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Phenolic acids and antioxidant activity of wild, feral and domesticated diploid wheats

Volkan A. Yilmaz [a], Andrea Brandolini [b, *], Alyssa Hidalgo [c]

[a] Department of Food Engineering, Faculty of Engineering, Ondokuz Mayis University, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Samsun, TR 55139, Turkey

[b] Consiglio per la ricerca e la sperimentazione in agricoltura e l'analisi dell'economia agraria, Unità di Ricerca per la Selezione dei Cereali e la Valorizzazione delle varietà vegetali (CRA-SCV), Via Forlani 3, 26866 S. Angelo Lodigiano (LO), Italy

[c] Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria 2, 20133 Milan, Italy

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ABSTRACT

The aims of this research were: a) to analyse the phenolic acid composition and the total polyphenol content of several wild, feral and domesticated *Triticum* accessions; b) to determine their antioxidant activity by means of diverse spectrophotometric assays (DPPH, FRAP, ABTS and DMPD); and c) to evaluate the effect of different solvents on bioactive compounds extraction and antioxidant assessment. Conjugated and bound phenolic acids were determined by HPLC, while total polyphenol content and antioxidant activity were assessed spectrophotometrically on the extracts obtained with different solvents from whole meal flours. The wild wheats showed the highest phenolic acids, total polyphenol content and antioxidant activity. Among the antioxidant activity assessment methods tested, DPPH, FRAP and ABTS provided similar discrimination of the samples, while DMPD gave different outcomes. The use of several extraction solvents allowed a better characterisation of the antioxidant

activity: acidified methanol and water saturated butanol are the most effective solvents for hydrophilic (phenolic acids and anthocyanins) and hydrophilic as well as lipophilic (carotenoids and tocols) antioxidants, respectively.

1. Introduction

The onset of many human diseases is favoured or exacerbated by cellular and molecular damages caused by reactive oxygen molecules and free radicals. Natural antioxidants, largely present in many plants, help to limit such damage and contribute to prevent mutagenesis, carcinogenesis, cardiovascular diseases, type 2 diabetes and age-related ailments thanks to their radical scavenging activities (Serpen et al., 2008b).

Wheat (*Triticum* spp.), the staple food crop in many parts of the world, is outstanding in terms of cultivated area and food production, and supplies 28% of the world's edible dry matter; in several developing countries contributes more than 60% of the daily calorie intake (Dixon et al., 2009). Therefore, the nutritional quality of its flour has a significant impact on human health and well-being worldwide. Wheat contains substantial quantities of antioxidants, including phenolic acids, flavonoids, anthocyanins, carotenoids, tocols, lignans and phytosterols/phytostanols (Hidalgo and Brandolini, 2014; Ward et al., 2008). Most bioactive compounds are unevenly distributed in the kernel; bran, endosperm and germ show different antioxidant capacities, bran having the highest (Fares et al., 2010). The phytochemical composition of wheat is modified by several factors, like genetic background, cropping environment and processing conditions. Recent researches have shown that some wild and ancient wheats are richer sources of phytochemicals than modern varieties (Giambanelli et al., 2013; Hidalgo and Brandolini, 2014), but further studies are desirable to better understand the potential of the wheat genepool and particularly of the diploid wild and feral *Triticum* species. In fact, two forms of wild einkorn can be recognised, the true wild form *Triticum monococcum* ssp. *thaoudar* and the feral (weedy) form *T. monococcum* ssp. *aegilopoides*, escaped from farming during the spread of agriculture (Schiemann, 1948). Another diploid wild wheat belongs to the species *Triticum urartu* Tum which is the direct ancestor of the A genome of polyploid wheats.

* Corresponding author.
E-mail addresses: wolqun@gmail.com (V.A. Yilmaz), andrea.brandolini@entecra.it (A. Brandolini), alyssa.hidalgovidal@unimi.it (A. Hidalgo).

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The correct determination of phytochemical compounds and of their antioxidant capacity is frequently a challenge, because of the complex matrix and properties of foods. Additionally, it is often very difficult to measure each antioxidant component separately, because of interactions and/or synergistic effects among them. As a consequence, several methods to evaluate the total antioxidant capacity have been proposed (Singh and Singh, 2008). Among them, spectrophotometric methods are very popular because of their simplicity, accuracy, sensitivity and high reproducibility. However the results obtained with diverse methods are often difficult to compare and sometimes downright different. Furthermore, studies that compare different extraction solvents are scarce, as in the articles generally only one solvent is used, and tested across antioxidant assays; when comparisons are available, they are generally limited to a reduced set of extraction methods.

The objectives of this study were therefore: a) to analyse the phenolic acid composition and the total polyphenol content of several wild, feral and domesticated wheat accessions; b) to determine their antioxidant activity by means of diverse spectrophotometric assays (DPPH, FRAP, ABTS and DMPD); and c) to evaluate the effect of different solvents on bioactive compounds extraction and antioxidant assessment.

2. Material and methods

2.1. Materials

Fifteen wheat accessions from different *Triticum* species and subspecies were analysed (Table 1). Among the ten diploid einkorns tested, six were wild (*T. monococcum* L. ssp. *thaoudar*), two were feral (*T. monococcum* ssp. *aegilopoides*) and two were domesticated (*T. monococcum* ssp. *monococcum*). Another diploid sample belonged to the wild species *T. urartu* Tum. The other accessions were two tetraploid *Triticum turgidum* L. (ssp. *dicoccum*, i.e. emmer, and ssp. *durum*, i.e. durum wheat) and two hexaploid *Triticum aestivum* L. (ssp. *spelta*, i.e. spelt, and ssp. *aestivum*, i.e. bread wheat). The domesticated wheats were used as controls.

All the accessions, cultivated in small plots (5 m^2) at Sant'Angelo Lodigiano (Italy) during the 2011–2012 cropping season following standard cultural practices, were hand-harvested at maturity. The ripe, brittle wild accessions were collected as soon as the top spikelets started disassembling.

After harvesting, the kernels were stored at 5 °C. Just before milling the seeds of *T. monococcum*, *T. urartu*, emmer and spelt were de-hulled with a M3B micro-thresher (Co.Mi.L, Rome, Italy); dehulling was not required for the free-threshing durum and bread wheat. The kernels were ground with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark), obtaining a whole meal flour with particle size < 200 μm, which was stored under vacuum at −20 °C for a maximum of 24 h. Dry matter (DM) content was determined by heating ~2 g whole meal flour at 130 °C ± 1 °C for 2 h, and computing the ratio between final and initial weight.

Table 1
Soluble conjugated, insoluble bound and total phenolic acids (mean ± s.e.; mg/kg DM).

Species	Genotype	Soluble conjugated	Insoluble bound	Total
T. monococcum				
thaoudar	ID752	$58.7^{c} \pm 2.5$	$696^{bc} \pm 22$	$755^{b} \pm 20$
thaoudar	ID754	$47.9^{de} \pm 0.0$	$664^{cd} \pm 20$	$712^{c} \pm 20$
thaoudar	ID753	$50.5^{d} \pm 1.2$	$592^{e} \pm 2$	$642^{d} \pm 3$
thaoudar	ID1280	$55.8^{c} \pm 0.7$	$582^{e} \pm 26$	$638^{d} \pm 25$
thaoudar	ID870	$50.4^{d} \pm 2.3$	$706^{bc} \pm 19$	$757^{b} \pm 17$
thaoudar	ID1211	$63.9^{b} \pm 0.8$	$766^{a} \pm 16$	$830^{a} \pm 17$
aegilopoides	ID227	$57.7^{c} \pm 2.4$	$651^{d} \pm 16$	$709^{c} \pm 19$
aegilopoides	ID228	$56.2^{c} \pm 1.0$	$739^{ab} \pm 17$	$795^{ab} \pm 16$
monococcum	ID396	$34.4^{g} \pm 0.6$	$482^{g} \pm 8$	$516^{g} \pm 7$
monococcum	ID493	$44.7^{e} \pm 2.0$	$524^{fg} \pm 6$	$568^{ef} \pm 8$
T. urartu	ID1277	$70.8^{a} \pm 1.0$	$760^{a} \pm 9$	$831^{a} \pm 8$
T. turgidum				
dicoccum	FAR262	$44.3^{e} \pm 0.7$	$506^{fg} \pm 2$	$551^{efg} \pm 1$
durum	Dylan	$28.2^{h} \pm 0.8$	$482^{g} \pm 6$	$510^{g} \pm 6$
T. aestivum				
spelta	FAR62	$39.7^{f} \pm 0.8$	$538^{f} \pm 5$	$578^{e} \pm 6$
aestivum	Blasco	$50.2^{d} \pm 1.5$	$483^{g} \pm 9$	$533^{fg} \pm 8$

Means within a column with at least one identical superscript do not differ significantly ($p > 0.05$) while means with various superscripts show a significant difference ($p \leq 0.05$).

2.2. Extraction and analysis of soluble conjugated and insoluble bound phenolic acids

The extraction of the soluble conjugated and the insoluble bound phenolic acids was performed as described by Brandolini et al. (2013). Briefly, exactly 0.5 g of whole meal flour were mixed with 15 mL of a methanol/acetone/water (7:7:6) solution. After 15 min in an ice bath under discontinuous vortexing and after centrifugation (11,178 *g*, 10 min, 8 °C) with a Centrikon K24 centrifuge (Kontron Instruments, Bletchley, UK), the supernatant was recovered; the extraction from the sediment was repeated twice more, and the three extracts were pooled.

2.2.1. Soluble conjugated phenolic compounds

The supernatant was evaporated under vacuum at 35 °C for 18 min with a rotator evaporator Laborota 4000 (Heidolph, Milan, Italy), and nitrogen flux for 1 min. The samples were then digested with 15 mL of 4 M NaOH under nitrogen for 4 h at room temperature and continuous shaking, brought to pH 1.5–2 with 6M HCL and extracted twice with 20 mL of diethyl ether/ethyl ether (1:1 v/v). The extracts were then clarified with sodium sulphate, filtered with glass fibre 110 μm (Whatman, Maidstone, England), evaporated as previously outlined, resuspended in 2 mL of methanol:-water (1:1 v/v) and filtered with a 0.22 μm PTFE membrane (Millipore, Carrigtwohill Co., Cork, Ireland).

2.2.2. Insoluble bound phenolic compounds

The sediment was digested with 15 mL of 4M NaOH under nitrogen for 4 h at room temperature and discontinuous vortexing, brought to pH 1.5–2 with 6M HCL and extracted twice with 20 mL of diethyl ether/ethyl acetate (1:1, v/v). After centrifugation (11,178 *g*, 10 min, 8 °C), the supernatants were clarified with sodium sulphate, filtered, evaporated as previously outlined, resuspended in 2 mL of methanol-water (1:1 v/v) and filtered with a 0.22 μm PTFE membrane (Millipore, Carrigtwohill Co., Cork, Ireland).

All extractions were performed in duplicate under dim light; the extraction tubes were wrapped with aluminium foil, to avoid sample degradation by photo-oxidation.

2.2.3. HPLC determination of individual phenolics

Soluble conjugated and insoluble bound phenolic acids were analysed by HPLC as described by Brandolini et al. (2013). The results are expressed as mg/kg DM.

2.3. Extraction procedures for total polyphenol content (TPC) and antioxidant activity analysis

Exactly 0.5 g of whole meal flour were extracted with 5 mL of different solvents: 80% ethanol (EtOH) (Liyana-Pathirana and Shahidi, 2006), methanol:acetone:water (7:7:6 v:v:v; MeOH:acetone:H_2O) (Brandolini et al., 2013) or methanol:water solution (80:20 v:v) acidified with 1% HCl (MeOH:HCl) (Fares et al., 2010) under agitation using vortex (30 s) and an orbital stirrer PTR-35