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Compositional profile and variation of Distillers Dried Grains with Solubles from various origins with focus on non-starch polysaccharides

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ABSTRACT

Corn-, wheat- and mixed cereal Distillers' Dried Grains with Solubles (DDGS) were investigated for compositional variability among DDGS origins, ethanol plants, and the relationship between corn and corresponding DDGS. A total of 138 DDGS samples were analyzed by use of Near Infrared Reflectance Spectroscopy for common constituents, while 63 DDGS samples along with 11 corn samples were characterized for their non-starch polysaccharide (NSP) content. The results indicated that the compositional profile of DDGS reflected the nutrient content of the parent grain but with a greater content of remaining nutrients (*e.g.* protein, fat, fibre and minerals) after fermentation of starch to ethanol. Corn DDGS differentiated from wheat DDGS by a greater content of fat ($P \leq 0.006$), insoluble-NSP ($P<0.001$), uronic acids ($P<0.001$), cellulose ($P=0.032$), and arabinose/xylose ($P<0.001$) – and uronic acid/xylose-ratio ($P<0.001$). Wheat DDGS differentiated from corn DDGS by a greater content of ash ($P=0.001$), soluble-NSP ($P<0.001$), and Klason lignin ($P<0.001$). Among the three sources of DDGS, the greatest variation was observed for the content of soluble-NSPs, especially soluble arabinoxylan. Based on the compositional profiles of the DDGS, principal component analysis allowed for a visual differentiation of corn DDGS from five different ethanol plants, indicating the potential of each ethanol plant to produce DDGS with consistent compositional characteristics. Furthermore, investigation of corn and corresponding DDGS indicated that the NSP fraction is modified during the fermentation process, especially arabinoxylan, by an increase in soluble arabinoxylan proportion in DDGS. In addition, the arabinose/xylose ($P<0.001$) and uronic acid/xylose-ratio ($P<0.001$) were greater for corn, compared with corresponding DDGS, indicating modifications of the endosperm arabinoxylan during the fermentation and drying process.

Abbreviations: ADF, acid detergent fibre; AH, acid hydrolysis; A/X, arabinose-to-xylose; Cel./NSP, cellulose-to-NSP; CV, coefficient of variation CF, crude fibre; CP, crude protein; DF, dietary fibre; DM, dry matter; DDGS, distillers dried grain with solubles; EE, ether extract; I-NSP, insoluble NSP; NCP, non-cellulosic polysaccharide; aNDFom, neutral detergent fibre assayed with heat stable amylase exclusive residual ash; NIRS, near infrared reflectance spectroscopy; NSP, non-starch polysaccharides; PCA, principal component analysis; S-Ara, soluble arabinose; S-NSP, soluble NSP; S-Xyl, soluble xylose; T-NSP, total NSP; UA/X, uronic acid-to-xylose.

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1. Introduction

Distillers' Dried Grains with Solubles (DDGS) is the major co-product from the dry-grind production of bioethanol from cereal grains. After the conversion of grain starch to ethanol during the fermentation process, the non-fermentable part remains, with an increase of all nutrients except starch compared with those in the parent grain. For both corn- and wheat DDGS an increase of approximately 3-fold of nutrients such as protein, fat, vitamins, minerals and fibre is observed (Widyaratne and Zijlstra, 2007; Stein and Shurson, 2009).

Compositional variability of DDGS from various bioethanol plants has previously been reported for both corn (Spiehs et al., 2002; Belyea et al., 2004; Batal and Dale, 2006; Liu, 2011), and wheat origins (Nyachoti et al., 2005; Bandegan et al., 2009; Cozannet et al., 2010). The variability in the chemical composition of DDGS is due to a number of factors including differences in processing technologies among the bioethanol plants, and variability in chemical composition of the grains (Liu, 2011; Olukosi and Adebiyi, 2013). The majority of the reported compositional profiles of DDGS have focused mainly on common constituents such as crude protein (CP), crude fibre (CF), neutral detergent fibre, acid detergent fibre (ADF), fat, minerals, and amino acids (Liu, 2011; Olukosi and Adebiyi, 2013). Non-starch polysaccharides (NSP) make up 25–30% of the DDGS, with the two major components of the NSP being arabinoxylan and cellulose. Arabinoxylan consists of D-xylose units joined by β-linkages substituted with arabinose residues along the chain (Kim et al., 2008). The substitution of arabinose occurs randomly allowing other substitutes such as D-glucuronic acid and acetyl groups to attach to the xylan backbone (Bedford, 1995). These random substitutes together with feruloylated arabinose residues induce the arabinoxylan cross-linking to form strong heterogeneous intermolecular complexes, affecting the potential for enzymatic degradation along with the potential encapsulation of nutrients within the cell wall (Hartley, 1973; Lequart et al., 1999; Lapierre et al., 2001; Piber and Koehler, 2005). Despite the high content of NSPs in DDGS detailed characterization of the NSPs in DDGS is limited as only a few reports describe the NSP composition (Widyaratne and Zijlstra, 2007; de Vries et al., 2013). Characterization of the NSP composition in DDGS of various origins may contribute to the overall understanding and interpretation of opportunities and limitations in DDGS degradation *in vitro* and *in vivo*, which is particularly useful for feed manufactures and enzyme producers targeting DDGS degradation.

The current study describes the compositional profiles and extent of variation in DDGS from three different grain origins; corn, wheat, and mixed cereal. A total of 138 DDGS samples from three sources of grain and 24 different bioethanol plants were analyzed based on the common constituents, with detailed NSP profiles of 63 DDGS samples. Multivariate data analysis was applied to the compositional data to determine grouping and separation of DDGS samples according to grain origins and bioethanol plants.

2. Materials and methods

2.1. Materials

A total of 138 DDGS samples were collected from 24 ethanol plants in the U.S. and E.U., covering the following three parent grain origins; corn, wheat, and a mix of cereal (mixed).

A total of 72 corn DDGS samples were collected from 21 different ethanol plants in the U.S.; 11 DDGS samples along with 11 corn samples were sampled over 11 months with one sample every month from a plant in Nevada (P1), 17 samples were sampled over 1½ months from a plant in Minnesota (P2), 10 samples were sampled over 20 days from another plant in Minnesota (P3), 10 samples were sampled over 20 days from a plant in Wisconsin (P4), 8 samples were sampled over 8 days from a plant in Iowa (P5), and 16 samples were supplied by a DDGS supplier in Iowa, representing samples from 7 plants in Minnesota, 4 plants in Iowa, 2 plants in Nevada, and 1 plant in Illinois, Indiana and Kentucky.

A total of 56 wheat DDGS samples were collected from 2 different ethanol plants in the E.U., with 46 samples from a plant in the U.K. sampled over a period of app. 3 months, and 10 samples from a plant in France sampled over 20 days.

Finally, 10 DDGS samples of a mixed grain origin were sampled over 20 days from a plant in Sweden. The parent grains used in the production of the mixed DDGS were wheat, triticale, barley, and rye. However, their individual proportion is unknown.

2.2. Chemical analysis

All 138 DDGS samples (72 corn, 56 wheat, and 10 mixed) and 11 corn samples were milled (0.5 mm) and scanned from 1100 to 2498 nm by near infrared reflectance spectroscopy (NIRS) on a FOSS NIRSystems 5000 (Foss). The spectral data were predicted by Aunir, AB Agri Ltd., UK, for the composition of: moisture, fat ether extract (EE), fat acid hydrolysis (AH), CP ($N \times 6.25$), CF, ash, starch, total sugars, aNDFom and ADF, using the calibration available for DDGS and corn, respectively.

A total of 63 DDGS samples (47 corn, 11 wheat, and 5 mixed) and 11 corn samples were quantified for total and soluble NSP content along with their constituent sugars by gas–liquid chromatography for neutral sugars and by a colorimetric method for uronic acids, with procedure and calculations according to Bach Knudsen (1997), with the modification that 2 mol/L sulfuric acid for 1 h was used to hydrolyze the non-cellulosic polysaccharides (NCP) rather than 1 mol/L sulfuric

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Table 1
Compositional profile of corn-, wheat-, and mixed cereal DDGS (g/kg DM).

	Corn DDGS			Wheat DDGS			Mixed DDGS		
	Mean[a]	Range[b]	S.D. (CV)	Mean[c]	Range[d]	S.D. (CV)	Mean[e]	Range[f]	S.D. (CV)
Moisture	87[g]	(65–124)	8 (0.10)	76[g]	(68–87)	2 (0.02)	110[h]	(74–127)	15 (0.14)
Fat (EE)	91[g]	(65–118)	15 (0.17)	52[h]	(44–65)	8 (0.16)	47[h]	(44–50)	2 (0.05)
Fat (AH)	111[g]	(84–135)	14 (0.13)	73[h]	(65–88)	8 (0.11)	69[h]	(66–74)	3 (0.04)
Crude protein	314[g]	(271–364)	21 (0.07)	334[g]	(303–379)	28 (0.09)	366[g]	(338–383)	13 (0.03)
Crude fibre	77[g]	(64–95)	6 (0.07)	67[g]	(55–88)	9 (0.14)	62[g]	(56–76)	6 (0.10)
Ash	71[g]	(54–90)	7 (0.09)	91[h]	(81–100)	4 (0.05)	87[g,h]	(80–102)	7 (0.08)
Starch	60[g]	(29–139)	27 (0.45)	40[g]	(<10–88)	42 (1.03)	24[g]	(<10–37)	9 (0.39)
Total sugars	90[g]	(54–126)	17 (0.19)	98[g]	(46–124)	22 (0.23)	126[g]	(99–142)	13 (0.10)
aNDFom	351[g]	(302–397)	24 (0.07)	306[g]	(273–342)	26 (0.08)	302[g]	(289–312)	9 (0.03)
ADF	101[g]	(89–119)	6 (0.06)	105[g,h]	(95–122)	8 (0.07)	120[h]	(115–123)	3 (0.02)

Based on near infrared reflectance spectroscopy. ADF, acid detergent fibre; AH, acid hydrolysis; EE, ether extract; aNDFom, neutral detergent fibre; S.D., standard deviation.
[a] Avg. of means from 21 plants.
[b] $N = 72$.
[c] Avg. of means from 2 plants.
[d] $N = 56$.
[e] Mean from 1 plant.
[f] $N = 10$.
[g,h] Means that do not share a letter are significant different at P<0.05.

acid for 2 h. Klason lignin was measured gravimetrically as the residue resistant to hydrolysis by 2 mol/L sulfuric acid (Bach Knudsen, 1997).

2.3. Calculations and statistical analysis

Content of NCP-glucose was calculated as

$$\text{NCP-glucose} = \text{NSP}_{\text{glucose}\,(2\,\text{mol/L}\,H_2SO_4)}$$

Content of cellulose was calculated as

$$\text{cellulose} = \text{NSP}_{\text{glucose}\,(12\,\text{mol/L}\,H_2SO_4)} - \text{NCP-glucose}$$

Total non-starch polysaccharides (T-NSP) as

$$\text{T-NSP} = \text{rhamnose} + \text{fucose} + \text{arabinose} + \text{xylose} + \text{mannose} + \text{galactose} + \text{NCP-glucose} + \text{uronic acids} + \text{cellulose}$$

Soluble NSP (S-NSP) as

$$\text{S-NSP} = \text{T-NSP} - \text{insoluble NSP(I-NSP)}$$

Dietary fibre (DF) as

$$\text{DF} = \text{T-NSP} + \text{Klason lignin}$$

A one-way ANOVA was applied for comparison among means of the DDGS compositions from various origins followed by a Tukey pair wise comparison with overall significance level at P=0.05, using Minitab 16 (Minitab Inc.). Data from the compositional analysis were categorized and analyzed by principal component analysis (PCA) with evenly spread 7-fold partial cross-validation after mean centering and unit variance scaling, using Evince 2.5.5 software (UmBio AB) to detect distributions and separations among the groups, as previously discussed (Shewry et al., 2013).

3. Results

3.1. Compositional variation in DDGS of various origins

The results of the compositional analyses of the DDGS are presented in Table 1. Common for all DDGS, aNDFom and CP represented the major fractions, with a large extent of the aNDFom fraction present as ADF. Corn DDGS contained a greater content of fat (AH) compared with wheat- and mixed DDGS with an increase of 38 g/kg DM (P=0.005) and 42 g/kg DM (P=0.019), respectively. Furthermore, a greater aNDFom content was observed in corn DDGS with an increase of 45 g/kg DM (P=0.057) and 49 g/kg DM (P=0.118) compared with wheat- and mixed DDGS, respectively. Wheat DDGS contained 20 g/kg DM (P=0.001) greater ash content than corn DDGS. Among the three sources of DDGS, the greatest coefficient of variation (CV) was observed for the content of starch, with CVs of 0.39–1.03. For both corn- and wheat DDGS, the second and third greatest CVs was observed for total sugars (0.19 and 0.23) and fat (EE) (0.17 and 0.16), respectively. Corn- and mixed DDGS

Table 2
Non-starch polysaccharide profile of corn-, wheat-, and mixed DDGS (g/kg DM).

	Corn DDGS			Wheat DDGS			Mixed DDGS		
	Mean[a]	Range[b]	S.D. (CV)	Mean[c]	Range[d]	S.D. (CV)	Mean[e]	Range[f]	S.D. (CV)
Total NSP									
Total	283[g]	(250–337)	20 (0.09)	262[g]	(242–291)	9 (0.04)	247[g]	(238–257)	8 (0.03)
Soluble	31[g]	(16–65)	8 (0.47)	67[h]	(53–80)	1 (0.02)	65[h]	(54–76)	8 (0.13)
Cellulose	67[g]	(52–91)	8 (0.16)	50[h]	(35–67)	16 (0.32)	54[g,h]	(51–59)	3 (0.06)
NCP									
Xylose									
Total	77[g]	(67–100)	7 (0.10)	86[g]	(70–93)	7 (0.08)	78[g]	(74–85)	5 (0.07)
Soluble	6[g]	(1–16)	3 (0.62)	23[h]	(15–32)	5 (0.22)	21[h]	(17–25)	3 (0.12)
Arabinose									
Total	62[g]	(56–72)	4 (0.07)	57[g,h]	(51–62)	0 (0.00)	49[h]	(46–52)	3 (0.05)
Soluble	7[g]	(2–15)	3 (0.45)	17[h]	(12–22)	3 (0.15)	14[g,h]	(12–17)	2 (0.13)
Glucose									
Total	28[g]	(21–44)	4 (0.13)	33[g]	(27–37)	1 (0.05)	28[g]	(27–31)	2 (0.05)
Soluble	3[g]	(0–16)	4 (1.90)	11[g]	(1–21)	10 (0.89)	8[g]	(3–13)	3 (0.46)
Mannose									
Total	17[g]	(12–20)	2 (0.12)	16[g]	(13–18)	2 (0.13)	20[g]	(18–22)	2 (0.09)
Soluble	6[g]	(4–9)	1 (0.19)	7[g]	(4–8)	1 (0.18)	13[h]	(11–15)	2 (0.13)
Galactose									
Total	15[g]	(13–21)	2 (0.11)	11[h]	(10–12)	1 (0.11)	10[h]	(10–10)	0 (0.02)
Soluble	3[g]	(2–5)	1 (0.29)	6[h]	(4–7)	1 (0.18)	6[h]	(6–6)	0 (0.05)
Uronic acids									
Total	16[g]	(14–20)	1 (0.08)	8[h]	(7–9)	1 (0.12)	7[h]	(4–8)	2 (0.26)
Soluble	5[g]	(3–6)	1 (0.11)	3[h]	(2–4)	0 (0.15)	3[h]	(2–4)	1 (0.19)
Klason lignin	25[g]	(15–47)	7 (0.26)	66[h]	(44–93)	21 (0.32)	82[h]	(74–90)	6 (0.07)
A/X ratio	0.80[g]	(0.71–0.85)	0.0 (0.05)	0.66[h]	(0.62–0.70)	0.1 (0.09)	0.63[h]	(0.61–0.67)	0.0 (0.04)
UA/X ratio	0.20[g]	(0.16–0.23)	0.0 (0.08)	0.09[h]	(0.08–0.11)	0.0 (0.21)	0.08[h]	(0.06–0.11)	0.0 (0.30)

A/X ratio, arabinose-to-xylose ratio; CV, coefficient of variation; NCP, non-cellulosic polysaccharides; NSP, non-starch polysaccharides; S.D., standard deviation; UA/X ratio, uronic acid-to-xylose ratio.
[a] Average of means from 21 plants.
[b] $N = 47$.
[c] Average of means from 2 plants.
[d] $N = 11$.
[e] Mean from 1 plant.
[f] $N = 5$.
[g,h] Means that do not share a letter are significant different at P<0.05.

had CVs on moisture content of 0.10 and 0.14, respectively, and wheat- and mixed DDGS had CVs on CF content of 0.14 and 0.10, respectively.

Detailed analysis of the NSP profile of 63 samples (47 corn DDGS, 11 wheat DDGS, and 5 mixed DDGS) is presented in Table 2. Corn DDGS had the greatest content of NSP (286 g/kg DM), followed by wheat- (262 g/kg DM), and mixed DDGS (247 g/kg DM). Wheat- and mixed DDGS did not differ (P>0.05) in S-NSP content (ranging from 53 to 80 g/kg DM), while a markedly lesser content (P<0.001) was observed in corn DDGS (16–65 g/kg DM). The distribution of constituent sugars for all three DDGS origins was in the order of xylose > arabinose > NCP-glucose > mannose > galactose. No difference was observed in arabinoxylan content (P=0.555) across the three DDGS origins with corn- (123–172 g/kg DM), wheat- (121–155 g/kg DM), and mixed DDGS (120–136 g/kg DM). Despite the comparable content of arabinoxylan, corn DDGS contained 5 g/kg DM (P=0.226) and 13 g/kg DM (P=0.022) more arabinose compared with wheat- and mixed DDGS, respectively, accompanied by a 8 g/kg DM lesser content of xylose compared with wheat DDGS, hence yielding a greater arabinose/xylose-ratio (A/X-ratio) in corn DDGS of 0.80 compared with that of wheat- (0.66, P<0.001), and mixed DDGS (0.63, P=0.002), respectively. Corn DDGS contained approximately twice as much uronic acids (16 g/kg DM, P<0.001), along with a greater cellulose content (67 g/kg DM) compared with both wheat- (50 g/kg DM, P=0.32), and mixed DDGS (54 g/kg DM, P=0.248). Furthermore, corn DDGS contained a markedly greater uronic acid/xylose-ratio (UA/X-ratio) of 0.20, compared with both wheat- (0.09, P<0.001), and mixed DDGS (0.08, P<0.001). The greatest Klason lignin content was observed in mixed DDGS (82 g/kg DM), compared with wheat- (66 g/kg DM, P=0.105), and corn DDGS (25 g/kg DM, P<0.001). All three DDGS origins had comparable contents of total mannose and galactose. Except the soluble mannose content (P<0.001), no differences in compositional profile were observed between wheat- and mixed DDGS (P>0.05). Regardless of DDGS origin, greater CVs were observed for the soluble constituent sugars compared with the total constituents. Soluble-NCP-glucose had greatest CVs with values of 1.90, 0.89, and 0.46, for corn-, wheat-, and mixed DDGS, respectively. Overall, corn DDGS had greater CVs for both total- and soluble constituents, followed by wheat- and mixed DDGS, respectively. For corn DDGS especially soluble xylose (S-Xyl) and soluble arabinose (S-Ara) had high CVs with values of 0.62 and 0.45, respectively.

The PCA illustrated a clear clustering and separation between corn DDGS to one side, and wheat- and mixed cereal DDGS on the other (Fig. 1A). The corresponding loading plot (Fig. 1B), indicated the components most responsible for the separation,

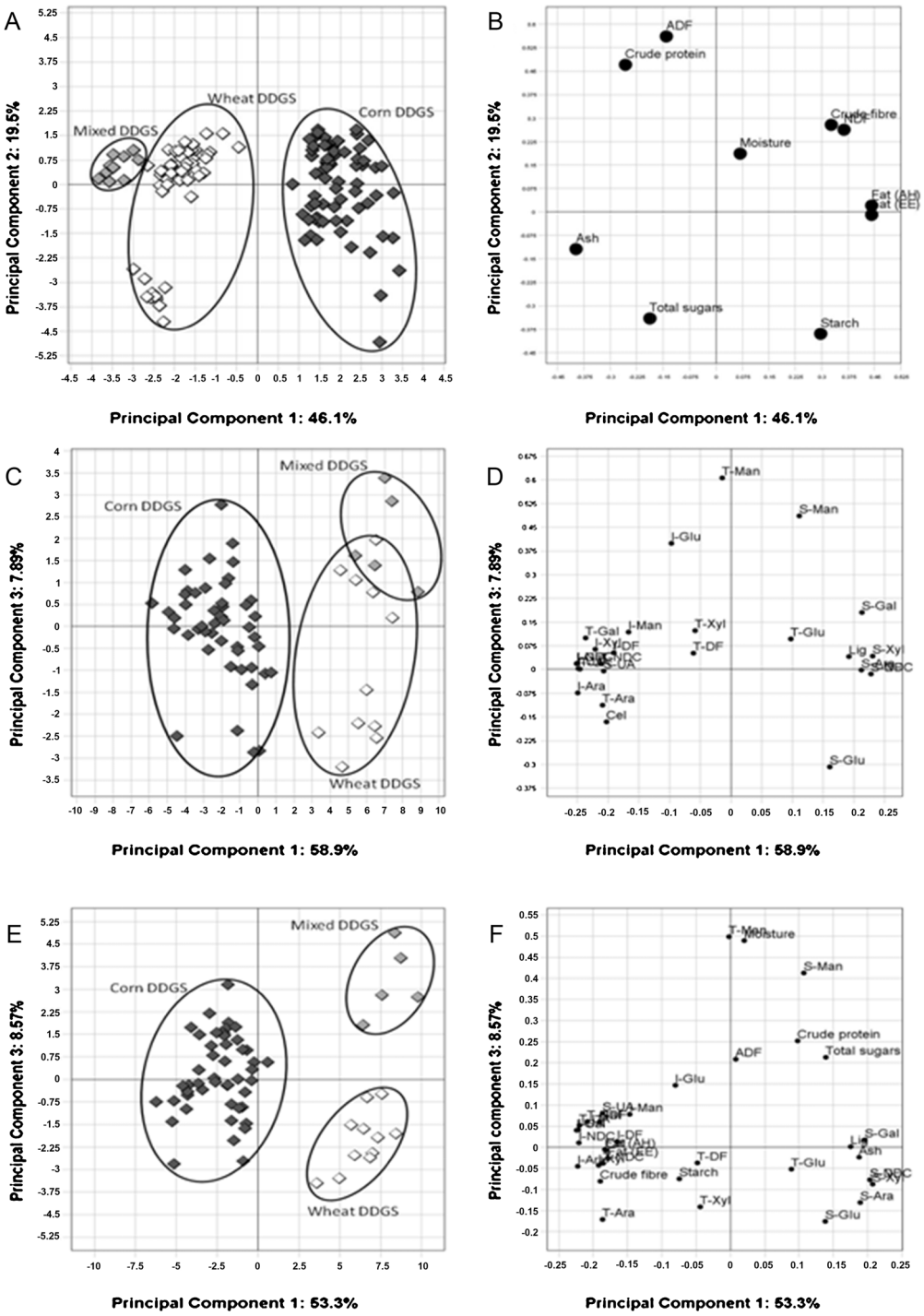

Fig. 1. Principal component analysis of DDGS samples of corn-, wheat, and mixed cereal origin: (A, B) scores and loadings plots of PCA model constructed from common constituent profiles as model variables ($N = 138$), (C, D) scores and loadings plots of PCA model constructed from NSP profile as models variables ($N = 63$), (E, F) scores and loadings of PCA model constructed from combined common constituents and NSP profiles as models variables ($N = 63$). ADF, acid detergent fibre; AH, acid hydrolysis; Ara, arabinose; Cel, cellulose; DF, dietary fibre; EE, ether extract; Gal, galactose; Glu, NCP-glucose; I, insoluble; Lig, Klason lignin; Man, mannose; NDC, non digestible carbohydrate; NDF, neutral detergent fibre; S, soluble; T, total; Xyl, xylose.

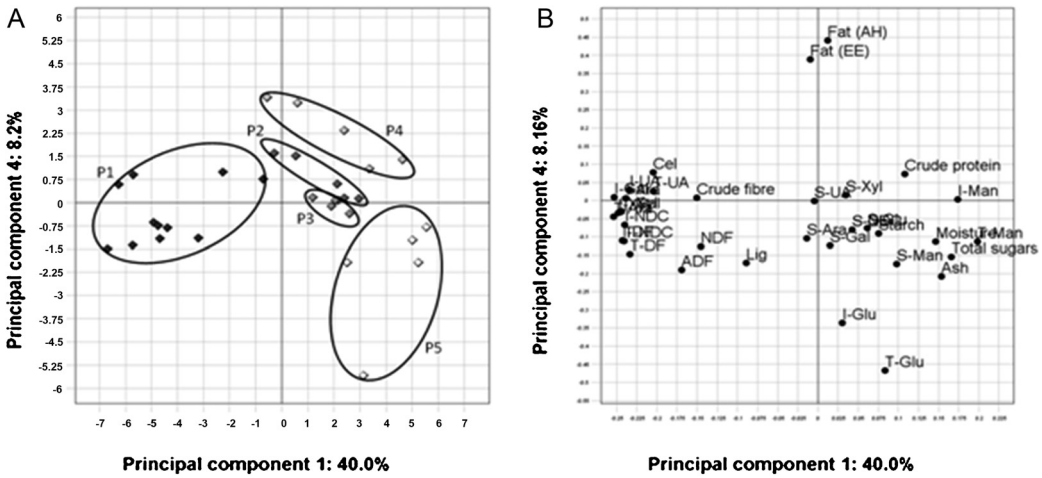

Fig. 2. Principal component analysis of corn DDGS samples originating from five different ethanol plants in the US: (A, B) scores and loadings of PCA model constructed from combined common constituents and NSP profiles as models variables ($N = 31$). ADF, acid detergent fibre; AH, acid hydrolysis; Ara, arabinose; Cel, cellulose; DF, dietary fibre; EE, ether extract; Gal, galactose; Glu, NCP-glucose; I, insoluble; Lig, Klason lignin; Man, mannose; NDC, non digestible carbohydrate; NDF, neutral detergent fibre; S, soluble; T, total; Xyl, xylose.

with corn DDGS differentiating due to a general greater content of fat, aNDFom, CF, and starch. Wheat and mixed DDGS differentiated from corn DDGS by a general greater content of ash, CP, and total sugars. The corn- and wheat DDGS samples indicated a comparable variation between individual samples in the score plot. The PCA model illustrated clear separation and clustering between the three different DDGS origins based on the NSP characteristics, with only a minor overlap between wheat- and mixed DDGS (Fig. 1C). The corresponding loading plot revealed the constituents with the greatest effect on the separations, with corn DDGS differentiating due to large content of insoluble- and total constituents along with greater content of cellulose, and wheat- and mixed DDGS differentiating due to greater content of soluble constituents and Klason lignin (Fig. 1D). A combined PCA model on both NIRS- and NSP compositional data on 63 samples, revealed the clearest separation among the three DDGS origins (Fig. 1E), compared with the two PCA models based individually on NIRS- and NSP compositional data (Fig. 1A and C). The corresponding loading plot (Fig. 1F), strongly underlined the constituents differing the most and being most responsible for the grouping between the three DDGS origins. Corn DDGS was greater in insoluble- and total NSP, cellulose, and starch, and wheat- and mixed DDGS greater in soluble NSPs, total sugars, and ash. Furthermore, the loading plot revealed that mixed DDGS differed from wheat DDGS by having a greater content of mannose, total sugars, ADF, and CP.

3.2. Compositional variation in corn DDGS from various ethanol plants

Table 3 presents the results of corn DDGS samples collected from 5 different ethanol plants; P1 ($N = 11$), P2 ($N = 17$), P3 ($N = 10$), P4 ($N = 10$), and P5 ($N = 8$), of which 56 samples were analyzed for common constituents, and 31 samples for the NSP profile. For starch content P1, P4, and P5 had the greatest CVs of 0.10–0.11. Furthermore, P1 and P5 had greater CVs in moisture content of 0.11 and 0.16, respectively, than P2–P4 and greater CVs were observed for total sugar content for P1 (0.09) and P2 (0.11). Across all of the common constituents, the five plants had overall average CVs of 0.07, 0.05, 0.03, 0.05, and 0.05, for P1, P2, P3, P4, and P5, respectively.

The five plants had varying CVs with regard to S-NSPs with values of 0.07–0.49. However, only small variation in CVs was observed for T-NSPs (288–325 g/kg DM) with values of 0.01–0.04 across all five plants. The CVs were relatively consistent for cellulose content among the different plants. On the other hand Klason lignin content differed with CVs of 0.13–0.22. The CVs with regard to soluble constituent sugars varied markedly between the five plants with overall CVs of 0.03–1.49. Each of the five plants had conserved A/X-ratios (0.71–0.79) with corresponding low CVs with values of 0.01–0.02, together with conserved UA/X-ratios (0.17–0.23). There was an individual grouping between the five plants, indicating a conserved compositional profile of DDGS from each ethanol plant (Fig. 2A), and a greater compositional variation of P1, followed by P5, P4, P2, and P3, respectively. The corresponding loading plot (Fig. 2B), illustrated that P1 differed from the remaining plants by a greater content of total- and insoluble NSP ($P<0.05$), cellulose ($P<0.05$, except when compared with P2), and uronic acid ($P<0.05$, except when compared with P4), P4 differed mainly by a greater content of fat(EE) ($P<0.05$), P5 differed based on a greater content of ash ($P<0.05$), total sugars ($P<0.05$), and total NCP-glucose ($P<0.05$), while P3 differed from P2 mainly by a greater content of fat (AH) ($P<0.05$).

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Table 3
Compositional profiles of corn DDGS from five ethanol plants (g/kg DM).

Sampling period	Plant P1 (*N* = 11; 11)[a]			Plant P2 (*N* = 17; 5)[a]			Plant P3 (*N* = 10; 5)[a]			Plant P4 (*N* = 10; 5)[a]			Plant P5 (*N* = 8; 5)[a]		
	11 months			1½ months			20 days			20 days			8 days		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
Moisture	80[e]	(65–91)	0.11	98[b,c]	(90–108)	0.05	103[b]	(95–109)	0.04	87[d,e]	(78–93)	0.05	90[c,d]	(78–124)	0.16
Fat (EE)	86[c]	(79–93)	0.06	87[c]	(81–101)	0.05	84[c]	(79–92)	0.04	92[b]	(83–103)	0.06	83[c]	(80–87)	0.02
Fat (AH)	106[c]	(101–115)	0.05	112[b]	(105–126)	0.04	106[c]	(102–114)	0.03	112[b]	(104–122)	0.05	105[c]	(100–108)	0.02
Crude protein	317[c]	(297–337)	0.05	351[b]	(337–364)	0.02	349[b]	(343–356)	0.01	320[c]	(309–333)	0.02	319[c]	(313–332)	0.02
Crude fibre	85[b]	(74–95)	0.08	76[d]	(73–82)	0.03	81[b,c]	(78–86)	0.03	83[b,c]	(80–86)	0.03	79[c,d]	(77–81)	0.02
Ash	68[d,e]	(62–73)	0.06	65[e]	(61–70)	0.04	70[c,d]	(68–73)	0.02	72[c]	(66–78)	0.05	83[b]	(74–90)	0.06
Starch	51[d]	(43–59)	0.10	69[b]	(65–78)	0.05	39[e]	(36–45)	0.07	58[c]	(49–69)	0.10	64[b]	(57–79)	0.11
Total sugars	72[d]	(62–87)	0.09	64[e]	(54–78)	0.11	89[c]	(86–95)	0.03	94[c]	(85–103)	0.06	102[b]	(92–114)	0.07
aNDFom	373[b]	(346–397)	0.04	345[d]	(330–363)	0.02	367[b,c]	(359–380)	0.02	359[c]	(350–368)	0.02	362[b,c]	(357–365)	0.01
ADF	113[b]	(108–119)	0.03	109[b]	(104–116)	0.03	111[b]	(108–114)	0.02	102[c]	(98–107)	0.03	105[c]	(99–114)	0.05
Total NSP															
Total	325[b]	(313–337)	0.02	305[c]	(329–349)	0.04	293[c]	(290–296)	0.01	288[c]	(280–299)	0.03	294[c]	(283–309)	0.04
Soluble	29[b]	(18–37)	0.19	37[b]	(18–65)	0.49	29[b]	(23–33)	0.15	25[b]	(23–27)	0.07	31[b]	(26–41)	0.18
Cellulose	79[b]	(74–91)	0.06	74[b,c]	(65–79)	0.08	67[c]	(64–70)	0.03	68[c]	(64–71)	0.04	68[c]	(64–76)	0.07
NCP															
Xylose															
Total	94[b]	(88–100)	0.04	88[c]	(84–91)	0.03	80[d]	(80–81)	0.01	79[d]	(74–85)	0.06	78[d]	(75–83)	0.04
Soluble	5[b]	(1–8)	0.48	8[b]	(1–16)	0.78	5[b]	(2–6)	0.33	4[b]	(2–6)	0.39	4[b]	(2–6)	0.34
Arabinose															
Total	69[b]	(65–72)	0.04	62[c]	(60–64)	0.03	63[c]	(62–63)	0.01	61[c]	(59–65)	0.05	59[c]	(57–62)	0.04
Soluble	7[b]	(3–9)	0.25	8[b]	(3–14)	0.54	7[b]	(5–7)	0.13	6[b]	(4–7)	0.19	7[b]	(6–8)	0.15
Glucose															
Total	27[c]	(22–29)	0.06	25[c]	(23–25)	0.04	28[c]	(27–29)	0.03	25[c]	(24–28)	0.07	34[b]	(29–44)	0.17
Soluble	1[b]	(0–5)	1.35	4[b]	(0–14)	1.41	2[b]	(0–5)	1.17	0[b]	(0–1)	1.49	3[b]	(0–8)	1.04
Mannose															
Total	17[d]	(14–18)	0.06	19[b,c]	(18–20)	0.04	18[b,c]	(17–19)	0.05	18[c]	(17–19)	0.04	20[b]	(19–20)	0.03
Soluble	7[c,d]	(5–8)	0.13	9[b]	(8–9)	0.09	6[d]	(6–7)	0.08	7[c,d]	(6–7)	0.06	8[b,c]	(8–8)	0.03
Galactose															
Total	20[b]	(18–21)	0.05	17[c]	(17–18)	0.03	17[c,d]	(17–17)	0.01	17[c,d]	(16–18)	0.04	16[d]	(15–17)	0.05
Soluble	3[b]	(2–4)	0.17	3[b]	(2–5)	0.31	3[b]	(3–3)	0.09	3[b]	(3–3)	0.03	3[b]	(3–4)	0.09
Uronic acids															
Total	19[b]	(18–20)	0.04	17[c]	(16–18)	0.06	18[c]	(17–18)	0.02	18[b,c]	(17–18)	0.03	17[c]	(16–17)	0.02
Soluble	5[b]	(5–6)	0.08	5[b]	(3–6)	0.26	5[b]	(5–5)	0.04	5[b]	(5–6)	0.07	5[b]	(4–6)	0.08
Klason lignin	38[b]	(28–47)	0.13	33[b]	(29–42)	0.18	30[b]	(23–41)	0.22	31[b]	(24–38)	0.18	34[b]	(27–42)	0.17
A/X-ratio	0.73[d]	(0.71–0.74)	0.01	0.71[e]	(0.70–0.72)	0.01	0.78[b]	(0.77–0.80)	0.01	0.77[b,c]	(0.75–0.79)	0.02	0.76[c]	(0.75–0.78)	0.01
UA/X-ratio	0.20[c]	(0.19–0.21)	0.03	0.20[c]	(0.17–0.22)	0.08	0.22[b]	(0.21–0.22)	0.02	0.23[b]	(0.22–0.23)	0.04	0.22[b]	(0.20–0.23)	0.05

ADF, acid detergent fibre; AH, acid hydrolysis; CV, coefficient of variation; EE, ether extract; NCP, non-cellulosic polysaccharides; aNDFom, neutral detergent fibre.
[a] *N* common constituents analysis; *N* NSP analysis.
[b–e] Means that do not share a letter are significant different at $P<0.05$.

Table 4
Compositional profile of corn and corresponding DDGS (g/kg DM).

	Corn (N = 11)			Corn DDGS (N = 11)			Ratio[a]
	Mean	Range	S.D. (CV)	Mean	Range	S.D. (CV)	
Moisture	139[b]	(130–146)	6 (0.04)	80[c]	(65–91)	9 (0.11)	–
Fat (EE)	35[b]	(34–37)	1 (0.03)	86[c]	(79–93)	5 (0.06)	2.4
Fat (AH)	39[b]	(37–41)	1 (0.04)	106[c]	(101–115)	5 (0.05)	2.7
Crude protein	83[b]	(75–91)	6 (0.08)	317[c]	(297–337)	15 (0.05)	3.8
Crude fibre	25[b]	(23–27)	1 (0.04)	85[c]	(74–95)	7 (0.08)	3.4
Ash	10[b]	(9–11)	1 (0.05)	68[c]	(62–7-3)	4 (0.06)	6.5
Starch	723[b]	(705–736)	11 (0.01)	51[c]	(43–59)	5 (0.10)	–
Total sugars	51[b]	(46–59)	4 (0.08)	72[c]	(62–87)	7 (0.09)	1.4
aNDFom	93[b]	(79–104)	8 (0.08)	373[c]	(346–397)	15 (0.04)	4.1
ADF	38[b]	(33–44)	3 (0.09)	113[c]	(108–119)	4 (0.03)	3.0
Total NSP							
Total	79[b]	(67–91)	7 (0.08)	325[c]	(313–337)	8 (0.02)	4.1
Soluble	6[b]	(2–10)	3 (0.39)	29[c]	(18–37)	6 (0.19)	4.5
Cellulose	17[b]	(14–20)	2 (0.12)	79[c]	(74–91)	5 (0.06)	4.6
NCP							
Xylose							
Total	23[b]	(20–27)	2 (0.09)	94[c]	(88–100)	3 (0.04)	4.0
Soluble	1[b]	(0–1)	1 (0.97)	5[c]	(1–8)	2 (0.48)	7.6
Arabinose							
Total	18[b]	(15–20)	1 (0.07)	69[c]	(65–72)	2 (0.04)	3.8
Soluble	1[b]	(0–2)	1 (0.63)	7[c]	(3–9)	2 (0.25)	5.8
Glucose							
Total	7[b]	(6–8)	1 (0.09)	27[c]	(22–29)	2 (0.06)	3.7
Soluble	1[b]	(0–1)	1 (0.97)	1[b]	(0–5)	2 (1.35)	2.4
Mannose							
Total	2[b]	(2–3)	0 (0.10)	17[b]	(14–18)	1 (0.06)	7.5
Soluble	1[b]	(1–1)	0 (0.14)	7[c]	(5–8)	1 (0.13)	6.8
Galactose							
Total	6[b]	(4–7)	1 (0.13)	20[c]	(18–21)	1 (0.05)	3.6
Soluble	1[b]	(1–2)	0 (0.38)	3[c]	(2–4)	1 (0.17)	2.5
Uronic acids							
Total	5[b]	(4–6)	1 (0.10)	19[c]	(18–20)	1 (0.04)	3.5
Soluble	1[b]	(1–2)	0 (0.19)	5[c]	(5–6)	0 (0.08)	3.5
Klason lignin	10[b]	(7–15)	2 (0.23)	38[c]	(28–47)	5 (0.13)	3.9
A/X-ratio	0.77[b]	(0.74.0.79)	0.0 (0.02)	0.73[c]	(0.71–0.74)	0.0 (0.01)	
UA/X-ratio	0.23[b]	(0.22–0.24)	0.0 (0.03)	0.20[c]	(0.19–0.21)	0.0 (0.03)	
Cel./T-NSP ratio	0.21[b]	(0.20–0.23)	0.0 (0.05)	0.24[c]	(0.20–0.23)	0.0 (0.05)	

ADF, acid detergent fibre; AH, acid hydrolysis; A/X ratio, arabinose-to-xylose ratio; CV, coefficient of variation; EE, ether extract; NCP, non-cellulosic polysaccharides; aNDFom, neutral detergent fibre; NSP, non-starch polysaccharides; UA/X ratio, uronic acid-to-xylose ratio.

[a] Average corn DDGS-to-corn ratio.

[b,c] Means that do not share a letter are significant different at P<0.05.

3.3. Compositional variation between corn and corresponding corn DDGS

The results of the compositional profile of corn and corresponding DDGS, sampled simultaneously from the ethanol plant over a period of 11 month, are presented in Table 4. For common constituents, DDGS had an increase in all components but starch, with average increase of 3.4-fold. The greatest increase was observed for the content of ash, aNDFom, and CP, whereas the least increase was observed for the content of total sugars, and fat, respectively. Furthermore, DDGS had greater CVs of fat and CF content compared with the parent grain, whereas corn on the other hand had greater CVs of CP, ADF, and aNDFom content, across the 11 months. Comparable CVs of ash and total sugar content was observed between corn and corresponding DDGS.

The NSP compositional profiles revealed that DDGS had a 4.1- and 4.5 times as high content of T-NSP and S-NSP as in corn, respectively. The S-Xyl and S-Ara content in DDGS increased to levels 7.6 and 5.8 times the content in corn, respectively. Furthermore, the content of total- and soluble mannose was markedly increased in DDGS compared with corn with values of 7.5- and 6.8-fold greater than in corn, respectively. The A/X- and UA/X-ratio were 0.04- (P<0.001) and 0.05 (P<0.001) units less in DDGS than in corn, respectively. On the other hand cellulose/NSP-ratio was 0.03 units greater (P<0.001) for DDGS than in corn. Corn DDGS had reduced CVs of T-NSP (0.06 units) and S-NSP (0.20 units) than the corresponding corn, and except for soluble NCP-glucose, this was the case for all constituent sugars, both total- and soluble content.

4. Discussion

In line with our expectations, the nutrient composition of DDGS varied between DDGS from both different ethanol plants and DDGS originating from different parent grains (Spiehs et al., 2002; Olukosi and Adebiyi, 2013). Furthermore, as expected,

we also observed that the nutrient composition of the DDGS in part reflected the composition in the parent grain, as only starch was removed during the fermentation process (Widyaratne and Zijlstra, 2007; Gibreel et al., 2011). The greater content of fat in corn DDGS compared with wheat DDGS, along with the greater content of protein in wheat DDGS than in corn DDGS corresponds to previous characterizations of content in parent grains (Belitz et al., 2009). Overall, the major constituent analysis of the DDGS corresponds to previous published results (Cromwell et al., 1993; Belyea et al., 2004; Widyaratne and Zijlstra, 2007; Kim et al., 2008; Liu, 2008). The cereals used in the production of the mixed DDGS are unknown, however the major cereal fraction is presumably wheat, considering the similar composition of wheat- and mixed DDGS, based on the PCA-model and compositional analysis.

Similar to previous studies describing the NSP profiles of corn and wheat (Bach Knudsen, 1997), and corn- and wheat DDGS (Widyaratne and Zijlstra, 2007), we also observed greater content of soluble NSPs in wheat DDGS compared with corn DDGS, and a greater content of insoluble NSPs in corn DDGS compared with wheat DDGS. It can be speculated that the slightly greater NCP-glucose content of wheat DDGS compared with corn DDGS is caused by the greater content of β-glucan present in wheat compared with corn, as described in literature (Bach Knudsen, 1997). However, it has been speculated that also β-glucan and mannans from yeast are present in the DDGS (de Vries et al., 2013). Both corn- and wheat DDGS contained similar amounts of total arabinoxylan (approximately 140 g/kg DM). However, the greater content of both arabinose and uronic acids present in corn DDGS compared with wheat DDGS, yielding greater A/X- and UA/X-ratios, indicate a more complex structure of the heteroxylan in corn DDGS than in wheat DDGS. This is in line with previous studies reporting a greater branch density and complexity of corn arabinoxylan compared with that of wheat (Bedford, 1995; Saulnier et al., 1995a; Jilek and Bunzel, 2013; Yang et al., 2013).

The markedly greater content of Klason lignin present in wheat DDGS compared with corn DDGS is also in line with previous reported results present in parent grains (Bach Knudsen, 1997; Bunzel et al., 2011). However, the Klason lignin content might overestimate the "true" lignin (complex phenolic polymers) content of the DDGS. Klason lignin is not a well defined chemical matter, but an empirical residue consisting of materials not solubilized by 12 mol/L sulfuric acid (Davin et al., 2008). Klason lignin determined in corn and wheat insoluble fibre have been shown to include, besides true lignin; N (proteins), residual fat and waxes, as well as cutin, with true lignin content equal to approximately 1/3 and 1/4 of the measured Klason lignin content for wheat and corn, respectively (Bunzel et al., 2011). However, other nitrogen sources than proteins, such as Maillard products, can potentially contribute to nitrogen content of the Klason lignin fraction. The Maillard reaction can occur between reducing sugars and lysine residues during the processing of DDGS, as a result of the addition of condensed solubles to the wet distillers cake during drying, consequently damaging the protein fraction (Fastinger and Mahan, 2006; Pahm et al., 2009; Kim et al., 2012). Furthermore, changes in the corn protein structure during the ethanol production have been described to induce a greater fraction of DDGS protein associated with the cell wall material, compared with that of corn (Yu and Nuez-Ortin, 2010). The herein presented high CV values of Klason lignin content in both corn- (0.26) and wheat DDGS (0.32) from the different ethanol plants along with their corresponding different processing technologies, further indicates the presence of non-lignin sources, such as Maillard products, may be present in the Klason lignin fraction of DDGS. Regardless of DDGS origin, the greatest CV values were observed for the S-NSP constituent sugar monomers. It can be speculated that this observation is related to the amount of condensed solubles added to the wet distillers cake during drying. However, it should also be noted that the relative small S-NSP content present could directly give rise to greater CVs. Furthermore, degradation of insoluble NSP occurring during the fermentation process could increase the content of soluble NSP, due to the presence of endogenous fibre degrading enzymes from yeast, exogenous fibre degrading enzymes added to increase ethanol yield, and mechanical- and heat (pre)treatment.

When sampled over a period of 8 days to 11 months the five ethanol plants showed, in spite of some variation, capability of producing DDGS with conserved compositional profiles, indicated by PCA models. The variation in starch content, especially observed for three of the five plants, is directly related to the effectiveness of the fermentation process at each individual ethanol plant, which varies according to process technology and fermentation time, yeast, and enzymes used in the production. Furthermore, the relatively high CV in moisture content is related to different intensities of the DDGS drying process, which is a crucial step in the DDGS production, due to the negative effects on nutritional value associated with excessive heat damage of especially lysine, and the high energy expenses associated with drying. Similarly large variation was observed for the content of Klason lignin, as discussed above. Despite the consistent content of total NSP and total constituent sugars from each individual ethanol plant, large variation was observed for the S-NSPs fractions, as discussed above. P1 showed greater overall variation in compositional profile, which could be explained by P1 having the far greatest sampling period of 11 months, hence probably encountering greater variation in raw material. Despite the narrowest sampling period of 8 days, P5 on the other hand also showed relatively large overall variation.

The simultaneous sampling of both corn and corresponding DDGS over 11 months allowed for investigating the changes in compositional profile after the fermentation and drying process. Although the corn and the DDGS were sampled simultaneously, it should be noted that the corn and DDGS did not originate from the same fermentation batch, which would be practically impossible to obtain. The observed changes between corn and corresponding DDGS may not be considered common for all ethanol plants, underlined by the presented compositional variations between the 24 corn DDGS plants. As expected, the DDGS reflected the nutrient content of the parent grain (Spiehs et al., 2002). All other constituents than starch were concentrated in the DDGS compared with corn, with an average 3.4-fold increase for the common constituents, similar to previous studies (Urriola et al., 2010; de Vries et al., 2013). The relatively small increase in fat content of 2.4–2.7 fold may indicate that the ethanol plant have extracted some of the oil from the thin stillage before condensing and blending with

the grain fraction and drying to make DDGS. The marked increase in mannose content in DDGS compared with corn, most likely originate from yeast cell wall mannans, as discussed previously (de Vries et al., 2013). Furthermore, a marked shift in solubility of xylose and arabinose was observed for DDGS, indicating a modification of the arabinoxylan fraction during processing. Corn showed a consistently greater content of substituted xylan with both greater A/X- and UA/X-ratios, compared with corresponding DDGS. Unpublished results from our research group indicate the A/X-ratio in corn differs among the different botanical grain fractions, with endosperm having a markedly greater A/X-ratio compared with that of corn bran, which is in line with previous published results in corn grain, flour and bran (Bach Knudsen, 1997; Rose et al., 2009). Similarly, UA/X-ratio has been reported greater in corn flour, compared with corn bran (Bach Knudsen, 1997). The shift in A/X- and UA/X ratio between corn and DDGS indicates modification of the greater substituted xylan (*e.g.* in endosperm) during the fermentation process. It must be noted, however, that the total content of arabinoxylan and uronic acids present in corn endosperm is very low compared with corn bran.

Though highly substituted, corn endosperm arabinoxylan is not cross-linked to the same degree as corn bran heteroxylans (Saulnier and Thibault, 1999; Bunzel et al., 2006). Hence, corn endosperm arabinoxylan is potentially more susceptible to environmental changes, such as heat processing and presence of fibre degrading enzymes and other accessory enzymes, which might lead to an increased degradation and solubilization of arabinoxylan. It can be speculated that the solubilized arabinoxylan is more susceptible to participate in Maillard reactions, by having a potentially larger fraction of reducing ends due to hydrolysis. The arabinoxylan parting in Maillard reactions would be unable to determine as part of the constituent sugars in the NSP-procedure used in this study, since they would most likely end up in the Klason lignin fraction. On the other hand, cellulose appear to remain unmodified during processing, due to its strong rigid structure and anchorage in the cell wall matrix (Van Eylen et al., 2011), which consequently leads to the greater cellulose/NSP-ratio for DDGS compared with corn in the current study. Endosperm arabinoxylan comprise approximately 20% of the total content of arabinoxylan in corn, calculated with data from Bach Knudsen (1997) and Watson (1987). Hence, considering that the endosperm arabinoxylan only comprise a minor fraction of the total arabinoxylan content of the grain, and that the cellulose fraction is concentrated in DDGS, the outcome will be that the majority of NSP remain unmodified or potentially more complex in DDGS than in the parent grain.

The use of multivariate data analysis, PCA, on compositional data of DDGS proved useful to visually distinguish between DDGS covering three different feedstock origins; corn, wheat, and mixed cereals. Furthermore, PCA was able to provide information concerning the most conserved components of each DDGS origin, hence the components most responsible for the individual clusters and groupings of DDGS. By combining both compositional data of the common constituents and NSP profiles of the DDGS, a more clear separation between the groups was observed.

DDGS has a reputation of having variable nutrient composition and protein quality, and a high content of mycotoxins, which has limited its use in swine feed (Stein et al., 2006; Pedersen et al., 2007; Anderson et al., 2012). High quantities of DDGS in feed increase the content of dietary fibre, associated with negative effects on nutrient digestibility. To increase nutrient digestibility of feed formulations containing DDGS, studies have investigated the effects of adding fibre degrading enzymes to animal diets containing DDGS (Jones et al., 2010; Yoon et al., 2010; Yáñez et al., 2011). However, the results are inconclusive. This may relate to the complexity of the cell wall matrix in corn DDGS, and that the most readily degradable arabinoxylan for the fibre degrading enzymes already have been modified during DDGS production, as discussed above. These observations indicate that the fibre degrading enzymes applied for degradation of corn DDGS need to be targeted towards highly complex substrates. With respect to enzymatic degradation, corn bran has been acknowledged as a recalcitrant substrate, as a consequence of the highly branched structure of the arabinoxylan (Saulnier et al., 2001; Agger et al., 2010). The cross-linking and complexity of the NSP matrix is influenced by many factors including; content of substituted xylan, ferulic acid cross-linking, and lignin and structural proteins interacting with arabinoxylan (Saulnier et al., 1995b). Despite the ethanol plants' capability of individually producing DDGS with a conserved NSP profile, variation in the degree of substituted xylan from plant to plant exists. For corn DDGS, the differences in A/X- (0.71–0.85) and UA/X-ratio (0.16–0.23) underline the heterogeneous structure of DDGS NSPs. It can be speculated that the enzymatic degradation potential of the DDGS NSPs is sterically hindered by a greater proportion of substituted xylan (Rose et al., 2009). Hence, the DDGS samples from 24 different ethanol plants analyzed in this study might yield different results in relation to digestibility and enzymatic degradation potential of the NSPs. Difference in animal performance may be observed when feeding either corn- or wheat DDGS, due to the observed large variation in NSP cell wall complexity between the two DDGS sources likely affecting digestibility. However, it should be noted that the overall degradation potential of the DDGS NSPs, is affected by a combination of several factors (as described above), a number of which not determined in this study.

5. Conclusion

The current study showed a large variability in the chemical composition of common constituents, and in non-starch polysaccharide profiles of corn-, wheat, and mixed DDGS. Despite variations, the major fractions across all origins were; CP and aNDFom with a large degree of the aNDFom fraction being ADF. All samples also had a large content of sugars, while corn DDGS contained more fat than the other two. Wheat- and mixed DDGS showed a markedly greater content of S-NSP than in corn, which on the other hand had greater contents of total- and insoluble NSPs. Of the NSP fraction, all samples had the greatest content of arabinoxylan. However, the A/X- and UA/X-ratio differed with greater ratios for corn DDGS than in the other two. DDGS samples from five different ethanol plants showed that each plant were capable, despite variations,

of producing DDGS with an individual conserved compositional profile. Finally, analysis of corn and corresponding DDGS samples showed that the content of substituted xylan and S-NSP is modified during the production of DDGS.

Conflict of interest

The authors declare that there are no conflicts of interest.

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