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Characterization of grain quality and phenolic acids in ancient wheat species (*Triticum* sp.)

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Summary

The matter of this study was to determine the phenolic acid profile of different ancient wheat, furthermore, to analyze the total phenolic content (TPC) with the Folin-Ciocalteu assay and the antioxidative capacity with the ORAC (oxygen radical absorbance capacity) assay. The concentration and composition of free, conjugated, and insoluble bound phenolic acids were analyzed in 20 accessions of wheat (*Triticum* sp.), including 16 ancient wheat and 4 bread wheat samples grown in Germany. Six phenolic acids were analyzed by HPLC, and ferulic acid (FA) was identified to be the abundant phenolic acid. The content of phenolic acids in total was comparable to bread wheat, ranging between 141.0 and 542.0 µg GAE/g (gallic acid equivalent/g) whole wheat flour. In the current study no significant distinction between the analyzed species could be observed. There was no significant impact by the *Triticum* species, neither on the total phenolic content by Folin-Ciocalteu nor on the antioxidative capacity by ORAC. Correlation analysis between ORAC values and total phenolic acids demonstrated a positive correlation ($r=0.469$, $p=0.05$). Phenolic acids, TPC and ORAC values of the analyzed ancient wheat samples were comparable to bread wheat.

Introduction

Wheat (*Triticum aestivum* L. ssp. *aestivum*) is one of the most important agricultural commodities worldwide with 670 million tons in 2009, and it is constantly rising (USDA, 2010). The domestication of wheat goes back to 10,000 BC, where wild species were taken into cultivation (Feldman, 2001; Nesbitt and Samuel, 1995). Wheat is one of the most important vegetable foods which provides human with basic nutrients (carbohydrates, proteins, vitamins, minerals). Besides basic nutrients, wheat caryopses contain secondary plant metabolites, such as carotenoids, flavonoids and phenolic acids. These compounds can have an influence on the utility value and can influence the quality characteristics of wheat products. Additionally, secondary plant metabolites are bioactive compounds because of their antioxidative capacity (Watzl and Rechkemmer, 2001; Slavin, 2003).

Einkorn (*T. monococcum* L.), emmer (*T. dicoccum* L.) and spelt (*T. aestivum* L. ssp. *spelt*) are ancient wheat species with low grain yields and non-threshable grain. Wild einkorn, an one-grained wheat, is known as the progenitor of cultivated diploid wheat, which later results in tetraploid wheat (emmer) and hexaploid wheat (spelt) (Feldman, 2001). In the last time interest in ancient wheat and a higher variety in nutrition grew in some countries, e. g. Germany. For instance, the harvested area of spelt rose from 2003 to 2009 more than 300 % in Germany (Statistisches Bundesamt, 2010). Einkorn, emmer and the hexaploid spelt are very robust and modest cereals which can be harvested under moderate environmentally conditions like poor soils and water deficiency (Stagnari et al., 2008). Thus, ancient wheat is recommended for organic production, where no synthetic fertilizers or pesticides are used to strengthen the plant.

One reason for increasing interest in ancient wheat is the consumer, who claims more biologically grown products because of their environmentally friendlier production (Zhao et al., 2007; Dangour et al., 2009). Another reason is the health beneficial-aspect of wheat products, especially of whole grain products. Investigations report that whole grain wheat products are reducing high blood pressure (Behall et al., 2006). Ancient wheat products often can be found as whole grain products, where the health aspect is higher than in white flour products.

Phenolic acids (PA), such as ferulic, *p*-cumaric, vanillic, sinapic, syringic, and caffeic acid are valuable antioxidativ ingredients in wheat (Mpofu, 2006). It is reported that phenolic acids inhibit lipid oxidation by scavenging free radicals such as hydroxyl radicals (Cuppett et al., 1997). The phenolic acid profile in ancient wheat subjects a high range of variation (Mpofu, 2006). In wheat are three different forms of phenolic compounds existing, free phenolic acids, soluble conjugated (e. g. esterified to sugars) and bound phenolic acids (e. g. esterified to cell walls). The predominant phenolic acid is ferulic acid (Hatcher and Kruger, 1997; Sosulski et al., 1982; Weidner et al., 1999).

The aim of this study is to quantitatively investigate phenolic acids, their composition, and their range of variation in ancient wheat. Furthermore, the determination of the antioxidative capacity as a nutritionally positive and health affecting potential in emmer, einkorn and spelt growing in Germany is carried out in this study.

Materials and methods

Wheat materials

In 2006 ancient wheat species were grown in field plots of the experimental station "Weilburger Grenze" of the Institute of Agronomy and Plant Breeding, (degree of longitude: 8° 39' 16" E degree of latitude: 50° 36' 12" N, altitude: 158 m). Climate conditions were characterized by the annual total precipitation of 600 mm and mean air temperature of 8.5 °C. The experiment was carried out on an alluvial soil which is characterized by a clay content (< 2 µm) of 33 %, and a silt content (2 - 63 µm) of 58 %. The pH value was 6.3, and the total carbon content (humus) of the soil (soil depth 0 - 30 cm) was 1.42 %. In total 20 accessions (species and/or cultivars) of the genus *Triticum* (*T.*) were obtained from the field experiment, including three of the section *monococcon* (diploid), seven of the section *dicoccoidea (*tetraploid*),* and 10 of the section *triticum (*hexaploid) (Tab. 1). No plant growth regulators (PGRs) were applied which supposably led to lodging during ripening.

Hulled wheat was dehulled automatically by a Saatmeister-Allesdrescher K35 (Kurt Pelz Maschinenbau, Germany). The dehulled and freethreshing samples were ground on a Cyclotec 1073 Sample Mill (Foss, Germany) with a 500 µm sieve to get an whole wheat flour. The flour was mixed to ensure homogeneity and was extracted subsequently.

Chemicals

Vanillic acid (VA), syringic acid (SYA), caffeic acid (CA), p-coumaric acid (PCA), ferulic acid (FA), as well as sinapic acid (SA)

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Tab. 1: Wheat material of the eight species with corresponding accessions

species [No.]	section	species	accession	Cv. [No.]
1	*monococcon*	*T. boeticum* L. subsp. *boeticum*	*hausknechtii*	1
			pseudoreuteri	2
2	*monococcon*	*T. monococcum* L. subsp. *monococcum*	*hohensteinii*	1
3	*dicoccoidea*	*T. turgidum* L. subsp. *dicoccoides*	*spontaneovillosum*	1
			artratum	2
			semicanum	3
4	*dicoccoidea*	*T. turgidum* L. subsp. *turgidum*	*griseo buccale*	1
			centigranum	2
			mirabile	3
5	*dicoccoidea*	*T. timopheevii* Zhuk. subsp. *timopheevii*	*timopheevii*	1
6	*triticum*	*T. aestivum* L. subsp. *spelta*	*albispicatum*	1
			durhamelianum	2
			arduini	3
7	*triticum*	*T. aestivum* L. subsp. *aestivum*	*erythrospermum*	1
			ferrugineum	2
			lutescens	3
			miturum	4
8	*triticum*	*T. aestivum* L. subsp. *compactum*	*humboldii*	1
			erinaceum	2
			pseudo-rubiceps	3

were purchased from Sigma-Aldrich (Taufkirchen, Germany). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Gallic acid, acetonitrile, acetic acid, acetone, ethyl acetate, and methanol were obtained from Roth (Karlsruhe, Germany). Water (gradient grade) was acquired by AppliChem (Darmstadt, Germany) and ethanol by Schmidt (Dillenburg, Germany). Sodium carbonate was purchased from Acros organics (New Jersey, USA).

Analytics

Analysis of Quality Parameters

Firstly, thousand grain weight ([g], ISTA 1999) and the proportion of germinated caryopses [%] were analyzed. According to ICC standard methods the following analyses were carried out: total nitrogen (Dumas method, ICC 167), falling number (Hagbert-Perten, ICC 107/1) and sedimentation test (ICC 116/1).

Extraction of Bioactive Compounds

The extraction of the grain samples was done in three separate fractions ((1) soluble free, (2) soluble conjugated, and (3) bound phenolic acids) as previously described by using the methods of ADOM and LIU (2002) and KRYGIER et al. (1982) with modifications. Briefly: 1 g whole wheat flour was extracted for one hour with (7:7:6, v/v/v) methanol/acetone/water and centrifuged. The supernatant provided fraction 1 and 2, the residue fraction 3. Before extracting all three fractions with ethyl acetate, bound and soluble conjugated phenolic acids had to undergo an alkaline pulping with 5 M NaOH. After evaporating the extracts they were resumed in 10 % acetonitrile and stored at -18 °C until analysis. The extracts were used for all further analyses (HPLC, Folin, ORAC).

Analysis of Phenolic Acids by HPLC

Six phenolic acids were identified by HPLC-DAD analysis on a C18 column (EC 250 x 4 Nucleodur Sphinx RP, 5 µm (MN)) (Fig. 1). For quantification of the hydroxybenzoic acids the diode array detection recorded at the wavelength of 250 nm and for the hydroxycinnamic acids at 290 nm. The method was modified according to ZIELINSKI et al. (2001) and WEIDNER et al. (2000). The temperature for the column was set to 25 °C. The injection volume was 100 µl and elution took place with a gradient mobile system: (A) acetonitrile, (B) acetic acid (0.5 %, pH 4.5) at 1 ml min^{-1} following the program: 0-14.5 min 8 % A, 15-30 min 10 % A, 31-40 min 80 % A and 40.5-45 min 8 %

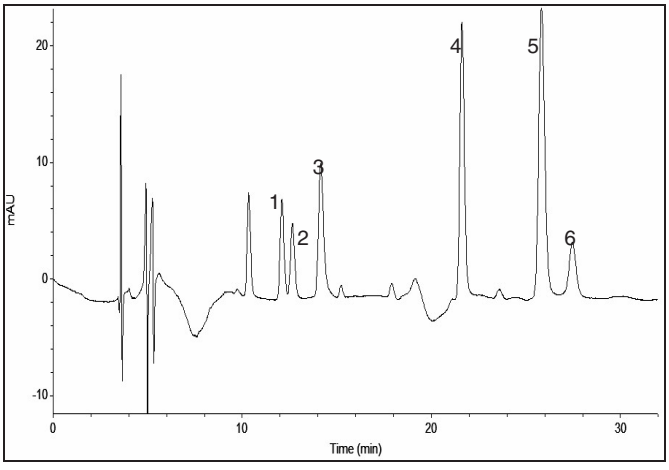

Fig. 1: HPLC chromatogram of a wheat sample with standards. Peak 1 vanillic acid (VA), 2 syringic acid (SYA), 3 caffeic acid (CA), 4 p-coumaric acid (PCA), 5 ferulic acid (FA), 6 sinapic acid (SIA)

A. The identification was made by retention times and UV/VIS spectra compared with commercially available reference compounds. The quantification of all six phenolic acids was done by a five point calibration curve, where the standards were added to a reference flour before extraction to exclude matrix effects. Results were expressed as µg/g and converted in µg GAE/g.

Total Phenolic Assay by Folin-Ciocalteu

The total phenolic content was determined by using the Folin-Ciocalteau micro method (WATERHOUSE, 2001) using gallic acid as a standard. This assay is electron transfer reaction based, which measures the sample's reducing capacity (HUANG et al., 2005). Folin-Ciocalteu reagent consists of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids. 40 µL of the sample extract was mixed with 3.16 ml aqua dest. and 200 µL Folin-Ciocalteu reagent was added. After 5 min 600 µL saturated sodium carbonate solution was added. The reaction blend was mixed and kept at 40 °C for 30 min in a water bath in darkness. Folin-Ciocalteu solution was reduced to blue oxides of tungsten and molybdenum and the absorbance was measured spectrophotometrically at 765 nm. The total phenolic content was expressed as gallic acid equivalent (GAE).

ORAC (oxygen radical absorbance capacity) Assay

The determination of the antioxidative capacity with the ORAC assay, described previously by HUANG et al. (2002), was operated on the 96-well plate fluorescence reader Fluoroskan (Fisher Scientific). The assay is hydrogen atom transfer reaction based and measures antioxidant capacity towards peroxyl radicals (HUANG et al., 2005). In general, 150 µl fluorescein (6-hydroxy-9-(2-carboxyphenyl)-(3*H*)-xanthen-3-on) was mixed with 25 µl of the sample extract and incubated at 37 °C for 30 min. To initiate the reaction the ROS-generator AAPH (2,2'-azobis(2-methylpropionamidine)dihy drochloride) had to be added and the fluorescence was measured at every 60 s for 90 min (excitation wavelength: 485 nm, emission wavelength: 538 nm). As a standard Trolox® (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a vitamin E analogue, was used. The antioxidative capacity was expressed as Trolox equivalents (TE).

Statistical Analysis

Results are reported as mean values and the statistical analysis was performed with PASW Statistics 18 (SPSS Inc., Chicago, IL). The following characteristics were statistically tested by the analysis of variance (ANOVA) in dependency on species with a general linear model: total phenolic content (Folin-Ciocalteu assay), antioxidative capacity (ORAC assay) and phenolic acid concentration (HPLC). Correlation analysis was performed by the Pearson test, bivariate.

Results

In this study grain quality parameters, concentrations of phenolic acids (TPA), the antioxidant capacity expressed as total phenolic contents (TPC) and ORAC values were analyzed. Despite this, the correlation between the concentration of phenolic acids, total phenolic content and ORAC values was calculated.

Grain Quality Parameters

Thousand grain weight (TGW) of the ancient wheat species ranged from 24.3 to 49.4 g. The tetraploid species *T. timopheevii* Zhuk. ssp. *timopheevii* showed highest TGW, therefore the tetraploid species showed in total the highest TGW followed by the hexaploid species. No significant differences were observed in this set of data (Fig. 2). The experiment was characterized by a very high germination level of the grain samples, which partly had visible symptoms of germination. *T. turgidum* L. ssp. *turgidum* showed in total the highest germination with more than 40 % of the analyzed caryopses. The species seem to have a significant influence on germination rate because the tetraploid species showed the highest germination followed by the hexaploid species (Fig. 2). In the case of the parameter falling number (FN) the observed values were very low (62 s) because of a high amylase activity by germination (Fig. 3). These low falling numbers indicate a very high level of degradation of starch in caryopses. For the quality parameter protein there was no significant difference identifiable. The relative proportion ranged between 12.4 and 20.4 % crude protein of the whole grain (Fig. 3). As opposed to crude protein significant differences were observed for the sedimentation test. The results ranged from 8 to 34 with the highest mean value for the tetraploid species, whereas one cultivar of *T. aestivum* L. ssp. *compactum* showed the highest value overall (Fig. 3).

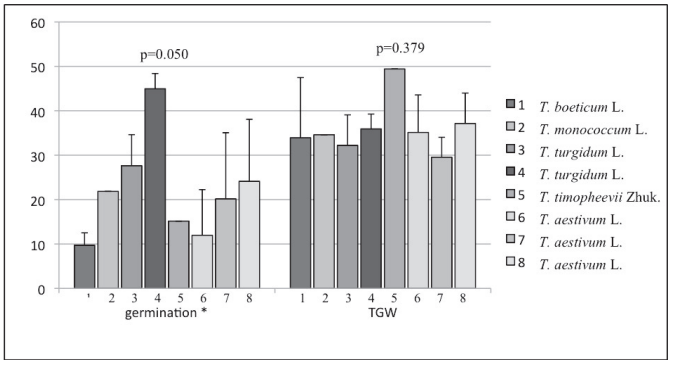

Fig. 2: Germination rate [%], and thousand grain weight (TGW) [g] of different wheat species (mean ± SD, α=0.05)

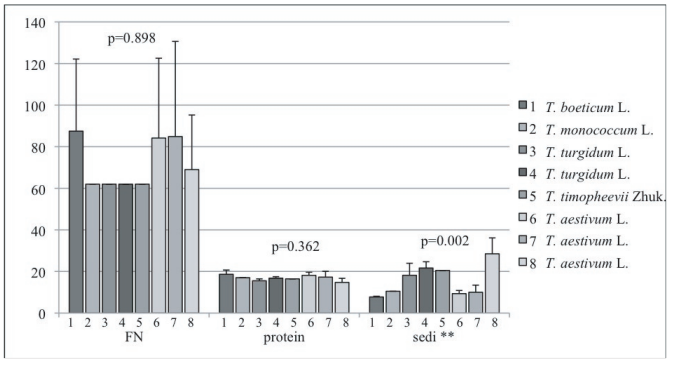

Fig. 3: Falling number (FN) [s], crude protein content [%], and sedimentation test of different wheat species (mean ± SD, α=0.05)

Phenolic Acids by HPLC

The mean value of TPA_total was 334.9 µg GAE/g (Tab. 2) and the individual concentration of the TPA_total in all accessions varied from minimum 141.0 µg GAE/g to maximum 542.0 µg GAE/g (not displayed). There was a large species variation with the highest concentration for *T. boeticum* L. ssp. *boeticum*. Neither between the species nor between the sections significant differences could be found for TPA_total or FA (Fig. 4). The value of the TPA consisted

of six detected phenolic acids: vanillic acid, syringic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid. 70 % of phenolic acids were found as cell wall esterified compounds (TPA 3) followed by 23 % of free esters (TPA 2) and 7 % of free compounds (TPA 1) (Tab. 2). The main phenolic acid was ferulic acid with about 65 % (217.9 µg GAE/g) (Tab. 3) of the phenolic acids (Fig. 4).

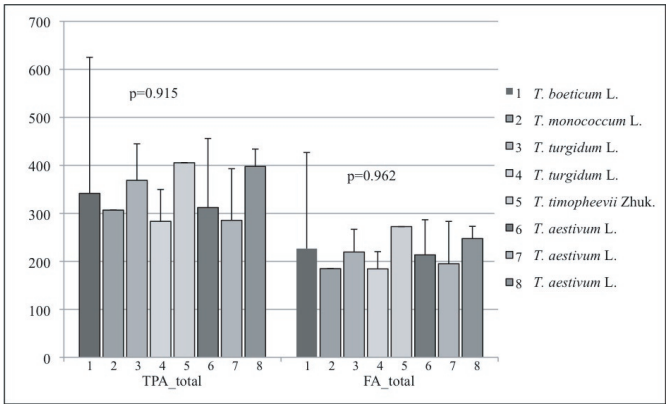

Fig. 4: Total phenolic acids (TPA_total) and total ferulic acid (FA_total) values [µg GAE/g] in different wheat species (mean ± SD, α=0.05)

Total Phenolic Assay by Folin-Ciocalteu

The TPC_total of the whole wheat flour (sum of all three fractions) measured by the Folin-Ciocalteu assay ranged from 2.3 to 2.6 mg GAE/g. Fraction 3 (TPC 3) was characterized by the highest total phenolic concentration (40.1 %) with a mean value of 1.0 mg GAE/g followed by fraction 1 (36.2 %) with a mean value of 0.9 mg GAE/g. The highest concentration of all samples was observed in fraction 1 (TPC 1) for *T. turgidum* L. ssp. *turgidum* with a value of 1.1 mg GAE/g. No significant difference between the species could be found (Fig. 5). A strong correlation between TPA_total and TPC_total values was calculated (r=0.847, p=0.00). There was also a strong relationship between TPA_total and FA_total (r=0.816, p=0.00) (Tab. 4).

ORAC (oxygen radical absorbance capacity) Assay

The antioxidant capacity by the ORAC assay ranged from 18.2 to 23.8 µmol TE/g whole wheat flour for the sum of all three fractions (ORAC_total). Highest ORAC values were measured in fraction 1 (ORAC 1) for the hexaploid wheat species *T. aestivum* L. ssp. *aestivum* with 11.1 µmol TE/g followed by the diploid *T. boeticum* L. ssp. *boeticum* and the tetraploid *T. turgidum* L. ssp. *dicoccoides.* The lowest value was observed in *T. timopheevii* Zhuk. ssp. *timopheevii* in fraction 2 (ORAC 2) with 3.0 µmol TE/g (Fig. 6). It was noticeable, that fraction 1 (ORAC 1) had the highest values holding 46.3 %

Tab. 2: Concentration of phenolic acids (TPA) [µg GAE/g] in caryopses of different wheat species

[No.]	species	TPA 1	SD	TPA 2	SD	TPA 3	SD	TPA_total	SD
1	*T. boeticum* L.	27.31 ±	15.66	81.75 ±	76.33	232.43 ±	191.55	341.49 ±	283.54
2	*T. monococcum* L.	15.38 ±	.	98.50 ±	.	192.89 ±	.	306.76 ±	.
3	*T. turgidum* L.	31.98 ±	13.39	88.75 ±	28.11	247.99 ±	46.86	368.71 ±	75.99
4	*T. turgidum* L.	14.92 ±	3.95	63.14 ±	10.82	205.20 ±	73.38	283.26 ±	66.34
5	*T. timopheevii* Zhuk.	21.67 ±	.	68.11 ±	.	315.51 ±	.	405.29 ±	.
6	*T. aestivum* L.	18.92 ±	15.06	68.61 ±	22.91	202.98 ±	84.76	312.02 ±	143.90
7	*T. aestivum* L.	22.76 ±	18.70	61.10 ±	7.69	201.45 ±	95.37	285.31 ±	107.59
8	*T. aestivum* L.	21.71 ±	5.71	87.58 ±	31.45	288.36 ±	27.47	397.65 ±	36.20
mean		21.83		77.19		235.85		334.87	
relative concentration [%]		6.52		23.05		70.43		100.00	
p (α=0.05)		0.823		0.859		0.818		0.915	

Tab. 3: Concentration of ferulic acid (FA) [µg GAE/g] in caryopses of different wheat species

[No.]	species	FA 1	SD	FA 2	SD	FA 3	SD	FA_total	SD
1	*T. boeticum* L.	5.09 ±	1.33	25.88 ±	25.05	195.48 ±	173.94	226.44 ±	200.32
2	*T. monococcum* L.	5.16 ±	.	25.73 ±	.	153.84 ±	.	184.73 ±	.
3	*T. turgidum* L.	5.86 ±	0.03	26.30 ±	6.08	187.20 ±	42.49	219.36 ±	47.41
4	*T. turgidum* L.	4.54 ±	0.93	26.99 ±	4.45	152.70 ±	39.14	184.23 ±	35.91
5	*T. timopheevii* Zhuk.	5.32 ±	.	23.39 ±	.	243.54 ±	.	272.26 ±	.
6	*T. aestivum* L.	4.86 ±	0.68	28.71 ±	4.68	213.86 ±	24.38	247.43 ±	25.42
7	*T. aestivum* L.	4.55 ±	1.91	30.02 ±	3.43	178.86 ±	71.98	213.44 ±	73.06
8	*T. aestivum* L.	2.77 ±	2.52	28.27 ±	2.95	163.89 ±	83.88	194.92 ±	88.21
mean		4.77		26.91		186.17		217.85	
relative concentration [%]		2.19		12.35		85.46		100.00	
p (α=0.05)		0.478		0.807		0.943		0.962	

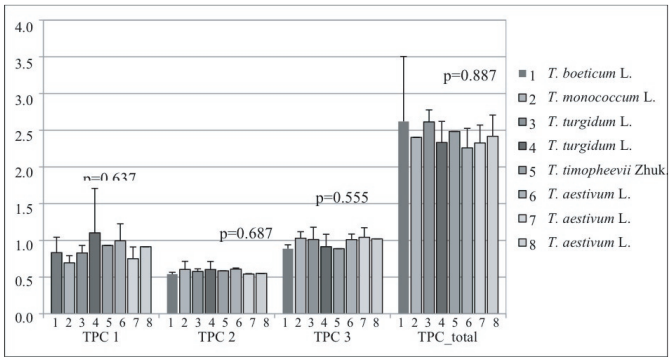

Fig. 5: Total phenolic content (TPC) [mg GAE/g] in different wheat species (mean ± SD, α=0.05)

of the ORAC values in total followed by fraction 3 (ORAC 3: 33.8 %). ORAC values were positively correlating with TPC_total on a moderate level (Tab. 4). A strong positive relationship between ORAC_total and fraction 1 of the TPC was observed (r=0.618, p=0.01) and no correlation between ORAC_total and fraction 2 and 3 of the TPC was found (Tab. 4).

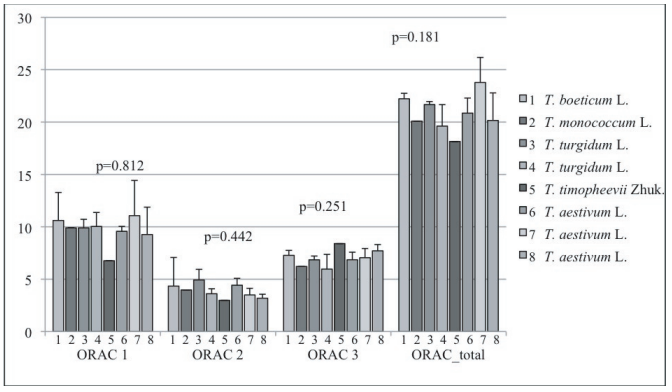

Fig. 6: Antioxidant capacity (ORAC) [µmol TE/g] in different wheat species for fraction 1, 2 and 3 (mean ± SD, α=0.05)

Tab. 4: Pearsons's correlation coefficients (r) between phenolic acids (TPA) and antioxidant capacity (TPC, ORAC) of different wheat species

	Phenolic acids				
fraction	TPA 1	TPA 2	TPA 3	TPA_total	FA_total
TPC_total	0.576 **	0.700 **	0.814 **	0.847 **	0.816 **
ORAC_total	0.618 **	0.182	0.116	0.469 *	0.371

* significant at $p < 0.05$
** significant at $p < 0.01$

Discussion

To our knowledge there are only a few studies published on the comparison of phenolic acid concentrations and the antioxidative capacity of ancient wheat (Li et al., 2008; Abdel-Aal and Rabalski, 2008; Serpen et al., 2009). For that reason the aim of this study is to evaluate the phenolic acid concentrations of different ancient wheat varieties (accessions) to learn more about those varieties. It is necessary to find out more about phenolic compounds and the antioxidative capacity in ancient wheat, as they are an alternative to bread wheat. Secondary plant metabolites, like phenolic acids, contribute to health promoting effects of wheat and consumers are increasingly interested in health promoting substances. Since the highest proportion of phenolic acids is located in bran and aleurone layer, the analysis of phenolic acids, total phenolic content and antioxidative capacity was carried out in whole wheat flour (Pussayanawin et al., 1988; Adom et al., 2005; Zhou et al., 2004; Anson et al., 2008).

The germination rate was relatively high, ranging between 0 and 48 % which resulted in low falling numbers with an average of 62 s. Low falling numbers are induced by the high degradation of starch during the process of germination. It can be supposed that humid conditions during grain ripening and a tendency of lodging led to germination before harvesting.

The analyzed phenolic acids (TPA_total), which result by addition of the concentrations of the three fractions, differed between 283.3 µg GAE/g for *T. turgidum* L. subsp. *turgidum* and 405.3 µg GAE/g for *T. aestivum* L. subsp. *compactum*. Einkorn (*T. monococcum* L. subsp. monococcum) showed the average concentration of 306.8 µg GAE/g and emmer (*T. turgidum* L. subsp. *dicoccoides*) 368.7 µg GAE/g. The phenolic acid concentrations ranged widely and they were comparable to those reported for bread wheat in the literature. For instance, Stracke et al. (2009) reported concentrations between 282 µg/g and 1262 µg/g in wheat samples, Zhou et al. (2005) analyzed the phenolic acid composition of hard red winter wheat bran extracts with low concentrations between 202.6 µg/g and 244.1 µg/g and Li et al. (2008) reported average contents of 615 µg/g (einkorn), 779 µg/g (emmer), and 579 µg/g (spelt).

Highest concentration of phenolic acids in the used ancient wheat samples showed fraction 3 (TPA 3, bound phenolic acids), which was also in line with literature (Stracke et al., 2009; Abdel-Aal et al., 2001). Phenolic acids are bound to hydrolysable tannins, lignins, cellulose and proteins which are mainly structural components of bran, building a protective layer to the seed. Phenolics, among phenolic acids, play a role in defending mechanisms against pathogens, parasites and predators (Liu, 2004). Also, they have antioxidant properties in plants (Parr and Bolwell, 2000; Graf, 1992). During cell elongation ferulic acid is proposed to increase wall extensibility (Graf, 1992). In this study about 70 % were bound to cell wall components, which was lower than in other studies, where up to 98 % were bound phenolic components (Stracke et al., 2009; Li et al., 2008). Free phenolic acids showed the smallest contribution to total phenolic acids ranging between 1 % and 2 % (Li et al., 2008; Abdel-Aal et al., 2001). In the present study fraction 1 (TPA 1) contributed 5 to 8 % to TPA_total, which was higher than in the other studies. Reasons for the ranging results could be that einkorn and emmer produce more free phenolic acids than bound forms to protect the plant material against antioxidative stress. If it is the case, that ancient wheat have a higher concentration of soluble free phenolic acids, the bioavailability and bioaccessibility could be higher than in bread wheat. E. g. free ferulic acid can be resorbed in the small intestine, whereas bound forms have to be released by bacterial hydrolytic enzymes during fermentation in the large intestine (Anson et al., 2009; Kroon et al., 1997; Zhao et al. 2003). Furthermore, different extraction methods are the reason for ranging amounts of phenolic compounds of the analyzed fractions, which leads to the suggestion, that a standardized method should be implemented.

Ferulic acid was the predominant phenolic acid with about 66 % in wheat, averaging between 185 µg GAE/g and 247 µg GAE/g. Highest concentration of ferulic acid was found in the hexaploid species (*T. aestivum*). The diploid species (*T. boeticum* and *T. monococcum*) and the tetraploid species (*T. turgidum* and *T. timopheevii*) could keep

up with the hexaploid ones. Bound ferulic acid (FA 3) contributed the highest concentration of the total ferulic acid in the analyzed ancient wheat samples. Ferulic acid is mostly bound to arabinoxylans and other indigestible polysaccharides restricting its release in the small intestine (Anson et al., 2009). In the present study the concentrations of ferulic acids were lower compared to those reported previously. Li et al. (2008) stated 298 µg/g for einkorn, 476 µg/g for emmer, 365 µg/g for spelt, and 395 µg/g for winter wheat. Stracke et al. (2009) found about 85 % (239-1072 µg/g) ferulic acid of the analyzed phenolic acids in total.

Total phenolic contents of the present ancient wheat samples were reported as gallic acid equivalents in mg/g whole wheat flour. The highest TPC was found in fraction 3 of the whole grain flour, where phenolic compounds exist in bound forms (Stracke et al., 2009; Abdel-Aal et al., 2001; Adom and Liu, 2002). In addition, there were high values of fraction 1. The Folin-Ciocalteu assay does not only display phenolics, it also reacts with nonphenolic substances such as vitamin C or other organic acids (Huang et al., 2005; Georgé et al., 2005). That could be an explanation for the high values of fraction 1, where further free phenolic and nonphenolic substances could be present. Other studies did not analyze those three fractions, as they divided into flour and bran. The values of the present study for the total phenolic contents in whole wheat flour (TPC_total: 2.4 mg GAE/g) were higher than in the literature reported for wheat samples. Adom et al. (2005) reported 176-195 µmol GAE/100 g (0.3-0.4 mg GAE/g) for the endosperm fraction of hexaploide wheat. Serpen et al. (2008) found total phenolic content values for einkorn with the average of 3.37 µmol/g (0.6 mg GAE/g), for emmer with 6.33 µmol/g (1.2 mg GAE/g) and for bread wheat with 4.36 µmol/g (0.8 mg GAE/g). The widely ranging total phenolic contents may be due to different extracting methods, such as inclusion of soluble free, soluble conjugated and bound phenolic acids. Dewanto et al. (2002) and Liyana-Pathirana and Shahidi (2006) drew a similar conclusion. In the current study there were no significant differences in total phenolic contents between the analyzed *Triticum* L. species. Einkorn and emmer were at about the same level of total phenolic contents as bread wheat. TPA_total could be attributed to TPC_total because of a strong correlation between the total phenolic acids and total phenolics (r=0.847, p=0.00).

Comparing the antioxidative capacity of the ORAC test in whole wheat flour with the literature was difficult because different ORAC assays and extraction methods had been applied (Moore et al., 2005; Zhou et al., 2007; Liyana-Pathirana and Shahidi, 2006; Okarter et al., 2010). In the present study ORAC values (ORAC_total) ranged from 15.1 to 25.2 µmol TE/g, which was lower than in most of other investigations of wheat reported in the literature. For instance, ORAC values of soft wheat cultivars investigated by Moore et al. (2005) ranged from 32.9 to 47.7 µmol TE/g. Slightly higher ORAC values of 51 to 96 µmol TE/g for whole wheat flours of different cultivars could be found by Orkater et al. (2010). In contrary much higher values of 3406 µmol TE/g defatted material were measured by Liyana-Pathirana and Shahidi (2006). The analyzed soft wheat cultivars of Zhou et al. (2007) varied between 15.5 µmol TE/g and 24.5 µmol TE/g, which is in line with the current study.

In the current study three separate fractions of the grain samples (soluble free, soluble conjugated, and bound phenolic acids) were used for HPLC and ORAC analysis. It could be observed that ORAC values in fraction 1 (ORAC 1, free phenolic acids) contribute 47.7 % to the total ORAC values and they are even higher than in fraction 3 (ORAC 3, bound phenolic acids). The bound fraction was reported to contribute about 86 % to the total ORAC (Liyana-Pathirana and Shahidi, 2006). However, in the present study fraction 1 of the total phenolic acids was correlating with the ORAC values (r=0.618, p=0.05), therefore the sum of all three TPA fractions were correlating with ORAC_total (r=0.469, p=0.04).

In conclusion, the current study showed, that TPA and TPC values of the applied ancient wheat samples were comparable to the values in the literature reported for bread wheat or spelt wheat. It could be supposed that phenolic acid contents have not been considerably changed during the evolutionary development of wheat although there is a genetic diversity between einkorn, emmer, spelt and bread wheat. Therefore, health-beneficial effects reported for bread wheat seem to be present in ancient wheat, too. The antioxidative capacity of ancient wheat, e.g. einkorn and emmer, is comparable to bread wheat which leads to the suggestion of using those wheat varieties as an alternative to bread wheat. Einkorn, emmer and spelt, especially used as whole grain flours, could be taken into consideration for human diets, with health benefits. But still, the low bioaccessibility of phenolic acids, especially of ferulic acid from cereals, has to be improved. Reasons for the wide variation of the phenolic acid concentrations within and between species have to be analyzed further. The effect of the germination rate on phenolic acids, total phenolic content and antioxidative capacity needs to be conducted in further studies.

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