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# Phenolic acid content in wheat grain (*Triticum* spp) of different genotypes

## Contenido de ácido fenólico (*Triticum* spp) de diferentes granos de trigo

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### Abstract

One of the most significant factors affecting resistance to fungal diseases in winter wheat genotypes is the content of phenolic compounds. A total of 100 winter wheat cultivars were investigated. The contents of five phenolic acids: ferulic, vanillic, syringic, vanillin, p-coumaricand free phenolic acids (FPA) were determined. Considerable variation was found both within tested cultivars. Among the investigated acids the greatest amounts were recorded for ferulic acid and its mean concentration was 975 µg $g^{-1}$, p-coumaric acid was characterised by a lower mean concentration (52 µg $g^{-1}$), while the lowest concentration was recorded for vanillin, amounting to 5 µg $g^{-1}$. Ferulic acid state 92.4% of total phenolic acids. Significant differences in content of ferulic acids between genotypes were observed. The group of winter wheats coming from different European countries was characterized by high variation in the contents of ferulic acid, ranging from 320 to 3103 µg $g^{-1}$. No significant correlations were observed between ferulic acid and other phenolic acids. Results provide the basis for further studies in this field.

**Keywords**
ferulic acid • phenolic acids • wheat

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## Resumen

Uno de los factores más importantes en la resistencia a las enfermedades fúngicas en genotipos de trigo de invierno es el contenido de compuestos fenólicos. Se investigó un total de 100 cultivos de trigo de invierno, se determinó sus contenidos de los cinco ácidos fenólicos: ferúlico, vanílico, siríngico, vainillina, p-cumárico y ácidos fenólicos libres (AFL) y se encontró una variación entre ambos cultivos probados. Entre los ácidos investigados, se detectó mayores cantidades de ácido ferúlico (concentración media de 975 µg $g^{-1}$), ácido p-cumárico (caracterizado por una baja concentración media (52 µg $g^{-1}$), mientras que la concentración más baja que se detectó fue de vainillina (a razón de 5 µg $g^{-1}$). El ácido ferúlico conforma el 92,4% de los ácidos fenólicos totales. Por otro lado, se observó diferencias significativas en el contenido de ácidos ferúlicos entre genotipos: el grupo de trigos de invierno procedente de varios países europeos tenía como característica una alta variación de contenidos de ácido ferúlico, del orden de 320 hasta 3103 µg $g^{-1}$. No se detectó correlaciones significativas entre el ácido ferúlico y otros ácidos fenólicos. En conclusión, los resultados de este estudio ofrecen la base para otras investigaciones con más profundidad en este ámbito.



**Palabras clave**
ácido ferúlico • ácidos fenólicos • trigo


## Introduction

Wheat is infested by many fungal diseases. In case of wheat this problem to the greatest extent pertains to grain contamination with mycotoxins produced by various species, of which the most important are fungi from the genus *Fusarium* (2, 15). This results in grain contamination with such toxins as deoxynivalenon (DON), nivalenol (NIV) (14).

Breeding towards resistance is the best prevention method. Although some genotypes are known to have resistance coming from spring cultivars such as Sumai 3 and Frontana, they exhibit disadvantageous yield traits. In view of such difficulty in obtaining resistant cultivars to *Fusarium* new genotypes are searched for both in Poland and worldwide, which could provide good germplasm for breeding towards resistance.

Some information indicates that also resistance to Fusarium Head Blight (FHB) in wheat may be to a considerable degree connected with the presence of phenolic acids in the spike and in kernels. Among phenolic acids in wheat grain ferulic acid is found in greatest amounts, which level depends on the cultivar and on kernel size (8, 10).

A dependence was indicated between ferulic acid found in wheat grain and resistance of wheat to fusariosis (17).

The aim of the presented investigations was to determine the contents of phenolic acids, particularly ferulic acid, in grain of winter wheat genotypes.

## MATERIAL AND METHODS

A total of 100 cultivars and lines of winter wheat were selected for analysis. They came from Polish breeding companies involved in wheat breeding and from the collection of winter wheat belonging to the Department of Genetics and Plant Breeding, the Poznań University of Life Sciences. Breeding companies, *i.e.* Małopolska Plant Growing Company - HBP Ltd. (MHR), Plant Breeding Company Smolice Ltd. (HRS), Danko Plant Breeding Company Ltd. (Danko HR), Poznań Plant Breeders Ltd., (PHR), Plant Breeding Company Strzelce Ltd. (HRSt) from their own breeding material selected for analyses genotypes with known resistance to fungal diseases. Genotypes selected from the collection of the Department of Genetics and Plant Breeding, PULS included cultivars and lines of winter wheat originated from different countries (Austria, Czech Republic, Holland, France, Germany, Russia and Hungary).

Based on literature data they were considered resistant to *Fusarium,* and they were obtained from the wheat collection of the National Small Grain Collection at the Agriculture Research Station in Aberdeen, belonging to the National Plant Germplasm System in the USA, and from dr Tomasz Góral, Plant Breeding and Acclimatization Institute in Radzików. Analyses were also conducted on wheat lines generated at the Department of Genetics and Plant Breeding (DG&PB) and coming from crossing of semi-dwarf English and French forms with leading Polish cultivars.

All grain samples used in the analyses were harvested in 2011 from genotypes growing at Wielkopolska conditions, at Agriculture Research Station "Dłoń".

The soil at the site is Luvisols (SgP, 2011) with pH ranged between 6.0 and 6.4. Weather conditions in thes pringvegetation in 2011 were as follows: the average temperature in April was 11.9°C, 15.5°C in May, June 19,0°C and in July 18.6°C. Total rainfall in the month of April was 14 mm, 37.5mm in May, 33.5mm in June and 169mm in July. The field experiment was conducted in 3 replications, while the plot size was $1m^2$.

From 3 replications, a bulk sample of 1 kg was collected, from which a sample of 100 g grain each was used in further analyses. The chemical analysis were conducted in 2 replication, and the mean of the replications is shown in the tables (table 1, page 4)

*2.1 Analysis of contents of free phenolic acids (FPA)*

Total of free phenolic acids was determined as described by Stuper-Szablewska *et al*., (2015).

*2.2 Determination of phenolic acids*

Contents of 5 phenolic acids was determinedas described by Stuper-Szablewska *et al.*, (2014).

*2.3 Statistical analysis*

Results were subjected to statistical analysis using the STATISTICA v. 8.0 software package. Means, range from the samples as well as the number and percentage of positive samples were determined. Moreover, values of Pearson's linear correlation coefficients were determined at the following significance levels, α=0.01, and α=0.001 (**, ***). In order to compare contents of analysed metabolites in samples the procedure of multiple comparisons using Tukey's method was applied.

**Table 1.** Mean, ranges and percentages of positive samples for concentrations of five identified phenolic acids and their totals as well as free phenolic acids (FPA).

**Tabla 1.** Media, rangos y porcentajes de muestras positivas para las concentraciones de cinco ácidos fenólicos identificados, sus totales y ácidos fenólicos libres (AFL).

| | **Ferulic $\mu g g^{-1}$** | **Vanilic $\mu g g^{-1}$** | **Syringic $\mu g g^{-1}$** | **Vanilin $\mu g g^{-1}$** | **p-cumaric $\mu g g^{-1}$** | **Phenolic acids (total) $\mu g g^{-1}$** | **FPA $\mu g g^{-1}$** |
|---|---|---|---|---|---|---|---|
| Mean | 975 | 17 | 5 | 6 | 52 | 1055 | 515 |
| Range | 320-3103 | 0-72 | 0-37 | 0-77 | 10-160 | 357-3186 | 220-843 |
| Percent of positive samples | 100% | 71% | 40% | 38% | 100% | 100% | 100% |

**Table 2.** Co-occurrence of phenolic acids.

**Tabla 2.** Co-ocurrencia de ácidos fenólicos.

| Co-occurrence | Percent of positive samples |
|---|---|
| Ferulic, vanilic, syringic, vanillin, p-cumaric | 11 |
| Ferulic, vanilic, vanillin, p-cumaric | 23 |
| Ferulic, vanilic, syringic, p-cumaric | 14 |
| Ferulic, syringic, vanillin, p-cumaric | 3 |
| Ferulic, vanillin, p-cumaric | 3 |
| Ferulic, syringic, p-cumaric | 9 |
| Ferulic, vanilic, p-cumaric | 19 |
| Ferulic, p-cumaric | 18 |

## Results

A total of 100 grain samples of winter wheat were analysed. In the samples five phenolic acids were identified: ferulic, vanillic, syringic, vanillin and p-coumaric acids (table1).

However, all the acids were presented in grain of only 11 genotypes (table 2).

Most frequently in the analysed samples (23%) four acids were found simultaneously: ferulic, vanillic, vanillin and p-coumaric acids as well as a combination of three acids: ferulic, vanillic and p-coumaric (table 2). In grain of all the genotypes two acids were detected: ferulic and p-coumaric acids.

Mean concentration of ferulic acid detected in 100% samples was 975 $\mu g\ g^{-1}$ while that of p-coumaric acid - also found in all samples - was 52 $\mu g\ g^{-1}$, with a considerable range from the sample observed in both cases. The presence of vanillin was found in grain of 38% tested genotypes, while syringic acid was found in grain of 40%.

At the same time in both cases the lowest mean content of these acids was recorded, amounting to 6 and 5 $\mu g\ g^{-1}$, respectively.

Calculated linear correlation coefficients showed that contents of ferulic acid and total phenolic acids were related to the highest degree, highly significantly (table 3, pág. 5).

**Table 3.** Matrix of internal correlations for concentrations of free phenolic acids (FPA), five identified phenolic acids and their totals.

**Tabla 3.** Matriz de correlaciones internas para las concentraciones de ácidos fenólicos libres (AFL), cinco ácidos fenólicos identificados y sus totales.

| | FPA | Ferulic | Vanilic | Syringic | Vanilin | p-cumaric |
|---|---|---|---|---|---|---|
| Ferulic | 0.4801** | | | | | |
| Vanilic | -0.0238 | 0.0732 | | | | |
| Syringic | 0.4193** | 0.1205 | -0.1679 | | | |
| Vanilin | 0.0232 | -0.0085 | 0.0551 | -0.0907 | | |
| p-cumaric | -0.0347 | -0.0284 | 0.0036 | 0.0426 | -0.0301 | |
| phenolic acids (total) | 0.4824** | 0.9974*** | 0.1065 | 0.1315 | 0.0129 | 0.0291 |

**. *** - Pearson's linear correlation coefficients at the significance level $\alpha = 0.01$, $\alpha = 0.001$.

**. *** - coeficientes de correlación lineal de Pearson en el nivel de significación $\alpha = 0,01$, $\alpha = 0,001$.

This dependence resulted from the fact that ferulic acid constituted 92.4% total phenolic acids. Significant correlations were also recorded between contents of ferulic acid, syringic acid and total phenolic acids and contents of FPA (table 3).

Conducted analyses of winter wheat grain showed very high variation in the analysed genotypes in terms of contents of ferulic acid (320-3103 μg $g^{-1}$) and total phenolic acids and FPA (table 1, page 4; table 2, page 4; table 3). Almost half of the genotypes: 47, had contents of ferulic acid within the range of 700-1000 μg, and only thirteen contained over 1500 μg. A very low content of ferulic acid <500 μg was found in only five genotypes.

## Discussion

Li *et al*. (2008) when analysing 130 genotypes of winter wheat found that the range from samples for ferulic acid was from 326 to 1171 μg $g^{-1}$ at the mean contents of 664 μg $g^{-1}$, while in this experiment the average for winter wheat was 974 μg $g^{-1}$. Significant differences between contemporary and old cultivars of *Triticum aestivum* and *T. durum* were reported by Heimler *et al.* (2010). Also the analysis of 58 genotypes from the Australian Winter Cereals Collection conducted by Wu *et al*. (2001) showed significant differences between genotypes in terms of contents of seven phenolic acids. Irmak *et al*. (2008) stated that even in isolines of cv. 'Pegaso' differing in glutenin protein subunits there were significant differences in PFA contents and total phenolic acids. Probably reports on a lack of significant differences between genotypes frequently result from a small number of analysed forms (3, 21). Amounts of ferulic, coumaric and vanillic acids comparable to those recorded in these experiment were observed in different wheat cultivars by other authors (11, 12).

Analogously to different publications on wheat phenolics, ferulic acid was the dominant phenolic acid in all wheat cultivars and accounted for 92.4% of total phenolic acids. Similarly, Irmak *et al*. (2008) and Jonnala *et al*. (2010) showed that ferulic acid constituted 92% and 95%, respectively, of total phenolic acids. Moreover, a high correlation was found between contents of ferulic acid and total contents of phenolic acids (12).

Significant differences in contents of ferulic acid and total contents of phenolic acids between spring wheat genotypes were found by Mpofu *et al*. (2006). Content of ferulic acid was low, amounting to 371-436 μmol gallic acid $100g^{-1}$ grain, while the total content of phenolic acids was high, amounting to 1709-1990 μg $g^{-1}$. In turn, Irmak *et al*. (2008) recorded that total phenolic acid content in cv. 'Pegas' was very high at 2795 μg $g^{-1}$ GAE. Kim *et al*. (2006) in wheat samples recorded concentrations of ferulic acid at an average level of 3420 μg $g^{-1}$.

Content of FPA was on average over two-fold lower than total phenolic acids. Greater differences were observed by Okarter *et al*. (2010), as in their study FPA content ranged from 255 to 499 μmol gallic acid $100g^{-1}$ grain, while total content of phenolic acids from 841 to 1099 μmol gallic acid $100g^{-1}$ grain, with significant differences between genotypes. In the analyses performed on 11 cultivars by Adom *et al*. (2003), FPA content was much lower (119-201 μmol gallic acid 100 $g^{-1}$ grain) than the total content of phenolic acids at 709-860 μmol gallic acid 100 $g^{-1}$ grain.

Considerable differences in contents of ferulic acid, observed in the conducted experiment, may be reflected in differences in resistance to *Fusarium.*

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