DDGS	PSEM[3]	CON	REN	SAN	PSEM	EI	EA	EI ×d	EA ×d
Ether extract, % as-fed[5]											
d 0	13.02[a]	5.01[b]	0.08	9.24[a]	9.20[a]	8.63[b]	0.11				
d 14	11.45[a]	2.86[b]	0.53	7.08	7.14	7.24	0.53				
d 28	8.98[a]	2.74[b]	0.53	5.98	5.86	5.75	0.55				
Mean[6]	10.21[a]	2.8[b]	0.51	6.53	6.50	6.50	0.09	<0.01	0.96	<0.01	0.05
Apparent mold, % fed as-fed[5]											
d 14	13.46[a]	24.2[b]	2.1	19.4	17.5	19.58	2.20				
d 28	46.08[a]	70.12[b]	3.2	56.33	57.81	60.17	3.60				
Mean[6]	29.77[a]	47.16[b]	2.3	37.86	37.66	39.88	2.50	<0.01	0.60	<0.01	0.68
Total mold count, cfu[5] × 10^6/g											
d 14	2.47	2.08	0.55	2.73	1.65	2.44	0.64				
d 28	11.36[a]	1.72[b]	0.62	6.93	7.45	5.25	0.71				
Mean[6]	6.91[a]	1.90[b]	0.42	4.83[x]	4.55[xy]	3.85[y]	0.47	<0.01	0.11	<0.01	0.12
Moisture, %[5]											
d 0	9.90[a]	10.39[b]	0.02	9.97[a]	9.96[a]	10.52[b]	0.03				
d 14	21.33[a]	19.66[b]	0.31	20.44	20.35	20.70	0.33				
d 28	23.05[a]	21.25[b]	0.32	22.02	22.04	22.38	0.35				
Mean[6]	22.19[a]	20.45[b]	0.23	21.23	21.19	21.54	0.26	<0.01	0.05	<0.01	0.66

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[x,y]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CON = no supplemental antioxidants; REN = Rendox-CQ (active ingredient: *t*-butylhydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 mg/kg of lipid; SAN = Santoquin-Q4T (active ingredient: ethoxyquin and TBHQ; Novus International, St. Louis, MO) at 1,500 mg/kg of lipid.

[2]EI = effect of ingredient; EA = effect of antioxidant.

[3]PSEM = pooled SEM.

[4]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration {[apparently moldy fraction/(apparently moldy fraction + apparently fresh fraction)] × 100}.

[5]Effect of time (d 14 vs. 28, $P < 0.05$).

[6]Main effect mean of ingredient or antioxidant, regardless of day.

were relatively small in magnitude (<2.5%) compared with the overall change during the 28 d of storage. The factors contributing to the difference in fatty acid profiles of DCO-1 and LO-DDGS are unclear, but they are likely related to oil extraction procedures and relative extraction rate of each fatty acid. However, little research has been conducted to elucidate those associations. Ultimately, DCO-2 and LO-DDGS may have had less peroxidation because they had less concentration of linoleic acid and PUFA. Linoleic acid is the predominant PUFA present in corn oil (NRC, 2012), and PUFA are highly susceptible to peroxidation relative to SFA (Belitz et al., 2009). The concentration of linoleic acid, linolenic acid, SFA, and MUFA did not differ ($P > 0.05$) across antioxidant treatments within each ingredient (Table 3). The PUFA concentration of HO-DDGS, LO-DDGS, and DCO-2 was not affected ($P > 0.05$) by antioxidant treatment, but PUFA content increased ($P < 0.05$) for DCO-1 treated with SAN relative to CON. However, the magnitude of this response was minor (approximately 0.7% relative difference).

Antioxidants such as TBHQ and ethoxyquin retard peroxidation (Wanasundara and Shahidi, 1994, 2005; Chen et al., 2014). However, the dietary addition of antioxidants to foods and animal feeds is regulated by the U.S. Food and Drug Administration. Specifically, BHT, BHA, TBHQ, or propyl gallate can be added at a maximum level of 0.02% of lipid, and ethoxyquin is limited to 0.015% of the diet (AAFCO, 2013). In the current experiment, REN and SAN inhibited peroxidation of lipids in DCO and DDGS through d 14 and 28. Adding either REN or SAN to all ingredients slowed peroxidation of fatty acid on d 14 and 28 compared with CON. However, ingredients treated with REN had a PV that was about 10% less ($P < 0.05$) than ingredients treated with SAN. Although the difference was relatively small in magnitude, other researchers have reported that TBHQ is more effective than ethoxyquin for stabilizing fish (Sanhueza et al., 2000) and soybean oil (Valenzuela et al., 2002). On d 14 and 28, the concentration of TBARS and AnV declined ($P < 0.05$) relative to CON by adding either REN or SAN, but there was no difference between the 2 antioxidants ($P > 0.05$).

The EE content of LO-DDGS (5.0%) was substantially less than HO-DDGS (13.0%), as intended by the experimental design (Table 4). Ether extract concentration on d 14 was greater ($P < 0.05$) for HO-DDGS treated with SAN compared with CON (data not shown), but there were no other effects of antioxidant on EE concentration within each ingredient. The EE concentration of both DDGS sources decreased ($P < 0.05$), with a 22% decrease in HO-DDGS and a 5.2% decrease in LO-DDGS, from d 14 to 28 (Table 4). The EE concentration negatively correlated ($P < 0.01$) with PV, TBARS, and AnV for both HO-DDGS ($r = –0.74$, $r = –0.72$, and $r = –0.58$, respectively) and LO-DDGS ($r = –0.80$, $r = –0.78$, and $r = –0.68$, respectively), suggesting that the lipid was being peroxidized. Additionally, the decrease in lipid concentration during storage may have been due to the visible mold growth that developed on all DDGS samples after 14 and 28 d of storage in the hot and humid environment. Reed et al. (2007) showed that the EE content of corn declined after experimental infection with mold and 8 wk of storage, indicating that mold metabolizes lipids. However, connections between mold growth and lipid peroxidation have not been elucidated. The total mold count was less ($P < 0.05$) for HO-DDGS treated with SAN relative to CON (data not shown), but the practical implications of this finding are unclear because there was no effect ($P > 0.05$) of antioxidant on apparent mold growth for HO-DDGS. In the current experiment, the apparent concentration of mold increased ($P < 0.05$) from d 14 to 28, and the apparent concentration of mold was greater ($P < 0.05$) on d 14 and 28 in LO-DDGS compared with HO-DDGS. However, the mechanisms underlying these differences are not clear. Elevated moisture content and temperature conditions promote mold growth (Milton and Pawsey, 1988). In the current study, moisture content of DDGS increased ($P < 0.05$) over time, but the moisture content of HO-DDGS exceeded ($P < 0.05$) that of LO-DDGS on d 14 and 28. Therefore, these findings are contrary to the positive correlation between moisture content and mold growth because HO-DDGS had greater moisture content (22.2%) but less apparent mold growth than lower moisture LO-DDGS (20.4%) averaged across d 14 and 28, respectively. It is likely that the combination of high RH and exposure to heat contributed to the development of mold growth in DDGS during storage. However, the total mold count was less ($P < 0.05$) on d 14 and 28 in LO-DDGS compared with HO-DDGS, which suggest that the mold fraction of LO-DDGS had reduced ability to generate new colonies compared with mold in HO-DDGS.

In conclusion, these data indicate that lipid peroxidation occurs when DCO and DDGS are exposed to 38.6°C and 94.0% RH during 14- and 28-d storage periods. These findings have important implications for storage, handling, and transportation of these ingredients under conditions of high temperature and RH. Lipids in HO-DDGS were more susceptible to peroxidation than LO-DDGS after 28 d of storage, and a small increase in MIU content of DCO appears to elicit significant increases in lipid peroxidation. Finally, the commercial antioxidants evaluated in this experiment had similar effectiveness in reducing lipid peroxidation in DDGS and DCO but did not completely prevent lipid peroxidation.

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