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Winter rye (*Secale cereale* L.) antioxidant capacity, total phenolic content and quality indices

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Abstract. Rye (*Secale cereale* L.) grain is an excellent raw material for healthy and tasty foods. Rye products are characterized by their unique composition and properties such as antioxidant capacity and total phenolic. The aim of the study was to assess radical scavenging capacity, total phenolic content, protein, starch content and falling number in rye varieties wholemeal. The trial included population winter rye varieties 'Kaupo', 'Amilo', 'Dankowskie Amber', 'Dankowskie Rubin', 'Inspector' and hybrid rye varieties 'SU Drive', 'SU Mephisto', 'SU Bendix', 'Brasetto', 'Palazzo' grown in Latvia. The antioxidant activity was determined using the DPPH (2.2-diphenyl-1-1-picrylhydrazyl radical) assay and total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteou method. The rye grain antioxidant capacity was estimated as Trolox equivalent, while the total phenolic content was expressed as gallic equivalents (GAE). 'Su Drive' rye variety contained the largest amount of total phenolic (average 208 mg GAE 100 g^{-1} DW), but the lowest – 'Inspector' rye variety 176 mg GAE 100 g^{-1} DW. In general, all rye samples tested in this study demonstrated similar level of antioxidant capacity (from 38.5 to 46.2 mmol Trolox eq. 100 g^{-1}). Statistically higher ($P < 0.05$) total phenols content and falling number had hybrid rye grains, compared to the population rye grains. In the present trial, the differences between hybrid varieties grains protein and *starch content* comparing to population varieties were not observed.

Key words: antioxidant capacity, phenolic, protein, falling number, rye, starch.

INTRODUCTION

Rye grain, like other cereal grains, is important part of human diet. Rye is second to wheat, the most commonly used grain, in the production of bread (Michalska, 2007). The most popular for baking bread is wholemeal rye flour. Whole grain rye, primarily its aleurone layer, germ and bran, has rich sources of phytochemicals including phenolic compounds (Zieliński & Kozlowska, 2000; Žilic, 2016). Thus, whole grains are believed to consume significant health benefits in the prevention of chronic diseases such as cardiovascular disease and diabetes due to the contribution of phenolic compounds (Van Hung, 2016). Among health-promoting phytochemicals found in cereal grain, phenolic compounds have gained much attention in many scientific research areas due to their strong antioxidant properties. Their concentration in rye grains are at least partly influenced by cultivar (Michalska et al., 2007). Generally, main phenolic compounds in rye grains are phenolic acids, flavonoids, coumarins, proanthocyanidins, stilbenes and lignans (Žilic et al., 2012). The phenolic content of cereal grains is considered to be particularly important for human health, given its antioxidant activity and high cereal consumption among the general population (Mazzoncini et al., 2015). Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and activity of pathogens. About 8,000 plant polyphenol compounds have been identified so far, whereas only some hundred occur in edible plants. They are in fruits, flowers, leaves, roots and stems, whereas external tissues include bigger amounts of these components (Manach et al., 2004). Antioxidant activity is defined as an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking the propagation step in oxidative chain reactions (Augšpole et al., 2018).

The environmental conditions are known to have significant influence on the end-use quality of cereal grains (Augšpole et al., 2018; Linina et al., 2019).

The quality index of rye wholemeal flour is protein and starch content, they are affected by meteorological conditions, fertilizers and varieties (Kunkulberga et al., 2017; Stockmann et al., 2019).

In Latvia, cereal maturation and harvesting can occur during rainfall period, which can often be a reason for a low grain quality and even grain sprouting in ears. The falling number is a quick and easy test used to evaluate the degree of starch hydrolysis in wheat and rye grain samples. That quality index describes the degree of dextrinization of starch caused by amylolytic enzymes, mainly α-amylase. Increased activity of the enzyme is observed during the pre-or post-maturity sprouting of grain (Antonenko et al., 2014; Linina & Ruza, 2015).

The yield and quality of grain is mainly determined by management techniques and environmental conditions; however, it can be influenced by the genetic basis (Zdubel et al., 2009; Stepien et al., 2016; Linina & Ruza 2018; Stockmann et al., 2019).

The aim of the study was to assess radical scavenging capacity, total phenolic content, protein and starch content and also falling number in varieties of rye wholemeal.

MATERIALS AND METHODS

Study fields

Field trials with winter rye population varieties 'Kaupo' (Latvia), 'Inspector' (Germany) 'Amilo', 'Dankowskie Amber', 'Dankowskie Rubin' (all from Poland) and hybrid rye varieties 'Brasetto' (Canada) 'SU Drive', 'SU Mephisto', 'SU Bendix', and 'Palazzo' (all from Germany) were carried out in Priekuli Research Centre, Institute of Agricultural Resources and Economic in 2017/2018. The soil type was sod-podzolic loam, with close to neutral acidity, medium high phosphorus and potassium, organic mater 2.0 g kg^{-1}. Winter rye was sown in 20 September after spring barley. Seeding rate of population winter rye varieties was 500 seeds per m^2, whilst of hybrid rye varieties – 200 seeds per m^2. Trial was arranged randomly in four replications, plot size: 16 m^2. In total, there were 40 trial plots. In autumn NPK 6:26:30 kg ha^{-1} was applied. In spring after resumption of vegetative growth was applied nitrogen N68 kg ha^{-1}, at the shooting stage was applied N 31 kg ha^{-1}. Winter rye was harvested on 3 August. A sampling procedure for gain quality evaluations was performed according to ICC 101/1 standard for obtaining a mean sample.

Weather conditions

The air temperature in 2018 April was close to long-term average data. May was 5 °C warmer (17 °C), which influenced plant growth and development. Average daily temperature in June was close to long-term average data. Temperature in July (grain filling period) was 20 °C (2 °C warmer compared to long-term average data). Precipitation in April was close to long-term average data. May was dry. In June precipitation was close to long-term average data. The weather in July was very dry. Elevated temperatures and low precipitation significantly influenced plant growth, yield and quality.

Analysis

The rye grains were analysed at the Latvia University of Life Sciences and Technologies in Grain and Seeds Research laboratory and in laboratory of the Department of Chemistry. All winter rye grain samples were ground in a Perten Laboratory Mill 3100 to obtain the wholemeal.

Total phenolic content. For total phenolic extraction 1.0 ± 0.001 g of finely ground rye samples were weighed in to volumetric flasks, 10 mL of extractant, a mixture of methanol, distilled water and hydrochloric acid (79:20:1) was added. The vials were shaken at +18 ± 1 °C for 60 min in the dark, then centrifuged for 10 min at 5,000 rpm. The total phenolic content of the rye extract was analysed spectrometrically according to the Folin-Ciocalteu (Dewanto et al., 2002). Briefly, 2.5 mL of FolinCiocalteu reagent (diluted 10 times with distilled water) was added to 0.5 mL of rye extract. The mixture was then incubated for 3 min, after which 2 mL of sodium carbonate (Na_2C0_3) (7.5 g L^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 1 hour of incubation at +20 °C temperature in dark for colour development, absorbance was measured at 765 nm using JENWAY 630 Spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry-matter of rye extract. Analyses were carried out in triplicate for each rye extract sample (Augspole et al., 2017).

Antioxidant capacity. The antioxidant capacity of rye samples was determined according to Afify (2012) with some modifications. Method is based on the radical scavenging ability in reaction with stable 2.2-diphenil-1-picrylhydrazyl (DPPH) free radical. 3.5 mL of DPPH solution (4 mg of DPPH reagent dissolved in 100 mL pure ethanol) was added to 0.5 mL sample extract. A solution was well mixed and stood in dark place at +20 ± 1 °C for 30 min. Absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The antioxidant capacity was expressed as TROLOX (6-hydroxy-2.5.7.8-tetramethylcroman-2-carboxylic acid) equivalent antiradical activity mmol Trolox equivalent (TE) 100 g^{-1} DW.

Winter rye wholemeal quality index– *protein content* (%) and *starch content* (%) were analysed by grain analyser Infratec NOVA (Denmark). In Latvia grain analysers are connected in a unified network. Latvian grain network is a complex administrative-technical solution, providing uniform operation of the grain analyzers in the grain trade companies. Technically it means that every individual grain analyser is regularly controlled and adjusted according to Master Infratec via communication network. Master Infratec is controlled and adjusted according to well-known standard methods.

The *falling number* was determined according to the standard LVS EN ISO 3093:2011. 'Wheat, rye and their flours. Determination of the falling number according to Hagberg-Perten', using Perten Instrument (Sweden) 'Falling number 1500' assessed at using 7 g wholemeal adjusted to a moisture of 15%.

Statistical analysis

Results are presented as means ± standard error. For each trait, mean and the coefficient of variation (V%) were determined. Trial data evaluation was done using one factor analysis of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$). Significant differences for rye grain total phenolic content and antioxidant capacity are marked with different letters in superscript: $^{A, B, C}$ and $^{a, b, c}$. Differences of the average antioxidant capacity, total phenolic content and quality index between population and hybrid rye varieties were determined by t-test: Two-sample assuming unequal variances.

RESULTS AND DISCUSSION

The mean of antioxidant capacity (AC), total phenolic content (TPC), protein content (PC), starch content (SC) and falling number (FN) in the rye wholemeal from population and hybrid varieties selected for study are shown in Table 1. The least variation of the starch content (average 61.0%), total phenolic content (average 183.5 mg 100 g^{-1} GAE), protein content (average 10.4%) was noticed in the population varieties, with coefficient of variation (V%) from 0.2 to 2.5%. The greatest variation of the antioxidant capacity and falling number were observed in population cultivars wholemeal: coefficient of variation respectively 6.7% and 6.1%.

Table 1. Winter rye grain average antioxidant capacity, total phenolic, protein content, starch content and falling number

	AC, mmol Trolox eq. 100g^{-1}	TPC, mg 100 g^{-1} GAE	PC, %	SC, %	FN, s
Population varieties					
Mean ± standard error	42.0 ± 1.3	183.5 ± 2.0	10.4 ± 0.1	61.0 ± 0.1	260.8 ± 7.1
min	38.5	175.9	10.1	60.9	249.0
max	46.2	187.8	10.7	61.2	288.0
V%	6.7	2.5	2.5	0.2	6.1
Hybrid varieties					
Mean ± standard error	42.1 ± 0.8	195.9 ± 4.1	10.0 ± 0.3	61.1 ± 0.1	276.8 ± 2.9
min	39.3	184.9	9.3	60.9	269.0
max	43.8	208.3	10.6	61.3	285.0
V%	4.3	4.7	5.7	5.7	2.3

AC – antioxidant capacity; TPC – total phenolic content; PC – protein content; SC – starch content; FN – falling number; V% – coefficient of variation.

Winter rye wholemeal *total phenolic content* (TPC) significantly varied between hybrid varieties ($P < 0.05$) and also between population varieties ($P < 0.05$) (Fig. 1). The total phenolic content from hybrid varieties' wholemeal ranged from 185 mg 100 g^{-1} GAE ('SU Mephisto') to 208 mg 100 g^{-1}GAE ('SUDrive'), in population varieties – from 176 mg 100 gAE ('Inspector') to 188 mg 100 gAE ('Kaupo'). Similar results were gained in Poland (Zieliński et al., 2007) where total phenolic content in wholegrain of winter rye varieties was: 216mg 100 g^{-1} GAE ('Amilo'), 237 mg 100 g^{-1} GAE ('Warko') and 218 mg 100 g^{-1} GAE ('Dankowskie Zlote') and also in Serbia: 177 (variety 'DK-R') to 215 mg 100 g^{-1} GAE ('Raša') (Žilić et al., 2011).

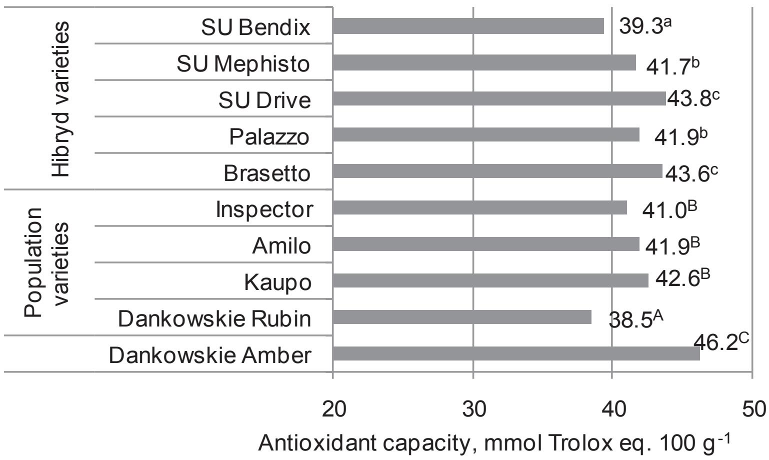

Figure 1. Total phenolic content (mg 100 g^{-1} GAE) in winter rye wholemeal (significantly different means are marked with different letters in superscript: [a, b, c, d, e,] – significant difference for rye grain hybrid varieties; [A, B, C] – significant difference for rye grain population varieties).

Winter wheat grains have a higher content of phenols comparing to rye. In the trial Skrajda et al. (2017) in Poland with 24 winter wheat varieties found that antioxidant capacity was from 296 to 359 mg 100 g^{-1} GAE. Total phenolic content and antioxidant capacity depend on the environmental conditions, management techniques and genetic trait of the variety (Zieliński et al., 2007).

In our investigation winter rye wholemeal *antioxidant* capacity (AC) significantly varied between hybrid varieties ($P < 0.05$) and also between population varieties ($P < 0.05$) (Fig. 2). In hybrid varieties the antioxidant capacity ranged from 39.3 mmol Trolox eq. 100g^{-1} ('SU Bendix') to 43.8 mmol Trolox eq. 100g^{-1} ('SU Drive'), in population varieties – from 38.5 mmol Trolox eq. 100g^{-1} ('Dankowskie Rubin') to 46.2 mmol Trolox eq. 100g^{-1} ('Dankowskie Amber'). Grains of hybrid rye varieties had statistically higher total phenolic content ($t_{stat.}2.68 > t_{crit.}1.94$).

Lower results were obtained in Poland (Zieliński et al., 2007) where in winter the antioxidant capacity of rye population varieties was: 14.7 mmol Trolox eq. 100 g^{-1} ('Amilo'), 15.2 mmol Trolox eq. 100g^{-1} ('Warko') and 14.3 mmol Trolox eq. 100 g^{-1} ('Dankowskie Zlote'). In Switzerland trial (Langenkämper et al., 2006) with wheat cultivar 'Titlis' antioxidant capacity in the wholemeal was 15.0 mmol Trolox eq. 100g^{-1}. Augspole et al. (2018) found that the antioxidant capacity of samples could be influenced by lots of factors, such as cultivation, production, storage conditions and test systems.

In another trial with peeled grains of rye was fixed lower total phenolic content (132 mg 100 g^{-1} GAE) and antioxidant capacity 20.0 mmol Trolox eq. 100 g^{-1} (Djordjevics et al., 2011), because whole rye grains contain germ and bran which are rich sources of total phenolic content and antioxidant capacity (Žilic et al., 2011).

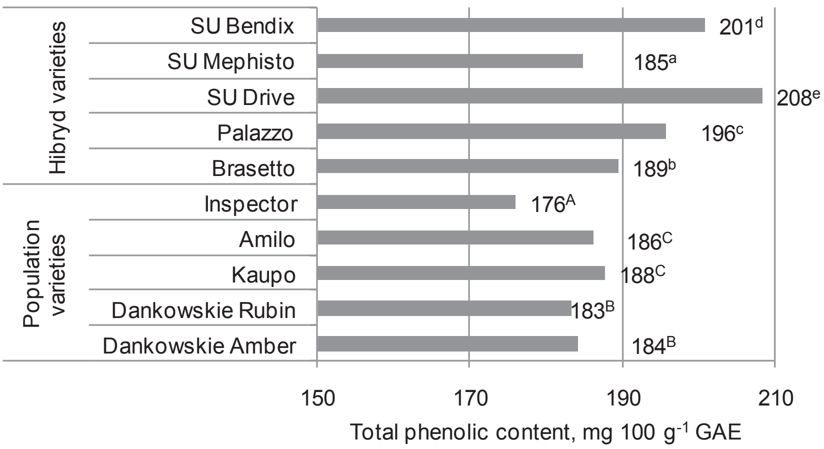

Figure 2. Antioxidant capacity (mmol Trolox eq. 100g^{-1}) in winter rye wholemeal (significantly different means are marked with different letters in superscript: $^{a, b, c,}$ – significant difference for rye grain hybrid varieties; $^{A, B, C}$ – significant difference for rye grain population varieties).

The protein content, starch content and falling number belong to an important criteria for the quality of rye grain (Linina & Ruza, 2015; Stepien et al., 2016). In our trial grain protein content, starch content and falling number significantly ($P < 0.05$) varied depending on the variety (except starch content for hybrid varieties). The results of analysed index are presented in Table 2. The *protein content* from hybrid varieties ranged from 9.3% ('Palazzo') to 10.6% ('SU Mephisto' and 'SU Bendic'), in population varieties – from 10.1% ('Dankowskie Amber') to 10.7% ('Dankowskie Rubin). Zielinski et al. (2007) showed that the average content of protein for population varieties were: 'Amilo' 8.7%, 'Dankowske Zlote' 10.3% and 'Warko' 11.6%, similar results were obtained in Estonia (Järvan et al., 2018) respectively 8.4% to 11.9%.

Starch is an important component of rye flour, ranging from 60.9% to 61.3% for both varieties.

In another investigation (Linina et al., 2019), the starch content in grain

Table 2. Winter rye wholemeal protein content, starch content and falling number

	PC, %	SC, %	FN, s
Hybrid varieties			
Brasetto	9.8	60.9	280
Palazzo	9.3	61.0	278
SU Drive	9.7	61.3	272
SU Mephisto	10.6	61.2	269
SU Bendix	10.6	61.1	285
$LSD_{0.05}$	0.04	0.12	ns*
Population varieties			
Dankowskie Amber	10.1	61.0	253
Dankowskie Rubin	10.7	61.2	249
Kaupo	10.2	61.0	252
Amilo	10.6	60.9	288
Inspector	10.6	60.9	262
$LSD_{0.05}$	0.12	0.13	7.14

PC – protein content; SC – starch content; FN – falling number; *ns – not significant.

of hybrid and population varieties of winter rye fluctuated over a larger range: from 58.8% to 64.8%, while in Poland (Zieliński et al., 2007) trial starch content was lower: from 53.3% (‘Warko’) to 55.7% (‘Amilo’).

In the present trial, the differences between hybrid varieties grains protein and *starch content* comparing to population varieties were not observed ($t_{stat.} < t_{crit.}$).

Hagberg falling number is the major quality attribute of rye grain. In 2018, the weather during the harvest was dry and sunny, therefore falling number for all winter rye samples was high. Falling number of hybrid varieties ranged from 269 s (‘SU Mephisto’) to 285 s (‘SU Bendic’), in population varieties – from 252 s (‘Kaupo’) to 288 s (‘Amilo’), which considerably exceeds the minimum requirements for grain processing in Latvia (120 s). In our investigation the falling number of grains was significantly ($P < 0.05$) different for population cultivar. Statistically higher falling number was obtained of hybrid rye varieties ($t_{stat.}2.08 > t_{crit.}2.01$).

Linina et al. (2019) in the previous study (2015–2017) discovered, that the falling number in hybrid and population varieties of winter rye was from 130 s to 305 s, in Estonian trial it was 62 s to 289 s (Järvan et al., 2018), while in Poland it was from 316 to 397 s (Michalska et al., 2007). The falling number is influenced mainly by meteorological conditions and varieties (Linina & Ruza, 2015; Kunkulberga et al., 2017).

Winter rye properties demonstrate that the quality of the studied varieties fit the requirements for high–grade rye for food consuption. The varieties ‘SU Mephisto’, ‘SU Bendic’, ‘Dankowkie Rubin’, ‘Amilo’ and ‘Inspector’ with higher protein content are more required for the cereal industry for further processing.

CONCLUSIONS

In our investigation total phenolic content, antioxidant capacity, protein, starch content and falling number are determined in winter rye population and hybrid varieties wholemeal.

Protein content and starch content, falling number, total phenolic content and antioxidant capacity in winter rye varied significantly depending on the cultivars. The greatest variations of the antioxidant capacity and falling number were observed in population varieties wholemeal, compared to the hybrid varieties. Statistically higher total phenolic content and falling number was in grain of hybrid rye varieties, compared to the population varieties. Differences between hybrids and population varieties of grain protein and starch content were not observed.

This study showed that whole rye grains, which are widely used in human consumption, contain high levels of total phenol content and show a relatively moderate antioxidant capacity. It can be suggested that in future more and more rye products will be available on market, especially those originated from wholemeal.

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