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Environmental and cultivar variability in composition, content and biological activity of phenolic acids and alkylresorcinols of winter wheat grains from a multi-site field trial across Europe

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ABSTRACT

Different factors such as the genotype, environmental conditions, temperature stress, solar radiation and others can influence the phytochemical status of plants. The concentration of phenolic acids and alkylresorciols (ARs) as well as their chemical composition and biological activity have been determined in twelve winter wheat cultivars grown at eight European locations. This was the first winter wheat multi-location field trial of the European Consortium for Open Field Experimentation (ECOFE). Extracts from grain were analyzed using a UPLC-PDA-ESI-MS system (phenolic acids), UPLC-PDA-MS/MS (alkylresorcinols) and TLC-DPPH$^{\bullet}$ test with ImageJ program (antiradical activity). The phenolic acid profile consisted of five hydroxybenzoic acid and four hydroxycinnamic acid derivatives, among which ferulic and sinapic acids were predominated. The ARs profile consisted of nine AR derivatives, among which 5-*n*-heneicosylresorcinol (C21:0) and 5-*n*-nonadecanylresorcinol (C19:0) were predominated. Our study showed significant differences in phenolic acids and AR content between wheat cultivars, as well as between locations. We observed a positive correlation between the biological activity of extracts and the total amount of phenolic acids and ARs. Two cultivars, Chambo and Julius (average of all sites) and samples from the Spanish site (average of all cultivars) showed the highest content and composition of nutritional substances.

1. Introduction

Ensuring the development of knowledge to generate enough high quality food for a growing world population with minimal environmental impact is one of the world's biggest global scientific and social issues. Long-term trends such as climate change, and the need for sustainable production of raw plant ingredients for manufacturing from plants, coupled with biological limits to crop productivity and limit resources, further increase this challenge. Fundamental to improving the understanding of cultivation and farming systems is an appropriate coordination of research facilities that allows an intensive study of the interactions among plant genotype, environment and farming, e.g. the study of plant cultivars in various farming practices and locations under

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Abbreviations

AR	alkylresorcinol (resorcinolic lipid)
C15:0	5-*n*-pentadecylresorcinol
C17:0	5-*n*-heptadecylresorcinol
C19:0	5-*n*-nonadecylresorcinol
C19:1	5-*n*-nonadecenylresorcinol
C21:0	5-*n*-heneicosylresorcinol
C21:1	5-*n*-heneicosenylresorcinol
C21:2	5-*n*-heneicosadienylresorcinol
C23:0	5-*n*-tricosylresorcinol
C25:0	5-*n*-pentacosylresorcinol
NaOH	sodium hydroxide
HCl	hydrochloric acid
TLC	thin-layer chromatography
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl radical
UPLC	ultra-performance liquid chromatography
PDA	photodiode array detector
MS	mass spectrometry
JPEG	joint photographic experts group
BBCH scale	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie scale
ANOVA	analysis of variance
LOQ	below limit of quantification
DM	dry matter
cv.	cultivar
loc.	location
ECOFE	European Consortium for Open Field Experimentation
SD	standard deviation
r	Pearson correlation coefficient

highly normalized conditions. The current organization of agricultural research with historically separate experimental farms for each institution do not meet the framework to study regional effects on growth and development of crops. The research presented here was conducted within the framework of the European Consortium for Open Field Experimentation (ECOFE) and involved renowned research institutions from several countries, stretching from the Mediterranean to Scandinavia and from Ireland to the eastern border of the European Union. This research network caters for the needs of 21st century crop science and has provided European scientists with access to an outstanding collaborative platform while providing them with a competitive advantage (Stützel et al., 2016). This collaboration has made it possible to select the most appropriate locations for particular experiments, and has also given European scientists a globally unique opportunity to systematically address key problems such as climate change mitigation and biological limitations to crop yields in a variety of environments.

Wheat genotype, environment, and possible interactions between genotype and environment are known to strongly influence antioxidant levels in grain. It has been described that factors such as genotype (Shewry et al., 2010), location, environmental conditions (Fernandez-Orozco et al., 2010) such as temperature stress (Moore et al., 2006), and solar radiation can strongly influence the contents of plants in relation to secondary molecules. Mpofu et al. (2006) reported that effects of environment had a stronger impact on phenolic compound concentrations than genotype, and that genotype × environment interactions had a significantly lesser effect on all parameters assessed than both main factors. Fernandez-Orozco et al. (2010) also found that environmental factors were more significant predictors of phenolic levels than genotypic diversity. Shewry et al. (2010) confirmed previous studies and showed that heritable variation in the amount of bioactive ingredients in wheat grains can be used by breeders to develop new cultivars with higher health value.

In Europe, wheat is the most important cereal species used for human consumption. Current estimates indicate that by 2050 crop yields of most key food crops (wheat, rice and barley) will have to be increased by at least 50% to maintain food supply. Plant natural products have great potential as nutrients and pharmaceuticals, which is still mostly unexplored. Wheat is considered to be an important source of polyphenols which are plant secondary metabolites with numerous health-promoting effects. The most important group of phytochemicals in wheat grains are phenolics, mainly phenolic acids. They are common dietary phytochemicals and have several different functions in plants (Ma et al., 2021). These compounds are used as biopesticides, bioherbicides, drugs and additionally have been recognized as important ingredients for human and animal health. Many of them exhibit biological activities such as anti-cancer and anti-inflammatory properties as well as high antioxidant activity. The predominant phenolic acid in wheat grain is ferulic acid, accounting for 85.3–89.3% of the total phenolic acids (Żuchowski et al., 2011; Abotaleb et al., 2020). Vanillic, sinapic, *p*-coumaric and *p*-hydroxybenzoic acids were identified in wheat grains, too. It has also been shown that organically produced spring and winter wheat cultivars had significantly higher concentrations of total phenolic acid content than conventionally grown wheat (Żuchowski et al., 2011).

Other, very important components of grains are alkylresorcinols (ARs, phenolic lipids), which are of attracting considerable interest as an important bioactive component of food, a potential biomarker of whole grain product consumption and play an important role in prevention and inhibition of human cancer as has been reported in observational and *in vitro* studies (Ma et al., 2021). The major ARs homologs in wheat grains are 5-*n*-heneicosylresorcinol and 5-*n*-nonadecylresorcinol. The levels of other ARs e.g. 5-*n*-heptadecylresorcinol, 5-*n*-tricosylresorcinol and 5-*n*-pentacosylresorcinol are considerably smaller (Zarnowski et al., 2004; Kowalska and Jędrejek, 2020). Significant variation was found in the ARs content of various wheat samples. In spring and winter wheat the content of ARs range from 471 to 995 μg/g (mean 680 μg/g) and 203–1272 μg/g (mean 796 μg/g), respectively (Kowalska and Jędrejek, 2020). According to Ross et al., 2003, the quantity of ARs in wheat flour were reported to be typically in the range of 300–700 μg/g. Andersson et al. (2010) showed that the average value of ARs for ten durum wheat cultivars was 399 mg/g DM, while for five spelt cultivars it was 605 mg/g DM.

The study was carried out to verify the hypothesis of how genetic and environmental factors (different European locations) can cause changes in the content of natural products. The aim of the study was to demonstrate differences in accumulation of selected bioactive compounds (phenolic acids and ARs) in grain between 12 winter wheat cultivars grown in different locations. Another objective was to investigate the antioxidant potential of phenolic acids and ARs and to correlate it with the total content of these compounds in wheat grain. There is a lack of information about composition, concentrations and biological activity of phenolic acids and ARs in different *Triticum aestivum* L. cultivars, cultivated across the main agro-climate zones. With this information, it will be possible to target breeding or engineering of plants with increased concentrations of these compounds in combination with higher yielding cultivars. The most desirable cultivars would be those containing high levels of bioactive components but with no interaction with the environment (weather conditions).

2. Materials and methods

2.1. Plant material

The research material consisted of 12 winter wheat cultivars (KWS Lilli, KWS Siskin, RGT Reform, Sobervio, Henrik, Hondia, JB Diego,

Julius, Benchmark, Bologna by SIS, Chambo, CH-Nara), with one cultivar chosen per institute as representative of locally grown wheat cultivars. Field experiments have been organized in eight different sites (Belgium, Germany-Hannover, Germany-Hohenheim, Ireland, Poland, Spain, Sweden, United Kingdom), to optimize environmental impact. More specific information about the agricultural practices (details of location, soil type and pH, date of sowing and harvest, fertilization, plant protection) are shown in Table S1. All locations were autumn sown and harvested at maturity. Seed was common to all locations, e.g. we distributed seed to each location from a common pool. Each location determined their own fertilizer and agrochemical inputs, according to needs and local practice. A split-plot experimental design. Wheat grains were harvested in 2018, at fully ripe stage (BBCH 89). After harvesting, the grains were milled using an Ultra Centrifugal Mill ZM 200 (Retsch, Germany). After milling the grain, whole-grain flour was obtained with a middling granulation of 0.8 mm and then used for chromatographic analysis. The plant material has been deposited at the Institute of Soil Science and Plant Cultivation-State Research Institute in Pulawy, Poland.

2.2. Chemicals

Methanol and acetonitrile hypergrade (>98%, MS-grade) were of the analytical purity grade, purchased from J.T. Baker (Deventer, Netherlands). Formic acid (LC-MS grade), 4-dodecylresorcinol, 5-*n*-pentadecylresorcinol and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) were supplied by Sigma-Aldrich, (St. Louis, MO, USA). Aluminium-backed silica gel 60 F254 plates and 2-propanol were purchased from Merck (Darmstadt, Germany). Acetone, *n*-hexane and ethyl acetate were obtained from Acros Organics BVBA (Belgium). Other reagents (sodium hydroxide, ascorbic acid, *m*-hydroxybenzoic acid, hydrochloric acid) were analytical grade, and were provided by commercial suppliers, including POCH S.A. (Gliwice, Poland) and Chempur (Poland). Water Milli-Q was obtained using a purification system (Millipore Corp., Molsheim, France).

2.3. Chemical analyses

2.3.1. Phenolic acids

2.3.1.1. Extraction of phenolic acids from wheat grains. For the extraction and hydrolysis of the ester-linked phenolic acids, the method applied previously by Żuchowski and coauthors was used (Żuchowski et al., 2011). Briefly, samples of milled wheat grains were defatted in Soxhlet apparatus with hexane. Four-hour long hydrolysis of 100 mg in 6 mL of 2 M NaOH under air condition, containing 1% of ascorbic acid as an antioxidant and 4 μg m-hydroxybenzoic acid as an internal standard, were performed. After hydrolysis, samples were acidified with 6 M HCl solution to a pH of approximately 2. Hydrolysates were centrifuged with 6500×*g* for 20 min. Supernatants were transferred to 15 mL falcon tubes and three times extracted with ethyl acetate. The extracts were evaporated to dryness and dissolved in 4 mL of 25% methanol.

2.3.1.2. Determination of phenolic acids. The phenolic acids in wheat flours were analyzed by Waters ACQUITY UPLC Chromatograph (Waters Corp., Milford, MA, USA), equipped with a PDA and a triple-quadrupole mass detector (Waters Corp.). Samples (50 mg/mL) were separated on a Waters ACQUITY UPLC® HSS C18 column (100 × 2.1 mm, 1.8 μm) at 30 °C. The mobile phase consisted of solvent A (0.1% formic acid in water Milli-Q) and solvent B (acetonitrile with 0.1% formic acid). Analytes were eluted using a combination of isocratic and gradient steps. The elution (0.50 mL/min) was carried out with a gradient of solvent B: 0.00–0.50 min, 8% B; 0.50–8.00 min, 8–20% B; 8.00–8.10 min, 20–95% B; 8.10–10.00 min, 95% B; 10.00–10.10 min, 95–8% B; 10.10–12.00 min, 8% B. The sample injection volume was 2.5 μL (full loop mode). The detection of phenolic acids was performed in the negative ionization mode, using a selected reaction monitoring method. The condition of MS analysis was published by Czaban et al. (2013). Concentrations of phenolic acids (μg/g of the grain) in wheat extracts were calculated on the basis of calibration curves (Table 1).

2.3.1.3. Antiradical activity of phenolic acids. TLC-DPPH$^{\bullet}$ test combined with Image Processing. The antiradical activity of wheat extracts was assessed by means of a TLC-DPPH$^{\bullet}$ test with the ImageJ software. This method, with modifications, has been found suitable for the analysis of complex samples, as shown in our previous publication (Rolnik et al., 2020). The 50 μL of the analyzed samples (50 mg/mL) and 2 μL of the standard compound (caffeic acid, 1 mg/mL) were applied on silica gel (60 F254, Merck) chromatographic plates. Next, the plates were developed in vertical chambers pre-saturated for 15 min with the optimized mobile phase consisting of acetonitrile: water: chloroform: formic acid in a ratio of 60:15:10:5 (v/v/v/v). The plates were developed at a distance of 90 mm and dried in a hood for 30 min before derivatization. Next, the TLC plates were immersed in 0.2% (w/v) methanolic DPPH$^{\bullet}$ solution, stored in the dark for 30 min, scanned, recorded in the form of JPEG image files, and further processed by means of an open source and free program, ImageJ (Rolnik et al., 2020), compiled at the National Institute of Health in the USA. The tests were performed in triplicate.

2.3.2. Alkylresorcinols (resorcinolic lipids)

2.3.2.1. Extraction of ARs from wheat samples. ARs from wheat flours were extracted according to Kowalska and Jędrejek (2020) with slight modifications. Air condition extraction of 1 g of not-defatted wheat grains by 40 mL of acetone was performed, adding 20 μL of 4-dodecylresorcinol in a 4 mg/mL concentration, as an internal standard. After 24 h of sonification at room temperature, the extract was centrifuged at 10 000 rpm^{-1}. The supernatant was removed and evaporated under reduced pressure. The dry sample was dissolved in 2 mL of 2-propanol and analyzed.

2.3.2.2. Qualitative and quantitative. UPLC – PDA – MS/MS analysis of ARs. The wheat grains were analyzed by a Waters ACQUITY UPLC Chromatograph (Waters Corp., Milford, MA, USA), equipped with a PDA and a triple-quadrupole mass detector (Waters Corp.). The samples (concentration of 0.5 g/mL) were separated on an ACQUITY BEH C8 (100 × 2.1 mm, 1.8 μm; Waters) column, which was maintained at 50 °C. The elution (0.50 mL/min) was carried out with a gradient of solvent B (methanol with 0.1% formic acid) in solvent A (water Milli-Q with 0.1% formic acid): 0.00–1.00 min, 75% B; 1.00–12.00 min, 75–96% B; 12.00–12.10 min, 96–100% B; 12.10–14.00 min, 100%; 14.00–14.05 min, 100–75% B; 14.05–16.00 min, 75% B. The injection volume was 5 μL (partial loop with needle overfill mode). The PDA operated in the range of 210–450 nm and the resolution was set at 3.6

Table 1
Parameters of the calibration curve for nine different phenolic acids.

No.	Phenolic acid	Calibration curve	R^2
1	protocatechuic acid	$y = -0.0254426\ x^2 + 1.46612\ x + 0.0137605$	0.997
2	*p*-OH-benzoic acid	$y = -0.0116753\ x^2 + 1.43904\ x + 0.164956$	0.997
3	vanillic acid	$y = 0.000116384\ x^2 + 0.194029\ x - 0.00311$	0.998
4	caffeic acid	$y = -0.0182712\ x^2 + 2.42109\ x + 0.436786$	0.995
5	syringic acid	$y = -0.0000546324\ x^2 + 0.259824\ x - 0.00264286$	0.986
6	*p*-coumaric acid	$y = -0.0165714\ x^2 + 2.05818\ x + 2.05818$	0.993
7	ferulic acid	$y = -0.000407832\ x^2 + 0.380126\ x + 3.30005$	0.994
8	sinapic acid	$y = -0.00315979\ x^2 + 0.55236\ x - 0.0620381$	0.998
9	salicylic acid	$y = -0.0338427\ x^2 + 3.26378\ x + 0.82676$	0.998

nm.

The MS analyses were performed applying a positive ion mode with electrospray ionization (ESI), using the condition which was published by Kowalska and Jędrejek (2020). Alkylresorcinols peaks were identified based on the UV, MS and MS/MS spectra. Quantitation of individual alkylresorcinols was based on the internal standard method and peak area calculated from UV chromatograms (275 nm, 5 points/s rate). 4-dodecylresorcinol (40 μg/mL) was used as an internal standard. A 5-*n*-pentadecylresorcinol was used as a group standard, for which a calibration curve $y = -0.0000553736x^2 + 0.36339x + 0.748474$, where y is the integrated peak area and x is the concentration in μg/mL, showed high linearity of response ($R^2 > 0.997$) within the tested range (25.00–250.00 μg/mL), prepared from triplicate analyses of standard working solutions at six different concentrations. Three independent chromatographic runs were performed for each sample. All data were acquired and processed using the Waters MassLynx 4.1 software (Waters Corp., Milford, USA). Identification of alkylresorcinols was performed with the help of data obtained by mass spectrometry and compared with literature data. The results are presented in Table 2 and Fig. 1.

2.3.2.3. Free radical scavenging activity of ARs. The antioxidant activity of alkylresorcinols in wheat grains was determined using a method developed earlier and first published in 2020 (Kowalska and Jędrejek, 2020).

2.4. Statistical analysis

The results were statistically analyzed using a Statistical Version 13.3. Analysis of variance for a complete randomization system was performed. The values represent the mean of data obtained from experiments with 3 replicates. The differences between cultivars, within one location, were evaluated by applying 1-way ANOVA, followed by a Tukey's test. The differences among locations, were evaluated by applying multi-way ANOVA using the General Linear Model and for mean separation the HSD Tukey test was used and shown using capital letters in the Tables. Statistical significance was declared at $P < 0.05$. For the statistical analysis of the experimental data, JMP statistical software (SAS Institute, USA) was used.

3. Results and discussion

3.1. Chromatographic analysis of phenolic acids

By the means of UPLC-PDA-ESI-MS system (Waters Corp., USA) five hydroxybenzoic (protocatechuic, *p*-OH-benzoic, vanillic, syringic, salicylic acid) and four hydroxycinnamic acid derivatives (caffeic, *p*-coumaric, ferulic, sinapic acid) were identified and quantified in the grain of twelve winter wheat cultivars, from eight European locations during the growing season 2018. The results are presented in Fig. S1. Comparison of results indicate that phenolic acids showed a high concentration variability (Table 3). The mean phenolic acid content, averaged over cultivars, ranged from 809.75 μg/g (Germany-Hohenheim) to 1016.19 μg/g of the grain DM (Spain). This high concentration in Spain may be the result of the country's climate (Lleida Region) which is characterized by mild winters and long, hot summers. It was reported that long day, radiation intensity and high air temperature are associated with a high content of bioactive substances and antioxidant activity of grains (Pu et al., 2019). This would be consistent also with previous studies performed by Fernandez-Orozco et al. (2010), which suggested that a range of environmental stresses such as high temperature during grain filling, solar radiation, drought and excess water can induce an increase of phenolic content. An increase in the content of free phenolic acids in wheat grain with increasing temperature during plant growth was also observed in three Canadian and three Australian genotypes grown in a controlled environment (Shamloo et al., 2017).

This increase of phenolic acids in wheat kernels under high temperature and solar radiation can be explained as a result of plant protection mechanisms against increased UV radiation. As indicated by Ma et al. (2021) increased amount of phenolic acids are an important plant defense factor for wheat plants growing under many different stress conditions such as, temperature, UV irradiation, nutrient deficiencies, high light intensity, insect, pathogens and herbivore attack as well as less selective herbicide treatments. There was no found significant differences in total phenolic acids between cultivars growing in Belgium, Germany-Hannover and Germany-Hohenheim. The wheat cultivars grown in these countries showed a similar phenolic acids content, which was lower than those obtained in Spain and Poland (Table 3).

The mean phenolic content for the twelve cultivars across the growing locations ranged from 821.95 μg/g (cv. Bologna by SIS, high yielding and hard kernel cultivar) to 961.78 μg/g (cv. Chambo) (Table 3). The cv. Chambo is distinguished by its high yield and resistance to rust. These concentrations correlate very well with previously published data. In the study of Horvat et al. (2020), with the five most cultivated wheat cultivars in Croatia, there were significant differences ($P < 0.05$) in phenolic acid content, in the range from 556 (cv. Kraljica) to 654 μg/g DM (cv. Katarina). Other studies showed that the total phenolic acid contents of 150 wheat genotypes grown in Hungary, in one location, ranged from 326 to 1171 μg/g DM (Li et al., 2008). This suggests the possibility of genetic variation, which may be of interest to plant breeders. In the investigation by Mpofu et al. (2006) showed that six wheat genotypes cultivated at four locations in Canada showed great diversity in terms of the total amount of six phenolic acids and their antioxidant activity. This is in agreement with the results of our study which also observed significant differences between locations.

Fertilizer amount was another factor that could have influenced the differences in the content of the tested compounds, as it differed in each location. Stumpf et al. (2019) reported that wheat grains contained more soluble phenolics under non-fertilized versus fertilized conditions. Fig. S1 shows the content of particular phenolic acids, in eight locations. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of the most abundant phenolic acids in wheat. It is a byproduct derived from metabolizing phenylalanine and tyrosine found in wheat (Abotaleb et al., 2020). Ferulic acid was found to be the main phenolic acid in all locations and cultivars. It constituted from 78.36% (Spain) to 88.50% (Ireland) of total phenolic acid content in particular locations. The average content of this compound in individual cultivars ranged from 82.53% (cv. KWS Lilli) to 85.11% (cv. Sobervio). The cultivar Chambo, grown in Ireland, had the highest content (1044.07 μg/g of the grain DM) of this compound. Ferulic acid content reported in this work was slightly higher than that the 648 μg/g DM published by Adom and Liu

Table 2
Identification of alkylresorcinol derivatives by UPLC-DAD-MS/MS with retention time and characteristic ions $[M + H]^+$ *m/z*.

No.	Alkylresorcinol derivatives	RT [min]	*m/z* $[M+H]^+$	Reference
1	5-*n*-pentadecylresorcinol (C15:0)	3.65	321	Knödler et al. (2007)
2	5-*n*-heptadecylresorcinol (C17:0)	4.96	349	Knödler et al. (2007)
3	5-*n*-nonadecenylresorcinol (C19:1)	5.32	375	Knödler et al. (2007)
4	5-*n*-heneicosadienylresercinol (C21:2)	5.59	401	Identification by MS
5	5-*n*-nonadecylresorcinol (C19:0)	6.30	377	Kowalska and Jędrejek (2020)
6	5-*n*-heneicosenylresorcinol (C21:1)	6.69	403	Identification by MS
7	5-*n*-heneicosylresorcinol (C21:0)	7.56	405	Kowalska and Jędrejek (2020)
8	5-*n*-tricosylresorcinol (C23:0)	8.70	433	Kowalska and Jędrejek (2020)
9	5-*n*-pentacosylresorcinol (C25:0)	9.72	461	Kowalska and Jędrejek (2020)

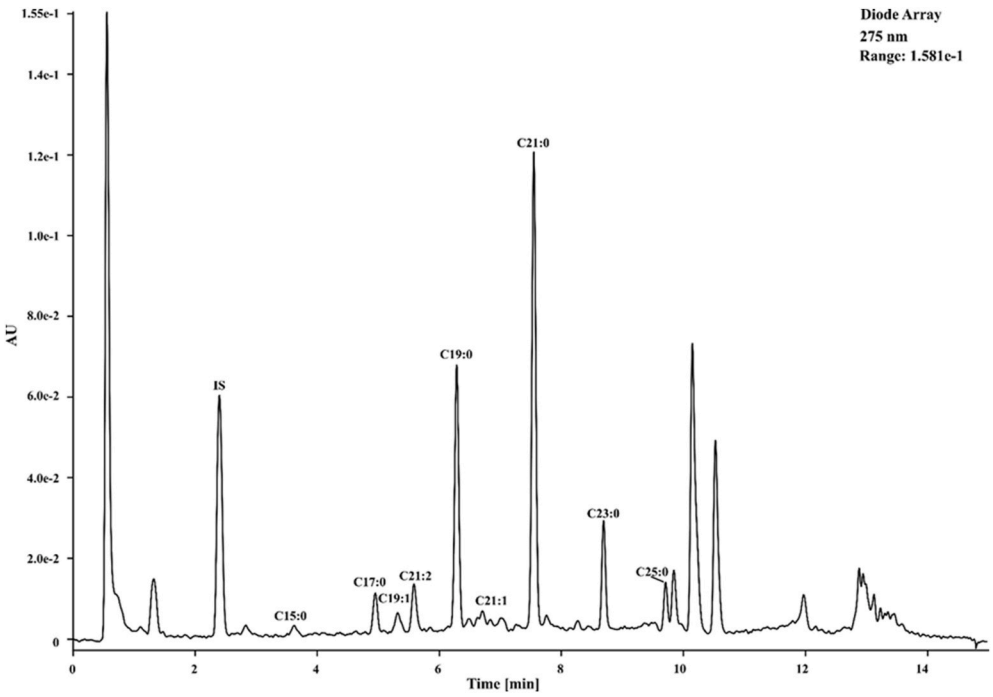

Fig. 1. UPLC-UV$_{275\ nm}$ chromatogram of a common UV spectrum for all alkylresorcinol derivatives determined in wheat cultivars: 5-*n*-pentadecylresorcinol (C15:0) (1), 5-*n*-heptadecylresorcinol (C17:0) (2), 5-*n*-nonadecenylresorcinol (C19:1) (3), 5-*n*-heneicosadienylresercinol (C21:2) (4), 5-*n*-nonadecylresorcinol (C19:0) (5), 5-*n*-heneicosenlyresorcinol (C21:1) (6), 5-*n*-heneicosylresorcinol (C21:0) (7), 5-*n*-tricosylresorcinol (C23:0) (8), 5-*n*-pentacosylresorcinol (C25:0) (9), dodecylresorcinol (IS – internal standard).

(2002) and this of 590 μg/g DM by Hernández et al. (2011). In Croatia's wheat cultivars (Horvat et al., 2020), ferulic acid was also the most abundant phenolic acid, with concentrations ranged from 424 (cv. Kraljica) to 482 μg/g DM (cv. Katarina), which was consistent with the findings of other authors (Stuper-;Szablewska et al., 2019). Mpofu et al. (2006) reported that the content of this acid in different cultivars ranged from 371 to 441 μg/g. The results of the study by Stumpf et al. (2019) showed that higher nitrogen fertilizer doses lead to a reduction in soluble ferulic acid content, but had no effect on insoluble ferulic acid and the total phenolics content.

In the present study, apart from ferulic acid, the sinapic, vanillic and caffeic acids were the most abundant in the different wheat cultivars. Sinapic acid, the second in terms of content, ranged from 5.11% (Sweden) to 10.28% (Spain) of total mean phenolic acid content at particular locations and from 5.58% (cv. Chambo) to 8.06% (KWS Siskin) of total mean phenolic acids content in individual cultivars. The cv. Julius, at the Spanish site, had the highest content (160.02 μg/g) of this compound. The mean highest levels of this acid was found in Spain (mean 104.46 μg/g) (Fig. S1). The content of the other acids was significantly lower. In particular, wheat cultivars grown in United Kingdom and Ireland contained much less vanillic, caffeic, syringic, *p*-coumaric and salicylic acid than on the other locations. Lower amounts of acids e.g. *p*-OH-benzoic, *p*-coumaric, syringic and salicylic acids were also present in American wheat cultivars as reported by Moore et al. (2006), in Canadian wheat samples (Shamloo et al., 2017), and Polish wheat grain (Żuchowski et al., 2011). According to Hernández et al. (2011) the content of phenolic acids levels are related to genotype. However, others have found that location effect (e.g. environmental factors) is more important (Fernandez-Orozco et al., 2010). In this study we demonstrated that the content of phenolic acids depends both on cultivar and environmental conditions.

3.2. Antiradical activity of phenolic acids fraction

Phenolic acids are recognized as superior natural antioxidants with potential health benefits (Chen et al., 2020). In specific terms, it is expected that dietary hydroxybenzoic and hydroxycinnamic acids should be associated with protection against coronary heart disease, cancer and inflammation, since epidemiological studies demonstrated an inverse correlation between dietary intake of phenolic acids and the appearance of such pathologies. The antiradical activity of phenolic acids was assessed by means of a TLC-DPPH$^{\bullet}$ test combined with an Image Processing Procedure (Fig. 2 a, c, d). Winter wheat cultivars grown in Spain had the highest antioxidant activity (mean 0.214 in relation to caffeic acid standard), followed by Poland (mean 0.208) and then Ireland (mean 0.199). Among all tested cultivars, in the eight locations, cv. Chambo showed the highest mean antioxidant activity (0.210). High antioxidant activity is a result of the presence of phenolic acid, especially ferulic, caffeic and *p*-coumaric, acid content (Table 3). Ferulic acid, the main phenolic acid in wheat, is thought to be the main contributor to the total antioxidant activity. Hydroxycinnamic acids have significant antioxidant activity because the CH═CH–COOH group plays a key role in conferring higher antioxidant activity to hydroxycinnamic acids than hydroxybenzoic acids with a COOH group (Ma et al., 2021). The 3,4-position of dihydroxylation on the phenolic ring in caffeic acid showed an increased antioxidant activity as compared to *p*-coumaric acid. Caffeic acid is predicted to have higher antioxidant activity because of the additional conjugation in the propene side chain, which may facilitate electron delocalization, via resonance, between the propene group and the aromatic ring (Chen et al., 2020). After DPPH$^{\bullet}$ scavenging, several half-quinones formed by ferulic acid can combine to form dimers, which increase the scavenging activity free radicals (Chen et al., 2020). In contrast, wheat cultivars grown in Belgium showed the lowest antiradical activity. Statistically significant differences ($P < 0.05$) in the antioxidant activity of phenolic acids of particular cultivars in the tested locations, were found (Table 3). Total values of phenolic acid were highly correlated to the total antiradical activity. It has been proven that of the antioxidants found in wheat, free and esterified phenolic acids appear to have the greatest potential for beneficial health effects (Abotaleb et al., 2020). In numerous studies on the antioxidant properties of phenolic acids, scientists have shown the dependence of these properties on the chemical structure, and more specifically they are related to the number of hydroxyl groups in a molecule and the degree of their esterification. In compounds with one hydroxyl group, the antioxidant activity is additionally increased by the presence of one or two methoxy groups in the ring (Farhoosh et al., 2016). Introduction

Table 3
Mean phenolic acids (μg/g of the grain ±SD), total phenolic acids concentration (μg/g of the grain ±SD) and antiradical activity (in relation to caffeic acid's activity = 1.00 ± SD) of twelve cultivars of wheat grown at **eight** locations[a].

	Protocatechuic acid	*p*-OH-Benzoic acid	Vanillic acid	Caffeic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Salicylic acid	Total	Antiradical activity
Locations											
Belgium	3.66de ±0.64	2.81bc ±0.69	24.31b ±2.27	21.22c ±3.21	17.08cd ±3.90	22.53b ±4.64	697.68ef ±84.73	52.74cd ±9.65	1.63b ±0.11	843.67d ±94.97	0.163e ±0.02
Germany-Hannover	3.93cd ±0.63	2.10d ±0.75	22.69c ±1.86	23.78b ±1.91	17.80c ±3.68	19.22d ±2.56	679.83f ±39.89	50.75de ±7.98	1.66 ab ±0.11	821.76d ±44.37	0.186cd ±0.02
Germany-Hohenheim	4.13c ±0.48	2.04de ±0.62	21.27d ±1.89	20.76c ±1.97	16.62d ±4.02	16.61e ±1.79	675.07f ±51.36	51.73d ±7.84	1.51c ±0.04	809.75d ±58.71	0.175de ±0.01
Ireland	1.26f ±0.26	1.65e ±0.47	7.83e ±0.88	12.61d ±1.52	13.58e ±1.18	10.04g ±1.33	820.92a ±165.75	58.07c ±15.71	LOQ	927.59b ±172.62	0.199bc ±0.01
Poland	3.55e ±0.58	2.52c ±0.77	23.05bc ±2.27	21.69c ±3.03	18.97b ±4.83	21.68bc ±4.68	766.98bc ±70.62	43.09f ±8.07	1.53c ±0.18	903.06b ±83.39	0.208 ab ±0.01
Spain	5.16a ±1.11	3.92a ±1.23	28.13a ±3.58	26.99a ±3.05	23.07a ±4.79	26.45a ±6.71	796.29 ab ±78.65	104.46a ±30.89	1.72a ±0.12	1016.19a ±111.44	0.214a ±0.03
Sweden	4.76b ±0.56	3.15b ±0.74	28.01a ±2.28	20.76c ±2.50	17.36cd ±3.39	19.92cd ±4.11	750.62cd ±61.94	45.62ef ±8.49	1.69 ab ±0.07	891.89bc ±68.58	0.197bc ±0.02
United Kingdom	1.39f ±0.20	2.95b ±0.67	9.05e ±0.84	13.49d ±2.02	9.15f ±2.42	14.18f ±1.73	724.77de ±85.72	69.57b ±18.81	LOQ	846.14cd ±101.31	0.175de ±0.02
Cultivars											
KWS Lilli	3.17e ±1.25	3.13abc ±1.37	22.16 ab ±9.04	21.11 ab ± 6.56	20.58a ±6.24	22.99a ±8.35	779.36abc ±135.11	70.13 ab ±34.31	1.22bcd ±0.81	944.30 ab ±168.08	0.207 ab ±0.02
KWS Siskin	3.14e ±1.29	3.36 ab ±0.92	19.25d ±7.51	21.21 ab ±4.81	14.76c ±3.48	18.21c ±6.44	733.21bcde ±59.61	71.44 ab ±18.39	1.26abc ±0.76	886.24bcde ±80.14	0.196abc ±0.04
RGT Reform	3.80bc ±1.37	2.37def ±0.80	23.35a ±7.31	21.30 ab ±4.41	19.79 ab ±3.30	17.93c ±4.95	729.38cdef ±71.26	55.34de ±19.16	1.35a ±0.58	874.85cdefg ±90.27	0.186bcdef ±0.02
Sobervio	4.17 ab ±1.68	1.95f ±0.93	20.41bcd ±7.78	18.59cd ±5.42	13.01d ±4.67	17.33cd ±4.85	704.53ef ±76.87	46.25f ±20.09	1.18cde ±0.71	827.80 fg ±107.47	0.173ef ±0.03
Henrik	3.21de ±1.35	2.18ef ±0.98	19.78d ±7.61	19.89bc ±5.27	13.28cd ±3.02	17.38cd ±5.79	699.18ef ±62.38	55.01de ±15.10	1.19cde ±0.71	831.50efg ±64.27	0.176def ±0.03
Hondia	3.26de ±1.31	2.65cde ±1.08	21.84abc ±7.98	18.56cd ±4.66	19.00b ±4.69	21.00 ab ±6.36	738.60bcde ±74.22	53.81ef ±24.82	1.20bcde ±0.72	880.32cdef ±81.37	0.193abcd ±0.03
JB Diego	3.59cd ±1.36	2.90bcd ±0.95	18.72d ±7.09	21.74a ±5.61	13.66cd ±3.38	17.58c ±4.67	754.05bcd ±108.87	73.16a ±20.31	1.26bc ±0.78	907.07bcd ±114.28	0.191abcde ±0.04
Julius	3.43cde ±1.36	2.51de ±1.12	19.26d ±7.65	20.65 ab ±4.77	13.77cd ±3.64	21.42 ab ±7.47	780.00 ab ±110.63	61.95cd ±39.72	1.22bcde ±0.73	924.61abc ±135.62	0.204abc ±0.04
Benchmark	3.05e ±1.26	2.45def ±0.74	20.28cd ±7.96	20.44 ab ±6.09	20.72a ±5.02	19.17bc ±7.74	739.93cde ±88.17	65.06bc ±29.74	1.21bcde ±0.73	892.70bcd ±119.05	0.187bcdef ±0.04
Bologna by SIS	4.46a ±2.19	3.48a ±0.93	21.62abc ±8.27	19.67bc ±3.69	13.94cd ±2.66	14.89d ±2.04	684.90f ±110.13	57.28de ±18.63	1.28 ab ±0.77	821.95g ±116.85	0.170f ±0.04
Chambo	3.29de ±1.31	2.13ef ±0.56	20.17cd ±7.17	22.03a ±5.93	20.10 ab ±5.05	21.01 ab ±6.48	817.78a ±123.46	53.70ef ±6.06	1.16de ±0.69	961.78a ±129.47	0.210a ±0.03
CH-Nara	3.08e ±1.24	2.41def ±0.54	19.71d ±7.25	17.35d ±3.74	19.89 ab ±5.20	17.61c ±3.15	705.88def ±100.57	53.17ef ±15.62	1.12e ±0.74	840.69defg ±98.49	0.184cdef ±0.03

LOQ-below limit of quantification.
[a] Means in the same column followed by different letters are significantly different ($P < 0.05$).

of an electron-donating group in the ortho position of an electron-donating alkyl or alkyl or methoxy group in the ortho position, increases the stability of the antioxidant properties of phenolic acids (Chen et al., 2020). Thus, sinapic acid with two methoxy groups is more active than ferulic acid with one methoxy group, which in turn is more active than *p*-coumaric acid (containing one hydroxyl group). The results of Adom and Liu (2002) indicate that phenolic acids from ready-to-eat wheat and wheat bran-based breakfast cereals have strong *in vitro* antioxidant activity and concentrations that could be derived from a standard portion of wheat cereal. Moreover, acidic conditions and enzymatic hydrolysis increase the solubility and activity of wheat phenols, suggesting that the digestion process may be relevant to altering the antioxidant capacity of wheat-based foods. Adom and Liu (2002) also found a higher antioxidant capacity in wheat than in oats and rice.

3.3. Identification and quantification of ARs

Using UPLC-UV-ESI-MS/MS analysis, nine AR derivatives were identified and quantified in winter wheat cultivars: 5-*n*-alkylresorcinols including 5-*n*-pentadecylresorcinol (C15:0), 5-*n*-heptadecylresorcinol (C17:0), 5-*n*-nonadecanylresorcinol (C19:0), 5-*n*-heneicosylresorcinol (C21:0), 5-*n*-tricosylresorcinol (C23:0), 5-*n*-pentacosylresorcinol (C25:0) and 5-*n*-alkenylresorcinols including 5-*n*-nonadecenylresorcinol (C19:1), 5-*n*-heneicosenylresorcinol (C21:1), 5-*n*-heneicosadienylresorcinol (C21:2) (Table 2). UPLC-$UV_{275\ nm}$ chromatogram of a common UV spectrum for all alkylresorcinol derivatives determined in the wheat cultivars studied is presented in Fig. 1. Results of quantitative analyses expressed as microgram per gram of the grain are presented in Fig. S2. An average content of total resorcinolic lipids over all cultivars were the highest in Spain (954.77 μg/g), while the lowest in Ireland (719.86 μg/g) and the United Kingdom (749.08 μg/g) (Table 4). These results correlate with the data presented by Ross et al. (2003) who showed that the amount of ARs in the wheat grains, cultivated in the United Kingdom, France, Germany ranged from 595 to 1429 μg/g and in Sweden from 200 to 1480 μg/g. Other studies have shown that in Swedish wheat the average total ARs content ranged between 227 and 639 μg/g (mean 412 μg/g) (Chen et al., 2020), while in selected Polish wheat grain it was found to be about 723 μg/g (Skrajda-Brdak et al., 2018) or 800 μg/g (Kulawinek et al., 2008). Tsirivakou et al. (2020) made a very valuable compilation of alcocresorcinol content in grains, flours and derived products of wheat cultivars, from different countries (e.g. Finland, France, Germany, United Kingdom, Sweden, Italy, Poland, Hungary, China, India) reported in the literature between 1988 and

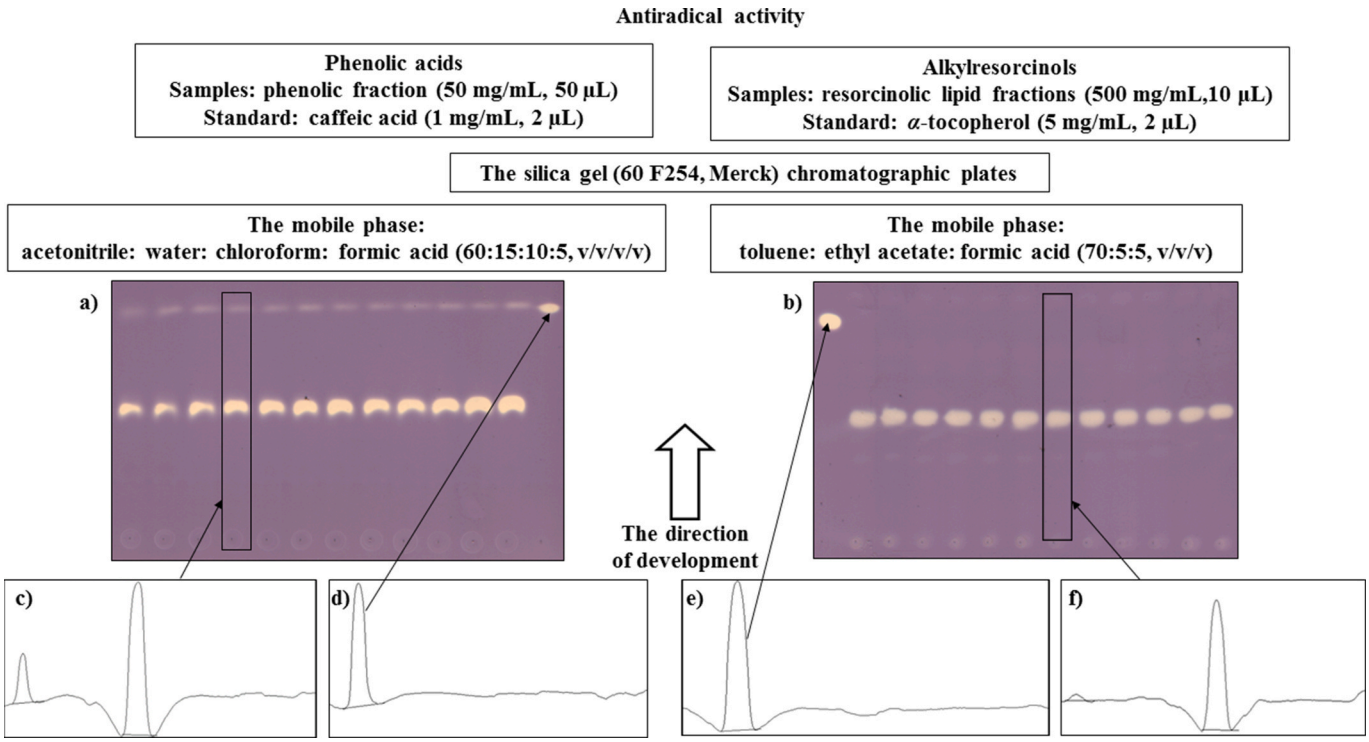

Fig. 2. The results of TLC-DPPH• test combined with Image Processing Procedure including: (a) TLC plate image for 12 winter wheat cultivars and caffeic acid standard, after processing; (b) TLC plate image for α-tocopherol and 12 winter wheat cultivars, after processing; (c) line profile plot obtained for one (cv. Sobervio) of the cultivars; (d) line profile plot obtained for caffeic acid; (e) line profile plot obtained for α-tocopherol (vitamin E equivalent); (f) line profile plot obtained for one (cv. JB Diego) of the cultivars.

2020. In summary they have shown that the biochemical profile of a plant does not depend solely on the genetic information but can be changed due to many environmental factors (e.g. climatic conditions or agronomy). This conclusion was further supported by several other research groups. The team of Ross et al. (2003) reported that several cultivars of *Triticum aestivum* L. showed AR variations from 200 to 1480 mg/g DM. Similarly, Andersson et al. (2010) showed a significant effect of location, cultivar and year on both individual and total AR homologue content ($P < 0.001$) in 26 wheat cultivars grown in Hungary, Poland, France and the United Kingdom. A warm and dry climate induced higher AR levels, while high rainfall, particularly during plant growth and grain filling caused lower levels. In the current study, it was shown that both the cultivar and the environmental conditions influenced the content of the analyzed compounds. An influence of cultivars (within one location) on ARs content was observed (Table 4). Considering the cultivars, for each location (except Poland), the cv Julius (high yielding milling cultivar) had the highest total AR contents (Fig. S2). The lowest average total ARs content in all locations was found in RGT Reform (quality wheat) (Table 4). Wheat cultivars differed significantly also in their individual AR content (Table 4). The most common ARs homologs were C21:0 and C19:0 derivatives, followed by C23:0, C21:2, C25:0, C17:0, C19:1. Similar effects in wheat samples were reported by Zarnowski et al. (2004) and Konopka et al. (2017). In our study, predominant ARs were C21:0, which made up 46.17% (Sweden) to 50.66% (Ireland) of the total mean ARs content in the particular locations. The average content of this compound in individual cultivars ranged from 43.81% (cv. JB Diego) to 51.52% (cv. Benchmark). The cv. Benchmark which was grown in Spain, had the highest content (547.74 µg/g) of this compound. Whereas, 5-*n*-nonadecanylresorcinol constituted from 26.42% (Poland) to 28.54% (Ireland) of the total mean ARs content in particular locations and from 23.09% (cv. Benchmark) to 29.55% (cv. Chambo) of total mean ARs content in individual cultivars. The cv. Julius, grown in Spain, had the highest content (328.03 µg/g) of this compound. The two identified alkylresorcinols (C15:0, C21:1) were detected below the limit of quantification (LOQ).

3.4. Free radical scavenging activity of ARs

The antiradical activity of resorcinolic lipids were assessed by means of a TLC-DPPH• test combined with the ImageJ program (Fig. 2 b, e, f). The α-tocopherol (used as a standard) and an appropriately selected mobile phase have confirmed that this method is suitable for the determination of ARs activity found in cereal kernels (Kowalska and Jędrejek, 2020). The quantitative antiradical activity results are presented in Fig. S1. They showed a strong positive correlation ($r = 0.76$) with total AR content and with C21:0 ($r = 0.71$) and C19:0 ($r = 0.57$) derivatives. The wheat cultivars grown in Spain had the highest antiradical activity (mean 0.218 in relation α-tocopherol's activity), followed by Belgium (mean 0.187) and Poland (mean 0.142). Wheat cultivars grown in the United Kingdom, Ireland and Germany-Hohenheim showed the lowest average antiradical activity. In the present study, among all tested cultivars, in 8 locations, Julius and Bologna by SIS showed the highest mean antioxidant activity of 0.172 and 0.168, respectively (Table 4). It was interesting to notice that specific cultivars, grown in different regions, possessed various antioxidant potentials based on this assay (Fig. S2). Taking into account the structural composition of the ARs molecule, it can be concluded that the length of the aliphatic side chain and the phenolic ring, significantly affect the antioxidant activity of these compounds. The antioxidant activity of ARs is stronger the longer the alkyl chain, which improves the fat solubility (Tian et al., 2020). The alkyl chain of ARs enables uptake into cellular membranes, whereas the phenolic 1,3-dihydroxybenzene ring provides the antioxidant capacity (Ross et al., 2003). Furthermore, the antioxidant potential of ARs as polyphenolic compounds is due to the possibility of their hydroxyl groups to donate H to the free radical or to donate one electron to the free alkyl radical. In this way, they can modify the redox state of the cell and protect DNA, RNA, proteins, carbohydrates, phospholipids and from peroxidation.

Table 4
Mean alkylresorcinols (μg/g of the grain ±SD), total alkylresoscinols concentration (μg/g of the grain ±SD) and antiradical activity (in relation to α-tocopherol's activity = 1.00 ± SD) of twelve cultivars of wheat grown at eight locations[a].

	C15:0	C17:0	C19:0	C19:1	C21:0	C21:1	C21:2	C23:0	C25:0	Total AR	Antiradical activity
Locations											
Belgium	LOQ	32.30b ±6.61	245.42b ±35.84	27.85bc ±7.46	414.97b ±53.81	LOQ	41.87 ab ±4.78	86.38bc ±14.81	33.23b ±7.93	882.01b ±97.42	0.187b ±0.02
Germany-Hannover	LOQ	30.34c ±5.69	231.30c ±30.50	26.99cd ±7.40	408.78b ±51.04	LOQ	32.93d ±3.96	86.14bc ±13.77	40.47a ±12.85	856.95bc ±90.58	0.141c ±0.02
Germany-Hohenheim	LOQ	29.88c ±7.00	220.51c ±45.24	24.17d ±7.13	364.31d ±55.36	LOQ	36.11c ±6.78	76.41ef ±14.52	28.85c ±7.43	780.24de ±121.80	0.122e ±0.01
Ireland	LOQ	27.23d ±5.16	205.42d ±30.34	27.30bc ±6.42	364.69d ±43.12	LOQ	22.71e ±4.41	72.51f ±10.87	LOQ	719.86f ±69.84	0.119e ±0.01
Poland	LOQ	28.94c ±5.67	223.17c ±30.87	31.22a ±7.71	399.47bc ±43.12	LOQ	36.47c ±6.66	88.41b ±13.11	36.99 ab ±8.16	844.67bc ±86.54	0.142c ±0.01
Spain	LOQ	34.49a ±4.99	257.92a ±28.76	32.87a ±9.39	444.00a ±56.02	LOQ	43.33a ±5.70	102.07a ±18.39	40.09a ±8.75	954.77a ±104.70	0.218a ±0.03
Sweden	LOQ	30.44c ±6.99	225.88c ±38.75	30.22 ab ±9.29	381.02cd ±47.90	LOQ	40.77b ±5.55	82.59cd ±10.64	34.42b ±6.66	825.34cd ±94.77	0.132d ±0.02
United Kingdom	LOQ	26.25d ±4.66	202.46d ±28.09	26.14cd ±6.13	365.63d ±38.72	LOQ	25.00e ±3.98	78.05de ±11.76	25.56c ±3.81	749.08ef ±61.90	0.136cd ±0.02
Cultivars											
KWS Lilli	LOQ	29.18c ±3.50	223.40de ±22.86	16.18g ±1.84	421.20abc ±47.09	LOQ	37.56bc ±8.39	95.08a ±14.16	34.28abc ±15.78	856.88bc ±103.44	0.164 ab ±0.05
KWS Siskin	LOQ	24.26d ±3.18	193.70f ±23.39	22.85ef ±5.50	388.28de ±41.07	LOQ	39.44b ±7.60	94.11 ab ±10.73	36.33a ±15.85	798.96cde ±94.39	0.148cde ±0.04
RGT Reform	LOQ	28.20c ±4.64	221.49de ±23.55	34.05 ab ±5.30	350.67f ±66.81	LOQ	35.83cd ±6.50	67.65e ±15.27	21.24e ±11.28	759.13e ±100.87	0.138f ±0.03
Sobervio	LOQ	28.74c ±2.09	214.51e ±13.44	20.60f ±3.20	369.46def ±21.86	LOQ	29.43 fg ±8.23	79.85d ±6.63	30.82abcd ±13.34	773.42de ±52.99	0.139ef ±0.02
Henrik	LOQ	29.29c ±5.87	213.35e ±41.73	34.36 ab ±5.30	358.33f ±63.79	LOQ	36.00cd ±6.59	77.75d ±17.54	28.21d ±13.97	777.29de ±146.83	0.135f ±0.03
Hondia	LOQ	29.86c ±3.45	229.58cde ±33.49	24.00ef ±5.88	381.00def ±38.73	LOQ	32.64e ±7.77	80.85cd ±9.55	26.83de ±11.85	804.77cde ±94.26	0.144cdef ±0.03
JB Diego	LOQ	37.75a ±4.48	241.68bc ±22.71	36.63a ±7.19	363.79ef ±31.91	LOQ	43.12a ±11.56	78.16d ±10.01	29.29bcd ±13.03	830.42bcd ±85.80	0.140def ±0.03
Julius	LOQ	38.65a ±6.09	285.22a ±43.35	37.58a ±4.78	443.99a ±57.62	LOQ	34.44de ±9.01	99.32a ±18.20	34.53 ab ±16.46	973.73a ±139.13	0.172a ±0.04
Benchmark	LOQ	21.21e ±4.98	188.89f ±33.14	31.12bc ±5.05	421.36 ab ±62.09	LOQ	36.19cd ±8.36	87.63bc ±18.83	31.53abcd ±20.07	817.93cde ±119.48	0.150cd ±0.04
Bologna by SIS	LOQ	30.25c ±4.05	232.84cd ±26.44	28.17cd ±5.16	435.66a ±50.66	LOQ	33.84de ±6.57	95.15a ±13.87	30.19bcd ±12.61	886.10b ±103.21	0.168a ±0.04
Chambo	LOQ	32.79b ±3.97	249.45b ±28.28	28.12cd ±3.46	395.19bcd ±37.49	LOQ	32.13ef ±8.84	77.70d ±8.98	28.80cd ±13.32	844.17bc ±87.46	0.154bc ±0.04
CH-Nara	LOQ	29.61c ±3.09	226.11cde ±22.08	25.08de ±4.12	389.60cde ±37.86	LOQ	27.44g ±6.24	74.89de ±10.11	25.70de ±15.18	798.44cde ±86.29	0.149cde ±0.04

LOQ-below limit of quantification.
[a] Means in the same column followed by different letters are significantly different ($P < 0.05$).

Although ARs do not have a hydroxyl group in the ortho-position, the other factor that may influence antioxidant efficacy in lipid complexes is lipophilicity, which permits better penetration into lipid micelles. Several *in vitro* studies have indicated that 5-alkylresorcinol has antioxidant activity, which is primarily due to the two intermediate positions of the hydroxyl groups on the benzene ring, which can generate protons and scavenge free radicals (Tian et al., 2020). Our results confirm that ARs exhibit weak antioxidant activity relative to different tocopherols, which is consistent with previous reports of Korycińska et al. (2009) as well as Kowalska and Jędrejek (2020). Korycińska et al. (2009) showed antioxidant activities of several ARs with chains lengths from C15:0 to C23:0 using the HT29 human colon cancer cells. Moreover, they were capable of greatly blocking copper-induced peroxidation of human small-density lipoprotein *in vitro*.

3.5. Genotype by environment interactions associated with phenolic acid and AR composition in wheat across Europe

As explained above, overall (average of all locations) Chambo and Julius were among the cultivars showing the highest contents of phenolic acids and alkylresorcinols, respectively. However, for all compounds the interactions between genotype and environment were significant. Table 5 present analysis of variance of phenolic acids and alkylresorcinols measured in eight locations and twelve bread wheat cultivars with significance of location, cultivar and interactions between location and cultivars effects. For example, in the phenolic acids profiles, cv. Chambo showed the best profile overall, however in some countries KWS Lili (two sites in Germany and in the United Kingdom) showed the highest contents instead of cv. Chambo. Moreover, for the alkylresorcinols profiles, cv. Julius was the best cultivar in most countries except in Germany and Poland where Bologna and Henrik, respectively, showed the best profiles. As reported elsewhere (Fernandez-Orozco et al., 2010) genotype by interaction is a major constrain to increase the genetic gain of these traits through breeding and the best strategy in these conditions is to test the highest economically viable number of sites to determine stability of the best observations in each sub-region of Europe.

4. Conclusions

The results presented here come from the first multi-location ECOFE field study of winter wheat. The differences in the accumulation levels of some bioactive compounds (phenolic acids and ARs) were investigated in wheat grain of different cultivars grown in each location as well as in wheat grain of cultivars grown under different conditions. The UPLC-PDA-ESI-MS method was applied for qualitative and quantitative

Table 5
Analysis of variance of phenolic acids and alkylresorcinols measured in eight locations and twelve bread wheat cultivars with significance of location, cultivar and interactions between location and cultivars (Loc.*cv.) effects. Degrees of freedom (DF), sum of squares (SS), F ratio and probability of F ratios (Prob > F) are shown.

Phenolic acid Source					Alkylresorcinol		
	DF	SS	F ratio	Prob > F	SS	F ratio	Prob > F
Protocatechuic acid					C17:0		
Location	7	198.56	366.20	<.0001	782.54	45.23	<.0001
Cultivars	11	30.25	34.87	<.0001	4063.58	146.79	<.0001
Loc. * cv.	77	26.65	3.32	<.0001	1699.41	6.64	<.0001
p-OH-Benzoic acid					C19:0		
Location	7	76.93	71.87	<.0001	42183.94	43.28	<.0001
Cultivars	11	36.58	21.36	<.0001	112780.36	72.32	<.0001
Loc. * cv.	77	44.80	2.83	<.0001	72933.52	5.06	<.0001
Vanillic acid					C19:1		
Location	7	5807.62	505.17	<.0001	1184.96	22.16	<.0001
Cultivars	11	142.08	7.72	<.0001	4785.95	55.94	<.0001
Loc. * cv.	77	324.52	1.91	0.0016	2013.34	2.54	<.0001
Caffeic acid					C21:0		
Location	7	2746.48	248.65	<.0001	114128.86	35.88	<.0001
Cultivars	11	201.18	11.38	<.0001	127342.90	25.02	<.0001
Loc. * cv.	77	563.86	3.45	<.0001	201326.36	4.28	<.0001
Syringic acid					C21:2		
Location	7	1172.18	151.44	<.0001	6563.27	208.04	<.0001
Cultivars	11	1284.84	103.75	<.0001	2142.93	42.45	<.0001
Loc. * cv.	77	489.07	4.27	<.0001	1539.83	3.30	<.0001
p-Coumaric acid					C23:0		
Location	7	3843.80	162.16	<.0001	12849.06	76.41	<.0001
Cultivars	11	606.08	15.98	<.0001	9456.87	35.15	<.0001
Loc. * cv.	77	1318.59	3.76	<.0001	13731.31	5.52	<.0001
Ferulic acid					C25:0		
Location	7	481691.87	61.22	<.0001	28128.06	243.84	<.0001
Cultivars	11	221593.34	17.60	<.0001	1184.30	6.42	<.0001
Loc. * cv.	77	969999.85	8.33	<.0001	5775.71	3.38	<.0001
Sinapic acid					Total		
Location	7	59336.42	282.53	<.0001	798012.10	68.38	<.0001
Cultivars	11	10117.94	30.11	<.0001	505284.84	27.06	<.0001
Loc. * cv.	77	26789.15	8.62	<.0001	694150.08	4.02	<.0001
Salycilic Acid					Antiradical activity		
Location	5	0.73	25.23	<.0001	0.18	503.31	<.0001
Cultivars	11	0.30	4.62	<.0001	0.02	34.71	<.0001
Loc. * cv.	55	0.86	2.23	0.0009	0.04	6.45	<.0001
Total							
Location	7	736864.10	63.59	<.0001			
Cultivars	11	288590.30	15.57	<.0001			
Loc. * cv.	77	1182339.30	6.89	<.0001			
Antiradical activity							
Location	7	0.05	35.75	<.0001			
Cultivars	11	0.02	11.03	<.0001			
Loc. * cv.	77	0.12	5.96	<.0001			

analyses and the TLC-DPPH$^{\bullet}$ method with ImageJ software was used for quantitative antioxidant analysis of both phenolic acids and ARs. In addition, the quantitative profiling of each identified compound was also carried out for all cultivars, and for all locations. The results indicate that ferulic acid was the major phenolic acid in wheat grain, and C21:0 and C19:0 were dominant among ARs, in all cultivars and locations. The highest total amount of phenolic acids and the strongest antiradical activity were detected in the cv. Chambo, but the highest total amount of ARs and the highest antiradical activity was identified in the cv. Julius. The mean values of the total levels of the studied compounds were significantly higher in Spain than in the other regions. The significant variation in the content of the studied compounds was due to cultivar, agronomic practices and climatic conditions. This suggests the possibility of selecting and breeding wheat cultivars with increased levels of phenolic acids and AR, in multi-location field trials aimed at obtaining additional health benefits for consumers. Furthermore, the variation in phenolic profile between wheat cultivars can be used to characterize wheat cultivars, helping to contribute to breeding programs for future cultivars with increased levels of beneficial compounds.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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