

Characterisation of Several Rye Cultivars with Respect to Arabinoxylans and Extract Viscosity

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Received: October 24, 2011

Accepted: December 6, 2011

Online Published: April 1, 2012

doi:10.5539/jas.v4n5p1

URL: <http://dx.doi.org/10.5539/jas.v4n5p1>

Abstract

Arabinoxylans are considered as the major limiting factor for use of rye in animal feeding. In this study, four selected winter rye cultivars were grown at nine locations and tested for arabinoxylans including total (TAX), soluble (SAX) and insoluble fractions (IAX), extract viscosity, falling numbers and crude protein contents. Amounts of TAX ranged on average between 89 and 103 g/kg wholemeal flour, and the extract viscosity between 2.6 and 5.1 cSt. The extract viscosity was significantly correlated with falling numbers and all three arabinoxylan fractions. Arabinoxylan contents and extract viscosity were influenced by the rye genotype. The anthocyanin free hybrid cultivar Helltop combined reduced TAX, SAX and IAX levels with low extract viscosity and high crude protein contents and seems to be more suitable for use in animal feeding than the other three cultivars tested in this respect.

Keywords: Rye, Arabinoxylans, HPLC, Extract viscosity, Falling numbers, Animal feeding, Crude proteins

Abbreviations: TAX, total arabinoxylans; SAX, water-soluble arabinoxylans; IAX, insoluble arabinoxylans; dm, dry matter; rpm, rotation per minute; HPLC, high pressure liquid chromatography; ASER, anthranilic acid ethyl ester reagent; ISTD, internal standard solution; THF, tetrahydrofuran

1. Introduction

Rye (*Secale cereale* L.) as one of the most important cereal crops in Central Europe is closely related to wheat however it has several economic advantages (Allen, 2002). Rye is more tolerant than wheat to frost and drought and it can grow on wide range of soil types including poor, sandy soils. In addition, rye has some nutritional benefits over wheat, since its content of minerals, lysine and dietary fibre is higher (Allen, 2002). Besides starch which comprises about 65% of the dry matter (dm), dietary fibre is the most abundant component in whole-grain rye with about 17% of dm (Bengtsson and Aman, 1990). Major non-starch polysaccharides (NSP) are arabinoxylans with about 8% of dm. They primarily consist of the two sugars L-arabinose and D-xylose (Boros *et al.*, 1993) and represent about 55% of total NSP in rye grains (Bengtsson and Aman, 1990; Knudsen and Laerke, 2010). Arabinoxylans are major components of the rye endosperm cell walls (Vinkx and Delcour, 1996), and consist of a linear backbone of (1-4)- β -D-xylopyranose residues partly substituted with α -L-arabinofuranosyl residues (Hansen *et al.*, 2003; Bengtsson *et al.*, 1992). The arabinoxylan content in rye is higher than that found for barley and wheat (Nyström *et al.*, 2008). Rye is an important source of dietary fibre in human diet (Hansen *et al.*, 2003; Anonymus, 2011) and regarded as a healthy food that can reduce the risk of cancers, coronary heart diseases and diabetes (Dongowski, 2007; Garcia *et al.*, 2007; Linko and Adlercreutz, 2005; Nyström *et al.*, 2008). In Europe rye is mainly cultivated as bread grain (Bengtsson *et al.*, 1992). Its arabinoxylans improve the baking quality (Saastamoinen *et al.*, 1989; Vinkx and Delcour, 1996), and preserve freshness and sensoric properties of bread through the formation of starch-arabinoxylan complexes (Jankiewicz and Michniewicz, 1987).

In Germany, about half of the rye grains harvested annually are used for animal feeding (Anonymus, 2011). However, the high level of antinutritive substances in rye such as NSP can reduce its digestibility and feeding quality, particularly for monogastric animals (Antoniou *et al.*, 1981). Above all, these are arabinoxylans forming highly viscous solutions in water (Bengtsson and Aman, 1990) associated with negative effects on the food uptake and growth reduction. Hence, arabinoxylans are considered to be the major limiting factor for unrestricted use of rye as feeding component (Antoniou *et al.*, 1981). Water-soluble and insoluble fractions of arabinoxylans

from rye incorporated in a wheat based diet caused growth depression of chicken by 20 and 55%, respectively (Antoniou *et al.*, 1981). In order to open new ways of using rye in animal feed rye cultivars with diminished arabinoxylan contents and low extract viscosities are needed, and rye breeding should pay more attention to this topic in the future. But, this requires efficient methods for the assay of arabinoxylans accumulated in rye grains with a high throughput of samples.

The aim of the present work was to evaluate German winter rye cultivars for parameters that influence the feeding quality of rye such as arabinoxylans comprising (i) total, (ii) water-soluble and (iii) insoluble fractions as well as extract viscosity, falling numbers and crude protein contents.

2. Materials and Methods

2.1 Rye Samples

The three hybrid winter rye cultivars Helltop (Year of registration, 2009), Minello (2008), Visello (2006) and the population variety Conduct (2006) were grown in field plots at nine locations, i.e. Gülzow, Neuhoof, Borwede, Hude, Hamerstorf, Rembserhof, Mayen-Rosenhof, Vipperow and Bornhof, situated all in different districts of the four German states Mecklenburg-Western Pomerania, Lower Saxony, Rhineland-Palatinate and Bavaria. The experiments were carried out in 2010 under uniform agronomic management (Fertilizer, herbicides etc.). The locations were distributed from the North to the South of Germany, and the soil types varied from sandy soil over loamy sand or sandy loam to silty loam. The long term average rainfall ranged from 549 mm at Bornhof to 820 mm at Rembserhof.

After harvest, grain samples of about 250 g per rye cultivar were randomly taken and analysed in the laboratory of the Institute for Resistance Research and Stress Tolerance, Experimental Station for Potato Research, Groß Lüsewitz situated in Mecklenburg-Western Pomerania, Germany. The rye grains were ground in a Laboratory mill 3100 (Perten Instruments, Hamburg, Germany) to pass a 0.8 mm sieve, and the wholemeal flour was used for the analyses as detailed below. All wholemeal samples revealed a dry matter content of about 88% and were stored at room temperature until analyses.

2.2 Assay of Total, Soluble and Insoluble Arabinoxylans

Chemicals and standard solutions: Anthranilic acid ethyl ester, dimethyl sulfoxide (DMSO), sodium cyanoborohydride, sodium hydroxide solution (50%, w/v), arabinose, xylose, galactose, glucose, rhamnose and fucose were supplied by Sigma-Aldrich (Taufkirchen, Germany). Sulfuric acid (1 mol/l), glacial acetic acid, sodium azide, formic acid and methanol were obtained from Merck (Darmstadt, Germany). Tetrahydrofuran (THF) was supplied by Lab Scan, (Gliwice, Polen) and 2-deoxy-D-glucose by Acros Organics (Geel, Belgium). Anthranilic acid ethyl ester reagent (ASER): 40 mg of sodium cyanoborohydride were dissolved in 700 μ l DMSO, before 55 μ l of anthranilic acid ethyl ester and 300 μ l of glacial acetic acid were added. Internal standard solution (ISTD): 40 mg of 2-deoxy-D-glucose were dissolved in 1 ml of deionized water. Sugar standards: 50 mg of arabinose, 50 mg of xylose, 10 mg of galactose, 1000 mg of glucose, 10 mg of rhamnose and 10 mg of fucose were dissolved in 10 ml of deionized water. For the assay of arabinoxylans the hydrolysis of rye samples was carried out according to Fengler and Marquardt (1988a) and the generated monosaccharides were fluorescent labelled following the method detailed by Bigge *et al.* (1995). Appropriate dilutions of the sugar standard were prepared, and 1 ml of each dilution was supplemented with 100 μ l of ISTD reagent and thoroughly mixed. 10 μ l of this solution were mixed with 100 μ l of ASER and heated in a thermo-block at 80 °C for 2 h. After derivatization, 1.9 ml of deionized water containing 1 mmol/l sodium azide were added, the solution was mixed and centrifuged at 20 000 \times g for 20 min at 4°C. The supernatant was used for HPLC analyses as described below.

In order to analyse total amounts of arabinoxylans (TAX) 400 mg of rye wholemeal flour were transferred into a 15 ml Falcon-tube, and suspended in 10 ml of 1 mol/l sulfuric acid using a laboratory shaker. The sample was hydrolyzed at 99 °C for 2 h on a thermomixer (Eppendorf, Wesseling-Berzdorf, Germany), cooled, and an aliquot of 1 ml was supplemented with 100 μ l of ISTD solution, mixed and centrifuged at 20 000 \times g for 20 min at 4 °C. 10 μ l of the supernatant were mixed with 100 μ l of ASER reagent, and heated in a thermo-block at 80 °C for 2 h. After derivatization, 1.9 ml of deionized water containing 1 mmol/l sodium azide were added, the solution was mixed and centrifuged at 20 000 \times g for 20 min at 4°C. The supernatant was used for HPLC analyses.

The extract samples for measurement of soluble arabinoxylans (SAX) were prepared according to Boros *et al.* (1993). 2 g of rye wholemeal flour were suspended in 20 ml of deionized water and shaken for 1 h at 20 °C. The

suspension was centrifuged at $12\,000 \times g$ for 10 min at 5 °C. The supernatant was used for the assay of soluble arabinoxylans, and in addition for measurement of the extract viscosity as detailed below.

For the analyses of SAX, 1 ml of the extract sample was supplemented with 0.5 ml of 3 mol/l of H₂SO₄, mixed and hydrolysed at 99 °C for 2 h on an Eppendorf-thermomixer. The hydrolysate was cooled, and supplemented with 50 µl of ISTD reagent, mixed and then centrifuged at $20\,000 \times g$ for 20 min at 4 °C. 20 µl of the supernatant were supplemented with 100 µl ASER reagent and heated in a thermo-block at 80 °C for 2 h. Afterwards, the sample was supplemented with 1.9 ml of water containing 1 mmol of NaN₃, mixed and centrifuged as detailed before. The supernatants were used for the HPLC assay performed in a HPLC-system consisting of a PU 980 pump, a gradient mixer LG 1580 (both from Jasco, Gross-Umstadt, Germany), a fluorescence detector RF-10AXL (EX, 340 nm; EM, 420 nm; Shimadzu, Duisburg, Germany) and an autosampler 465 (Kontron Instruments, Neufahrn, Germany). Sugar separation was carried out on a Synergi Polar-RP 80A column (150 × 3 mm; 4.0 µm; Phenomenex, Aschaffenburg, Germany) equipped with an RP pre-column, at a flow rate of 0.7 ml/min. The injection volume was 10 µl for TAX and 20 µl for SAX. The column temperature was maintained at 25 °C.

Solvent (A): 900 ml of 0.1 mol of formic acid adjusted to pH 3.85 with NaOH (50%, w/v) were mixed with 200 ml of THF. Solvent (B): 200 ml of formic acid (pH 3.85) were mixed with 800 ml of methanol. The following gradient was used: 0 - 13 min, 100% A; 13 - 16 min, 0 - 20% B; 16 - 18 min, 20 - 100% B; 18 - 22 min, 100% B; 22 - 24 min, 0 - 100 % A; 24 - 27 min, 100% A. Analyses of arabinoxylans were performed in duplicate with standard deviation (S.D.) ≤ 4%. Software Shimadzu Class VP was used for data analyses. The amounts of arabinose and xylose present in the extract samples were summarized for expression of TAX and SAX, respectively.

In order to determine insoluble arabinoxylans (IAX) an indirect method was used, i.e. contents of IAX were calculated as differences between total and water-soluble arabinoxylans (IAX=TAX-SAX). Amounts of total, soluble and insoluble arabinoxylans were expressed in grams per kilograms of rye wholemeal flour.

2.3 Assay of Extract Viscosity

Extract samples used for the viscosity assay according to Boros *et al.* (1993) were prepared as described for measurement of SAX. Aliquotes of the water-extract samples were applied to a ViscoSystem AVS 350 instrument (Schott-Geräte, Ludwigshafen, Germany) equipped with a capillary viscosimeter, and connected with a ViscoDoser AVS 20 piston buret (Schott-Geräte) which enabled an automated viscosity assay. The latter was carried out at 30 °C, using an extract volume of 2 ml per assay. Measurements of extract viscosity were performed in duplicate with S.D. ≤ 5%. The instrument software package (Win Visco) was used for data analyses, and the viscosity was expressed in centistokes (cSt).

2.4 Assay of Falling Numbers

The Hagberg Falling Number (HFN) was measured in a falling number system FN 1400 (Perten Instruments GmbH, Hamburg, Germany) according to the method described by Barnard (2001) using 7.0 g rye wholemeal flour per assay and an extract volume of 25 ml. Falling numbers were expressed in seconds (s). All measurements were performed in duplicate with standard deviation ≤ 5%.

2.5 Assay of Crude Proteins

The concentration of crude proteins was determined according to Kjeldahl (conversion factor, 6.25) using 0.5 g of rye wholemeal flour for each assay. Measurements were performed in duplicate with standard deviation ≤ 5%. Amounts of crude protein were expressed in grams per kilograms of wholemeal flour.

2.6 Statistical Analysis

Differences in TAX, SAX, IAX, extract viscosity, falling numbers and crude protein contents between the four winter rye cultivars were tested using the SAS 9.2 statistical package (PROC GLM, Tukey-test, SAS Institute Inc., Cary, NC, USA). In this case, the locations were arranged in nine replications. In order to test the differences between the locations, the rye cultivars were arranged in four replications. $P < 0.05$ was considered significant.

Correlations (Pearson) between individual quality parameters were calculated using the SAS 9.2 statistical package (Procedure CORR). Data obtained for the four rye cultivars and nine locations were involved in the calculations ($n = 36$). $P < 0.05$ was regarded to be statistically significant.

3. Results

The acid hydrolysis of rye samples according to Fengler and Marquardt (1988a) combined with a fluorescent labelling of the released monosaccharides (Bigge *et al.* 1995) and a HPLC assay enabled a good separation of the individual sugars as indicated by the chromatogram presented in figure 1. Separation of arabinose and xylose as the two major sugars of arabinoxylans on a reverse phase column could be improved through supplementation of tetrahydrofuran to solvent (A) instead of methanol or acetonitrile generally used by other authors, e.g. Known & Kim (1995).

3.1 Total, Soluble and Insoluble Arabinoxylans

Contents of total arabinoxylans ranged on average between 89 g/kg for cultivar Helltop and 103 g/kg for Minello (Table 1). Helltop revealed the lowest amounts of TAX in grains derived from Rembserhof with a value of 82 g/kg. At all nine locations this cultivar had less TAX than Conduct, Minello and Visello, which all three displayed their lowest TAX contents at Mayen-Rosenhof with a value of 91, 97 and 95 g/kg, respectively (Fig. 2A). TAX correlated significantly with SAX, IAX and extract viscosity (Table 2).

The four cultivars also varied in contents of soluble arabinoxylans with values ranging on average between 16 g/kg for Helltop and 22 g/kg for Visello (Table 1). Helltop displayed lower amounts of SAX than the other three cultivars at all locations (Fig. 2B). Its values ranged between 15 g/kg for Borwede and 18 g/kg for Vipperow. Visello, on the other side, achieved its highest SAX content at Gülzow with an appropriate value of 25 g/kg. SAX correlated significantly with TAX, IAX and extract viscosity (Table 2).

On average, amounts of insoluble arabinoxylans ranged from 72 g/kg for Helltop to 83 g/kg for Minello (Table 1). Generally, Helltop exhibited lower amounts of IAX in its grains than Conduct, Minello and Visello, a tendency observed at all locations (Fig. 1C). IAX values of cultivar Helltop ranged between 66 g/kg (Rembserhof) and 78 g/kg (Hude) and that of Minello between 78 g/kg (Mayen-Rosenhof) and 88 g/kg (Rembserhof). IAX correlated significantly with TAX, SAX and extract viscosity (Table 2).

The differences in TAX, SAX and IAX between Helltop and the other three rye cultivars were all statistically significant (Table 1). Furthermore, it was important that the differences in TAX, SAX and IAX between the locations were statistically not significant (Table 3).

3.2 Extract Viscosity

As expected, the four rye cultivars varied notably in their extract viscosity (Table 1). Of the four cultivars, Visello achieved the highest extract viscosity with a mean value of 5.1 cSt. Its individual values ranged between 3.0 cSt for the location Gülzow and 8.2 cSt for Bornhof (Fig. 2D). The differences in extract viscosity between Visello and the other three cultivars Minello, Conduct and Helltop were all statistically significant (Table 1). Helltop exhibited the lowest extract viscosity among the four cultivars, i.e. on average (Table 1) and at all nine locations (Fig. 2D). Its individual values ranged from 1.9 cSt (Gülzow) to 4.0 cSt (Bornhof). The differences in extract viscosity between Helltop and the other three cultivars were all statistically significant (Table 1). The extract viscosity correlated significantly with falling numbers, TAX, SAX and IAX (Table 2).

All four cultivars revealed their lowest extract viscosity at Gülzow and their highest levels were concordantly found for Bornhof (Table 3; Fig 2D). The differences in extract viscosity between these two locations were statistically significant. In addition, Mayen-Rosenhof differed significantly in this respect from Bornhof (Table 3).

3.3 Falling Numbers

On average, Visello displayed the highest falling numbers among the four rye cultivars (Table 4). Individual values of this cultivar ranged between 105 s for Gülzow and 399 s for Bornhof. The differences in falling numbers between Visello and the other three cultivars Helltop, Conduct and Minello were all statistically significant. Helltop had the lowest falling numbers among the four cultivars with a mean value of 222 s and individual values ranging from 62 s (Gülzow) to 341 s (Bornhof) (Table 4), and it differed significantly in this respect from Visello, however, not from Conduct and Minello. The falling numbers were significantly correlated with the extract viscosity (Table 2).

All four cultivars exhibited their lowest falling numbers at Gülzow, while the highest values were found for Bornhof (Tables 3 and 4). The differences in falling numbers between these two locations were statistically significant. In addition, Bornhof differed significantly with respect to falling numbers from Rembserhof, Mayen-Rosenhof and Vipperow (Table 3).

3.4 Crude Proteins

Helltop exhibited on average the highest crude protein contents among the four rye cultivars, and differed significantly in this respect from Visello and Minello (Table 4). Its individual values ranged between 93 g/kg for Rembserhof and 129 g/kg for Hude. Visello had on average the lowest amounts of crude proteins with values ranging from 80 g/kg for Rembserhof to 120 g/kg for Hude (Table 4). Visello differed significantly in its protein level from Helltop and Conduct. Correlations between crude proteins and all other parameters were statistically not significant (Table 2).

The four cultivars exhibited concordantly the highest crude protein contents at the location Hude, while their lowest levels were found for Rembserhof (Table 4). Differences in proteins between these last two locations were statistically significant. In addition, Hude differed significantly in this respect from Gülzow, Borwede, Hamerstorf, Mayen-Rosenhof, Vipperow and Bornhof (Table 3).

4. Discussion

In animal feeding the utilization of rye could be extended, provided that amounts of antinutritive substances such as arabinoxylans can be reduced (Antoniou *et al.*, 1981; Ragaee *et al.*, 2001). In this study, four new German winter rye cultivars were analysed for arabinoxylans comprising total, soluble and insoluble fractions, extract viscosity, falling numbers and crude protein contents - all parameters that influence the feeding quality of rye.

4.1 Total, Soluble and Insoluble Arabinoxylans

The procedures described in section material and methods enabled serial analyses of the three arabinoxylan fractions in rye with reliable results and a high throughput of samples (Fig. 1), a fact which is significant for breeding of rye cultivars with low arabinoxylan contents. Concerning the HPLC assay, it should be pointed out that addition of tetrahydrofuran to solvent (A) instead of methanol or acetonitrile generally used by other authors, e.g. Known and Kim (1995), lead to a better separation of arabinose and xylose as the two major sugars of arabinoxylans. Amounts of arabinoxylans including total (Bengtsson and Aman, 1990; Hansen *et al.*, 2003; Jankiewicz and Michniewicz, 1987; Vinkx and Delcour, 1996) soluble and insoluble fractions (Antoniou *et al.*, 1981; Fengler and Marquardt, 1988a; Salmenkallio-Marttila and Hovinen, 2005) of the four rye cultivars involved in the present work were in agreement with results of other authors. Similarly as reported elsewhere, contents of arabinoxylans including TAX, SAX and IAX were influenced by the rye genotype (Table 1; Fig. 2A-2C) (Bengtsson *et al.*, 1992; Saastamoinen *et al.*, 1989; Smulikowska and Nguyen, 2001). For example, total amounts of arabinoxylans accumulated in grains of the four cultivars ranged between 82 g/kg for cultivar Helltop and 108 g/kg for Minello when both were grown at Rembserhof (Fig. 2A).

The hybrid cultivar Helltop exhibited on average (Table 1) and at all nine locations the lowest TAX values among the four cultivars (Fig. 2A). This was an important result, since arabinoxylans bind large amounts of water and form highly viscous solutions reducing the digestion of nutrients and their absorption in the intestinal tract (Bengtsson *et al.*, 1992; Knudsen and Laerke, 2010) associated with weight reduction in animals (Antoniou *et al.*, 1981). Above all, water-soluble fractions of arabinoxylans cause a raise in viscosity (Antoniou *et al.*, 1981; Bengtsson and Aman, 1990) reducing the nutrient efficiency in the diet (Misir and Marquardt, 1978; Fengler and Marquardt, 1988b). This may also be supported by the fact, that besides TAX and IAX also SAX correlated significantly with the extract viscosity (Table 2). In this context it should be mentioned that Helltop again had significantly lower amounts of SAX than Conduct, Minello and Visello, (Table 1; Fig. 2B). The SAX content in Helltop grains derived from Mayen-Rosenhof amounted only 15 g/kg, while Visello (Gülzow) was higher in its level with a value of 25 g/kg (Fig. 2B). Similar significant differences were noticed for IAX with appropriate values ranging between 66 g/kg for Helltop (Rembserhof) and 88 g/kg for Conduct (Bornhof) and Minello (Rembserhof) (Fig. 2C). Among the four cultivars, Helltop again ranked on the lowest level in its IAX, i.e. on average (Table 1) and at all nine locations (Fig. 2C).

Overall, the results indicated that concentrations of arabinoxylans including all three fractions, i.e. TAX, SAX and IAX were reduced in Helltop (Table 1; Fig. 2A-2C). Accordingly, Helltop could be more suitable for animal feeding than the other three winter rye cultivars involved in the study. In this context it should be mentioned, that concentrations of all the three arabinoxylan fractions were significantly influenced by the genotype (Table 1), but not by the location (Table 3). Hence, the desired quality traits expressed in Helltop grains seem to be relatively independent from the environment.

4.2 Extract Viscosities, Falling Numbers and Crude Protein Contents

The extract viscosity is another important quality factor for cereals used in animal feeding (Antoniou *et al.*, 1981; Ragaee *et al.*, 2001). A high extract viscosity is associated with reduced nutrient utilization and weight reduction

in animals as already mentioned (Misir and Marquardt, 1978; Fengler and Marquardt, 1988b; Ragaee *et al.*, 2001). It was interesting therefore that the variation in extract viscosity (Table 1; Fig. 2D) among the four rye cultivars was stronger than that found for the three arabinoxylan fractions (Fig. 2A-2C). Appropriate values ranged from 1.9 cSt for Helltop (Gülzow) to 8.2 cSt for Visello (Bornhof). Again Helltop was outstanding and displayed the lowest extract viscosity among the four cultivars (Table 1), a tendency observed at all nine locations (Fig. 2D). On average, Helltop had two-fold lower extract viscosity than Visello. Its low extract viscosity was associated with reduced arabinoxylan contents and can be regarded as another advantage of this cultivar, especially with respect to a potential use in animal feeding. In Visello a high extract viscosity (Table 1; Fig. 2D) was combined with high amounts of SAX in its grains (Table 1; Fig. 2B). As already mentioned, the extract viscosity correlated significantly with all three arabinoxylan fractions, including SAX (Table 2). This is a result that may support the notion of Boros *et al.* (1993) who suggested the extract viscosity as an indirect marker for concentrations of soluble arabinoxylans accumulated in rye grains. Accordingly, measurement of extract viscosity which is relatively simple compared to HPLC assays could be used for pre-selection of genotypes with low SAX contents in rye breeding.

Falling numbers are known to be closely related with the extract viscosity (Barnard, 2001; Ragaee *et al.*, 2001; Kucerová, 2009) and were determined therefore as another quality factor of rye. Also in the present experiments the falling numbers correlated significantly with the extract viscosity (Table 2). Falling numbers are an indirect measure of α -amylase activity and indicate the baking quality of rye. For example, in Finland the falling number of rye flour used for bread making was mentioned to be 120 s (Salmenkallio-Marttila and Hovinen, 2005). Appropriate values of the four German rye cultivars ranged from 62 s for Helltop and Conduct both grown at Gülzow and 399 s for Visello grown at Bornhof (Table 4). With it, they varied stronger and were higher in their maximum than those of the rye cultivars grown in Finland (Salmenkallio-Marttila and Hovine, 2005). Visello had on average the highest falling numbers and differed significantly in this respect from Conduct, Minello and Helltop which again ranked on the lowest level among the four rye cultivars (Table 4).

Apart from crude fibre, proteins are major quality factors of feeds, since they are needed for the supply of animals with essential amino acids (González-Martin *et al.*, 2006). The crude protein contents ranged between 80 g/kg for Visello (Rembserhof) and 129 g/kg for Helltop (Hude). It was interesting, that Helltop achieved the highest level in proteins among the four cultivars (Table 4). This tendency was in contrast to those found for its extract viscosity, falling numbers, IAX, SAX and TAX contents which all ranked on a low level in this cultivar. Doubtless, such high amounts of crude proteins can be seen as another advantage of the hybrid cultivar Helltop with respect to a potential use in animal feeding. It should be mentioned in this context that crude protein contents were influenced by the rye genotype and the location, similarly as it was observed for the extract viscosity and falling numbers (Tables 1, 3, 4).

5. Conclusions

In order to widen the use of rye in animal feeding, it is necessary to find genotypes with reduced arabinoxylan contents and low extract viscosity. The anthocyanin free hybrid cultivar Helltop may fulfil these requirements, since it combined relatively low amounts of TAX, SAX and IAX with low extract viscosities and falling numbers, but high crude protein contents. Such combination in quality traits might be optimal with respect to a potential use as component in animal diet. Apart from the last aspect, Helltop could be an interesting candidate for involvement in breeding of new rye cultivars with low arabinoxylan contents and extract viscosity. Successful breeding efforts in this direction may be helpful to increase the field of using rye for different purposes.

Acknowledgements

We thank C. Leesch and M. Jennerjahn for technical assistance.

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Table 1. Amounts of total, soluble and insoluble arabinoxylans as well as extract viscosity of the four rye cultivars grown at nine locations (Means and standard error; n = 9 locations). Harvest 2010

Cultivar	Arabinoxylans (g/kg)			Extract viscosity (cSt)
	Total	Soluble	Insoluble	
Helltop				
Mean	88.5 ^a	16.2 ^a	72.3 ^a	2.61 ^a
Error	1.3	0.4	1.2	0.21
Conduct				
Mean	101.4 ^b	20.0 ^b	81.4 ^b	3.85 ^b
Error	1.4	0.7	1.4	0.29
Minello				
Mean	103.1 ^b	20.4 ^{bc}	82.7 ^b	4.41 ^b
Error	1.2	0.6	1.0	0.49
Visello				
Mean	100.6 ^b	21.7 ^c	78.9 ^b	5.14 ^c
Error	1.2	0.5	1.2	0.54

Cultivar means followed by different letters within the same column differ significantly at $P < 0.05$

Table 2. Correlations between quality parameters of the four rye cultivars grown at nine locations. Harvest 2010

Parameter	Protein contents	Falling numbers	Extract viscosity	Arabinoxylans		
				Total	Soluble	Insoluble
Protein content	1.00					
Falling number	0.16	1.00				
Extract viscosity	-0.08	0.70*	1.00			
Total arabinoxylans	-0.09	0.20	0.56*	1.00		
Soluble arabinoxylans	-0.19	-0.15	0.43*	0.73*	1.00	
Insoluble arabinoxylans	-0.03	0.33	0.52*	0.94*	0.45*	1.00

* Significant at $P \leq 0.01$; n = 36

Table 3. Amounts of total, soluble and insoluble arabinoxylans as well as extract viscosity, falling numbers and crude protein contents of the four rye cultivars grown at nine locations (Means and standard error; n = 4 cultivars per location). Harvest 2010

Location	Arabinoxylans (g/kg)			Extract viscosity (cSt)	Falling numbers (s)	Crude proteins (g/kg)
	Total	Soluble	Insoluble			
Gülzow						
Mean	97.2 ^a	22.0 ^a	75.1 ^a	2.52 ^b	74 ^c	100.5 ^{bcd}
Error	4.3	1.7	2.9	0.22	10	2.6
Neuhof						
Mean	97.5 ^a	18.6 ^a	78.8 ^a	4.68 ^{ab}	302 ^{ab}	114.0 ^{ab}
Error	3.3	0.6	2.8	0.68	19	4.1
Borwede						
Mean	98.9 ^a	18.3 ^a	80.6 ^a	3.79 ^{ab}	301 ^{ab}	92.9 ^{cd}
Error	3.4	1.4	2.4	0.57	14	3.2
Hude						
Mean	102.0 ^a	20.0 ^a	81.9 ^a	4.51 ^{ab}	341 ^a	123.1 ^a
Error	2.8	1.2	1.6	0.66	17	3.1
Hamerstorf						
Mean	98.1 ^a	19.5 ^a	78.5 ^a	4.55 ^{ab}	294 ^{ab}	100.6 ^{bcd}
Error	3.2	1.4	1.8	0.68	19	2.6
Rembserhof						
Mean	98.0 ^a	19.0 ^a	79.1 ^a	3.43 ^{ab}	195 ^{cd}	86.9 ^d
Error	5.7	1.1	4.6	0.39	23	2.6
Mayen-Rosenhof						
Mean	92.5 ^a	17.9 ^a	74.5 ^a	2.81 ^b	223 ^{bc}	99.7 ^{bcd}
Error	2.2	1.2	1.3	0.31	17	2.5
Vipperow						
Mean	101.0 ^a	21.5 ^a	79.5 ^a	3.49 ^{ab}	122 ^{dc}	102.4 ^{bc}
Error	3.8	1.4	2.5	0.42	28	3.2
Bornhof						
Mean	100.6 ^a	19.6 ^a	81.0 ^a	6.23 ^a	365 ^a	99.3 ^{bcd}
Error	3.0	1.4	3.1	0.98	12	4.1

Location means followed by different letters within the same column differ significantly at $P < 0.05$

Table 4. Falling numbers and crude protein contents of the four rye cultivars grown at nine locations. Harvest 2010

Location	Falling numbers (s)				Crude proteins (g/kg)			
	Helltop	Conduct	Minello	Visello	Helltop	Conduct	Minello	Visello
Gülzow	62	62	69	105	104.8	103.5	100.5	93.1
Neuhof	257	294	308	351	121.2	120.5	109.4	104.9
Borwede	275	282	314	335	100.9	93.9	91.4	85.4
Hude	313	331	391	329	129.1	127.2	116.0	120.0
Hamerstorf	300	262	269	345	101.9	107.5	96.2	96.8
Rembserhof	171	208	148	252	92.5	89.1	85.8	80.1
Mayen-Rosenhof	210	187	226	269	103.1	104.5	97.1	93.9
Vipperow	71	78	153	187	108.4	106.8	99.5	94.9
Bornhof	341	357	362	399	104.5	107.9	91.5	93.2
Mean	222 ^a	229 ^a	249 ^a	286 ^b	107.4 ^a	106.8 ^a	98.6 ^b	95.8 ^b
Error	34	35	36	31	3.7	3.9	3.1	3.8

Cultivar means followed by different letters within the same line differ significantly at $P < 0.05$

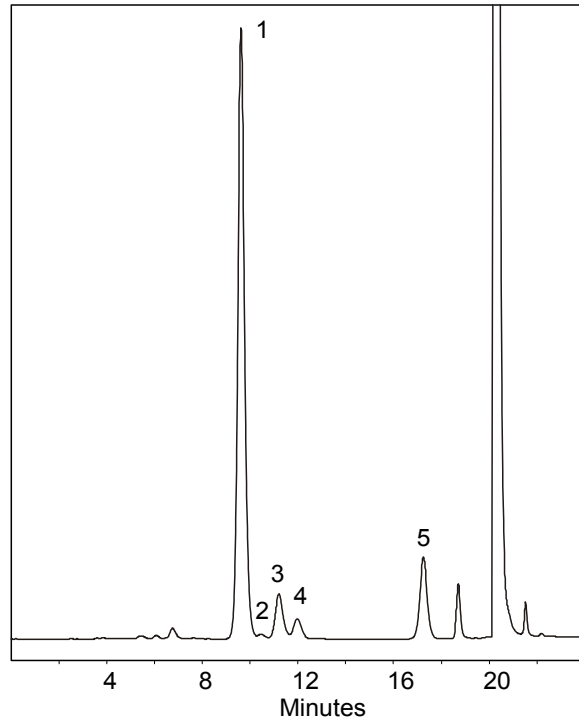
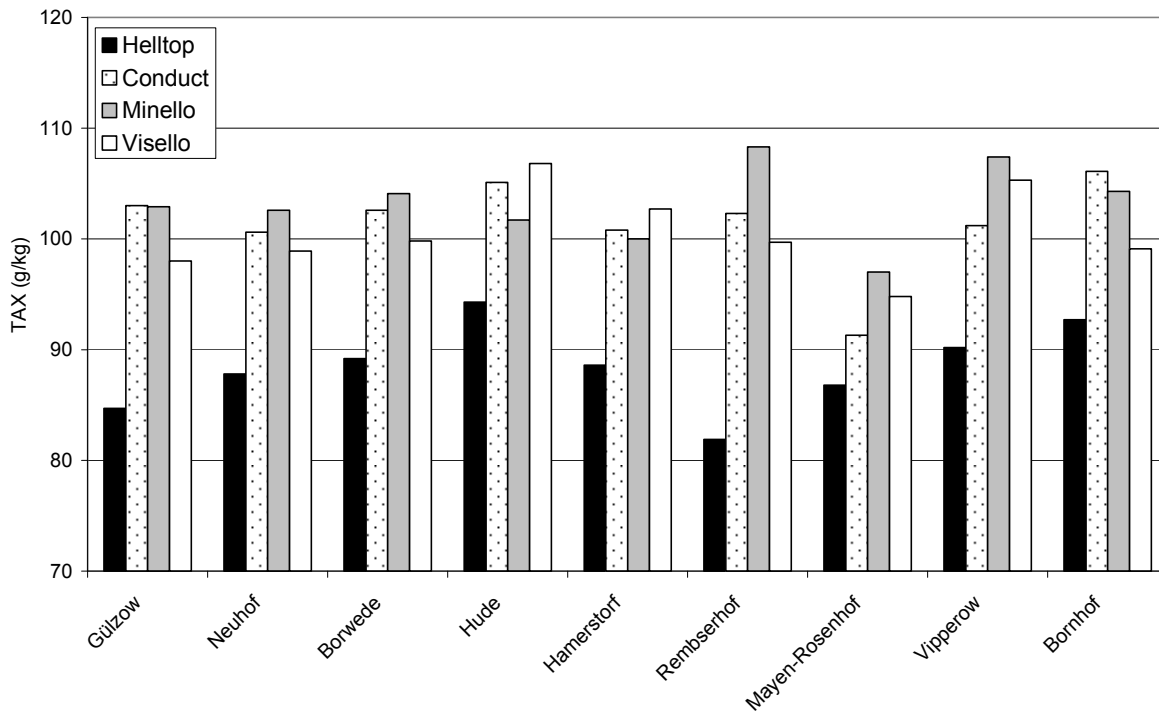
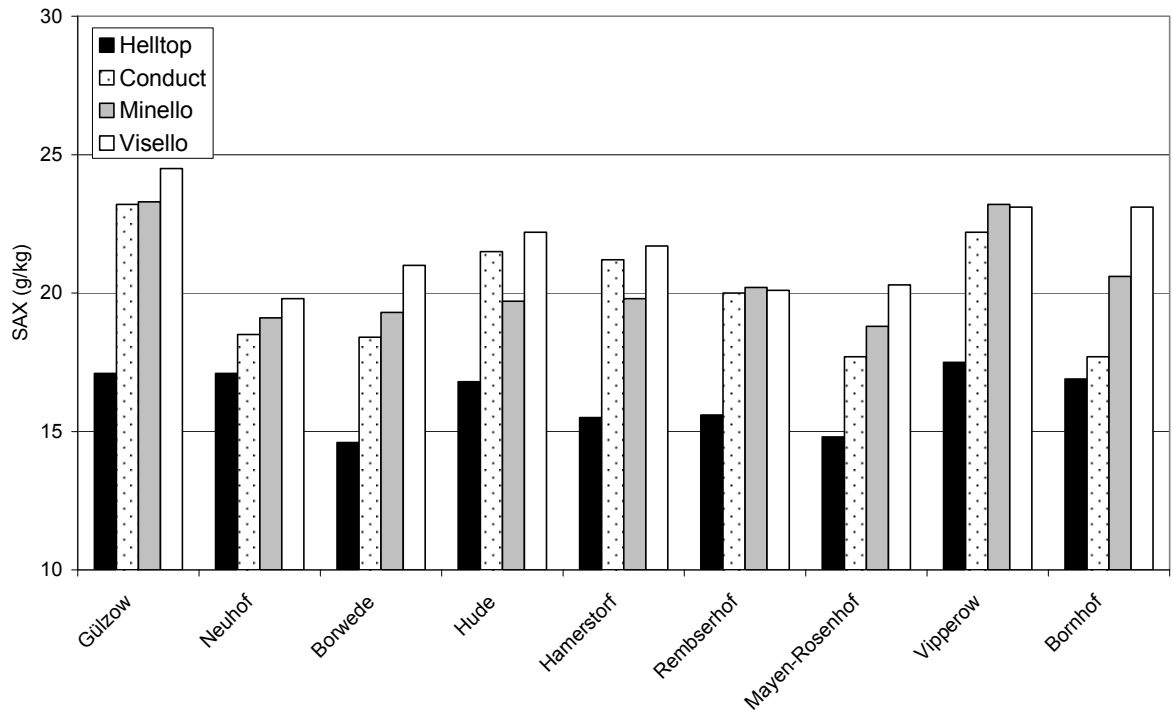


Figure 1. HPLC chromatogram for total arabinoxylans present in grains of cultivar Helltop grown at Hude in 2010. Peak ID: 1 = Glucose, 2 = Galactose, 3 = Xylose, 4 = Arabinose, 5 = ISTD

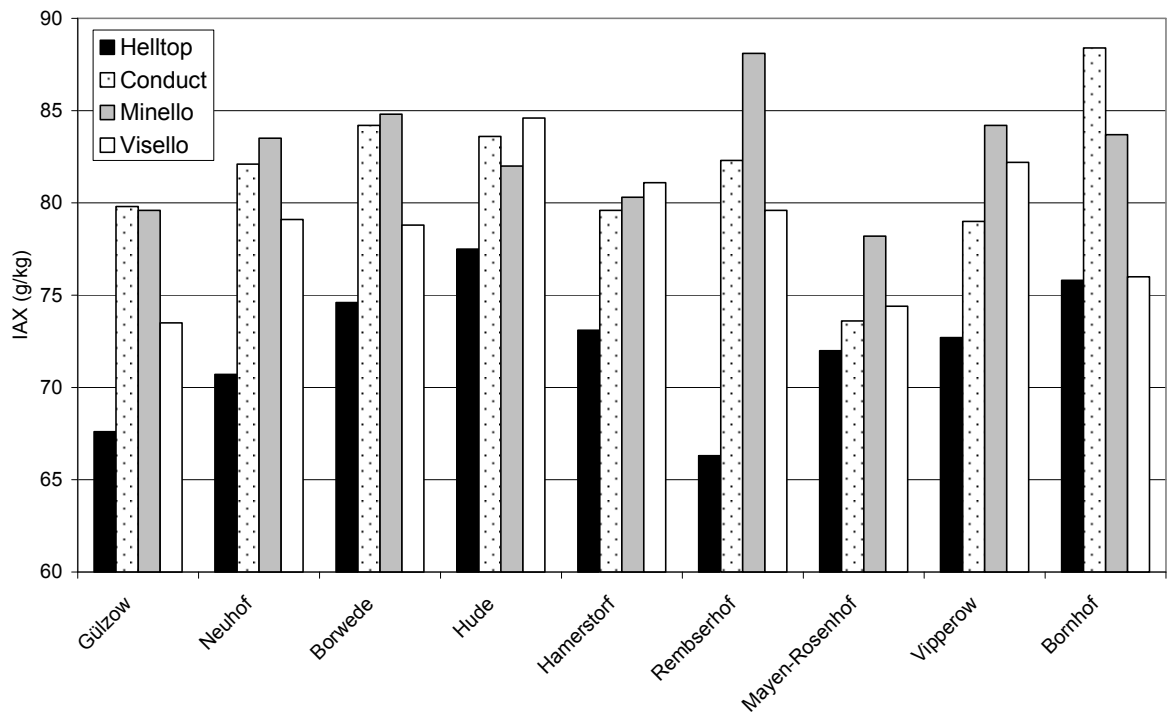
2A



2B



2C



2D

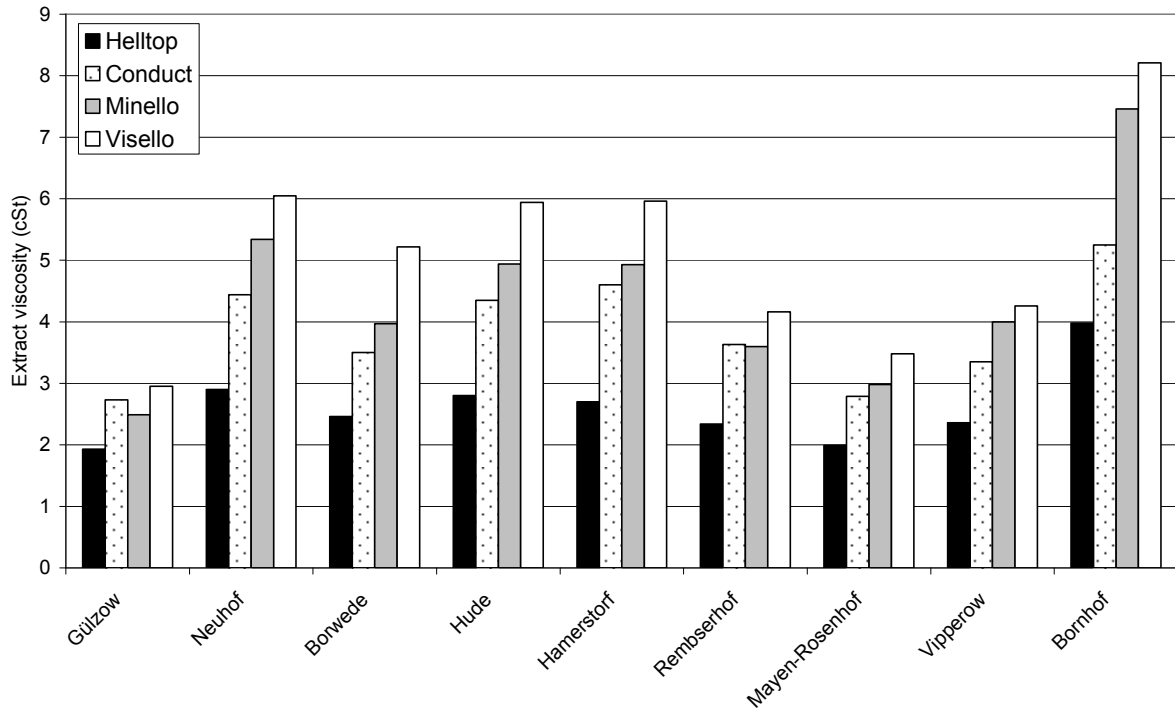


Figure 2. Arabinoxylan contents including total (A), water-soluble (B) and insoluble fractions (C) as well as extract viscosity (D) of the four rye cultivars grown at nine locations in 2010