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Concentration of Phenolic Compounds and Phenolic Acids of Various Spelt Cultivars in Response to Growing Years

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Abstract: The aims of this study were to evaluate and compare the radical scavenging activities (DPPH), phenolic concentrations and concentrations of selected phenolic acids (PAs) of spelt cultivars and breeding lines with common wheat in a three-year controlled field experiment under conditions of organic farming. No significant variations were observed in the total and free DPPH of Altgold, Ebners Rotkorn, Ostro and PN-1-36 with common wheat. The total DPPH ranged from 52.13% to the lowest value of 44.01% in Franckenkorn. Total, free and bound phenolic concentrations were the highest for common wheat (1902.55 µg FAE g^{-1} DM of total), while all spelt cultivars achieved significantly lower values (from 1434.94 µg FAE g^{-1} DM in Franckenkorn to 1650.22 µg FAE g^{-1} DM in Ebners Rotkorn). Bound phenolic compounds represented 86.3% of the total ones. An extremely dry and warm ripening period had a negative impact on the synthesis of phenolic compounds. The highest concentration of total PAs was observed in spelt Ebners Rotkorn (681.75 µg g^{-1} DM) and the lowest in common wheat (396.05 µg g^{-1} DM). The total share of free and bound PAs was 5.7% and 74.8%, respectively. The extremely dry and very warm grain filling period had a more evident negative impact on the concentration of free PAs compared to bound forms. The dominant free PA was ferulic (70.48%), followed by syringic (9.30%), p-HBA (5.59%), sinapic acid (5.40%), salicylic (4.18%), p-coumaric acid (3.22%) and caffeic acid (1.93%). Spelt cultivar Ebners Rotkorn was distinguished by the highest concentration of free and bound forms of PAs.

Keywords: spelt; common wheat; phenolic acid profiles; DPPH; phenolic compounds; organic farming

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1. Introduction

Spelt is an ancient hexaploid wheat species (AABBDD; 2n = 6x = 42) and the ancestor of the free-threshing common wheat that used to be common in Northern Europe [1]. The recent interest in "organic" and "natural" products resulted in the "rediscovery" of such ancient wheat owing to its nutritional value, high starch resistance and healing properties, which make it particularly useful for the treatment of diseases such as high blood cholesterol, colitis and allergies [2]. Furthermore, spelt has the capacity to grow on poor soils with little input, as well as its increased tolerance to biotic and abiotic challenges such as diseases, insects, extreme temperature (cold and heat), drought and salinity [3]. Consumers demand spelt wheat because it is more nutritious than common wheat [4].

Wheat species, especially ancient ones, are rich sources of antioxidant compounds, such as phenolic acids, flavonoids, carotenoids, tocopherols, phytosterols and vitamins, as well as water soluble antioxidants that have low molecular weights. A wide range of compounds, including those that are physiologically active and exhibit antioxidant capabilities, such as free radical scavengers, reducing agents and quenchers of the production of singlet oxygen, are found in whole grains of cereals [5,6]. Literature data are available based on the nutritional value of spelt wheat and spelt wheat-based products but not much is known about its antioxidant content and activity. The use of such species also presents more opportunities due to current trends towards organic and low-input agriculture and the growing demands for organic food products [7].

Phenolic compounds are antioxidants that occur naturally and have various beneficial health protection effects, such as anti-allergic, antiviral, anti-inflammatory and anti-mutagenic properties [8,9]. The interaction between antioxidant molecules and radicals reduced the DPPH radical absorption which is caused by antioxidants [10]. A study of whole wheat grains showed that phenolic compounds have a high antiradical power by scavenging free radicals, preventing lipid peroxidation and account for most of the total antioxidant activity [11,12]. Phenolic compounds can be found in free, soluble conjugated and bound forms, where the bound form is the most abundant among phenolic acids [13]. Ferulic acid accounts for up to 90% of the total phenolics in spelt seeds and 99% of it is present in the bound form [14]. Phenolic compounds are mainly located in the outer layers of the kernel, such as the aleurone, pericarp and embryo cell walls [15,16]. Phenolic acids and flavonoids are the most studied class of phytochemical compounds extracted from plants [17]. It is important to note that the environmental conditions may also affect the secondary plant metabolism and influence the accumulation of phenolic compounds during kernel development [18]. The production and accumulation of phenolic compounds in the wheat grain depended on the wheat genotype and the climatic and growing conditions [19]. The main phenolic acids (PAs) that were identified as allelopathic agents are 4-hydroxybenzoic, vanillic, coumaric and ferulic acids [20].

Various studies have shown that cereals organically grown have more antioxidants, phenols and zinc, but less protein, nitrates, nitrites and cadmium than those grown conventionally [21,22]. Moreover, some farmers choose to grow ancient tall-stemmed spring wheat varieties (from before the 1960s) that have more minerals (zinc, copper and selenium) in their grains than the modern short-stemmed ones using an organic farming approach [23]. The putative ancestral and primary gene pool of ancient wheat species can be helpful for the improvement of the health-related traits of modern wheat (durum and bread wheat) [2,24]. A similar study suggested that the high antioxidant potential and health benefits of evaluated ancient wheat cultivars and breeding lines make them an important source of phenolic acids in the human diet [25]. Published research studies demonstrating the phytochemical concentrations, compositions and profiles of PAs in different wheat species and cultivars are still sparse; furthermore, the results are often contradictory. In some research, the average level of total PAs of emmer was the highest, while others reported the highest concentration in spelt [2,24,25]. The high variability and higher concentrations of individual phenolic acids, e.g., ferulic (148.67–764.04 μg/g), p-coumaric (5.06–54.09 μg/g) and total phenolic content (2.06–8.11 μmol GAE/g) of ancient wheat were found compared to bread and durum wheat [10]. Intensive knowledge regarding the impact of the genotype, year and interaction on certain phenolic compounds might help to enhance breeding initiatives to develop ancient wheat grain richer in specific health components. However, there is less evidence in the literature about the impact of the cropping environment on the variance in phenolic acids and comparisons between these species, such as common and durum wheat under organic agriculture.

This study aimed to examine ancient wheat spelt and the differences among tested cultivars and breeding lines in comparison with common wheat. This study measured and compared the levels of (a) free, bound, total phenolic compounds; (b) free, bound, total phenolic acids; and (c) reducing capacity using DPPH assay. These compounds were evaluated under an organic management system and varying weather conditions during three growing seasons.

2. Materials and Methods

2.1. Field Trial, Experimental Design, Plant Material

Field experiments were conducted at the experimental base of the Faculty of Agrobiology and Food Resources in Nitra, Slovakia (48°19′ N, 18°07′ E) on a haplic luvisol. The design of the experiment was a randomized complete block in four replicates. The location has a continental climate and belongs to a warm agro-climatic region, with an arid subregion with a mild winter. The average long-term (1951–2000) annual temperature is 9.9 °C, and the annual long-term precipitation 547.6 mm. Temperature (t) and precipitation data (p) calculated during the flowering and ripening periods were compared with long-term data, as given in Supplementary Table S1. Six winter spelt cultivars (Altgold, Ebners Rotkorn, Oberkulmer Rotkorn, Ostro, Rubiota, Franckenkorn), one spelt breeding line (PN-1-36) and one common wheat winter variety (Laudis) were grown in crop rotation after pre-crop common pea; the experiment was managed under the organic system (without synthetic fertilizers and pesticides). After pre-cropping and ploughing to the depth of 0.22 m and during growing seasons, mechanical weed control was used. Crop nutrition was based on the use of manure in the amount of 30 t per hectare. The sowing dates were between the 15th and 21st of October, and spelt was hand-harvested in July (16th in 2015, 21st in 2016, 14th in 2017) at a grain humidity below 14%.

Harvested spikes of spelt were dehulled with the laboratory equipment KMPP 300 (JK Machinery, Prague, Czech Republic), hand-cleaned and sieved with a laboratory sieve shaker (Retsch GmbH, type AS 200, Haan, Germany). Grains under the sieve—smaller than 2.2 mm (remainders)—were not taken for further experimental procedures.

2.2. Sample Preparation and Extraction Procedure

The analyses of all antioxidant compounds were performed as previously described in Lacko-Bartošová et al. [25]. Briefly: wholegrain meal was prepared by grain milling on a FQC-109 laboratory mill with a 250 µm sifter. The wholegrain meal was defatted with hexane two times and filtrated. Phenolic extracts (free, bound) were prepared from defatted samples using 80% methanol; after centrifugation, the supernatant was used for the free phenolics determination. Next, the bound phenolics were released from cereal residua by alkaline hydrolysis. After sonification, the samples were extracted by diethylether and extracts were reconstituted in 60% methanol.

2.3. Determination of Phenolics and DPPH Reducing Capacity

Briefly: Fiolin–Ciocalteu's reagent was used for the determination of free and bound phenolics. The absorbance of supernatants was measured at 765 nm by the spectrophotometer UV-1800 (SHIMADZU, Kyoto, Japan). Free and bound phenolics were expressed as ferulic acid equivalent (FAE) per gram of dry sample. The reducing capacity (radical scavenging activity) was quantified against 2,2-diphenyl-1-picrylhydrazil hydrate (DPPH) radical, and the absorbance was measured at 515 nm by the spectrophotometer UV-1800. The reducing capacity was calculated as follows: % DPPH = $[(A_B - A_C)/A_B] \times 100$. A_B was the absorption of the blank sample (t = 0 min), A_C = the absorption of the tested sample (t = 30 min), and the control was methanol.

2.4. Phenolic Acid (PA) Quantification

The quantitative analysis of sinapic, syringic, salicylic, ferulic, caffeic, p-hydroxybenzoic and p-coumaric acids in free and bound forms was performed using HPLC/MS/MS system (AGILENT 1260, Santa Clara, CA, USA) with a DAD detector, Triple Quadrupole 6410 MS/MS detector as also reported in Lacko-Bartošová et al. [25]. The concentrations of individual phenolic acids in wholegrain meals were calculated in µg/g of dry matter. All analyses were performed in four analytical replicates.

2.5. Statistical Analysis

The statistical analysis of all analytical data was performed by the STATISTICA software version 14.0 (TIBCO Software Inc. Tulsa, OK, USA). After an analysis of variance (ANOVA) and the determination of differences between the experimental factors by F-test, the significance of the mean differences was assessed using Fisher's least significant difference test (LSD). The analytical data are presented as the means and standard deviations. Pearson's correlation coefficients were used to evaluate the relationships between the parameters under study.

3. Results and Discussion

3.1. DPPH Reducing Capacity

In the DPPH radical scavenging approach, a component with considerable antioxidant activity efficiently traps the radical, preventing its growth and subsequent chain reaction [26]. The capacity of reducing DPPH is determined by the decrease in absorbance which is caused by antioxidants. The present study indicated that all tested cultivars and breeding line of spelt and common wheat demonstrated that their radical scavenging activity had significant variations (Figure 1). There were inconsequential differences observed in the antioxidant properties between cultivars and the breeding line of spelt and common wheat. Significantly no differences were observed in both total and free DPPH radical scavenging activity of common wheat Laudis (20.58% of free and 51.72% of total) compared to Altgold, Ebners Rotkorn, Ostro and PN-1-36. These spelt cultivars and common wheat achieved the highest values, whereas Oberkulmer Rotkorn, Rubiota and Frackenkorn accounted for the lowest values among all. The DPPH scavenging activity of bound phenolic extracts was higher compared to free ones, ranging from 31.14% (common wheat) to 25.62% (Franckenkorn). The ANOVA results did not strictly differentiate the bound radical scavenging activity of *Triticum aestivum* (31.14%) with the cultivars Altgold, Ebners Rotkorn, Ostro, Rubiota and PN-1-36. Hence, these cultivars showed their ability to achieve the level of common wheat Laudis in scavenging the DPPH radicals. The lowest inhibition was found for cultivar Franckenkorn (25.62%). According to Abdel-Aal and Rabalski [27], a higher DPPH scavenging capacity and total phenolic concentrations were found in spelt compared to common wheat, while other studies reported no significant differences in antioxidant/phytochemical levels between these species [28]. Wheat antioxidants' ability to scavenge DPPH may vary based on the quantity of specific bioactive components in extracts and their synergistic effects. The findings by Sandhu et al. [29] reported the range of DPPH radical scavenging activity from 13% to 22% in wheat. In our experiment, a significant effect of the growing year was found for all free, bound and total DPPH scavenging activity (Figure 2), with the highest level in 2015 and 2017 (54.03%, 50.03% of total DPPH). Despite relatively significant genetic weighting, climatic parameters (rainfall and temperature) also impact the concentration of antioxidant compounds [30]. Some researchers reported an increase in antioxidant synthesis in cereals when grown under water stress and at higher average temperatures during the grain-filling stage [31]. We can confirm these findings for spelt and common wheat cultivars as, in 2016, the significantly lowest DPPH values were determined. The grain filling period in 2016 was characterized by the highest precipitations (from June until harvest, with 92.0 mm of rainfall) and the most favorable weather conditions compared to 2015 and 2017 harvest years, which can be characterized as dry and very warm (Table S1). The influence of harvest year on the total variation was high and accounted for 74.4% for the total DPPH, 75.7% for bound and 66.5% for free DPPH. According to Lu et al. [32], these environmental factors had a greater and more significant impact on the health-beneficial components and antioxidant characteristics of selected cereals. As a result, oxidative stress promotes the production of phenolic compounds, which function as phytoalexins and protect cell wall integrity [33].

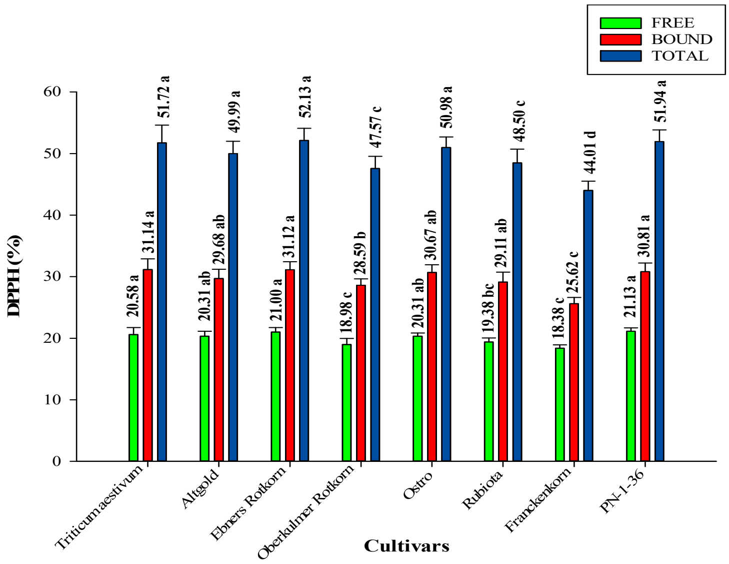

Figure 1. Free, bound and total DPPH (%). Values are the means for four replicates and three years of experiments. Values followed by the same letters (among free, bound, total DPPH separately) are not significantly different at $p < 0.05$.

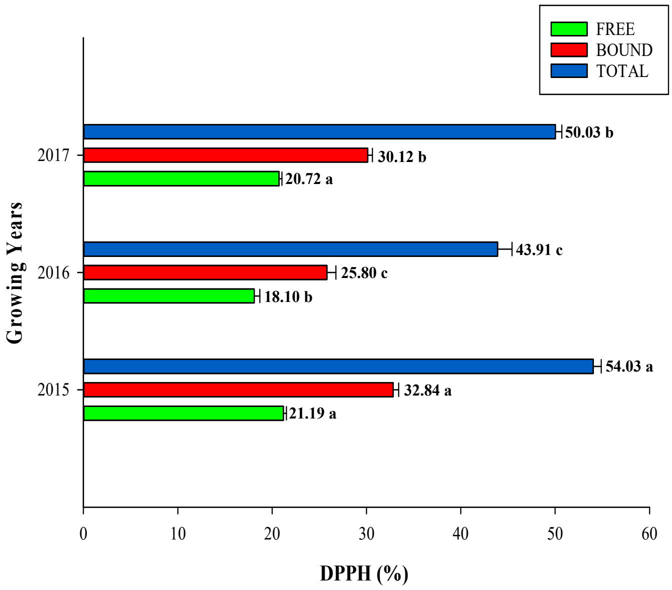

Figure 2. Free, bound and total DPPH (%). Values are the means for four replicates, three years of experiments and all cultivars. Values followed by the same letters (among free, bound, total DPPH separately) are not significantly different at $p < 0.05$.

3.2. Total Phenolic Concentration (TPC)

Phenolic compounds are secondary metabolites which are synthesized by plants during their growth and in reaction to stress conditions. They are efficient oxygen radical scavengers because they have a lower electron reduction potential than oxygen radicals. Thea analysis of variance indicated that the concentrations of free, bound and total phenolic compounds were significantly affected by all tested cultivars and the breeding line of spelt and common wheat. The highest concentration of total phenolic compounds was determined in common wheat Laudis (1902.55 µg FAE g^{-1} DM), while all spelt cultivars achieved lower values ranging from 1434.94 µg FAE g^{-1} DM (Franckenkorn) to 1650.22 µg FAE g^{-1} DM (Ebners Rotkorn) (Figure 3). Nevertheless, the differences between spelt cultivars and breeding lines were significant. Among seven spelt cultivars, the highest concentration of phenolic compounds was detected for Ebners Rotkorn and PN-1-36, while the lowest was achieved for Oberkulmer Rotkorn, Rubiota and Franckenkorn (1434.94–1478.82 μg FAE g^{-1} DM). Results indicated that common wheat Laudis (271.35 μg FAE g^{-1} DM) had the highest free phenolic concentration. Across spelt cultivars and breeding lines, ANOVA outputs showed no substantial differentiation in the free and bound phenolic concentration of Ebners Rotkorn, Ostro and PN-1-36, with the highest values compared to other spelt cultivars. Oberkulmer Rotkorn, Rubiota and Franckenkorn had the lowest concentrations of free and bound phenolics. The contribution of free phenolics to the total ranged from 10.9% in Franckenkorn up to 15.6% in common wheat Laudis. Bound phenolic compounds represented 86.3% of the total phenols, and the content of bound phenolics was found to be higher in all cultivars than the content of free ones. The highest concentration was found in common wheat (1631.20 μg FAE g^{-1} DM), whereas all spelt cultivars had significantly lower values ranging from 1244.08 μg FAE g^{-1} DM in Franckenkorn to 1426.50 μg FAE g^{-1} DM in Ebners Rotkorn. Ebners Rotkorn was the dominating one out of all spelt cultivars. Differences in the content and distribution of phenolics can be obtained by the breeding process; in our study, the advanced breeding line PN-1-36 achieved one of the highest levels of DPPH and free, bound and total phenolics among spelt cultivars. Inter-genotypic variability in spelt cultivars and the breeding line of the total phenolics was 13.1%. A higher value was found in wheat cultivars (18.5%) by Ma et al. [34]. Zrckova et al. [35] also determined a higher concentration of total phenolics in common wheat (702.15 mg GAE g^{-1} DM) compared to spelt (694.99 mg GAE g^{-1} DM). On the contrary, according to Wang et al. [22], spelt flour had a significantly higher antioxidant activity and phenolic concentrations than common wheat and the differences were greater in wholegrain than in white flour. In our study, a significant, positive but weak correlation was found between the total DPPH and total phenolics (r = 0.414), as well as between the bound DPPH and bound phenolics (r = 0.367) and free DPPH and free phenolic concentration (r = 0.373). Contrary to our results, other researchers have observed positive and strong relationship between the total phenolic content and antioxidant activity [36,37]. However, the weak correlation in our study may indicate that other factors, such as the type, structure and distribution of phenolic compounds, as well as the extraction and measurement methods, may influence the antioxidant activity of the samples. Further research is needed to elucidate the mechanisms and interactions between phenolic compounds and DPPH radicals in spelt cultivars. Research data on antioxidant compounds in wheat species grown under organic cropping systems are still limited, even though this knowledge is vital for both breeding and organic farming. In our experiment, the significant impact of weather conditions on the total phenolic concentration values was determined. The highest levels of free, bound and total phenolic concentrations were found in 2017, followed by 2016 and 2015 (Figure 4). During 2015, the ripening period was extremely dry (only 17.2 mm of rainfall was measured) and very warm, which negatively influenced the synthesis of all phenolics. In spite of the significantly lowest concentrations of phenolics in 2015, in this year, the DPPH scavenging capacity was high, at the level of 2017. These outputs are partially compatible with the findings of Lachman et al. [38], where substantial variances were observed between two growing years of emmer, einkorn and bread wheat cultivars due to less rainfall and higher temperatures during the cereal ripening stages. Similarly, Stracke et al. [39] found significant differences in the TPC of the wheat grain in function of the weather conditions, Hernández et al. [40] reported the effect of genotype. Others however, have discovered that environmental factors are more crucial [41]. Factors influencing the concentration of phenolic compounds in wheat species may have potential in food manufacturing process, since they delay food degradation caused by oxidation [42]. The identification of the majority of phenolic compounds in cereals (particularly ancient species) offers novel possibilities for the breeding and commercialization of value-added cultivars high in beneficial components, as well as their use in functional food. In the present study, the significant impact of interaction cultivars × growing year (C × Y) indicated that cultivars responded differently to environmental conditions and were better suited to specific environments. Shamanin et al. [43] reported the potential effect of the genotype on the antioxidant capacity of wheat and indicated that the breeding process may bring significant variations in the phenolic concentration.

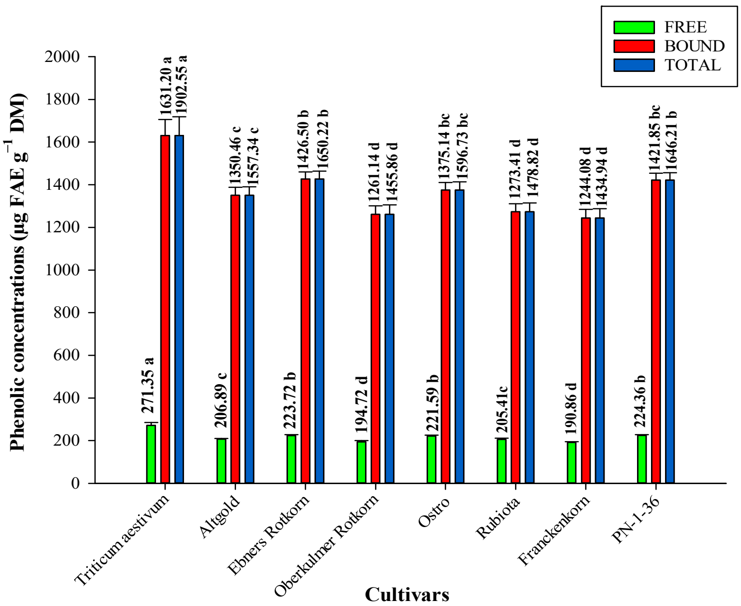

Figure 3. Free, bound and total phenolic concentrations. Values are the means for four replicates and three years of experiments. Values followed by the same letters (among free, bound and total phenolic concentrations separately) are not significantly different at $p < 0.05$.

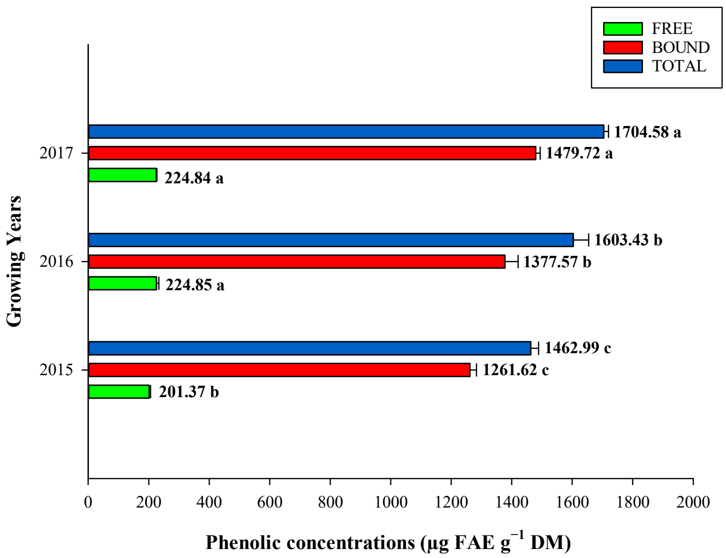

Figure 4. Free, bound and total phenolic concentrations. Values are the means for four replicates, three years of experiments and all cultivars. Values followed by the same letters (among free, bound and total phenolic concentrations separately) are not significantly different at $p < 0.05$.

3.3. Total Phenolic Acids (TPAs)

Statistical analyses showed that the concentrations of free, bound and total (as the sum of free and bound forms) phenolic acids were significantly affected by all tested cultivars and breeding lines of spelt and common wheat ($p < 0.001$), growing years and their interaction. The highest concentration of total phenolic acids was observed in Ebners Rotkorn (681.75 µg g^{-1} DM), followed by Altgold and the lowest in Ostro (450.05 µg g^{-1} DM) across all spelt cultivars (Figure 5). In comparison to these cultivars, the significant influence was less evident on the TPAs of Rubiota, Franckenkorn and PN-1-36. The total phenolic acid concentration of common wheat Laudis (396.05 µg g^{-1} DM) achieved the lowest value. The quantities of free PAs varied between 29.50 and 52.86 µg g^{-1} DM in the analyzed samples. Results showed that, statistically, the least variations were observed in the free PA concentrations in common wheat Laudis (29.50 µg g^{-1} DM) and Ostro (28.90 µg g^{-1} DM), both of which attained the lowest values among all cultivars and breeding lines. However, no statistical variations were found in the free PAs of Altgold, Franckenkorn and PN-1-36. The highest concentration was determined for Ebners Rotkorn. The overall contribution of free PAs on the total PAs was 5.7%.

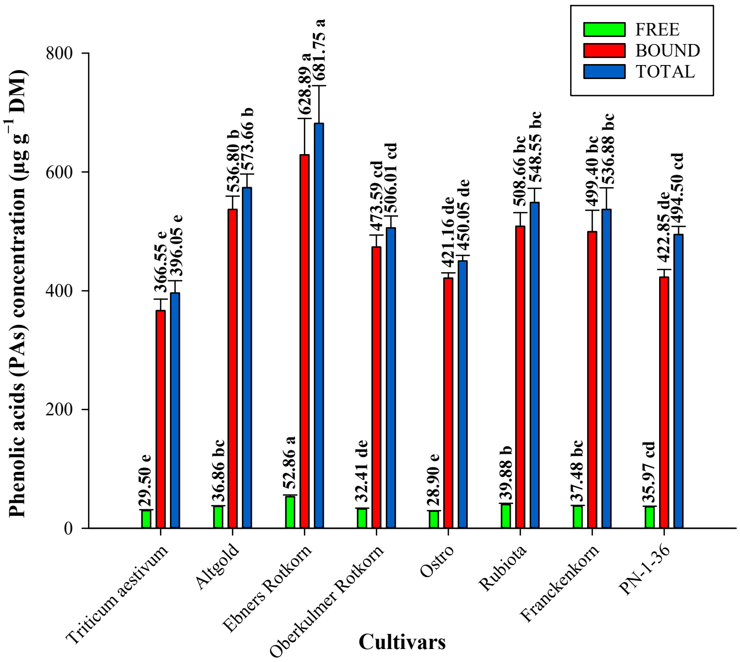

Figure 5. Concentrations of free, bound and total phenolic acids. Values are the means for four replicates and three years of experiments. Values followed by the same letters (among free, bound and total PAs separately) are not significantly different at $p < 0.05$.

The bound PAs were also found highest for spelt Ebners Rotkorn (628.89 µg g^{-1} DM) followed by Altgold (536.80 µg g^{-1} DM) and lowest for common wheat (366.55 µg g^{-1} DM) with high significant differences between the tested samples. There were no substantial variances observed between the bound PAs of Rubiota, Frackenkorn and Oberculmer Rotkorn. The average share of bound PAs on the total concentration of PAs was high and represented 74.8%. Similar findings were reported in the studies of other researchers [44], where bound forms of PAs accounted for a large portion of all PAs, whereas free PAs contributed up to 10% of total PAs. According to Li et al. [45], the amounts of phenolic acids in the various phenolic acid fractions showed a trend where bound phenolic acids accounted for around 77% of the total phenolic acid concentration, whereas free PAs made up between 0.5 and 1%. In our study, Ebners Rotkorn had the highest free, bound and total phenolic acid concentrations across all spelt cultivars. Our study showed that the effect of the growing year on free, bound and total PAs differed, with the highest content in 2017 (Figure 6). In 2015, when extremely dry and very warm weather occurred during grain filling stage, the lowest concentration of free PAs was recorded. The most favorable meteorological conditions during 2016 resulted in the lowest concentration of bound and total PAs. There was a significant effect of interaction between the cultivars and growing year (C × Y), which indicated the differential responses of cultivars to climatic conditions. The impact of the growing year on the phenolic acid concentrations has still been insufficiently studied and requires more attention.

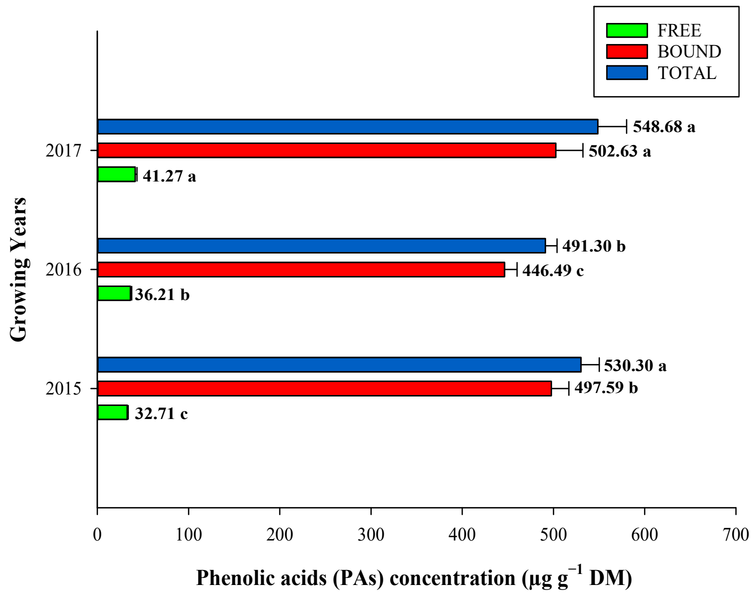

Figure 6. Concentrations of free, bound and total phenolic acids. Values are the means for four replicates, three years of experiments and all cultivars. Values followed by the same letters (among free, bound and total PAs separately) are not significantly different at $p < 0.05$.

3.4. Individual Phenolic Acids

In this study, individual phenolic acid concentrations were investigated for free and bound extracts. The tested phenolic acids were ferulic, p-hydroxybenzoic (p-HBA), p-coumaric, syringic, sinapic, salicylic and caffeic. Statistical analyses showed that the concentrations of individual free and bound phenolic acids were significantly affected by all tested spelt cultivars, breeding lines and common wheat ($p < 0.001$). Table 1 indicates the free PA composition of the wheat samples; the patterns of free PAs differed, depending on the cultivars and breeding line. It was expected that ferulic acid (70.48%) was the principal and predominant phenolic acid of free PAs, ranging from 19.85 µg g^{-1} DM (Ostro) to 37.58 µg g^{-1} DM (Ebners Rotkorn) among all spelt cultivars. In common wheat Laudis, the value 19.87 µg g^{-1} DM was quantified, which was also one of the lowest of all samples. No significant variations were observed in the free individual phenolic acids of Rubiota and Frankenkorn except salicylic acid, which was the highest for Rubiota. The second dominating PAs was syringic acid, with an average 9.30% share of total free PAs, followed by p-HBA (5.59%), sinapic acid (5.40%), salicylic (4.18%) and p-coumaric acid (3.22%), while the lowest share was found for caffeic acid (1.93%). The significantly lowest concentrations of free ferulic acid (19.87 µg g^{-1} DM), p-HBA (1.53 µg g^{-1} DM) and salicylic acid (1.06 µg g^{-1} DM) was determined in common wheat Laudis. Spelt cultivar Ebners Rotkorn was characterized by the substantially highest concentration of ferulic acid (37.58 µg g^{-1} DM), p-HBA (3.15 µg g^{-1} DM), syringic (5.34 µg g^{-1} DM) and sinapic acid (2.73 µg g^{-1} DM). The pattern of p-coumaric acid was different, and equal levels were measured for five cultivars (Altgold, Ebners Rotkorn, Rubiota, Franckenkorn, PN-1-36) out of eight. The ideal bioavailability of free ferulic acid in cereals was determined by Anson et al. [46]. It was mostly absorbed through the small intestine; hence, free extractable polyphenols are very significant for phenolic antioxidant consumption. According to our findings, the considerably greater levels of free phenolic acids in all spelt cultivars compared to common wheat could provide health benefits in the human diet. There is a lack of evidence on the occurrence of free phenolic acids in ancient wheat, and an additional in-depth study using a range of ancient and current wheat species and cultivars has been encouraged by Shewry and Hey [28]. The growing season had considerable influence on all individual free PAs. The significantly highest concentrations of all PAs were noted in 2017, when the maturation period was characterized by dry May and June, but rainfall occurrence was not limited.

The unusually dry and warm ripening stage in 2015, resulted in the significantly lowest concentration of free ferulic and sinapic acids; p-HBA, syringic and salicylic acid concentrations were at the level of 2016. It can be concluded that differentiated weather conditions during three years of experiments supported the synthesis of particular phenolic acids and affected their total concentrations in wholegrain flour. There was also a significant effect of interactions between all cultivars and growing years (C × Y) at $p < 0.001$. The effect of cultivars, growing years and interaction cultivars x growing years can be further clarified by ANOVA F-values, the higher F-value and the greater the influence of the experimental factor. In almost in all free PA concentrations, the effect of the growing year was greater compared to cultivars and interactions (C × Y), except free p-HBA and caffeic acids, where the effect of cultivars predominated the effect of growing years (Table 2).

Table 1. Free individual PA concentrations (µg g^{-1} DM) in wholegrain meal.

Species, Cultivars, Breeding Line	Ferulic Acid	p-HBA [1]	p-Coumaric Acid	Syringic Acid	Sinapic Acid	Salicylic Acid	Caffeic Acid
T. aestivum, Laudis	19.87 ± 4.25 e	1.53 ± 0.29 cd	1.05 ± 0.21 b	3.30 ± 0.58 bcd	2.08 ± 0.37 c	1.06 ± 0.22 e	0.61 ± 0.15 d
T. spelta							
Altgold	25.09 ± 0.86 cd	2.07 ± 0.16 b	1.29 ± 0.04 a	3.82 ± 0.34 b	2.48 ± 0.13 b	1.29 ± 0.09 cd	0.83 ± 0.03 c
Ebners Rotkorn	37.58 ± 2.58 a	3.15 ± 0.21 a	1.26 ± 0.20 a	5.34 ± 0.58 a	2.73 ± 0.50 a	1.84 ± 0.15 b	0.95 ± 0.10 b
Oberkulmer Rotkorn	23.35 ± 0.88 d	1.80 ± 0.12 c	0.90 ± 0.10 c	3.21 ± 0.37 cde	1.39 ± 0.27 f	1.23 ± 0.06 de	0.53 ± 0.08 ef
Ostro	19.85 ± 0.54 e	1.46 ± 0.07 d	0.89 ± 0.07 c	3.51 ± 0.20 bc	1.46 ± 0.19 ef	1.16 ± 0.03 de	0.56 ± 0.03 de
Rubiota	29.13 ± 1.29 b	2.14 ± 0.18 b	1.32 ± 0.09 a	2.72 ± 0.59 def	1.81 ± 0.39 d	2.22 ± 0.13 a	0.55 ± 0.10 def
Franckenkorn	27.50 ± 0.81 bc	2.22 ± 0.15 b	1.39 ± 0.10 a	2.48 ± 0.49 f	1.68 ± 0.30 de	1.74 ± 0.06 b	0.48 ± 0.8 f
PN-1-36	24.76 ± 0.79 cd	2.08 ± 0.06 b	1.39 ± 0.05 a	2.97 ± 0.08 def	2.24 ± 0.70 c	1.45 ± 0.06 c	1.19 ± 0.13 a
p cultivars (C)	***	***	***	***	***	***	***
2015	23.32 ± 0.78 c	2.02 ± 0.14 b	1.09 ± 0.06 b	2.88 ± 0.24 b	1.24 ± 0.12 c	1.48 ± 0.10 b	0.66 ± 0.08 b
2016	26.83 ± 1.21 b	1.92 ± 0.08 b	0.99 ± 0.04 c	2.73 ± 0.21 b	1.73 ± 0.11 b	1.41 ± 0.75 b	0.58 ± 0.03 c
2017	27.51 ± 1.45 a	2.22 ± 0.11 a	1.46 ± 0.06 a	4.64 ± 0.24 a	2.98 ± 0.16 a	1.58 ± 0.07 a	0.89 ± 0.04 a
p year (Y)	***	***	***	***	***	***	***
p C × Y	***	***	***	***	***	***	***

[1] *p*-hydroxybenzoic acid. All values are the means ± standard deviations (SD) for four replicates and three years of experiments. Values in the columns (among cultivars and years) followed by the same letter are not significantly different at $p < 0.05$. F-test from ANOVA significant at *** $p < 0.001$.

Table 2. ANOVA F-values indicating the influence of experimental factors on free and bound phenolic acids.

	Ferulic Acid	p-HBA [1]	p-Coumaric Acid	Syringic Acid	Sinapic Acid	Salicylic Acid	Caffeic Acid
Free PAs							
Cultivar (C)	45.7	82.9	67.9	39.5	95.3	89.3	368.0
Growing year (Y)	59.2	12.5	229.9	290.8	605.2	160.9	234.6
C × Y	8.1	25.8	33.7	38.4	80.5	25.2	104.6
Bound PAs							
Cultivar (C)	27.8	61.8	65.0	19.9	110.9	40.2	78.9
Growing year (Y)	69.4	121.7	114.9	67.2	711.3	229.7	311.6
C × Y	17.9	31.4	62.2	23.3	134.5	43.1	62.2

[1] *p*-hydroxybenzoic acid.

In our study, PAs in bound forms represented the majority of all PAs in the cereals. The results revealed that, when all cultivars and breeding lines were analyzed together, the bound ferulic acid accounted for 95.40% of the total bound PAs, followed by p-coumaric acid (2.71%), p-HBA (0.72%), syringic acid (0.71%) and caffeic (0.53%), whereas sinapic (0.41%) and salicylic acid (0.42%) had the lowest concentrations (Table 3). Spelt cultivar Ebners Rotkorn was distinguished from all the others by the highest concentration of all individual bound PAs except salicylic. Common wheat Laudis had the substantially lowest bound PA concentrations of ferulic (345.17 µg g^{-1} DM), salicylic (1.44 µg g^{-1} DM) and p-HBA (3.33 µg g^{-1} DM) acids, while the remaining individual bound phenolic acids of Laudis also achieved one of the lowest values. All the other spelt cultivars were lower in the quantity of all the analyzed individual-bound phenolic acids than in Ebners Rotkorn. Statistical analyses revealed that there were no strict differences observed in the concentrations of bound ferulic acid, p-HBA and p-coumaric acid of Altgold, Rubiota and Frackenkorn. The bound phenolic acids are closely associated with the seed's fibrous components, such as lignins, cellulose, arabinoxylans and other indigestible polysaccharides, which restrict their release in the small intestine [47]. The concentrations of all bound individual phenolic acids were found to be significantly affected by the growing year; moreover, highly significant interactions among cultivars (breeding lines) and growing years were determined for all bound PAs. The highest concentrations for all bound phenolic acids were observed in 2017 (except for salicylic acid) which was also found for free individual phenolic acids. The concentrations of the analyzed bound PAs showed significant differences in 2015 and 2016; however, the effect of dry and warm weather in 2015 was different compared to free PAs, when the negative impact on the concentrations of p-HBA, p-coumaric, sinapic, salicylic and caffeic acids was evident. The content of ferulic and syringic bound PAs was at the level of 2017. The maturation period of 2016 was not favorable for bound ferulic and syringic acids. According to the ANOVA F-values, the impact of the growing year on the concentration of all bound individual PAs was higher compared to cultivars and the C × Y interaction; therefore, the environmental factor's effect was greater than the selection of cultivars. The effect of environmental factors (rainfall, air temperature) was investigated in several studies, but the results were inconsistent. There was a negative correlation found between the high temperature and total phenolic concentrations [48], while other researchers, e.g., Alexieva et al. [49], observed that soluble phenols in wheat were directly proportional to drought and ultraviolet stressors. Depending on the crop species, climate, meteorological factors, agricultural systems and agri-technological methods like crop rotation and tillage, as well as genotypes, the metabolic pathways may have a variable impact on the concentration of secondary metabolites [35].

Table 3. Bound individual PA concentrations (μg g^{-1} DM) in wholegrain meal.

Species, Cultivars, Breeding Line	Ferulic Acid	p-HBA [1]	p-Coumaric Acid	Syringic Acid	Sinapic Acid	Salicylic Acid	Caffeic Acid
T. aestivum, Laudis	345.17 ± 63.4 e	3.33 ± 0.40 c	9.36 ± 2.39 c	3.10 ± 0.72 c	2.01 ± 0.33 b	1.44 ± 0.20 d	2.14 ± 0.47 cd
T. spelta							
Altgold	506.63 ± 22.45 b	3.59 ± 0.37 bc	15.90 ± 0.64 a	3.36 ± 0.12 c	2.54 ± 0.10 a	1.51 ± 0.03 cd	3.28 ± 0.16 b
Ebners Rotkorn	594.62 ± 57.35 a	4.73 ± 0.67 a	16.23 ± 2.53 a	4.62 ± 0.58 a	2.57 ± 0.55 a	2.38 ± 0.13 b	3.73 ± 0.37 a
Oberkulmer Rotkorn	452.44 ± 20.19 bcd	2.76 ± 0.45 d	10.30 ± 1.23 c	2.93 ± 0.34 cd	1.39 ± 0.26 c	1.73 ± 0.20 c	2.05 ± 0.33 cd
Ostro	402.29 ± 9.39 de	2.55 ± 0.37 d	7.44 ± 0.84 d	3.55 ± 0.49 bc	1.44 ± 0.14 c	1.58 ± 0.13 cd	2.30 ± 0.25 c
Rubiota	478.38 ± 22.33 bc	3.46 ± 0.49 bc	15.70 ± 0.53 a	4.12 ± 0.37 ab	1.83 ± 0.37 b	2.89 ± 0.14 a	2.28 ± 0.29 c
Franckenkorn	469.99 ± 35.68 bc	3.81 ± 0.27 bc	17.06 ± 1.00 a	2.32 ± 0.37 d	1.53 ± 0.19 c	2.76 ± 0.30 a	1.94 ± 0.29 d
PN-1-36	430.89 ± 12.07 cd	3.86 ± 0.14 b	12.90 ± 0.44 b	3.44 ± 0.10 c	2.56 ± 0.07 a	1.73 ± 0.05 c	3.00 ± 0.08 b
p cultivars (C)	***	***	***	***	***	***	***
2015	475.49 ± 18.82 a	2.12 ± 0.19 c	11.45 ± 1.01 c	3.56 ± 0.25 a	1.47 ± 0.14 c	1.72 ± 0.11 c	1.76 ± 0.15 c
2016	428.41 ± 12.51 b	3.79 ± 0.07 b	13.21 ± 0.54 b	2.69 ± 0.21 c	1.65 ± 0.12 b	2.43 ± 0.15 a	2.89 ± 0.13 b
2017	476.24 ± 27.80 a	4.61 ± 0.25 a	14.67 ± 1.05 a	4.02 ± 0.20 a	2.82 ± 0.18 a	1.86 ± 0.09 b	3.19 ± 0.18 a
p year (Y)	***	***	***	***	***	***	***
p C × Y	***	***	***	***	***	***	***

[1] *p*-hydroxybenzoic acid. All values are means ± standard deviation (SD) for four replicates and three years of experiment. Values in columns (among cultivars and years) followed by the same letter are not significantly different at $p < 0.05$. The F-test from ANOVA was significant at *** $p < 0.001$.

Non-traditional wheat cultivars have usually been acknowledged as being healthier than modern wheat [50]. But, there are insufficient data to support the increased health advantages of ancient wheat, which are impacted by genetically determined changes in grain composition when compared to common wheat [51]. These changes may be more likely as a result of environmental factors as well as interactions between environmental variables and genotypes. Spelt wheat is distinguished by a high degree of environmental adaptability [52]. Furthermore, ancient species are produced using ecologically sustainable ways of production, and therefore these species may provide an alternative selection. Research knowledge may help to improve breeding attempts to generate cereal grains with high health benefits and nutritional qualities under organic agriculture [7]. In recent years, due to the superior nutritional content and quantity of ancestral genes, spelt has regained popularity among consumers, farmers and breeders [53].

4. Conclusions

The results showed that the investigated spelt cultivars and breeding lines had a strong antioxidant capacity and health advantages as an important source of phenolic acids in the human diet. We showed that phenolic contents, radical scavenging abilities and phenolic acids profiles in both free and bound forms are significantly affected by spelt cultivars and breeding line, growing years and their interactions. These compounds are highly responsive to cultivars and genotypic variations. The results of the experiment showed that the influence of the growing year was more pronounced on the concentration of free phenolic acids than on the bound ones. Meteorological parameters, i.e., rainfall with air temperature during the ripening period, were indirectly proportional to the phenolic acid concentrations. Proper and in-depth understanding and research of the variability sources of such significant bioactive components in spelt cultivars could provide crucial knowledge for further breeding programs to produce health-beneficial varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13102024/s1, Table S1: Meteorological data during ripening periods compared to long-term average data (LTA); Table S2: The set of analytical data used for the evaluation of the effects of species (spelt and common wheat), cultivars, breeding line and growing years on phenolic compounds, DPPH, individual phenolic acids in free, bound and total forms.

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