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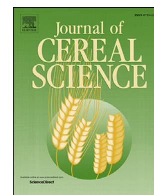

Variation of wheat grain lipid fraction and its antioxidative status under the impact of delayed sowing

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This study determined the profile of hydrophobic phytochemicals (steroid-like components and lipophilic phenolics) and antioxidant potential (DPPH and Rancimat tests) of wheat grain in relation to sowing date (standard vs. delayed) using six cultivars of *Triticum aestivum* wheat grown in Poland. The study found that "sowing time" generally had a low impact on sterols, carotenoids, squalene and total lipophilic phenolics in wheat grain. The highest impact of "sowing time" was noted for α-tocopherol, C19:0 alkylresorcinol and campesterol. Delayed sowing reduced their content by up to 9%. The antioxidant potential of grain extracts obtained by the use of water-saturated butanol (WSB) was mostly cultivar-dependent (depending on assay: DPPH 56.3% and Rancimat 75.1% of explained variation, respectively). Wheat grain WSB extracts increased rapeseed oil induction time by up to 21% and their antioxidant capacity was up to 1.24 μM TEg^{-1}.

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1. Introduction

Plant-origin food contains an array of phytochemicals with various health benefits. For example, wheat grain is a good source of antioxidants, which can prevent or limit the consequences of free radicals' action that contributes to the etiology of cardiovascular and inflammatory diseases, cataracts, carcinogenesis, ageing, etc. (Liu et al., 2012).

In total, wheat grain contains up to 2700 μg g^{-1} of phytochemicals (Konopka et al., 2012) that differ in affinity to hydrophobic and amphiphilic food phases. Hydrophobic compounds constitute up to 75% of their total (Shewry and Ward, 2012) and create so-called a non-saponifiable fraction, composed mostly of alkylresorcinols and steroid compounds (hydrocarbons, ketones and sterols) (Prinsen et al., 2014). In detail, the wheat grain non-saponifiable fraction is composed of sterols (mainly β-sitosterol), tocochromanols (mainly α-tocopherol and β-tocotrienol), carotenoids (mainly lutein) (Shewry and Ward, 2012; Chen et al., 2015), phenolic lipids known as alkylresorcinols (1,3-dihydroxyl-5-n-alkylbenzene derivatives) (Kulawinek et al., 2008), squalene (Spanova and Daum, 2011) and hydrophobic derivatives of phenolic acids (Huang et al., 2015). In grain tissue these compounds are localized mostly in storage oil bodies, various cell membranes, and protective coat layers (Konopka et al., 2012).

The characteristics of grain phytochemicals may be varied both by genotype (G) and environment (E), although the force of their individual impacts is still under investigation. The broad research of 150 wheat genotypes tested in the HEALTHGRAIN project determined the main impact of G on the accumulation of compounds such as tocols (ca. 80% impact), sterols (ca. 55% impact) and alkylresorcinols (ca. 60% impact) (Shewry and Ward, 2012). In contrast, Beleggia et al. (2013) conducted research in which they compared conventional vs. organic farming systems for durum wheat (three years of vegetation varied by rainfall and temperatures) and noted that grain tocols and sterols were mostly varied by E (up to 98% for most phytosterol homologues). Similarly, Lv et al. (2013), with the use of 10 soft red winter wheat varieties grown in Maryland (USA), stated that carotenoids were mostly affected by E (ca. 45% of total variance), while tocols were mostly affected by G × E interaction (up to 72% of total variance). Similarly, the results of Lu et al. (2015) showed that among soft winter wheat E had a generally stronger influence than G on α-tocopherol, δ-tocopherol and total tocopherol content. In this study the accumulation of grain tocols and

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carotenoids was positively correlated with precipitation and temperature during plant vegetation. This short introduction points out that environment can greatly affect grain composition. In this regard the term "environment" may mean variation in fertilization and protection regimes, in climate (temperature, rainfalls, sun light) and in inhabiting pests and microbiota presence. All these conditions may act as plant stressors and may stimulate the synthesis of secondary metabolites as a plant resistance/defence strategy in grain. Grain phytochemicals participate in a defence strategy, so their high variability between different grain genotypes/samples may be at least partially explained by such action. A striking example of this is the approx. 40-fold higher accumulation of flavonoids in wheat grain infected by *Fusarium culmorum* (Buśko et al., 2014).

The overall quantity of antioxidative compounds in a food sample creates its antioxidant potential (status/index/capacity/activity). The antioxidant potential of different wheat grain samples and grain end-products varies from below 1 μmol TE g^{-1} (Lavelli et al., 2009) to above 140 μmol TE g^{-1} (Chen et al., 2015). This variation results mostly from the phytochemical composition of the sample, but is affected at least partly by the method of extraction of active compounds and quantification procedures. For ingested food it also depends on the possibility of antioxidant release in the digestive tract. This is especially important for complex matrices, such as seeds and grains, in which phytochemicals are entrapped by cell compartments and organelles (Delgado-Andrade et al., 2010). Hydrophobic antioxidants are generally more easily released from the plant matrix than phenolic acids, which are strongly associated with the lignocellulose polymers of cell walls. This may explain the almost 2-fold more active extract of lipophilic compounds (dichloromethane-soluble) than extracts of compounds soluble in 80% methanol from grain samples (Konopka et al., 2014). This points out that the total antioxidant capacity of grain and its end-products may be balanced by the interaction of amphiphilic and hydrophobic components (Delgado-Andrade et al., 2010).

The end use quality of wheat grain can be modified by manipulation of sowing date (Motzo et al., 2007). This plant cultivation regime may affect grain productivity and resistance against pests. Variation of sowing time, by the modifications of rain and thermal conditions during grain filling, may affect grain protein content. In general, late sowed grain had lower grain weight but higher protein content (Motzo et al., 2007). However, the impact of sowing time on grain phytochemicals is poorly explored. The aim of this study was to determine the hydrophobic phytochemicals in wheat grain, as well as its antioxidant potential in DPPH and Rancimat tests in relation to two terms of sowing (standard vs. delayed date) using six cultivars of common wheat.

2. Material and methods

2.1. Sample preparation

Six *Triticum aestivum* cultivars – Bombona (spring wheat) and Oxal, KWS Ozon, KWS Dacanto, Meister, and Forkida (winter wheats), differentiated by term of sowing, were included in the study. A field-plot experiment was conducted in 2013 in south-eastern Poland. According to Polish classification used cultivars belong to premium quality (Bombona), high quality (Oxal), and bread quality (rest of cultivars). Wheat was sown in 20 m^2 plots. Standard fertilization regimes were as follows: nitrogen (N) and potassium (K_2O) at 100 kg ha^{-1} and phosphorus (P_2O_5) at 60 kg ha^{-1}. Plots were divided by the term of sowing: 1) standard sowing date; 2) delayed sowing date – two weeks after standard date. After harvesting, about 100 g grain samples were manually cleaned of all foreign materials and broken kernels, and stored at 8 ± 2 °C. Before further analyses, the required amount of grain was removed from the refrigerator and equilibrated at a temperature of 21 ± 1 °C and moisture of 40 ± 8% RH for at least 48 h. Prior to chemical analyses the grain was ground in a type A10 IKA Labortechnik mill (Staufen, Baden-Wurttemberg, Germany) to obtain particles smaller than 300 μm.

2.2. Preparation of wheat grain extracts

Water-saturated butanol (WSB) was used for the extraction of total lipids. Extraction was conducted from the ground grain using a procedure described by Konopka et al. (2012). Collected extracts were evaporated to dryness at temperatures below 50 °C in a vacuum evaporator (Büchi, type R-210; Büchi Labortechnik, Flawil, Switzerland) and stored in the dark at a temperature of 8 °C before further analyses.

2.3. Fatty acid composition

The fatty acid composition of compounds in WSB extracts was determined by GC chromatography. Methyl esters were prepared according to the method described by Roszkowska et al. (2015), and their analysis was carried out with a GC 8000 FISONS chromatograph equipped with a DB-225 capillary column (30 m × 0.25 mm × 0.25 μm), helium as the carrier gas, and a flame-ionization detector. Fatty acids were identified according to the retention time determined for fatty acid standards (Sigma-Aldrich, Poznań, Poland) and quantified based on an addition of heptadecanoic acid (C17:0) as an internal standard.

2.4. Sterols and squalene

The content of sterols was determined by the GC/MS method. The sterols were extracted from WSB extract according to the method described by Roszkowska et al. (2015). The analysis of sterols was performed using the GC-MS QP2010 PLUS manufactured by Shimadzu (Kyoto, Japan). Sterols were separated on a ZB-5ms capillary column (30 m × 0.25 mm × 0.25 μm) (Phenomenex, Torrance, CA, USA), and with helium as a carrier gas with a 0.9 mL min^{-1} flow rate. The temperatures were as follows: injector – 230 °C, column – 70 °C increased to 230 °C at 15 °C min^{-1}, and to 310 °C at 3 °C min^{-1}, and maintained for 10 min, GC-MS interface – 240 °C, ion source – 220 °C. Electron energy was set as 70 eV. The total ion current (TIC) mode was used for quantification (100–600 m/z range). Sterols were identified by comparison with the mass spectral library, and their content was determined based on the concentration of the internal standard, and was expressed as μg of 5α-cholestane per 1 g of grain. The content of squalene was determined and quantified simultaneously with sterols.

2.5. Alkylresorcinols

The content of alkylresorcinols was determined according to the method described by Sampietro et al. (2009). WSB extract was dissolved in methanol and colour reaction was performed by adding 0.05% Fast Blue RR reagent (4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt) diluted with methanol 1:5) and 10% K_2CO_3 solution to each extract. Absorbance measurements were carried out after 20 min at 480 nm using a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, Cambridge, United Kingdom). The content of alkylresorcinols was calculated using a standard curve prepared for olivetol. The composition of alkylresorcinols was determined with the use of the GC-MS QP2010 PLUS, manufactured by Shimadzu. The conditions of

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