11

Hulless Barley – A Rediscovered Source for Functional Foods Phytochemical Profile and Soluble Dietary Fibre Content in Naked Barley Varieties and Their Antioxidant Properties

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1. Introduction

1.1 The history of the use of barley

The beginning of agriculture is dated back to 10,000 years ago in the Pre-Pottery Neolithic Near East. Barley (*Hordeum vulgare* L.) is one of the cereal founder crops and it is believed that first plant domestication took place within the Fertile Crescent (Lev-Yadun et al., 2000). *Hordeum vulgare* L. subsp. *spontaneum* (wild barley) is said to be the ancestor of today's barley. The spread of barley most likely started in present-day Israel, northern Syria, southern Turkey, eastern Iraq and western Iran. With the movement of civilizations accompanied by the establishment of trade routes the use and cultivation of barley reached Europe. Barley was a popular food in ancient Greece and Italy and used as an ingredient for preparing porridge or unleavened bread. Greek and Roman scholars such as Hippocrates or Pliney the Elder, respectively, considered barley as a healthy and nourishing food and barley gained as well recognition for medical treatments. In the ancient Rome, gladiators believed that barley could increase strength and stamina and thus preferred it to other cereals. Barley reached Spain around 5,000 BC and spread then over today's Germany and France. Indications of domestication of barley on the British Isles date back until 3,000 BC and one millennium later, barley was introduced to Northern Europe. Probably due to the nourishing properties and the ruggedness of the crop, barley became a major food especially for poor people throughout history (Newman & Newman, 2005).

In 2009, barley was the twelfth most important agricultural commodity of the world in terms of production. After maize, wheat and paddy rice it was the fourth most important

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cereal crop (FAO, 2011). The annual production was about 152 million tons, of which the Russian Federation produced around 17.9 million tons followed by France, Germany and Ukraine with 12.9, 12.3 and 11.8 million tons, respectively. In the European Union, barley still attains distinction as the second cereal crop after wheat.

Barley falls botanically into the family of the grasses (*Poacea*=*Gramineae*), tribe *Triticeae*, genus *Hordeum*. It is a diploid (2n=2x=14) mainly self-pollinating crop. In contrast to domesticated variations wild barley has a brittle spike where the ripe spikelets separate from each other easily; this favours the spread of the seed but complicates harvest. Non-brittle variations can be harvested unproblematically but survive only under domestication. A barley head consists of triplets of spikelets that are alternatively arranged on the rachis. Due to the morphology of the spikelets one can distinguish between two- and six-rowed barleys. Two-rowed barleys have two lateral unfertile spikelets in each triplet and thus only two rows of fertile spikelets. In six-rowed barley all six spikelets are fertile (Takahashi, 1955; Zohary & Hopf, 2000).

Cultivated barley can as well be classified by the form of their caryopsis. In hulless (syn. hull-less or naked) barley varieties the expression of the recessive naked caryopsis gene (*nud*, nudum) prevents the intergrowth of husks and caryopsis. Consequently, the kernels thresh free and lemma and palea do not adhere to the caryopsis at maturity. This is why hulless barley (*Hordeum vulgare* var. *nudum*) requires no further dehulling for the production of food. Cultivation of hulless barley is as old as that of hulled barley but is less common worldwide due to significantly lower yields and only minor breeding activities (Atanassov et al., 2001; Pandey et al., 2006). Compared to hulled barley, the free-threshing character of hulless barley proportionally increases contents of protein and the limiting amino acids lysine and thereonine, respectively, (Baidoo & Liu, 1998; Bhatty, 1999) and as well levels of β-glucan but lowers contents of insoluble dietary fibre components (Xue et al., 1997; Baidoo & Liu, 1998).

1.2 Importance of barley as food ingredient

Starch is the major component in barley kernels amounting 60 – 70% of the dry matter. Starch itself is composed of two types of glucose polymers namely the highly branched amylopectin and the linear amylose. According to the proportions of amylose and amylopectin barley can further be classified: normal naked barley contains 25 – 30% amylose, waxy varieties less than 15% whereas high-amylose cultivars more than 35% amylose (Ajithkumar et al., 2005; Bhatty, 1986; 1999). Among the waxy cultivars there are also zero-amylose waxy barleys that contain no amylose (Izydorczyk et al., 2000). Both polymers strongly influence the quality of the obtained products, where amylopectin is responsible for the crystallinity of starch and excellent water absorbing properties (Bhatty, 1997).

Hulless barley is a good source of dietary fibre providing soluble and insoluble dietary fibre fractions (Bhatty, 1999; Izydorczyk et al., 2000). Mixed-linkage (1→3),(1→4)-β-D-glucans (hereafter termed as β-glucan) are a major part of the soluble dietary fibre (SDF) in barley. Nevertheless, its content generally underlies a natural fluctuation depending on the variety and conditions before and after harvest (Ehrenbergerová et al., 2008). Together with

arabinoxylans, a fraction of partly soluble nonstarch polysaccharides (NSP) occurring in the cell walls, β-glucan has a great impact on cereal processing and product properties. In Europe, the European Food Safety Authority (EFSA) has come to the decision that a claim like "regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations" will be allowed (EFSA, 2009). Until then, the interest of food producers and consumers in using hulless barley for food purposes may increase, although up to now, barley flour is hardly used for human consumption. It is assumed that barley β-glucans have the same effects than oat β-glucans. In order to bear the claim, EFSA demands a quantity in food of at least 3 g/day of β-glucans from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed β-glucans in one or more servings.

On the other side of the coin, the higher the β-glucan content in barley flours the worsen are the baking qualities, due to the high water binding capacity and thus decreasing the water availability for the gluten network (Gill et al., 2002). Furthermore, high β-glucan contents must not necessarily go along with increased health enhancing properties unless the extractability and/or digestibility are not considered (Xue et al., 1997).

1.3 Phytochemicals in barley

Over the last decade, fruits, vegetables and cereals have been studied for various bioactive compounds such as phenolics and flavonoids as health promoting and disease preventing effects were found in both *in vitro* and *in vivo* studies. Phytochemicals, per definition, are substances that are likely to contribute to health or are essential for the maintenance of health (Cooke et al., 2002). In contrary to vitamins or minerals, there is no evidence that they are essential or even required to sustain life. It is hypothesised that phytochemicals that are located in the fibre matrix, in addition to or instead of the fibre itself, are responsible for the reduced risk of various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (Jacobs & Steffen, 2003). Thus, the consumption of whole grains is seen as health-promoting (Slavin, 2003), because whole-grains and products thereof present a good source of natural antioxidants (Decker et al., 2002). Polyphenols constitute together with carotenoids the only dietary antioxidants present in the colon in valuable concentrations (Scalbert & Williamson, 2000; Manach et al., 2004). The release of phenolics in the colon may explain the inverse association between whole-grain consumption and incidence of certain chronic diseases (Andreasen et al., 2001; Kern et al., 2003). Moreover, an apparent increase of antioxidant release during enzymatic incubation was found in *in vitro* studies (Pérez-Jiménez & Saura-Calixto, 2005; Nagah & Seal, 2005; Menga et al., 2010).

Barley grains contain a wide range of phenolic acids, which are either derivatives of benzoic acid (C_6-C_1) or cinnamic acid (C_6-C_3). In general, higher levels of phenolic compounds were reported for barley and oat compared to wheat and rye (Zielinski & Kozlowska, 2000). Ferulic acid is the most abundant phenolic acid present in cereals, representing up to 90% of total polyphenols (Sosulski et al., 1982). Also Hernanz et al. (2001) and Naczk & Shahidi (2006) reported that ferulic acid is the dominant free phenolic acid in barley seeds. The composition of phenolic acids in 30 barley varieties was investigated by Yu et al. (2002) where they found varying levels of benzoic and cinnamic acids. Phenolic compounds are

said to exhibit one or more of the following roles: free radical scavenger, reducing agent, potential producer of prooxidant metals and quencher of singlet oxygen formation.

Anthocyanins in wheat and barley are found either in the pericarp or the aleurone layer causing purple and blue hues of kernel colour, respectively. The black pigmentation of the lemma and pericarp of barley, however, is described to be due to melanin-like pigment (Woodward, 1941; Lundqvist et al., 1996) which may overlap other pigments. Black kernel colour due to melanin-like pigment is unknown to wheat species.

Lutein and zeaxanthin are the two main carotenoids identified in barley (Goupy et al., 1999; Panfili et al., 2004). The electron rich chain makes them effective radical scavengers (Cooke et al., 2002) and inhibit free radical propagation reactions such as lipid peroxidation. Lutein and zeaxanthin are responsible for the coloration of the macula lutea ('yellow spot') of the retina, the area of maximal visual acuity. Hence, dietary lutein and zeaxanthin are supposed to protect against age-related macular degeneration and cataract (Seddon et al., 1994; Beatty et al., 2000). Furthermore, lutein and zeaxanthin possibly act together with other bioactive compounds against cancer, cardiovascular risk and other diseases (Wahlqvist & Wattanpenpiaboon, 1998; Mares-Perlman et al., 2002; Calvo, 2005). Vitamin E or tocopherols are also present in markedly concentrations (Cavallero et al., 2004; Andersson et al., 2008).

2. Materials and methods

2.1 Barley samples

Twenty-nine barley varieties were grown at the experimental station Groß-Enzersdorf (16°35′ E; 48°13′ N) in 2007, 2008 and 2009. The varieties were sown at the beginning of March and harvested mid July. Monthly precipitation and mean temperature of the growing seasons at the growing site for the three years are shown in Fig. 1. Post-harvest treatment included sifting via air classification. Barley samples were stored at 4 °C until use. Samples were milled directly before analyses using a laboratory mill (MF10 basic with a 1 mm sieve, IKA, Austria) to obtain wholemeal samples.

Twelve coloured naked barley varieties were further selected to study the distribution of the compounds of interest within the different milling fractions. These varieties were milled with a MLU 202 roller mill (Bühler, Switzerland). The grain was not conditioned before milling and the feed rate for milling was approximately 5 kg h^{-1}. Six flour fractions (B1-B3, C1-C3) from the starchy endosperm were collected and merged to give a straight-run white flour. Brans and shorts were collected separately. All millstreams were stored at 4 °C until use.

2.2 Analyses

2.2.1 Agronomic traits

Data were collected for thousand kernel weight (TKW, g), hectolitre weight (HLW, kg hL^{-1}) and seed plumpness (SP25, %). HLW was measured by a ¼ L chondrometer. Counting of seeds was done by a Contador machine (Pfeuffer GmbH, Kitzingen, Germany) and weighed. SP25 was determined by sieving 100 g of grain with a Sortimat laboratory machine (Pfeuffer GmbH, Kitzingen, Germany). Grains remaining on the 2.5 mm sieve were considered as plump.

2.2.2 Basic chemical composition

Moisture and ash content of the barley flour was determined according to AOAC approved standard methods 940.56 and 920.153, respectively (AOAC, 1995). Crude protein (PROT) was determined according to the ICC standard method 105/2 using the factor 5.83×N for conversion, whereas total starch content was determined according to the ICC standard method 168 (ICC, 2001).

2.2.3 Dietary fibre

Mixed linkage β-glucan (BG) (AOAC method 995.16) content was determined in barley wholemeal samples using a commercially available test kit (Megazyme, Bray, Ireland).

Total pentosanes (PENT) and water-extractable pentosanes (we-PENT) were analysed according to the method described by Douglas (1981). Briefly, for determination of we-PENT, 25 mg sample were suspended in 10 mL water and extracted for 2 h on a rotary shaker. One millilitre of the aqueous phase was mixed with 5 mL of the reaction solution (consisting of 110 mL glacial acid, 2 mL HCl conc., 5 mL phloroglucinol solution (20% (w/v) in ethanol) and 1 mL 1.75% (w/v) glucose solution) and incubated for 25 min in a boiling water bath and shaken from time to time. After cooling, the we-PENT content was calculated from the difference of the absorbances at 510 and 552 nm according to a xylose calibration curve (2 g L^{-1}). To determine total pentosanes (PENT) content, 5 mg of the sample was suspended in 2 - 4 mL water and incubated with the reaction solution.

2.2.4 Secondary plant metabolites

Extraction of free and bound phenolic compounds

Free and bound phenolic acids were extracted according to the methods of Adom et al. (2003) and Mattila et al. (2005) with minor modifications. Briefly, 0.15 g of barley flour was extracted twice with 80% aqueous methanol for 30 min. Supernatants were pooled after centrifugation at 2500*g* for 10 min and represented the crude extracts of free phenolics. Samples were stored at -20 °C until HPLC analysis and filtered prior injection into the HPLC.

To extract the bound phenolic compounds, the above obtained residues were blended with sodium hydroxide to reach a final concentration of 2 M NaOH. Samples were vortex mixed and incubated in the dark on a shaker (Rotator, VWR, Austria) overnight (16 h). The mixture was brought to pH 3 with hydrochloric acid. After centrifugation, supernatants and residues were extracted separately three times with ethyl acetate. The combined ethyl acetate fractions were evaporated to dryness and stored at -20 °C until analysis. Before quantification, bound phenolic compounds were dissolved in 50% methanol.

Anthocyanin content

Anthocyanins were extracted with acidified methanol (methanol and 1 M HCl, 85:15, v/v) with a solvent to sample ratio of 1:10 for 30 min on a magnetic stirrer and then separated by centrifugation. The supernatants were collected and kept in the dark and cold (+4 °C) until procedure. The residues were twice re-extracted under the same conditions, and supernatants

of all three cycles were combined. Total anthocyanin content (TAC) was calculated using a calibration curve of cyanidin-3-O-glucoside (C-3-glc) as reference. Extracts were measured at 525 nm in a U-1100 spectrophotometer (Hitachi, Tokyo, Japan). Contents were expressed as mg C-3-glc equ. per 100 g, dm (Abdel-Aal & Hucl, 1999).

Extracts were further proceeded to quantify different anthocyanidins. Therefore, samples were kept at -30 °C overnight and the precipitates were separated via centrifugation at 2500*g* thereafter. The solvent was removed at 40 °C using a rotary evaporator and the resulting anthocyanin concentrates were re-suspended in methanol prior to HPLC analysis.

Carotenoid content

Five gram of barley flour and 0.02 g butylated hydroxytoluene (BHT) were weighed into amber glass flasks. After addition of 20 mL of the extraction solvent (MeOH:ethyl acetate:petroleum ether, 1:1:1, v/v/v), samples were extracted on a magnetic stirrer for 2 h and then filtrated to recover the extract and the residue. The extraction procedure was repeated twice using the residue. The collected combined supernatants were transferred into a separation funnel and washed three times with a saturated NaCl-solution (37.5% NaCl in water). Remaining water in the organic phase was thereafter removed with sodium sulphate and the extract was evaporated until dryness at 35 °C. Samples were further kept under a stream of nitrogen to avoid any oxidation, sealed and immediately stored at -20 °C until analysis. Prior analysis, samples were thawed, solubilised in tetrahydrofuran (THF) and transferred into amber glass HPLC vials and kept under nitrogen-stream before closing.

HPLC analysis

The profile of anthocyanidins and their corresponding glycosides as well as phenolic compounds and carotenoids were determined using a HPLC system (Shimadzu, Korneuburg, Austria) consisting of a SPD-M10AVP photodiodearray detector, chromatogram integrator, LC-10ADVP pump and online degasser. Data signals were processed on a PC running the LC solution Multi software (Shimadzu, Korneuburg, Austria). Analytical separation of anthocyanidins and phenolic acids was carried out using a Phenomenex Luna 250×4.6 mm, 5 µm (HPLC Services, Breitenfurt, Austria) column, whereas carotenoids were separated on a Vydac 201TP54, C18 5µm, 250×4.6 mm (MZ-Analysentechnik GmbH, Mainz, Germany) column.

For the determination of anthocyanidins, dissolved anthocyanidin concentrates were passed through a 0.45 µm PTFE-filter and a 20 µL aliquot of the sample solution was injected. Elution for anthocyanidins was executed under gradient conditions with (A) 4.5% formic acid in water and (B) acetonitrile. The solvent gradient was programmed as follows: 10% B at 0 min, increasing to 12% within 9 min, to 13% within the next 7.5 min, 25% within the next 13.5 min, 90% within the next 15 min, holding at 90% for 5 min, followed by a decrease to 10% within the next 5 min, before equilibration at 10%. The solvent flow rate was set at 0.8 mL min^{-1} and the chromatogram was recorded at 520 nm (anthocyanidins and glycosides) at 35 °C. Peak areas were used for all calculations. Identification of compounds was done by comparing the retention time and the UV spectra with those of pure substances. DAD response was linear for all anthocyanidins and anthocyanins within the

calibration range of 0.05 – 40.0 μg mL^{-1}, with correlation coefficients exceeding 0.999. Coefficients of variation for sample replicates were consistently below 10%.

Phenolic acids were determined as follows: Twenty microliter of sample were injected into the column and eluted under gradient conditions performed with 0.05% trifluoroacetic acid in water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The solvent gradient was programmed as follows: at 0 min, 10% B; increasing from 3 to 15 min to 15% B; 25 min, 20% B; 30 min, 40% B; 36 – 40 min, 80% B; decreasing thereafter to 10% B within the next 4 min and equilibrating before the next injection. The flow rate was 1.0 mL min^{-1}. Analyte detection was at 260 nm for 4-OH-benzoic and vanillic acid and at 280 nm for caffeic, *p*-coumaric and *trans*-ferulic acid. DAD response was linear for all phenolic acids within the calibration range of 0.06 – 125.0 μg mL^{-1}, with correlation coefficients exceeding 0.999. Phenolic acids in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds. Coefficients of variation for sample replicates were consistently below 10%.

Carotenoids were separated under isocratic conditions with methanol:acetonitrile (9:1, v/v) and 1 mL min^{-1} flow rate. The temperature was set at 25 °C, and lutein (LUT) and zeaxanthin (ZEA) were detected at 450 nm. Peak areas were used for all calculations. Identification of compounds was done by comparing the retention time and the UV spectra with those of pure substances.

2.2.5 Determination of the antioxidant capacity

Extracts containing the free and bound phenolic compounds were analysed for their total antioxidant capacity by the ABTS radical cation scavenging assay (Trolox equivalent antioxidant capacity (TEAC)) and the ferric reducing antioxidant power (FRAP) assay. To distinguish between the two extracts, the terms TEACf and FRAPf were used for the extract containing the free phenolic acids, whereas TEACb and FRAPb refer to the extract containing the bound phenolic acids.

The total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu reagent (Singleton et al., 1999) and was termed thereafter as TPCf or TPCb for free and bound phenolics, respectively. An appropriate dilution of extracts (120 μL) was added to 600 μL of freshly diluted 10-fold Folin-Ciocalteu reagent. 960 μL of sodium carbonate solution (75 g L^{-1}) was added to the mixture after 2 min reaction time. The absorbance of the resulting blue colour was measured at 760 nm against a blank after 5 min of reaction at 50 °C. Ferulic acid was used as standard, and TPC was expressed as mg ferulic acid (FA) equ. per 100 g, dm.

The TEAC assay was analysed following a modified method of Pellegrini et al. (2003) and Moore et al. (2005). A stable stock solution of ABTS radical cation was produced by reacting a 7 mmol L^{-1} aqueous solution of ABTS with 2.45 mmol L^{-1} potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 – 16 h before use. On the day of analysis, an ABTS radical cation working solution was obtained by diluting the stock solution in ethanol to an absorbance of 0.70 ± 0.02 AU at 734 nm. Hundred microliters of extract were mixed with 1.25 mL of the ABTS working solution and

absorbance was red at 734 nm after a 1 min reaction time. Results were expressed as TEAC in mmol of Trolox per kg, dm.

The FRAP assay is based on the reduction of the Fe^{3+}–TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 595 nm (Benzie & Strain, 1999). Briefly, 0.2 mL of sample extract was mixed with 1.3 mL of the FRAP reagent. Absorption was measured at 595 nm in a spectrophotometer (U-1100, Hitachi, Japan) after 30 min of incubation at 37 °C. The FRAP reagent was prepared daily and consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v). FRAP values were obtained by comparing the absorption change in the test mixture with doses obtained from increasing concentrations of Fe(III) and expressed as mmol of Fe(II) equ. per kg, dm.

2.2.6 Statistical analysis

Data were reported as mean ± standard deviation for at least duplicate extracts. Statistical analysis were carried out using STATGRAPHICS Centurion® XV (StatPoint Technologies Inc., Warrenton, VA), SAS® 9.2 (SAS Institute Inc., Cary, NC) and/or GenStat® 13th Ed. (VSN International Ltd., Hemel Hempstead, UK) software packages. Linear mixed model analysis of variance was carried out for the multi-year data (2007-2009) with genotypes as fixed effects and year and genotype by year interaction (G×Y) as random effects. Model selection was based on the corrected Akaike information criterion (AIC). Significance of random effects was tested by the log-likelihood ratio test. Multivariate analyses were performed with collected data from 2008, i.e. principal component analysis (PCA) and canonical discriminant analysis. Multiple mean comparisons were carried out at the 5% significance level using the Duncan's multiple range test.

3. Results

3.1 Description of investigated hulless barley genotypes

Twenty-nine spring barley genotypes with different countries of origin, two- or six-rowed types as well as different seed colours were selected for this study. HB803 and Pronghorn (Washonubet) are white waxy genotypes, whereas all others were of normal starch type. Pronghorn was produced by crossing Wanubet and Shonubet and is currently filed as patent (Patent No. 20090064357).

The seed colour of barley genotypes is dependent on different pigments which are located in different seed layers. A great diversity from yellowish-white to blue, purple and black is reported in the literature. The interest in re-introducing coloured varieties is closely associated with their possible health promoting effects. Most of black and blue genotypes came from Ethiopia, a country rich in coloured barley germplasm. Apart from Purple Nudum, all purple classified genotypes are 6-rowed and bear the term "black" in their name. As they originated in majority from China, it is anticipated that the investigated genotypes have been selected from Chinese landraces. After their first propagations in 2006, all genotypes have been classed according to their seed colour. As the colour appeared dark-purple to black, they were finally carried on as purple (Table 1).

Id	Variety name	Accession No.	Origin[1]	Rows	Colour	TKW[2]	HLW	SP25	PROT	BG
1	SNG04		AT	2	Black	38.3	76.4	40.44	11.57	4.84
2	Ae 13 dunkel	BVAL358117	ET	6	Black	37.2	77.0	46.49	11.78	4.63
3	Naked Black	BVAL358163	ET	2	Black	31.6	73.5	20.34	10.35	4.78
4	Violaceum 2	C661	CN	6	Black	30.6	78.2	44.25	12.28	5.00
5	Ethiopia 179	E360 (Clho9978)	ET	2	Black	37.8	77.6	44.02	14.16	4.45
6	Ethiopia 12	E604 (CI967, PI24901)	ET	2	Black	38.2	74.1	24.28	15.11	4.74
7	Black Naked		SY	2	Black	26.3	76.6	11.99	12.79	4.33
8	Murasaki Hadaka	J307	JP	6	Black	40.0	76.0	41.73	12.86	5.11
9	Digersano		IT	2	White	37.2	77.9	30.97	12.20	3.88
10	HB803 [2]		CA	2	White	45.7	67.9	63.62	12.53	5.05
11	Hora	BVAL350010	DE	2	White	37.2	69.9	50.37	12.14	3.86
12	Lawina		DE	2	White	38.7	75.8	44.66	14.41	4.43
13	Taiga	BVAL350017	DE	2	White	37.6	75.4	43.63	12.77	4.92
14	Pronghorn (Washonubet) [2]		US	2	White	31.0	71.8	13.61	11.51	5.97
15	Himalayense Type 5	C651	CN	6	Blue	34.7	76.6	38.42	13.31	4.84
16	Debre Zeit AES 2 (12-20-3)	E515	ET	2	Blue	38.9	75.2	22.15	13.13	5.81
17	Addis Ababa 56 (3-10-1b)	E550	ET	6	Blue	30.1	73.9	13.79	14.30	4.93
18	Ethiopia 96	E632	ET	6	Blue	25.8	75.3	13.02	13.43	4.73
19	Ederle Sel. Blau	GE040	AT	6	Blue	28.7	74.1	18.46	14.71	4.23
20	Indian 3	I311	IN	6	Blue	30.7	75.4	12.92	14.06	4.78
21	Gho 1 (1392)	N308	NP	6	Blue	28.5	77.9	37.97	10.77	4.50
22	Sama 9 (1462)	N624	NP	6	Blue	30.1	75.0	26.40	12.41	4.59
23	Black Hull-Less	HOR11402	CN	6	Purple	33.4	73.8	37.33	15.06	5.45
24	Schwarze Nackte Kraftborn	HOR2199	DE	6	Purple	32.7	78.5	46.69	13.88	5.33
25	Black Hull-less	HOR2593 (PI24849)	CN	6	Purple	36.4	73.7	40.78	14.81	5.10
26	Schwarze Chinesische	HOR3727	CN	6	Purple	32.9	78.9	38.13	12.40	4.50
27	Purple Nudum	HOR4024 (Clho2250)	PK	2	Purple	33.7	81.3	29.29	13.52	5.71
28	Black Hulless	HOR4940 (Taastrup 625)	US	6	Purple	36.0	76.1	43.05	14.02	4.98
29	Lih Dhanra Gal (1459)	N023	NP	6	Purple	33.6	78.2	58.23	12.32	5.65
	Average S.E.D.					*2.6*	*2.8*	*11.53*	*1.40*	*0.50*

[1] AT, Austria; CN, China; DE, Germany; ET, Ethiopia; IN, India; NP, Nepal; PK, Pakistan; SY, Syria; US, Uni
kernel weight (g); HLW, hectolitre weight (kg·hL^{-1}); SP25, seed plumpness (% of seeds that remain on a 2.5 mm
content (%); BG, beta-glucan content (%); TPC, total phenolic content (mg FA equ.·100 g^{-1} dm); TAC, total a
equ.·100 g^{-1} dm); LUT, lutein content (ppm); ZEA, zeaxanthin content (ppm). [3] waxy genotypes.

Table 1. Description of the investigated spring hulless barley genotypes and their least square mean
traits (2007-2009)

3.2 Analyses of three consecutive harvests

In 2007, sowing of spring cereals was followed by a six weeks period without rainfall resulting in reduced plant emergence and development of stands with the consequence of lower performance of spring barley in regard to agronomic traits. The establishment of stands was also limited in 2008 due to the absence of sufficient rainfall in March and April. At anthesis (end of May), during the soft dough stage of grain filling and at harvest (mid July), the rainfalls were higher than the average. The climatic conditions of 2009 were comparable with 2007 in spring, whereas sufficient rainfall was available in June and July (Fig. 1). In a previous study, no correlation was found for the precipitation between 2006 and 2007, whereas the mean temperatures showed a strong association. Therefore, the limiting factor for a sustainable spring crop production in eastern Austria is the unequal distribution of rainfall from year to year (Bokore, 2008).

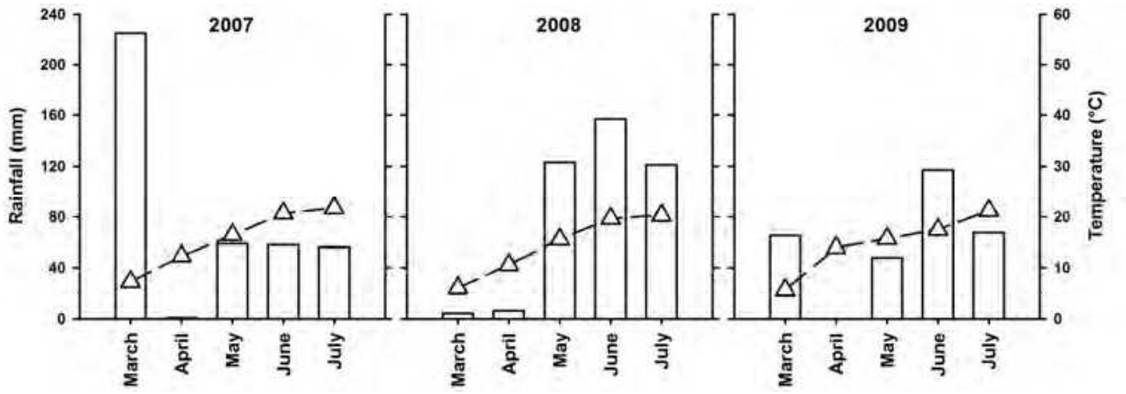


Fig. 1. Precipitation (illustrated as bars) and mean temperature (displayed as triangles) at the experimental site over the investigation period

Mixed model ANOVA revealed significant differences among genotypes (Table 1). Mean TKW and HLW of three consecutive harvests varied within genotypes and ranged from 26 – 46 g and 68 – 81 kg hL^{-1}, respectively. Highest TKW on a three years average was found for the white waxy genotype HB803 followed by the black Murasaki Hadaka and the white Lawina. Only a small range within HLW was observed, where highest values were found in the group of purple genotypes (Schwarze Nackte Kraftborn, Schwarze Chinesische and Purple Nudum). Highest seed plumpness (SP25) was recorded for HB803, Hora and the purple Lih Dhanra Gal. Regarding the investigated quality traits, highest protein values were found in the group of purple genotypes (Black Hull-less) and the blue Ederle Sel. Blau. β-Glucan content ranged from 4 to 6% in average, where the highest value belong to Pronghorn, a white waxy genotype followed by Debre Zeit AES 2 (5.8% dm) and Purple Nudum (5.7% dm). TPCf differed between the sample set widely, where the lowest levels were recorded for black genotypes and the highest for the white and blue group. By far the highest TAC was found for the purple genotypes, followed by the blue and black genotypes. LUT and ZEA were quantified using HPLC, and highest levels were found for the black genotypes SNG04, Violaceum 2 and Murasaki Hadaka.

Eticha et al. (2011) recently published a comparison of agronomic and quality traits of 13 diverse anthocyanin pigmented wheat genotypes. Concerning random effects, they found

significant greater variance components for G×Y than that of the year effect for all traits with the exception of heading date.

An excerpt of the gross composition of whole meal samples of the 29 barley genotypes, harvested in 2008 is presented in Table 2. Available starch was the major constituent (43 - 65% dm), whereas the protein content ranged from 8.7 to 13.3% dm.

Earlier studies indicated that hulless barley genotypes are richer in β-glucan compaired to their hulled counterparts. In this study, the β-glucan content of the 29 hulless barley genotypes ranged from 3.2 (Hora) to 6.1%. With 4.8 and 5.6% β-glucan the waxy genotypes HB308 and Pronghorn were significantly higher than the average of this sample set, but lower as the purple variety Purple Nudum (Table 2). There seems to be no relationship between kernel size and the content. The variation in the β-glucan content might underlie other genetic variations and is strongly determinated by the variety. Generally, high-amylose and waxy hulless barleys tend to contain more β-glucan than zero-amylose and regular hulless barleys. More precisely, waxy barley cultivars possess higher levels of water and acid extractable β-glucans than regular barleys (Ajithkumar et al., 2005; Izydorczyk et al., 2000). Our results are consistent with previously published studies (Andersson et al., 2008; Gao et al., 2009).

Total pentosanes (PENT) in barley wholemeal flours varied greatly between 4.1 and 9.2% dm (Table 2). With that wide range, barley genotypes are between the ranges of wheat and rye. Holtekjølen et al. (2006) found generally higher values for hulless genotypes and this observation was later on confirmed within the HEALTHGRAIN diversity screen (Andersson et al., 2008). Water-extractable pentosanes (we-PENT) were a minor constituent ranging from 0.1% (Taiga) to 1.1% (Purple Nudum).

Wholemeal samples were as well extracted for free and bound phenolic acids and the Folin Ciocalteu reagent was used to measure the total phenolic content (TPC). Antioxidant properties were determined from both extracts by estimating the radical scavenging abilities using the stable ABTS radical (thereafter termed TEACf and TEACb) and the reducing abilities of Fe(III). The latter results were expressed as FRAPf and FRAPb (Table 2). Highest TPCf and TPCb were found for the blue genotypes Debre Zeit AES 2 and Addis Ababa 56, followed by the white genotype Lawina. Interestingly, both TPC of the free and the bound fraction ranged between 200 and 500 mg FA equ. 100 g^{-1} and thus are at the first glance in contrast with findings from Verardo et al. (2011) and Madhujith & Shahidi (2009) who observed higher phenolic contents in the bound fraction. However, similar observations were found with samples of 2009, when twelve genotypes were milled and the three millstreams were analysed for their content of phytochemicals. Mean average of TPCf was higher than TPCb for the flour (grouped according to seed colour) (Fig. 3 & 4) but not for the shorts and bran fraction, whereas the content of quantified phenolic acids determined by HPLC showed clearly higher contents in the bound fraction for almost all detected phenolic acids. Thus, components co-extracted with the phenolic compounds may have interfered with the test reagent. Similarly, no differences between free and bound phenolics were found in terms of reducing abilities of Fe(III), but with $ABTS^{\bullet+}$ scavenging capacities.

None of the attributes correlated with TPCf, whereas highly significant (p<0.0001) correlations were obtained for the antioxidant properties and the TPC of the bound phenolic fraction, i.e. TEACb correlated with TPCb (r=0.74) and with FRAPb (r=0.82). Correlation was as well found between FRAPb and TEACb (r=0.81). Comparable correlation coefficients for phenolic

ID[1]	PROT[2]	ASH	STARCH	BG	PENT	we-PENT	TPCf	TPCb	FRAPf	FRAPb	TEACf
1	12.0	0.82	54.6	4.5	6.0	0.54	243.3	211.4	22.9	18.8	4.19
2	9.7	1.39	52.8	4.5	5.3	0.54	284.2	261.4	31.2	22.7	4.96
3	8.3	1.36	48.3	4.7	4.1	0.27	216.9	219.2	23.1	18.0	4.30
4	9.6	0.85	54.8	4.4	8.3	0.71	365.5	427.6	40.6	36.3	13.70
5	11.5	0.91	55.2	4.3	5.0	0.44	246.5	210.5	25.7	19.0	4.28
6	13.4	1.01	50.3	4.4	5.8	0.27	306.0	271.4	35.8	28.3	5.56
7	10.7	1.68	49.7	4.1	6.5	0.56	294.5	222.4	35.1	25.1	5.11
8	11.5	0.94	53.8	4.3	5.7	0.43	472.8	231.6	27.4	20.2	3.60
9	8.8	0.83	64.8	3.2	4.5	0.50	249.9	204.5	37.9	19.8	4.76
10	10.6	0.68	59.5	4.6	4.1	0.24	459.3	278.3	39.8	19.4	4.12
11	9.2	0.82	59.1	3.2	4.1	0.24	460.0	241.1	44.2	21.0	3.67
12	10.4	0.93	51.1	4.8	4.7	0.39	514.2	262.9	40.5	28.6	8.30
13	12.3	0.91	49.7	4.7	4.5	0.12	482.6	311.4	45.1	23.6	11.30
14	8.7	0.99	54.8	5.6	4.8	0.49	314.2	233.4	49.2	22.1	9.07
15	10.8	0.90	51.3	4.8	7.8	0.75	490.5	247.9	32.1	31.4	5.53
16	10.8	0.83	54.0	5.3	5.3	0.26	526.7	224.9	35.4	25.4	4.69
17	11.8	4.74	52.3	4.2	5.7	0.30	534.1	251.1	34.7	25.5	4.84
18	10.9	2.57	55.3	4.1	6.2	0.57	512.5	292.2	32.3	26.7	4.89
19	13.6	1.06	52.3	3.8	8.4	0.88	495.4	349.5	27.6	32.3	5.83
20	12.3	1.03	50.1	4.4	8.2	0.42	515.6	254.8	33.8	24.1	4.56
21	9.1	3.73	55.7	4.5	8.5	0.68	364.0	317.9	26.7	31.0	5.82
22	9.5	0.81	44.9	4.4	7.4	0.86	465.4	344.9	28.0	33.7	5.99
23	11.1	0.92	56.3	5.1	6.0	0.52	239.8	261.3	27.5	28.5	5.35
24	12.2	2.00	51.8	4.4	9.2	0.94	311.2	299.8	34.7	34.0	6.09
25	10.6	1.11	55.0	4.8	5.4	0.71	266.1	294.1	27.9	30.4	5.62
26	13.5	1.10	46.8	6.1	9.1	0.42	323.4	367.9	35.3	38.0	13.62
27	10.9	0.84	55.7	4.5	6.9	1.13	262.8	304.3	27.6	30.7	5.52
28	12.4	0.92	42.7	4.5	8.2	0.92	276.4	289.3	27.7	31.5	5.65
29	11.1	1.17	48.7	4.6	8.6	0.78	438.0	363.8	47.3	40.7	12.51
	10.9	*1.31*	*52.8*	*4.5*	*6.0*	*0.52*	*376.9*	*277.6*	*33.7*	*27.1*	*6.3*
	8.2	*0.68*	*47.7*	*3.2*	*4.3*	*0.10*	*216.9*	*204.5*	*22.9*	*18.0*	*3.6*
	13.6	*4.74*	*64.8*	*6.1*	*7.6*	*1.10*	*534.1*	*427.6*	*49.2*	*40.7*	*13.7*
	13.30	*70.03*	*8.52*	*12.85*	*16.96*	*46.73*	*29.18*	*19.75*	*21.24*	*22.87*	*45.73*

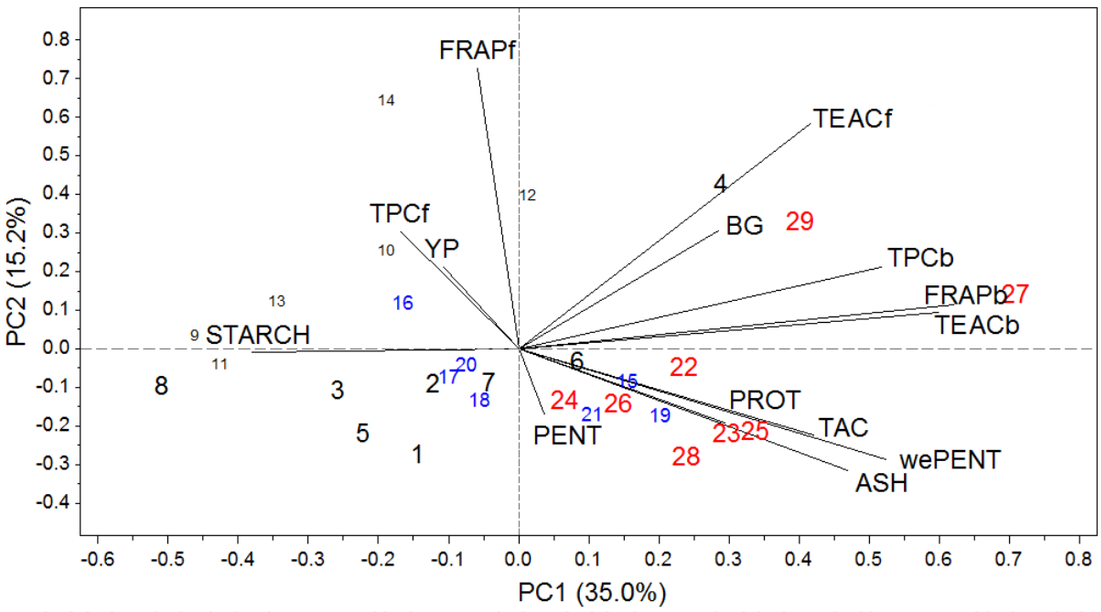
[1]Identication of varieties according to Table 1. [2]PROT, crude protein content (%); ASH, ash content (%); STARCH glucan content (%); PENT, total pentosan content (%); we-PENT, water-extractable pentosanes (%); TPCf, total p (mg FA equ. ·100 g^{-1} dm); TPCb, total phenolic content of bound phenolics (mg FA equ. ·100 g^{-1} dm); FRAPf, ferri free phenolics (mmol Fe(II) kg^{-1}); FRAPb, ferric reducing antioxidant power of bound phenolics (mmol Fe(II) kg antioxidant capacity of free phenolics (mmol TAA kg^{-1}); TEACb, Trolox equivalent antioxidant capacity of boun total anthocyanin content (mg C-3-O-glc equ. ·100 g^{-1} dm); YP, sum of lutein and zeaxanthin content (mg kg^{-1})

Table 2. Chemical composition of the different hulless barley varieties (harvest 2008). All data are ex

compounds and antioxidant activity were obtained for durum and common wheat (Adom et al., 2003; Siebenhandl et al., 2007), whereas an earlier study with three common wheats reports no correlation between TPC and the radical scavenging capacity (Yu et al., 2002).

3.3 Multivariate analysis

Principal components (PCs) analysis revealed four principal components with eigenvalues >1. Variation explained by these four PCs was 35.1, 16.0, 12.2 and 9.1%. The biplot of PC1 and PC2 is demonstrated in Figure 2. From the biplot it can be seen that on the one hand TPCb, FRAPb and TEACb are highly correlated (acute angles between the respective vectors of similar size), whereas TPCf, FRAPf and TEACf show no to small correlations. The well-known negative correlation between STARCH and PROT can be seen by the opposite direction of the variable vectors. BG shows no correlation to PROT, STARCH or PENT. The biplot also reveals a certain grouping of genotypes according to their seed colour. White seeded genotypes are mainly located in the upper left part of the plot, black seeded types in the lower left part (with the exception of Violaceum 2), blue aleurone types are located in the lower central part and purple types are located in the lower right part of the plot.

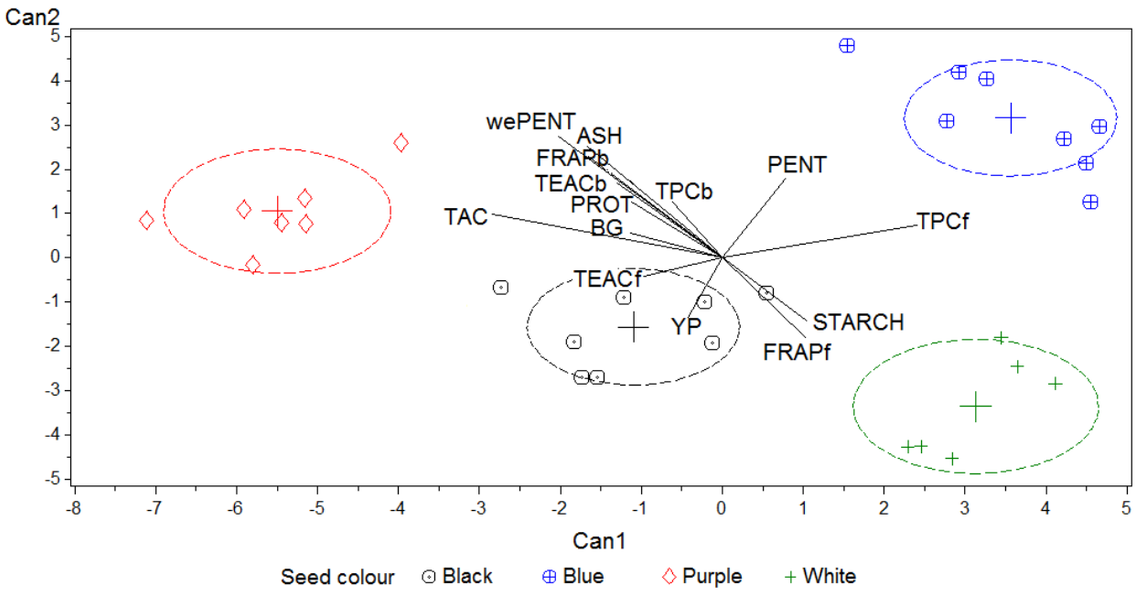


The length of each variable vector is proportional to its contribution to separating the genotypes, and the direction of the vector indicates its relative contribution to PC1 and PC2. Genotypes are presented with their ID as indicated in Table 1, where black letters were used for black genotypes, blue letters for blue, red letters for purple and small black letters for the white ones. Abbreviations used for traits are according to Table 2.

Fig. 2. Biplot of spring barley genotypes and variables

According to the canonical discriminant analysis the seed colour groups can be significantly separated by the data from the chemical analysis. Highly significant first (Can1) and second (Can2) discriminant functions were observed. The plot of discriminant scores is shown in Figure 3. It is obvious that along Can1 three colour groups can be separated, i.e. purple, black and white together with blue. Can1 is mainly determined by TAC and TPCf. While

white and blue seeded genotypes are overlapping along Can1 they can be significantly separated along Can2, which is mainly influenced by we-PENT, ASH and FRAPb. Thereby, discriminant analysis confirmed impressively the separation of seed colour groups which was already suggested by PCA.



The length of each variable is proportional to its contribution to separating the seed colour classes, and the direction of the vector indicates its relative contribution to the Can1 and Can2 linear combinations. Abbreviations for variables see Table 2.

Fig. 3. Plot of canonical discriminant scores with 95% confidence regions for the mean (big cross) of each seed colour class

3.4 Comparison of milling fractions of different genotypes

The milling process was done using a roller mill. Six flour fractions (B1-B3, C1-C3) from the starchy endosperm were collected and merged to give a straight-run white flour. Brans and shorts were collected separately. The investigated twelve genotypes showed differences in the flour yield. Generally, the flour yields were very low (in average 29%) with higher yields of shorts (65%) and bran (6.6%) (Table 3). White coloured hulless barley varieties had the highest flour yield, whereas the proportion of bran was smallest in the group of black genotypes.

3.4.1 Distribution of phenolic acids

As shown in Fig. 4, all colour groups had in common that the flour fraction showed the lowest content of phenolic compounds and antioxidant properties, followed by the shorts and bran fraction. It is visible from the shapes of the boxes that all colour groups contained genotypes with higher and lower attribute levels. In general, bran levels were approximately three to five times higher than the corresponding flour and all millstreams differed significantly from each other. Within the different milling fractions and using the seed colour as criterion, a few significant differences were found in respect to phenolic

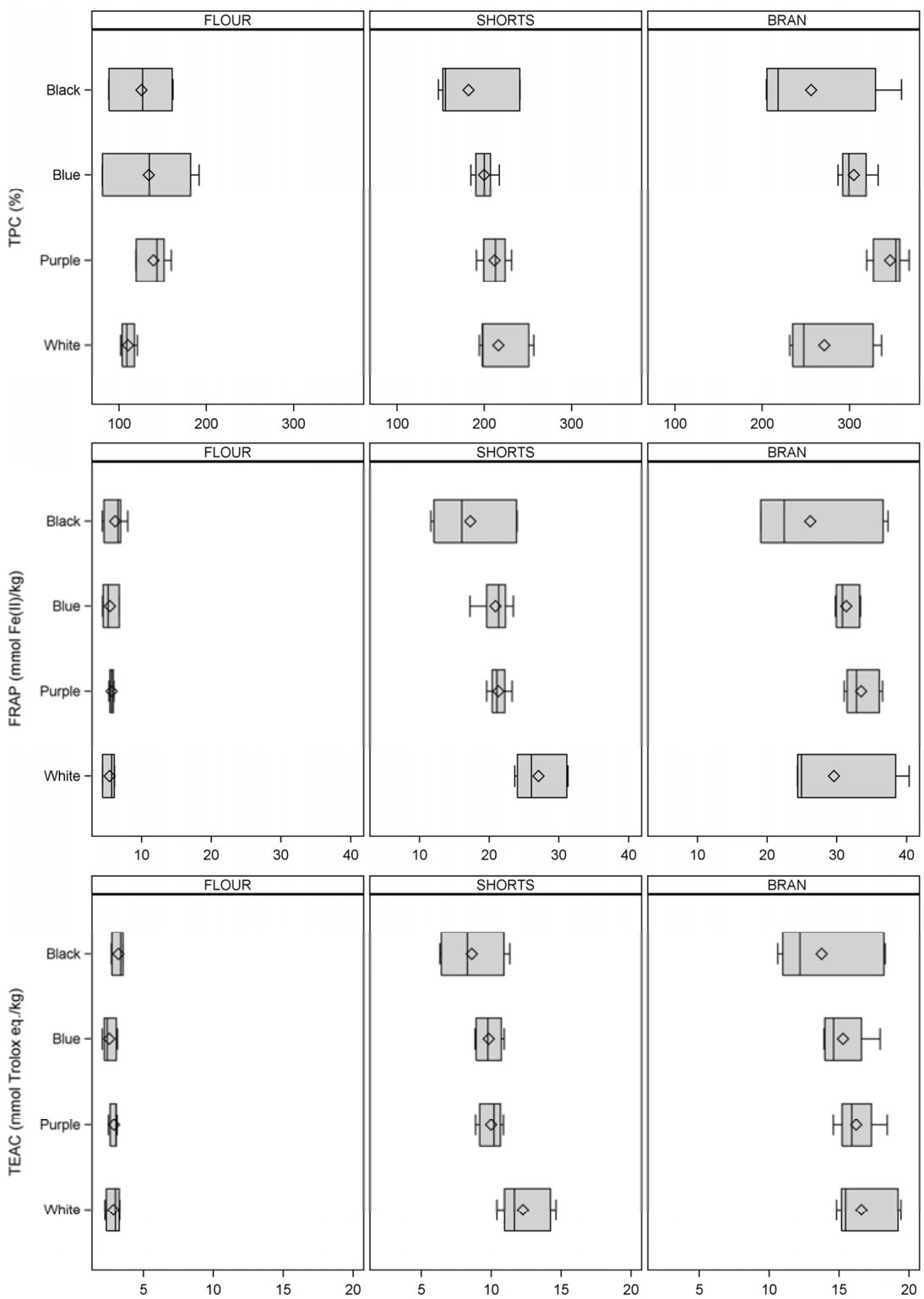


Fig. 4. Variation of free phenolic acids and their antioxidant capacities in regard to grain colour and milling fraction

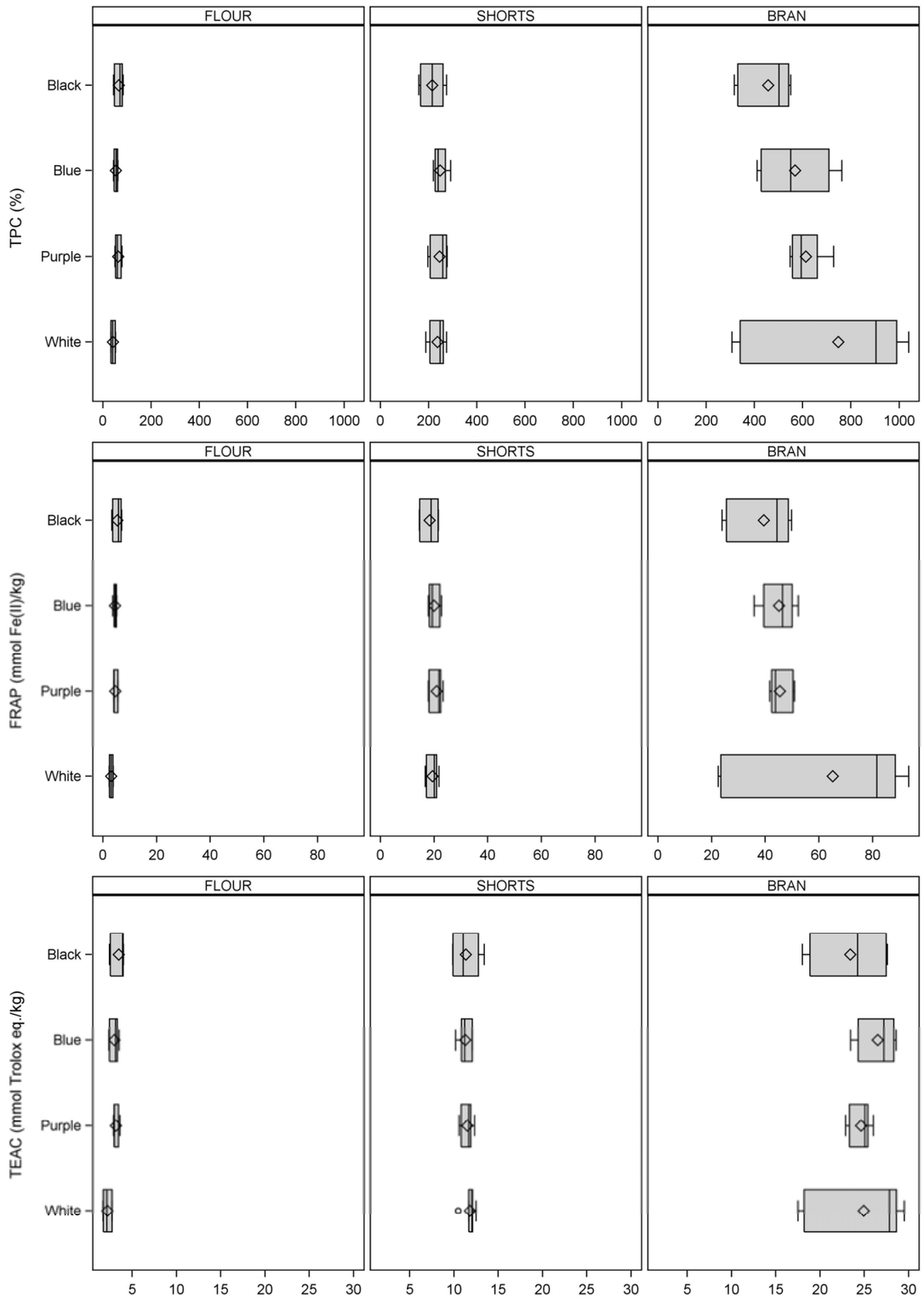


Fig. 5. Variation of bound phenolic acids and their antioxidant capacities in regard to grain colour and milling fraction

ID	Genotype	Flour (%)	Shorts (%)	Bran (%)
4	Violaceum 2	23.5	72.1	4.4
5	Ethiopia 179	26.8	68.3	4.9
7	Black Naked	25.4	72.1	2.5
10	HB803	36.2	54.1	9.7
11	Hora	41.8	54.0	4.2
12	Lawina	36.3	55.7	8.0
15	Himalayense Type 5	25.0	61.0	14.1
17	Addis Ababa 56	24.4	72.6	2.9
21	GHO 1	26.4	67.3	6.3
25	Black Hulless	36.3	59.1	4.6
27	Purple Nudum	21.1	68.8	10.1
29	Lih Dhanra Gal	23.1	68.8	8.1

Table 3. Flour yield of twelve different hulless barley genotypes

content (TPC) and antioxidant properties (FRAP, TEAC), although the ranking varied with the analysis: within the flour fraction, black genotypes differed significantly from the others in respect to their ability to scavenge the ABTS radical (p=0.0086). Regarding the bran fractions, a significant difference was found for TPC, where the purple ones yielded in the highest content and differed significantly from the others. Differences between the genotypes were found within each milling fraction with different orders of rankings but following the same trend. Flours with high antioxidative properties or high yield on phenolic substances were genotypes like Black Naked (ID 7) and Addis Ababa 56, whereas flours of the genotypes Himalayense Type 5 and HB803 had the lowest values. Lawina and Black Naked were highest in shorts and bran fraction, and the purple Lih Dhanra Gal in the bran fraction. Low values were found for the black Ethiopia 179 and Violaceum 2.

It is difficult to compare the phenolic acid profile and the antioxidant properties compiled by different researchers, as each group uses a different methodology to extract and measure the effect. Throughout the literature, the phenolic content correlated significantly with the antioxidant capacity. Thus, there is no doubt that phenolic compounds are mainly responsible for the antioxidant properties in cereals (Perez-Jimenez & Saura-Calixto, 2005). Furthermore, a high correlation was as well found for the total phenolic content, determined with the Folin-Ciocalteu reagent and test systems using DPPH- and ABTS radicals as well as the reducing ability as in the FRAP assay.

Sharma & Gujral (2010) estimated the phenolic content in barley ranging from 2.14 to 2.36 µg FA g^{-1} flour and 3.57 – 5.02 µg g^{-1} in bran. Madhujith et al. (2006) reported a range of 0.17 to 6.26 mg FA g^{-1}, depending on genotype and fraction, and Menga et al. (2010) found varying levels of TPC and TEAC, when comparing a methanolic extract with an extract after enzymatic digestion.

Residues after the extraction of the free phenolic compounds were used to isolate the bound phenolic compounds after an alkaline hydrolysis. As it was observed for the free phenolic compounds, differences were found between the milling fractions, where the flour fractions were lowest in the phenolic content and the antioxidant properties (Fig. 5). Comparing the four colour groups, significant differences were only found for the flour fraction, whereas the

shorts and the bran fraction did not differ in any of the investigated attributes. It is the group of white genotypes which was lowest, followed by the blue, purple and black ones. The more detailed examination of differences between the genotypes revealed a similar picture as was already observed for the free phenolic compounds. Flours with high phenolic content and antioxidant properties derive from the genotypes Violaceum 2, Black Naked (ID 7) and Purple Nudum with a three-fold difference to genotypes with lower expression of these attributes, e.g. Lawina and HB803. In compliance with the flour fractions, the genotype Purple Nudum showed high levels of phenolic compounds and antioxidant properties in the shorts fractions. A different picture was observed when genotypes were classed by the bran fraction. Here, the white genotypes Hora and HB803 exceeded the investigated set of genotypes two- to threefold in terms of FRAP and TPC levels. The occurrence of higher levels of bound phenolic compounds is in agreement with Nordkvist et al. (1984), Verardo et al. (2008) and Madhujith & Shahidi (2009).

In addition to the determination of the TPC with the spectrophotometric method, extracts were as well analysed by HPLC in order to quantify individual phenolic acids. The phenolic acids quantified in this study included 4-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid and ferulic acid (Table 4). Generally, free phenolic acids comprised a very small proportion of the quantified phenolic acids. Highest content was found for the blue genotype Himalayense Type 5. Bound phenolic acid content made up more than two thirds of the total content. Ferulic acid was by far the abundant phenolic acid and the difference between the flour and bran fraction was in average 10fold. Highest content was found in the waxy HB803 and Purple Nudum, whereas Lawina and Ethiopia 179 contained fewest of all. *p*-Coumaric acid was the second phenolic acid and was significantly higher in HB803 and Hora.

3.4.2 Distribution of anthocyanins

Bran, shorts and flour fractions of the black, purple and blue genotypes were screened and compared for their total content of anthocyanins (TAC) and their individual anthocyanins. In general, a sharp decline in the content of anthocyanins was observed with the increase in endosperm in the millstream. Black Hull-less (ID 25) and Purple Nudum were the only genotypes which contained malvidin-3-O-glucoside and apigeninidin. Sorghum is the only plant known to contain significant quantities of luteolinidin and apigeninidin (both 3-deoxyanthocyanidins), which are not commonly found in higher plants. First indications by their existence were given by Nip & Burns (1969; 1971) who isolated apigeninidin and luteolinidin from the seeds of red and white sorghum varieties. Both 3-deoxyanthocyanidins are also reported as the major anthocyanidins from black sorghum varieties (Awika et al., 2004). High contents of cyanidin-3-O-glucoside were found as well in Purple Nudum. Delphinidin was dominant in the black genotype Violaceum 2 (Table 5) but also present in purple genotypes like Black Hull-less (ID 25), Purple Nudum and Lih Dhanra Gal. Kim et al. (2007) reported that cyanindin 3-glucoside was predominant in the purple group, followed by peonidin-3-glucoside and pelargonidin 3-glucoside, whereas delphinidin 3-glucoside was highest in the blue and black group. Thus, our results basically display the same observations. The black 6-rowed genotype Violaceum 2 was salient in respect to the position in the biplot (Fig. 2), as it was displayed in the upper right corner together with the purple representatives Lih Dhanra Gal and Purple Nudum. It is therefore an apposite argument to question the taken classification as the stem of the name contains as well an indication for

purple colour. Probably, the kernels appeared black due to the high content of anthocyanins or the latter have been overlaid by some other darker pigments. On the other hand, the tested Violaceum 2 line could be the result of outcrossing between a purple and a black genotype during a cycle of multiplication.

ID[1]	Free/Bound	Fraction[2]	4-OH-benzoic acid	vanillic acid	*p*-coumaric acid	ferulic acid	caffeic acid	ID	Free/Bound	Fraction	4-OH-benzoic acid	vanillic acid	*p*-coumaric acid	ferulic acid	caffeic acid
4	free	B	n.d.	1.4	n.d.	5.0	n.d.	12	free	B	30.3	2.6	0.8	5.8	8.2
		S	n.d.	1.1	0.1	4.0	n.d.			S	29.6	2.5	0.4	8.6	14.1
		F	5.2	2.0	0.1	3.3	6.6			F	15.2	1.1	0.2	5.4	7.1
	bound	B	6.6	25.6	74.7	1854.1	10.6		bound	B	11.9	15.9	82.3	887.1	17.4
		S	4.8	11.2	10.7	852.3	3.9			S	5.0	8.2	17.9	639.5	2.5
		F	0.6	3.4	3.2	212.3	1.3			F	1.2	1.0	2.9	67.8	0.4
5	free	B	1.6	1.5	n.d.	5.9	n.d.	15	free	B	12.9	3.4	0.3	5.8	3.9
		S	20.4	3.4	5.2	5.4	19.5			S	9.6	1.6	0.3	8.3	9.2
		F	10.4	0.1	2.1	1.4	6.9			F	5.9	0.6	0.4	3.7	6.1
	bound	B	6.8	14.0	27.9	936.8	8.1		bound	B	4.7	11.3	39.0	1342.3	23.2
		S	4.1	8.2	7.9	510.3	3.6			S	5.4	9.0	11.4	707.5	3.0
		F	0.6	1.7	1.8	110.2	0.7			F	n.d.	1.0	1.5	92.7	1.1
7	free	B	4.0	9.3	1.8	20.7	n.d.	17	free	B	21.6	2.1	0.7	7.3	5.8
		S	1.2	8.0	0.9	4.1	n.d.			S	22.0	0.7	0.1	7.0	9.8
		F	1.3	1.2	0.5	4.6	4.4			F	14.3	0.2	0.4	4.3	10.2
	bound	B	10.1	28.1	353.4	1534.5	14.0		bound	B	12.1	27.6	170.3	1587.7	25.5
		S	6.2	12.9	22.2	790.3	14.3			S	3.1	5.3	14.0	563.7	12.5
		F	0.6	2.2	3.9	184.7	0.17			F	0.3	1.5	3.6	135.6	0.7
10	free	B	1.1	3.8	4.7	6.2	2.3	25	free	B	1.3	8.2	4.2	15.9	n.d.
		S	3.2	8.8	1.7	3.4	4.0			S	0.6	11.5	1.2	10.1	n.d.
		F	n.d.	2.7	0.7	1.9	3.9			F	n.d.	1.4	0.5	3.9	n.d.
	bound	B	14.1	43.7	1733.6	2311.2	26.3		bound	B	13.8	22.7	38.8	1618.6	7.6
		S	5.8	11.2	167.4	759.7	4.9			S	5.8	10.2	14.9	867.1	4.4
		F	1.1	1.1	17.7	74.0	0.7			F	0.5	2.0	2.8	142.2	0.9
11	free	B	5.3	14.2	11.2	18.0	6.3	27	free	B	4.3	6.6	6.0	16.6	n.d.
		S	2.0	5.8	2.1	10.9	19.9			S	2.1	6.9	1.5	11.7	n.d.
		F	0.2	1.9	0.7	2.4	10.7			F	0.1	2.0	0.7	5.2	n.d.
	bound	B	11.3	44.7	1427.7	1996.1	20.9		bound	B	13.2	26.2	52.2	2025.2	6.5
		S	4.2	11.1	87.3	805.9	6.2			S	6.8	13.8	11.0	997.6	2.8
		F	0.7	2.0	15.2	102.6	0.7			F	1.4	3.9	2.5	220.2	1.0

[1] ID number according to Table 1. [2] F, flour fraction; S, shorts; B, bran fraction.

Table 4. Content and composition of free and bound phenolic acids (in ppm) in the different millstreams of barley genotypes

ID[1]	Fraction[2]	Delph-3-O-glc[3]	Cyan-3-O-glc	Delph-3-O-rham	Pel-3-O-glc	Mal-3-O-glc	Luteo	Api	Cyan	Delph	Peo	Mal
4	B	+[4]	+	+	•		+	+	•	+++	•	
	S	+	•	•	•		+	+	•	++	•	•
	F	•	•	•	+		•	•	+	+	•	•
5	B	•	•		•		•		•	•	•	•
	S	•	•		•		•		•	•	•	
	F	•	•		+				•	•	•	•
15	B	+	+	•	•		+	•	•	++		
	S	•	•	•	•		+	•	•	+		
	F	•	•		+		+	•	+	•	•	•
17	B	+	•	•	•		+	•	•	++	•	•
	S	+	•	•	•			•	•	+	•	•
	F	•	•		•			•	+	•	•	•
21	B	+	•	•	•			+	+	++	•	
	S	•	•	•	•			•	•	+	•	
	F	•	•		•			•	•	•	•	
25	B	+	+	•	+	+	++	+	+	+++	•	
	S	•	+	•	•	+	+	+	+	++	•	
	F	•	•		+		•	+++	+	•	•	•
27	B	•	+++		++	++	+	+	+	++	+	•
	S	•	++		+	+	+	+	•	+	+	•
	F	•	+		+		•	+++	•	•	•	•
29	B	+	•	•	•		•	+	•	++	•	
	S	•	•	•	•		•	•	•	+	•	
	F	•	•	+	•			•	+	•	•	•

[1] ID number according to Table 1. [2] F, flour fraction; S, shorts; B, bran fraction. [3] Delph, Delphinidin; Cyan, Cyanidin; Pel, Pelargonidin; Mal, Malvidin; Luteo, Luteolinidin; Api, Apigeninidin; Peo, Peonidin.[4] • = in traces, + = 1.0 – 3.0 ppm, ++ = 3.1 – 5.0 ppm, +++ = > 5.1 ppm.

Table 5. Distribution of various anthocyanins (ppm) within the three milling fractions of barley genotypes

4. Conclusion

The basic function of food to satiate and to provide macro- and micronutrients has become in the Western industrialised countries less and less important. With increasing prosperity and/or educational knowledge additional attributes are demanded by consumers who search for sensorial sensations or tie their consumption of food with social prestige or ethical reasons. Among these supplementary functions of food the health-awareness of consumers

is used to a high degree by the food and marketing industry for the promotion of existing and newly developed food products.

Thus, scientists work together interdisciplinary to study the biochemical mechanisms of food in order to quantify possible health benefits. Since the late 1980s, most studies have mainly focused on the polysaccharide moiety, while the potential role of whole grain antioxidants was considered less up to now. The newly introduced term "dietary fibre-antioxidants" assumes that the beneficial effects attributed to the cereal dietary fibre are not only due to the polysaccharide moiety, but also to the associated polyphenolic compounds (Vitaglione et al., 2008).

These phytochemicals as well as the insoluble fraction of dietary fibres are located in the outer parts of the kernel and thus are removed within the milling process when gaining refined white flour. Barley grains differ considerably from those of other cereals in regard to their chemical constitution, as a large proportion of the soluble dietary fibre is located in the endosperm. Hence, this valuable fraction will not be lost by using normal milling practise for the production of flour. A recent experiment showed that the baking quality of hulless barley flour is sufficient to bake pure barley bread. Thus, hulless barley is an interesting alternative for commonly used grains and could contribute to a higher diversity in human nutrition (Kinner et al., 2011).

In this context, the phytochemical content and total antioxidant capacity of 29 hulless barley genotypes with different seed colours were investigated. Significant differences in the content of anthocyanins and carotenoids, and in reducing power were observed between genotypes, and between the different millstreams of the respective plant material. From the results of this study, and from other studies previously carried out (Siebenhandl et al., 2007; Bokore, 2008; Menga et al., 2010) it can be concluded that on the one hand genetic and environmental factors have a major impact on the phytochemical profile and the antioxidant activity, but on the other hand also postharvest treatments such as fractionation of the raw material can have a major influence. Thus, it is anticipated that the development of barley cultivars for human consumption should focus on breeding for specific end uses (Hang et al., 2007). Due to significant environmental influences on many phytochemicals genotypes with a stable performance across a wide range of environments are necessary to assure a continuous quality for food processors. Before a specific breeding programme can be started, the already existing germplasm has to be evaluated to identify possible genotypes which could serve as donors of valuable traits in cross breeding programmes.

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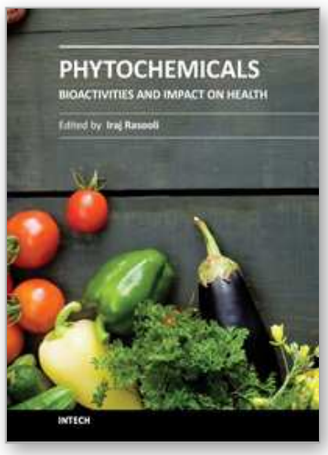

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Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose - or reasonably might be expected to pose - a significant risk to human health at current low levels of intake when used as flavoring substances. Due to their natural origin, environmental and genetic factors will influence the chemical composition of the plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products.

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