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Evaluation of value-added components of dried distiller's grain with solubles from triticale and wheat

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ABSTRACT

This study focused on the detection of value-added co-products in dried distiller's grain plus soluble (DDGS), a possibility that could open new avenues for further processing and marketing of DDGS and improving economic sustainability of ethanol industry. Varieties of triticale, wheat and two benchmarks, CPS wheat and Pioneer Hi-Bred corn, were fermented using two very high gravity (VHG) fermentation approaches: jet-cooking and raw starch processing (STARGEN fermentation). DDGS from STARGEN fermentation could be promising sources of value-added co-products. Pronghorn triticale DDGS (STARGEN fermentation) had the highest concentration of sterols (3.7 mg/g), phenolic compounds (13.61 mg GAE/g), and β-glucan (2.07%). CDC Ptarmigan DDGS (STARGEN fermentation) had the highest concentration of tocopherols and tocotrienols (107.0 μg/g), 1.93% of β-glucan, and 53.0 mg/g of fatty acids. AC Reed DDGS (STARGEN method) showed 1.97% of β-glucan. This study shows that proper choice of fermentation approach and feedstock for ethanol production could improve commercial quality of DDGS.

1. Introduction

In western Canada and Europe, wheat is used as a feedstock for fuel ethanol production (Boland, 1995). Triticale is an alternative crop for the production of fermentable starch (Wang et al., 1997, 1998). As a feedstock, triticale is able to grow in more marginal lands (Çiftci et al., 2003) and its genetic modification does not carry the same trade implications as wheat.

In the dry-grinding process for ethanol production, α-amylases and glucoamylases enzymes have traditionally been used for the pre-treatment of grains prior to fermentation. α-amylase decreases mash viscosity by breaking the α-(1,4)-glucosidic bonds of starch, producing smaller dextrin chains (Park and Rollings, 1994). This process, i.e., liquefaction, is done at high temperatures of 90–120 °C (Wang et al., 2007) with direct steam injection (jet-cooking). During subsequent saccharification, these dextrins are converted into fermentable sugars by glucoamylase. Recently, a new generation of starch hydrolyzing enzymes that are effective at cold temperatures was developed, for example, STARGEN 001 that is a cocktail of modified α–amylase and glucoamylase (Wang et al., 2007).

In ethanol industry, fermentation produces a co-product known as dried distiller's grain with solubles (DDGS). Since only starch and sugars are converted into ethanol, non-fermentable components in cereal grains are concentrated by a factor of two to three in DDGS (Weigel et al., 1997; Gibreel et al., 2009). Currently, the vast majority of the DDGS is used as an ingredient for livestock feeds (Kelsall and Lyons, 2003). However, the rapid increase in ethanol production capacity has resulted in an excess of DDGS (Fastinger et al., 2006) and identification of new value-added uses of DDGS is becoming essential for the ethanol industry to remain profitable. Cereal grains contain nutrients such as linoleic acid, dietary fiber such as β-glucan and antioxidants (e.g. vitamin E) that as a part of a diet may reduce risk factors for coronary heart disease (Truswell, 2002) and provide other health benefits (Charalampopoulos et al., 2002). These nutrients are concentrated in the DDGS; however, their concentration and thus potential for extraction has been poorly described.

The objective of this study was to detect higher value chemicals and co-products with potential commercial value in DDGS of spring and winter wheat (six cultivars) and triticale (two cultivars) resulting from ethanol fermentation in comparison to corn and CPS wheat. Simultaneous saccharification and fermentation (SSF) was used with very high gravity (VHG) mashes. Enzymatic treatment

Abbreviations: DDGS, dried distiller's grain with solubles; SSF, simultaneous saccharification and fermentation; VHG, very high gravity fermentation (fermentation that contains 27 grams or more of solids/100 grams of mash); GAE, gallic acid; DM, dry matter; GE, gross energy.

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Table 1
Fermentation data of grains by VHG fermentation methods[A].

Grain name	Total starch concentration (%)	Fermentation method	Residual starch (%)[B]	Ethanol concentration (% v/v)	Fermentation efficiency (%)[C]
Pioneer Hi-Bred corn	62.1	VHG jet-cooking	0.6^{efghi}	13.8^{def}	88.7^{bc}
		VHG STARGEN	4.5^{a}	14.5^{abcde}	84.5^{bcd}
CPS wheat	56.2	VHG jet-cooking	0.4^{fghi}	11.9^{fg}	91.3^{ab}
		VHG STARGEN	1.0^{cdefg}	13.3^{def}	85.2^{bcd}
Ultima triticale	53.8	VHG jet-cooking	0.2^{hi}	11.1^{g}	88.5^{bc}
		VHG STARGEN	0.7^{defghi}	11.9^{fg}	80.6^{cde}
Pronghorn triticale	58.9	VHG jet-cooking	0.2^{i}	13.0^{ef}	84.1^{bcd}
		VHG STARGEN	0.3^{ghi}	14.4^{abcde}	85.5^{bcd}
AC Reed wheat	61.7	VHG jet-cooking	1.9^{b}	15.4^{a}	81.0^{cde}
		VHG STARGEN	$0.9^{cdefghi}$	14.6^{abcd}	83.9^{bcde}
AC Andrew wheat	59.8	VHG jet-cooking	0.4^{fghi}	13.8^{bcde}	83.8^{bcde}
		VHG STARGEN	1.0^{cdefgh}	14.2^{abcde}	85.2^{bcd}
Average Ptarmigan wheat	63.5	VHG jet-cooking	0.3^{fghi}	15.1^{bc}	98.2^{a}
		VHG STARGEN-	$0.9^{cdefghi}$	15.0^{abc}	85.0^{bcd}
Large Ptarmigan wheat	63.5	VHG jet-cooking	1.6^{bc}	15.4^{ab}	97.2^{a}
		VHG STARGEN	0.7^{defghi}	14.1^{abcde}	80.0^{de}
CDC Ptarmigan wheat	66.1	VHG jet-cooking	1.5^{bcde}	13.6^{cde}	80.0^{de}
		VHG STARGEN	1.3^{bcde}	14.9^{abc}	82.4^{cde}
Small Ptarmigan wheat	63.8	VHG jet-cooking	1.13^{bcdef}	15.5^{a}	75.8^{e}
		VHG STARGEN	0.6^{efghi}	14.2^{abcde}	81.6^{cde}
SEM			0.14	0.27	1.44
P-value					
Grain			<0.001	<0.001	<0.001
Fermentation			<0.001	0.031	0.031
Grain × Fermentation			<0.001	<0.001	<0.001

[A] Within a column, means without a common superscript differ ($P < 0.05$).
[B] Residual starch (%) was calculated relative to the total mass of starch available at the start of fermentation.
[C] Fermentation efficiency (%) was calculated by dividing D by E where: D = gm of ethanol produced per 100 gm of starch involved in fermentation × 100 and E = 56.7 [which is the amount of ethanol (in gm) theoretically expected from complete hydrolysis and fermentation of 100 gm of starch].

coupled with "jet-cooking" and the alternative raw starch hydrolysis, based on the application of the STARGEN 001 enzyme system at 55 °C, was utilized for the generation of small amount of glucose during a short pre-saccharification step prior to fermentation. Elucidating the effects of fermentation processing steps on the recovery of value-added components in the resulting DDGS was another key objective described below.

2. Methods

2.1. Grain samples

Two triticale samples (cultivars Ultima and Pronghorn) were supplied by Alberta Agriculture and Rural Development (Lacombe, Alberta, Canada). Two samples of spring wheat (cultivars AC Reed, AC Andrew) were provided by Agriculture and Agri-Food Canada (Lethbridge, AB, Canada). Winter wheat samples of Large Ptarmigan, Average Ptarmigan, and Small Ptarmigan were provided by Western Agriculture Lab. Ltd. (Saskatchewan, Canada), whereas CDC Ptarmigan was provided by McDougall Acres (Saskatchewan, Canada). As benchmarks, CPS wheat (Alberta Agriculture and Rural Development; Barrhead, Alberta, Canada) and Pioneer Hi-Bred corn (Pioneer Hybrid Ltd.; Chatham, Ontario, Canada) were used. Grain was ground in a Jacobson-Carter Day Cutler-hammer mill (using a 1.98 mm sieve) or in a Retsch mill (model ZM 100, using a 0.5 mm sieve). Ground grain samples were stored in air-tight plastic bags at room temperature.

2.2. Enzymes, reagents, and chemicals

The STARGEN 001 (α-amylase and glucoamylase blend for processing uncooked starch), Optimash TBG (viscosity reducing) and Fermgen (protease) enzymes were provided by Genencor International (Palo Alto, CA, USA). Viscozyme Barley (viscosity reducing), Viscozyme Wheat (viscosity reducing), Liquozyme SC (α-amylase), and Spirizyme (glucoamylase) enzymes were obtained from Novozymes (Franklinton, NC, USA). Megazyme kits (Megazyme, Bray, Ireland) were used for starch and β-glucan determination. SuperStart *Saccharomyces cerevisiae* was provided by Ethanol Technology (Milwaukee, WI, USA). Urea was purchased from Fisher Scientific.

2.3. Preparation of the mash for fermentation

2.3.1. Preparation of mashes for VHG jet-cooking fermentation

Grain mashes were prepared for VHG jet-cooking fermentation using ground grains (1.98 mm, a particle size similar to what generally utilized in industry) as previously described (Gibreel et al., 2009).

2.3.2. Preparation of mashes for VHG STARGEN fermentations

The grains were milled to a particle size of 0.5 mm (this size was based on the recommendation of Genencor International to achieve optimal starch hydrolysis). The mashes were prepared as previously described (Gibreel et al., 2009).

2.4. Fermentation processes

In general, 2–3 kg of the mash of each grain (27 to 30% solids) was fermented in duplicate for 72 h in a 5-L high performance Minifors bioreactor (Rose Scientific Ltd., Mississauga, Ontario, Canada). The transfer of mashes into heat-sterilized (121 °C, 20 psi for 1 h) bioreactors and different fermentation experiments were carried out as described by Gibreel et al. (2009). Tests for microbial contamination were performed at three different stages as previously reported (Gibreel et al., 2009). Fermentation efficiency (%) was defined as the ratio between the actual and the theoretical ethanol yield × 100. The actual ethanol yield was calculated as

Table 2
Concentrations of sterols detected in various grains and their corresponding DDGS[A].

	Concentration of sterol (mg/g)[B]				
Grain type and fermentation method	Sitosterol	Stigmasterol	Campesterol	Unknown[C]	Total
Pioneer Hi-Bred corn*	0.56^{jk}	0.07^{ghi}	0.20^{fm}	0.46^{hijk}	1.29^{j}
DDGS (VHG jet-cooking)**	1.75^{a}	0.23^{a}	0.66^{a}	1.99^{a}	4.63^{a}
DDGS (VHG STARGEN)**	1.77^{a}	0.21^{a}	0.66^{a}	1.42^{bcdef}	4.06^{a}
CPS wheat*	0.30^{kl}	Trace	0.07^{o}	0.28^{jk}	0.65^{k}
DDGS (VHG jet-cooking)**	0.76^{ij}	Trace	0.21^{m}	0.81^{ghij}	1.78^{i}
DDGS (VHG STARGEN)**	0.88^{ghi}	Trace	0.24^{m}	0.91^{fghi}	2.03^{hi}
Ultima triticale**	0.47^{kl}	Trace	0.15^{o}	0.33^{jk}	0.95^{jk}
DDGS (VHG jet-cooking)**	1.16^{cde}	0.05^{ghi}	0.38^{def}	1.05^{defgh}	2.64^{f}
DDGS (VHG STARGEN)**	1.36^{bc}	0.06^{ghi}	0.42^{def}	1.14^{cdefg}	2.98^{d}
Pronghorn triticale*	0.36^{kl}	Trace	0.13^{o}	0.27^{jk}	0.76^{k}
DDGS (VHG jet-cooking)**	1.22^{cd}	0.06^{ghi}	0.45^{def}	1.20^{bcdefg}	2.93^{de}
DDGS (VHG STARGEN)***	1.52^{b}	0.11^{ghi}	0.49^{b}	1.59^{abc}	3.71^{b}
AC Reed wheat*	0.32^{kl}	Trace	0.09^{o}	0.28^{jk}	0.69^{k}
DDGS (VHG jet-cooking)***	0.93^{ghi}	Trace	0.27^{jkl}	1.07^{defgh}	2.27^{gh}
DDGS (VHG STARGEN)**	1.05^{def}	0.06^{ghi}	0.30^{jkl}	1.24^{bcdefg}	2.65^{f}
AC Andrew wheat*	0.29^{kl}	Trace	0.10^{o}	0.25^{jk}	0.64^{k}
DDGS (VHG jet-cooking)**	0.83^{ij}	Trace	0.28^{jkl}	0.97^{defgh}	2.08^{hi}
DDGS (VHG STARGEN)****	1.00^{efgh}	0.06^{ghi}	0.32^{jkl}	1.19^{bcdefg}	2.57^{fg}
Large Ptarmigan wheat*	0.32^{kl}	Trace	0.10^{o}	0.30^{jk}	0.72^{k}
DDGS (VHG jet-cooking)**	0.99^{efgh}	0.06^{ghi}	0.34^{hij}	1.20^{bcdefg}	2.59^{fg}
DDGS (VHG STARGEN)**	1.07^{defg}	0.08^{ghi}	0.35^{def}	1.68^{ab}	3.18^{cd}
Average Ptarmigan wheat*	0.28^{kl}	Trace	0.09^{o}	0.36^{jk}	0.73^{k}
DDGS (VHG jet-cooking)**	0.96^{efgh}	0.05^{ghi}	0.32^{jkl}	1.27^{bcdefg}	2.60^{fg}
DDGS (VHG STARGEN)**	1.12^{def}	0.08^{ghi}	0.36^{def}	1.53^{abcd}	3.09^{cd}
CDC Ptarmigan wheat**	0.30^{kl}	Trace	0.10^{o}	0.36^{jk}	0.76^{k}
DDGS (VHG jet-cooking)**	0.91^{ghi}	0.06^{ghi}	0.31^{jkl}	1.31^{bcdefg}	2.59^{fg}
DDGS (VHG STARGEN)**	1.07^{def}	0.08^{ghi}	0.36^{def}	1.46^{abcd}	2.97^{d}
Small Ptarmigan wheat*	0.34^{kl}	Trace	0.11^{o}	0.38^{jk}	0.83^{k}
DDGS (VHG jet-cooking)**	1.16^{cde}	0.09^{ghi}	0.39^{def}	1.45^{acde}	3.09^{cd}
DDGS (VHG STARGEN)**	1.25^{cd}	0.09^{ghi}	0.41^{def}	1.52^{abcd}	3.27^{c}
SEM	0.04	0.003	0.009	0.09	0.05
P-value					
Grain	<0.001	<0.001	<0.001	<0.001	<0.001
Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001
Grain × Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001

[A] *Grain types involved in VHG fermentation processes; **, DDGS preparations recovered from two methods of VHG fermentation: VHG jet-cooking and STARGEN fermentation.
[B] Trace, <0.05 mg/g was detected. Within a column, means without a common superscript differ ($P < 0.05$).
[C] Number of peaks of unknown sterols was in the range of 3 and 4 for initial grains and the DDGS samples, respectively.

ethanol produced (in gm) per 100 gm of starch involved in fermentation. The value of the theoretical ethanol yield utilized was 56.7 [which is the amount of ethanol (in gm) theoretically expected from complete hydrolysis and fermentation of 100 gm of starch].

2.5. Ethanol and DDGS analyses

Ethanol was analyzed with 1-butanol internal standard using gas chromatography using a Restek Stabilwax–DA column and the conditions previously described (Gibreel et al., 2009). Following fermentation with yeast, DDGS samples were prepared in two stages as previously described (Gibreel et al., 2009). The DDGS samples were analyzed for macro-nutrients, *in vitro* energy digestibility, sterols, fatty acids, phenolic compounds, tocopherols, and tocotrienols using the methods described by Gibreel et al. (2009). Total and available lysine concentrations were analyzed at the University of Missouri (AOAC, 1995).

2.6. Statistical analyses

Data were analyzed using either the general linear model of SAS when all effects were fixed or the mixed model (Statistical Analysis Systems, 2003) when random and repeated effects were present (Wang and Goonewardene, 2004). The main effects included grain type, fermentation method and their interaction in a 10 × 2 factorial arrangement. The viable yeast count data was analyzed as a repeated measures design with grain type, fermentation method and interaction as fixed effects and time as a repeated effect. The sample within grain type and fermentation method was the experimental unit. The variance–covariance structure was chosen based on the Scharzs' Bayesian criterion. Least squares means were estimated and separated using the pdiff option when fixed effects were significant ($P < 0.05$).

3. Results and discussion

3.1. Comparison of the ethanol yields of grains

Theoretically, 100 g of starch is expected to produce 56.7 g of ethanol as a maximum yield, but in practice only 90–93% of the theoretical yield is obtained (Ingledew, 2008). For each grain, actual ethanol yield resulting from the fermentation was calculated based on experimental ethanol concentration (% v/v) and total starch content of the grain. The calculated ethanol yield was then

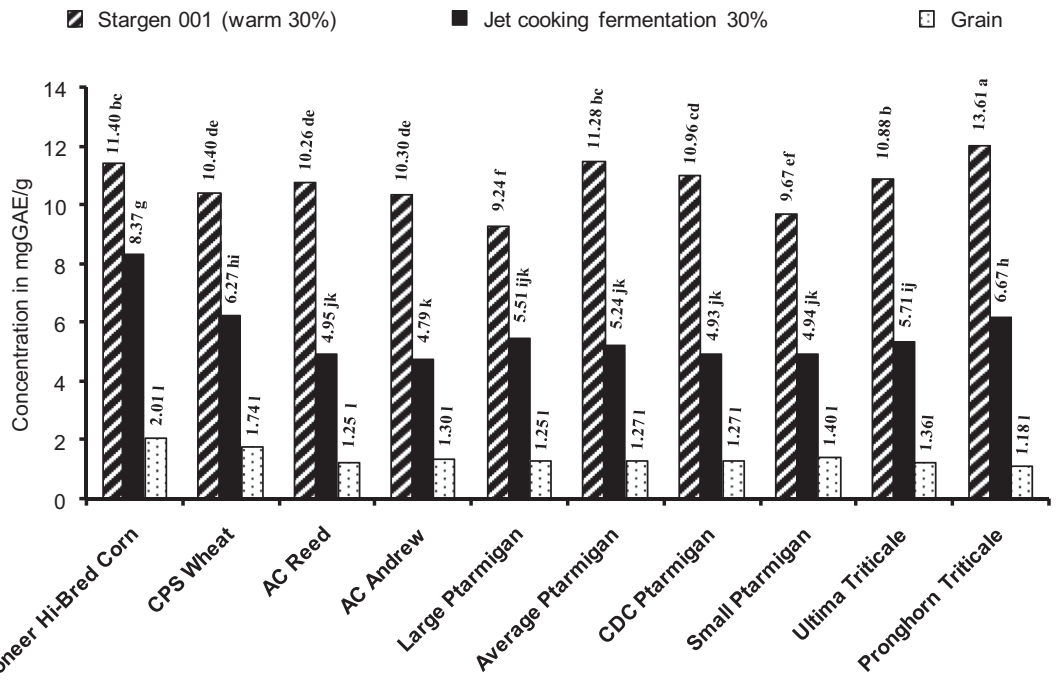

Fig. 1. Concentration of free phenolic compounds (in mg of gallic acid/g of sample) detected in the grains and their corresponding dried distiller's grain plus solubles recovered from various fermentation runs. Data are averages from two independent fermentations. The standard error of the mean based on experimental error (SEM) was 0.15. *P* value was <0.001 for grains, fermentation, and grain and fermentation interaction.

used to determine fermentation efficiency (Table 1). For most grains examined, the efficiency of VHG jet-cooking fermentation did not differ from that of VHG STARGEN fermentation ($P > 0.05$). This ruled out the possibility that the use of milled grain with a larger particle size for VHG jet-cooking fermentation compared to the size used for VHG STARGEN fermentation would reduced the efficiency of VHG jet-cooking fermentation. One exception was Small Ptarmigan that had the lowest efficiency for VHG jet-cooking fermentation. The decrease in efficiency of jet-cooking fermentation in that case could be due to the Maillard reaction between amino acids and free glucose during the jet-cooking resulting in consumption of some reducing sugars and free amino acids that otherwise would have been available for fermentation (Göğüş et al., 1998). Interestingly, jet-cooking process impacted the fermentation efficiency of Average and Large Ptarmigan leading to higher efficiency ($P < 0.05$) than VHG STARGEN fermentation (Table 1).

In general, fermentation efficiencies of triticale and wheat did not differ from those of the benchmarks.

3.2. Analysis of value-added components in grains and DDGS

Compared to the initial grains, the concentration of the value-added components increased 3-5-fold in all grain DDGS. This increase is mainly due to consumption of the starch during fermentation. For most grains, VHG STARGEN fermentation was associated with an increased recovery of value-added components compared to jet-cooking fermentations probably due to thermal destruction of these compounds in the latter.

3.2.1. Analysis of phytosterol content

The most abundant sterols were sitosterol and campesterol, whereas stigmasterol was detected at very low concentrations (Table 2). Utilization of the starch fraction, enzymatic treatment, and acidic conditions during fermentation could have facilitated the release of sterols from naturally-occurring glycosylated conjugates (Kesselmeier et al., 1985). This would explain why sterols were detected in the DDGS of some grains only but not before fermentation. DDGS of corn and Pronghorn triticale had higher ($P < 0.05$) concentrations of sterols relative to the other grain DDGS. Among spring and winter wheat varieties, the four Ptarmigan samples had the highest total concentration of sterols compared to CPS wheat as a benchmark ($P < 0.05$). For most grains, VHG STARGEN fermentation resulted in high concentrations of sterols ($P < 0.05$) compared to jet-cooking fermentation.

In general, the total sterol concentrations were higher in wheat and triticale DDGS than in CPS wheat DDGS but lower than in corn DDGS.

3.2.2. Analysis of the free phenolic compounds

The total concentrations of the phenolic compounds detected in different DDGS from VHG STARGEN fermentations were 34–120% higher ($P < 0.05$) than those detected after jet-cooking with the greatest difference determined in the DDGS of CDC Ptarmigan, Average Ptarmigan, AC Andrew, AC Reed, and Pronghorn triticale. The DDGS from VHG STARGEN fermentation of Pronghorn triticale had the highest ($P < 0.05$, Fig. 1) yield of free phenolic compounds than all other grain DDGS samples. In this study, the lower concentration of free phenolics detected after jet-cooking fermentation compared to STARGEN fermentations could be due to the impact of heating during jet-cooking. Previous studies showed that heating can result in interaction of the phenolic hydroxyl groups with food components such as protein and minerals to form insoluble complexes which will be inaccessible for detection (Matuscheck et al., 2001). Also, heating phenolic compounds can lead to polymerization into condensed phenolics, decreasing the number of assayable phenolic hydroxyl groups (Matuscheck et al., 2001).

The concentrations of phenolic compounds in the DDGS of triticale and wheat (represented by AC Reed, Average ptarmigan, and CDC ptarmigan) from VHG STARGEN fermentation were similar to those in the benchmark DDGS. For jet-cooking fermentation, corn DDGS had higher concentration of phenolic compounds compared to CPS wheat, triticale and wheat. However, the concentrations of

Table 3
Concentrations of fatty acids detected in various grains and their corresponding DDGS.

Grain type and fermentation method[A]	Concentration of fatty acid (mg/g)[B]				
	Linoleic acid	Oleic acid	Palmitic acid	Others[C]	Total
Pioneer Hi-Bred corn**	20.3^{h}	11.1^{c}	5.0^{k}	2.8^{k}	39.2^{ij}
DDGS (VHG jet-cooking)**	36.1^{a}	18.4^{b}	11.7^{bc}	6.6^{b}	72.8^{a}
DDGS (VHG STARGEN)**	37.7^{a}	19.9^{a}	12.6^{a}	7.9^{a}	78.1^{a}
CPS wheat**	11.0^{ij}	2.3^{i}	3.5^{h}	1.6^{h}	18.4^{j}
DDGS (VHG jet-cooking)***	23.3^{ef}	4.8^{fg}	8.9^{f}	4.6^{ef}	41.6^{fgh}
DDGS (VHG STARGEN)**	25.2^{de}	5.5^{ef}	9.8^{ef}	5.3^{de}	45.8^{def}
Ultima triticale*	9.1^{ij}	2.2^{lm}	2.6^{m}	1.4^{k}	15.3^{j}
DDGS (VHG jet-cooking)**	19.7^{h}	4.8^{ijk}	6.9^{jk}	4.5^{j}	35.9^{i}
DDGS (VHG STARGEN)**	20.3^{h}	4.6^{jk}	6.7^{k}	4.7^{hij}	36.3^{hi}
Pronghorn triticale*	8.0^{j}	1.7^{m}	2.9^{m}	1.2^{k}	13.8^{j}
DDGS (VHG jet-cooking)**	23.8^{fg}	4.5^{k}	8.3^{hi}	5.2^{fghi}	41.8^{fg}
DDGS (VHG STARGEN)**	27.4^{cd}	5.0^{ijk}	10.3^{cde}	5.8^{cdef}	48.5^{cd}
AC Reed wheat*	11.4^{ij}	2.5^{m}	3.4^{m}	1.2^{k}	18.5^{j}
DDGS (VHG jet-cooking)**	23.9^{efg}	5.3^{hij}	9.0^{gh}	4.8^{ghij}	43.0^{efg}
DDGS (VHG STARGEN)***	27.4^{cd}	6.2^{gh}	10.5^{cd}	5.7^{def}	49.8^{cd}
AC Andrew wheat**	10.6^{ij}	2.3^{m}	3.2^{m}	1.2^{k}	17.3^{j}
DDGS (VHG jet-cooking)**	21.2^{gh}	4.6^{jk}	7.9^{ij}	4.5^{j}	38.2^{ghi}
DDGS (VHG STARGEN)**	26.0^{def}	5.5^{hi}	10.0^{defg}	5.4^{efg}	46.9^{def}
Large Ptarmigan wheat*	10.6^{ij}	2.9^{l}	2.8^{m}	1.4^{k}	17.7^{j}
DDGS (VHG jet-cooking)**	25.9^{def}	7.1^{ef}	9.1^{fgh}	5.9^{bcde}	48.0^{cde}
DDGS (VHG STARGEN)**	26.8^{cde}	7.6^{de}	9.2^{efgh}	6.1^{bcd}	49.7^{cd}
Average Ptarmigan wheat*	11.7^{i}	3.1^{l}	3.1^{m}	1.4^{k}	19.3^{j}
DDGS (VHG jet-cooking)**	26.0^{def}	6.7^{fg}	9.0^{ghi}	5.7^{def}	47.4^{de}
DDGS (VHG STARGEN)**	31.3^{b}	8.4^{d}	11.1^{bc}	6.4^{bc}	57.2^{b}
CDC Ptarmigan wheat*	11.1^{ij}	3.0^{l}	2.9^{m}	1.3^{k}	18.3^{j}
DDGS (VHG jet-cooking)**	25.3^{def}	6.8^{efg}	8.5^{hi}	5.5^{def}	46.1^{def}
DDGS (VHG STARGEN)****	29.2^{bc}	8.0^{d}	10.1^{cdef}	5.7^{def}	53.0^{bc}
Small Ptarmigan wheat*	10.7^{ij}	2.7^{l}	2.8^{m}	1.3^{k}	17.5^{j}
DDGS (VHG jet-cooking)**	24.9^{def}	6.8^{efg}	8.6^{hi}	5.5^{def}	45.8^{def}
DDGS (VHG STARGEN)**	26.8^{cde}	7.1^{ef}	9.2^{efgh}	5.8^{cdef}	48.9^{cd}
SEM	0.53	0.16	0.19	0.12	0.97
P-value					
Grain	<0.001	<0.001	<0.001	<0.001	<0.001
Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001
Grain × Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001

[A] *Grain types involved in VHG fermentation processes; **, DDGS preparations recovered from two smethods of VHG fermentation: VHG jet-cooking and STARGEN fermentation.
[B] Within a column, means without a common superscript differ ($P < 0.05$).
[C] Other fatty acids included polyunsaturated fatty acids such as alpha-linolenic acid in addition to arachidic acid, gadoleic acid, behenic acid, and nervonic acid.

phenolic compounds in triticale DDGS from jet-cooking fermentation did not differ from CPS wheat DDGS as a benchmark.

3.2.3. Analysis of fatty acids

By far, the most abundant fatty acids detected in this study were linoleic, oleic, and palmitic acid (Table 3). Among the six varieties of wheat, the DDGS of Average Ptarmigan and CDC Ptarmigan showed the highest concentrations of fatty acids, regardless the fermentation type ($P < 0.05$, Table 3). With the exception of corn, Ultima triticale, Large Ptarmigan, and Small Ptarmigan, the type of fermentation impacted ($P < 0.05$) the total concentrations of fatty acids detected in the DDGS with the highest concentration detected after VHG STARGEN fermentation.

Regardless of the fermentation approach, fatty acid concentration in corn DDGS samples was superior ($P < 0.05$) to the DDGS of wheat, triticale and CPS wheat. However, wheat DDGS (represented by the four ptarmigan samples) had higher concentrations of fatty acids compared to the DDGS of triticale and CPS wheat as a benchmark.

3.2.4. Analysis of the tocopherols and tocotrienols

Similar to CPS wheat (as a benchmark), β-tocotrienol was the dominant tocotrienol type identified in the unfermented grains and in the DDGS of all varieties of spring and winter wheat and triticale (Table 4). However, β-tocotrienol was not detected in corn and α-tocotrienol was the dominant form. In the case of tocopherols, the α type was dominant for all grains except corn. The predominant tocopherol in corn was γ-tocopherol. The γ type of tocopherols and tocotrienols was found almost exclusively in corn, the only exception being the DDGS of Pronghorn triticale (0.8 μg/g, Table 4), which had a relatively small amount of γ-tocopherol. Small amounts of δ tocopherol were detected in the initial corn grain and in the DDGS of corn, Ultima and Pronghorn triticale from both jet-cooking and STARGEN fermentations

Regardless of fermentation method, CDC Ptarmigan DDGS had the highest concentrations ($P < 0.05$) of both tocopherols and tocotrienols compared to the two benchmarks. VHG STARGEN fermentation increased ($P < 0.05$) recovery of tocopherols and tocotrienols in the case of AC Reed, AC Andrew, Average Ptarmigan, and CDC Ptarmigan compared to jet-cooking fermentation. The exposure of grain to high temperature during jet-cooking could decrease the recovery of tocopherols and tocotrienols due to thermal destruction (Gassmann and Schneeweiss, 1959; Håkansson and Jägerstad, 1990). The DDGS of CPS Wheat, Ultima and Pronghorn triticale from VHG jet-cooking fermentation showed higher concentrations of tocopherols and tocotrienols ($P < 0.05$).

Table 4
Concentrations of vitamin E components such as tocopherols and tocotrienols in various grains and their corresponding DDGS[A].

Grain type and fermentation method	Concentration of vitamin E component (μg/g)[B]						
	Tocotrienol			Tocopherol			Total
	α	β	γ	α	β	γ	
Pioneer Hi-Bred corn*	6.6^{fg}		2.3^{de}	6.6^{efg}		24.2^{b}	42.2^{ghi}
DDGS (VHG jet-cooking)**	11.5^{f}		4.4^{b}	12.6^{a}		52.5^{a}	85.3^{cd}
DDGS (VHG STARGEN)**	11.6^{f}		5.3^{a}	11.5^{ab}		56.9^{a}	90.6^{bc}
CPS wheat**	4.9^{g}	13.3^{b}		5.6^{efgh}	3.7^{ab}		27.5^{jklm}
DDGS (VHG jet-cooking)**	5.3^{g}	19.7^{a}		4.4^{gh}	3.9^{a}		33.4^{ijkl}
DDGS (VHG STARGEN)	4.2^{g}	16.1^{bc}		3.6^{h}	2.7^{c}		20.1^{mn}
Ultima triticale*	3.3^{mno}	8.3^{op}		6.0^{klmn}	5.8^{hijk}		23.3^{lmn}
DDGS (VHG jet-cooking)**	6.1^{jk}	14.6^{jkl}		11.1^{gh}	8.0^{ghi}		44.7^{gh}
DDGS (VHG STARGEN)**	4.2^{mno}	9.6^{no}		7.1^{kl}	5.9^{ijk}		27.7^{klm}
Pronghorn triticale*	2.6^{o}	5.1^{p}		3.2^{n}	1.9^{mn}		13.6^{n}
DDGS (VHG jet-cooking)**	13.1^{a}	16.4^{ijk}		22.2^{b}	7.5^{ghij}	0.8^{d}	59.1^{f}
DDGS (VHG STARGEN)**	8.3^{ghi}	14.4^{kl}		10.4^{ghi}	5.4^{jk}	0.8^{d}	40.3^{hi}
AC Reed wheat*	3.2^{mno}	11.3^{mno}		8.2^{ijk}	4.8^{kl}		27.4^{klm}
DDGS (VHG jet-cooking)**	7.1^{hij}	22.3^{g}		13.9^{ef}	8.2^{gh}		51.6^{fg}
DDGS (VHG STARGEN)**	9.6^{defg}	28.2^{f}		20.8^{b}	13.4^{cd}		72.0^{e}
AC Andrew wheat*	3.0^{o}	11.8^{mno}		9.6^{hij}	5.9^{ijk}		30.3^{jkl}
DDGS (VHG jet-cooking)**	5.2^{kl}	19.6^{gh}		12.3^{fg}	9.2^{fg}		46.2^{gh}
DDGS (VHG STARGEN)**	8.4^{fgh}	28.2^{f}		21.5^{ab}	13.5^{cd}		71.7^{e}
Large Ptarmigan wheat**	2.9^{o}	13.8^{klm}		5.4^{mn}	5.2^{k}		27.3^{klm}
DDGS (VHG jet-cooking)**	8.0^{hi}	32.7^{e}		16.6^{d}	12.1^{de}		69.4^{e}
DDGS (VHG STARGEN)**	8.5^{efgh}	35.5^{de}		14.2^{ef}	14.5^{bc}		72.7^{e}
Average Ptarmigan wheat**	3.1^{no}	16.0^{ijk}		7.7^{jkl}	6.1^{hijk}		32.8^{ijkl}
DDGS (VHG jet-cooking)**	8.3^{ghi}	35.5^{de}		19.4^{bc}	13.1^{cd}		76.3^{e}
DDGS (VHG STARGEN)**	9.9^{def}	42.1^{c}		20.6^{b}	16.1^{ab}		88.6^{bc}
CDC Ptarmigan wheat**	4.1^{mno}	18.9^{hi}		8.5^{ijk}	6.1^{hijk}		37.6^{hij}
DDGS (VHG jet-cooking)**	12.9^{ab}	48.2^{b}		21.1^{ab}	12.1^{de}		94.3^{b}
DDGS (VHG STARGEN)**	12.1^{abc}	53.5^{a}		23.5^{a}	18.0^{a}		107.0^{a}
Small Ptarmigan wheat*	4.5^{mn}	17.5^{hij}		8.0^{ijk}	4.8^{kl}		34.8^{ijk}
DDGS (VHG jet-cooking)**	9.9^{de}	33.7^{e}		16.1^{de}	10.8^{ef}		70.5^{e}
DDGS (VHG STARGEN)**	10.4^{cd}	39.0^{cd}		16.8^{cde}	11.1^{def}		77.2^{de}
SEM	0.27	0.54	0.06	0.43	0.40	0.65	1.62
P-value							
Grain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Grain × Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[A] *Grain types involved in VHG fermentation processes; **DDGS preparations recovered from two methods of VHG fermentation: VHG jet-cooking and STARGEN fermentation.
[B] Within a column, means without a common superscript differ ($P < 0.05$).

In general, corn DDGS had higher concentrations of tocopherols and tocotrienols (regardless of the fermentation method) compared to CPS wheat, triticale, and wheat. However, the concentrations of tocopherols and tocotrienols in the DDGS of wheat and triticale (represented by Pronghorn) surpassed those in the DDGS of CPS wheat as a benchmark.

3.2.5. Analysis of β-glucan fiber

The DDGS of Pronghorn triticale, AC Reed, and CDC Ptarmigan from VHG STARGEN fermentation contained more β-glucan (2.07, 1.97, and 1.93%, respectively) compared to the DDGS of other grains including the benchmarks (data not shown). The DDGS of Pronghorn triticale, AC Reed, and CDC Ptarmigan from VHG STARGEN fermentation could be used as high dietary fiber food ingredients.

In general, β-glucan concentrations in the DDGS of CPS wheat, triticale (represented by Pronghorn), and wheat were higher than those in corn DDGS.

3.3. Analysis of the nutritional characteristics of DDGS

In vitro energy digestibility was used as an estimate for energy digestibility in swine. The focus on characterizing energy is because this is the most important cost component in feed formulation for livestock (Regmi et al., 2008).

The DDGS of corn was highest in crude fat (Table 5), reflecting the high fat content in corn (Widyaratne and Zijlstra, 2007), but was lowest in crude protein and *in vitro* energy digestibility. Among cereal grains, DDGS of Ultima triticale from VHG STARGEN fermentation was highest in crude protein content. Therefore, the DDGS of Ultima has the potential for increased value if used as a flour support in baked goods.

Determination of the percent of total lysine to protein of a food or feedstuff provides useful information about nutritional value because lysine is the first limiting amino acid for swine in most diets (Young, 2008). The percentage of available lysine relative to total lysine correlates with the biological value of food or feedstuff (Hackler and Stillings, 1967). The term available lysine describes the unmodified lysine that is absorbed in a form usable for protein synthesis, catabolism or conversion (Rutherfurd et al., 2006). Compared to other grains and the benchmarks (Table 6), Pronghorn triticale had the highest total lysine to protein ($P < 0.05$), and available lysine to total lysine did not differ. Fermentation of the grains led to a slight increase in total lysine to protein partly due to consumption of the starch fraction during fermentation and to increased level of lysine derived from yeast biomass (Choi et al., 2009). This study shows that the jet-cooking method was accompanied with an increase in total lysine to protein in the case of Large Ptarmigan, CDC Ptarmigan, and Pronghorn DDGS compared to their corresponding DDGS from STARGEN fermentation. This might be explained by heat-induced unfolding and dissociation of some proteins in AC Reed and CDC Ptarmigan wheat resulting in higher measured total

Table 5
Impact of grain source, type of fermentation, and percentage of solids on nutritional characteristics of DDGS.

Grain type	Nutritional characteristics %[A]								
	Fermentation method (VHG)	Ash	Moisture	Crude protein	Crude fiber	Crude fat	*In-vitro* DM digestibility	*In-vitro* GE digestibility	GE (Mcal/g)
Pioneer Hi-Bred corn	jet-cooking	5.6^{ab}	8.9	32.0^{j}	4.6^{efg}	12.5^{b}	53.7^{f}	49.7^{e}	5.6^{b}
	STARGEN	4.9^{b}	7.2	30.8^{j}	4.1^{fgh}	15.5^{a}	56.4^{f}	54.8^{d}	5.8^{a}
CPS wheat	jet-cooking	4.8^{b}	4.2	43.7^{bc}	4.2^{fgh}	5.2^{fghi}	70.3^{bcd}	67.7^{bc}	5.2^{def}
	STARGEN	5.8^{ab}	6.1	43.1^{bcde}	5.1^{def}	4.9^{ghij}	70.9^{b}	68.5^{ab}	5.2^{def}
Ultima triticale	jet-cooking	4.7^{b}	2.9	45.3^{b}	5.1^{def}	3.5^{j}	67.3^{de}	65.7^{bc}	5.2^{ef}
	STARGEN	5.2^{ab}	4.5	49.0^{a}	3.4^{h}	3.9^{ij}	74.5^{a}	71.6^{a}	5.3^{cde}
Pronghorn Triticale	jet-cooking	5.5^{ab}	4.1	39.0^{hi}	3.6^{gh}	5.5^{efgh}	70.7^{bc}	67.4^{bc}	5.4^{def}
	STARGEN	5.7^{ab}	8.5	40.4^{fgh}	4.5f^{g}	5.6^{defgh}	70.4^{bc}	68.5^{ab}	5.5^{bc}
AC Reed wheat	jet-cooking	5.2^{ab}	6.2	39.6^{ghi}	6.6^{abc}	5.5^{efgh}	68.9^{bcde}	66.2^{bc}	5.3^{cdef}
	STARGEN	5.0^{ab}	4.0	42.9^{bcdef}	5.7^{cde}	6.7^{cde}	68.9^{bcde}	66.2^{bc}	5.3^{cde}
AC Andrew wheat	jet-cooking	6.0^{ab}	6.6	41.0^{defgh}	5.9^{cd}	4.7^{hij}	67.8^{cde}	66.1^{bc}	5.2^{ef}
	STARGEN	5.6^{ab}	5.0	43.6^{bcd}	5.8^{cd}	5.31^{efghi}	69.5^{bcde}	67.5^{bc}	5.3^{cdef}
Large Ptarmigan wheat	jet-cooking	5.0^{ab}	6.6	40.5^{efgh}	7.3^{a}	5.3^{efghi}	68.0^{bcde}	66.7^{bc}	5.4^{bcde}
	STARGEN	5.5^{ab}	4.1	42.5^{cdef}	6.4^{abc}	6.6^{cdef}	68.6^{bcde}	64.2^{c}	5.1^{f}
Average Ptarmigan wheat	jet-cooking	4.4^{b}	7.6	41.9^{cdefg}	6.7^{abc}	5.7^{cdefg}	67.2^{e}	66.1^{bc}	5.4^{bcde}
	STARGEN	4.5^{b}	4.8	43.9^{bc}	5.8^{cd}	7.0^{cd}	69.1^{bcde}	65.5^{bc}	5.4^{bcd}
CDC Ptarmigan wheat	jet-cooking	5.2^{ab}	6.8	37.7^{i}	7.0^{ab}	4.9^{ghij}	66.7^{e}	65.0^{bc}	5.3^{cdef}
	STARGEN	6.6^{a}	6.9	43.0^{bcdef}	6.0^{bcd}	7.1^{c}	69.4^{bcde}	65.7^{bc}	5.4^{cde}
Small Ptarmigan wheat	jet-cooking	5.1^{b}	6.8	39.2^{hi}	7.2^{a}	5.4^{efgh}	66.6^{e}	64.8^{c}	5.3^{cdef}
	STARGEN	4.7^{b}	5.2	42.8^{bcdef}	5.9^{cd}	6.4^{cdefg}	67.2^{e}	66.4^{bc}	5.4^{cde}
SEM		0.29		0.46	0.19	0.26	0.53	0.63	32.50
P-value									
Grain		0.003		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fermantation		0.211		<0.001	<0.001	<0.001	<0.001	0.001	0.002
Grain × Fermantation		0.045		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[A] GE, gross energy; DM, dry matter. Within a column, means without a common superscript differ ($P < 0.05$).

Table 6
Total and available lysine detected in the grains and their corresponding DDGS.

Grain Name	Percentage of total and available lysine[A]		
	Grain	DDGS (STARGEN)	DDGS (jet-cooking)
Pioneer Hi-Bred corn	2.6^{l} (88.8^{n})	4.4^{a} (93.5^{n})	3.8^{bcd} (89.2^{n})
CPS wheat	2.6^{l} (90.0^{n})	3.3^{fgh} (93.2^{n})	3.2^{hgi} (91.7^{n})
Ultima triticale	3.2^{ghijk} (93.5^{n})	3.1^{hijk} (95.0^{n})	3.3^{fghi} (91.1^{n})
Pronghorn Triticale	3.6^{cdef} (95.2^{n})	3.3^{fghi} (94.5^{n})	4.0^{b} (91.4^{n})
AC Reed wheat	2.6^{l} (95.9^{n})	3.2^{ghijk} (93.8^{n})	3.4^{efg} (92.7^{n})
AC Andrew wheat	2.5^{l} (92.0^{n})	3.1^{hijk} (94.6^{n})	3.2^{ghijk} (95.1^{n})
Large Ptarmigan wheat	2.6^{l} (N/A)	3.1^{hijk} (94.3^{n})	3.8^{bcd} (92.5^{n})
Average Ptarmigan wheat	2.9^{jkl} (93.7^{n})	3.2^{ghij} (94.5^{n})	3.3^{fgh} (92.3^{n})
CDC Ptarmigan wheat	3.1^{ghijk} (96.5^{n})	3.1^{ijk} (95.0^{n})	3.9^{bc} (92.4^{n})
Small Ptarmigan wheat	2.9^{kl} (96.6^{n})	3.4^{efg} (94.6^{n})	3.6^{de} (92.6^{n})

[A] Values are expressed as percentage of total lysine relative to the crude protein content. Means in parenthesis are percentage of available lysine relative to total lysine. Within a column, means without a common superscript differ ($P < 0.05$).

lysine values than those of the native, unheated proteins (Kwok et al., 1998).

Corn DDGS from STARGEN fermentation was superior to CPS wheat, triticale and wheat in the concentration of total lysine to protein. However, DDGS samples of CPS wheat, wheat, and triticale from STARGEN fermentation had comparable concentrations of total lysine to protein. The DDGS of corn, triticale (represented by Pronghorn), wheat (represented by small ptarmigan, large ptarmigan, and CDC ptarmigan) from jet-cooking fermentation had higher concentrations of total lysine compared to CPS wheat (Table 6). The type of fermentation did not impact ($P > 0.05$) the available lysine to total lysine detected in the DDGS. The DDGS of corn, CPS wheat, triticale, and wheat did not differ in the concentration of available to total lysine.

4. Conclusion

This study shows that utilization of the new "raw starch hydrolysis" technology has the potential to improve the recovery of value added components in DDGS compared to the jet-cooking approach indicating that the proper choice of fermentation process could improve commercial quality of DDGS. The appropriate feedstock and fermentation approach utilized in ethanol industry should be carefully chosen to ensure that an ethanol plant can produce several unique value-added components on site. This study could be the first report providing complete data regarding the impact of fermentation approach on the concentration of total lysine and on its bioavailability in DDGS.

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