

ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt



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ABSTRACT

Many analytical methods for antioxidant determination in foodstuffs and raw materials based on various principles have been published so far. However, not all of them are applicable to barley and malt. The results of total antioxidant capacity (TEAC) of barley and malt obtained with methods based on ability to eliminate radicals of 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) were compared and statistically evaluated. The average TEAC of barley determined using ABTS and DPPH ranged from 2.1 to 2.5 $\mu\text{mol g}^{-1}$ and from 1.2 to 1.7 $\mu\text{mol g}^{-1}$, respectively. The TEAC ranges in malt were 2.7–3.0 $\mu\text{mol g}^{-1}$ (ABTS) and 1.8–2.6 $\mu\text{mol g}^{-1}$ (DPPH). TEAC of barley and malt were affected by the weather conditions (year), variety and application of Zn^{2+} fertilizer. The ABTS and the DPPH methods represent an effective tool for the assessment of antioxidant capacity of spring barley and malt and the factors having an impact on it.

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1. Introduction

An involvement of oxidative stress in pathogenesis of various disorders and diseases has attracted much attention of scientists and general public. In order to follow a healthy life style as a prevention of many diseases, the content of antioxidants in a diet should be considered as one of the main criteria (Niki, 2010). Besides, antioxidants also play an important role in food shelf life. For instance, they inhibit or prevent oxidation of lipids resulting in formation of undesirable aromatic compounds, and protect valuable food components such as proteins and vitamins (Bamdad and Chen, 2013). Antioxidants in barley and hops are responsible for the sensoric stability of beer and suppression of aldehydes formation originating in radical reactions of fatty acids, amino acids and higher alcohols (Zhao et al., 2013).

Barley (*Hordeum vulgare* L.) is one of the ancient cereal crops. This crop, in the past mainly used for food purposes, has currently

its place in both food and feed industries. Due to high content of biologically active compounds barley has become of a high interest as a commodity for production of functional food (Omwamba and Hu, 2009). However, still, barley is mainly used for malt and beer production (Newman and Newman, 2008).

Phenolic compounds are considered as a main group of compounds responsible for desirable antioxidant properties (Zhao et al., 2006). They are formed as secondary metabolites in planta either commonly during the growth or as a response to stress (infection, injury, UV radiation) or both (Ondrejovic et al., 2009). A wide range of phenolic antioxidants of barley involves phenolic acids (benzoic and cinnamic acid derivatives), proanthocyanidins, chinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Goupy et al., 1999). The content of polyphenols in grains is closely related to the protein amount (decreasing by increasing protein content). Total amount of polyphenols is ranging from 0.1% to 0.6% of dry matter depending on many other factors, e.g. barley variety, growing locality and weather (Kosař et al., 2000). Phenolic antioxidants are present in free and bound forms in barley grains mainly located in the husk and aleurone layer. Enzymatic processes during grain germination of the malting process can release the bound forms from cell wall structures, and thus improve the

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extraction efficiency of phenolic acids. In addition, an increase of the friability of grain layer tissues during kilning can also contribute to higher extraction rate of phenolic compounds. Also other compounds, such as sugar reductions and melanoidins, originating through the Maillard reaction contribute to malt antioxidant capacity (Inns et al., 2011; Goupy et al., 1999; Maillard et al., 1996; Čechovská et al., 2012).

Antioxidant capacity of barley and malt is considered as one of the most important parameters related to the quality of final products. Beers produced from malts of higher antioxidant capacity showed better sensoric quality and stability. These malts had higher values of final attenuation and soluble nitrogen, and better prediction of filterability (Mikyska and Prokeš, 2009).

So far, a lot of analytical methods have been developed for the assessment of antioxidant properties of plant materials. In general, they are based either on the ability to eliminate the radicals (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS; 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl, DPPH; Oxygen Radical Absorbance Capacity, ORAC; Photochemiluminescence, PCL) or reduction potentials of compounds (Ferric Reducing Antioxidant potential, FRAP); voltammetry; High Performance Liquid Chromatography with Electrochemical Detection, HPLC-ECD (Holasová and Fiedlerová, 2011). Analytical methods based on the deactivation of radicals can be divided on the methods using Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET). The outcomes differ in reaction kinetics and side reactions appearance. HAT and SET co-occurs and their balance is characterised by antioxidant structure and pH value (Gülçin, 2012).

Most of the natural antioxidants occur in complex mixtures. The mixture components can react differently with the individual radicals and/or influence each other either synergistically or antagonistically. Therefore, the term „Total Antioxidant Capacity“ (TAC) has been introduced for the characterisation of antioxidant properties of plant materials. TAC expresses an amount of free radicals which can be eliminated by antioxidants present in a sample (Niki, 2010). TAC expressed as an equivalent of Trolox (synthetic derivative of vitamin E) is defined as Trolox Equivalent Antioxidant Capacity (TEAC) (Niki, 2010).

Although, the advantages and disadvantages of the analytical methods have been discussed several times in the literature, so far, none of the methods could have been assigned as the most suitable for determination of antioxidant properties (Sharma and Singh, 2013).

The aim of this study was to: (i) determine the total antioxidant capacity of barley and malt produced thereof using ABTS and DPPH methods, (ii) assess the applicability of the used methods for barley and malt antioxidant capacity determination, (iii) statistically evaluate the factors influencing the antioxidant capacity of barley and malt, (iv) discuss the relations between the antioxidant capacity and barley grain parameters, and malting quality index parameters.

2. Material and methods

2.1. Experimental setup

Spring barley (*Hordeum vulgare* L.) harvested in the years 2009–2011 on the experimental plot of Mendel University in Brno (Zabčice, N49°01' E16°37') were analysed. The dominating soil type of the field is middle heavy gleic fluvisols (Flg). Zabčice locality belongs to the warmest regions of the Czech Republic. It is specified as warm, moderately dry with moderate winter and short sunshine during vegetation (Ehrenbergerová et al., 2010).

Six varieties of spring malting barley (Aksamit, Bojos, Jersey, Prestige, Radegast and Sebastian) were grown in three replicates, each on 12 m². Each replicate was analysed once. Description of

these barley varieties is given in the [Supplementary material 1](#). In addition, an effect of foliar application of Zinran fertilizer (50% Zn, 4.7% S) used in two growing stages: Zn1 (DC 31) and Zn2 (DC55) (Meier, 2001), on the technological parameters of barley grains was studied (Cerkal et al., 2010).

2.1.1. Malting

Micro-malting tests were carried out at the Research Institute of Brewing and Malting, Plc. (RIBM), Malting division Brno. Raw grains were cleaned and graded by sieving. For analyses and malting, first grade (kernels larger than 2.5 mm) was used. Malting technology for Pilsner malt production was used.

2.1.2. Determination of barley and malt quality parameters

Barley samples were determined for Thousand grain weight (TGW), Starch content (STA), Total protein content in barley (TNB) and Germinative energy (GER). The following parameters were evaluated to prove the malt quality: Extract yield (EXT), Relative extract at 45°C (RE 45), Diastatic power (DP), Apparent final attenuation (FER), Total protein content in malt (TNM), Kolbach index (KI), Malt friability (FRI), and β -glucans (BG) (EBC Analysis committee, 2009). Malting quality index (MQI) was calculated according to Psota and Kosař, 2002. Average levels of barley and malt qualitative parameters in the years of 2009–2011 are given in the [Supplementary material 2](#).

2.2. Determination of antioxidant capacity

2.2.1. Sample preparation

Barley and malt samples (30 g) were ground on a laboratory grinder QC 124 (Mezos Ltd, Hradec Kralove, Czech Republic) with sieve holes of 0.8 mm. 25 g of a ground sample placed into a mashing bin were properly mixed with 225 ml of deionised water. Afterwards, samples were mashed in 12-place mashing bath (Bender a Hobein Ltd, Bruchsal, Germany) at 45°C for 15 min. Cooled-down samples were filtered through a Whatman Grade 41 filter paper (Whatman, Maidstone, The United Kingdom) and the obtained supernatant was stored at –20 °C.

2.2.2. ABTS method

A solution of cation-radical ABTS^{•+} was prepared using the reaction mixture of 5 ml of aqueous solution of ABTS (CAS 30931-67-0, Sigma-Aldrich, Saint Louis, USA) at concentration of 7 mmol l⁻¹ and 88 μ l of 140 mmol l⁻¹ K₂S₂O₈ (Merci, Brno, Czech Republic). The final working solution of ABTS^{•+} was obtained after the reaction time from 12 to 16 h under the laboratory temperature at darkness. The ABTS^{•+} reagent was diluted with acetate buffer (0.1 mol l⁻¹; pH 5, Merci, Brno, Czech Republic) to achieve the absorbance of 0.700 \pm 0.02 at 734 nm. For the spectrophotometric measurement of the samples, 1 ml of diluted ABTS^{•+} and 100 μ l of sample were mixed. Calibration curve was prepared using Trolox at the concentration range of 50–350 μ mol l⁻¹ in methanol (CAS 53188-07-1, Merci, Brno, Czech Republic). As a negative control 1 ml of diluted ABTS^{•+} and 100 μ l of acetate buffer were used. The absorbance (A) was acquired at 734 nm after 10 min using Helios Gamma 9423 UVG 1000E (Thermo Electron Corporation, Madison, WI, USA).

The content of antioxidants in the sample is determined as a percentage decrease of colour intensity (% of inhibition) related to the negative control sample (Equation (1)). The total antioxidant capacity of barley and malt samples was expressed as an equivalent of Trolox (TEAC) per 1 g of dry matter of a sample (μ mol g⁻¹).

$$\% \text{ inhibition} = \frac{A_{\text{negative control}} - A_{\text{sample}}}{A_{\text{negative control}}} \times 100 \quad (1)$$

2.2.3. DPPH method

A working solution of DPPH (CAS 1898-66-4, Sigma-Aldrich, Saint Louis, USA) at the concentration of $186 \mu\text{mol l}^{-1}$ was prepared by dilution with methanol-acetate buffer (1:2 mixture of acetate buffer (0.1 mol l^{-1} ; pH 4.3) and methanol). 1.9 ml of the DPPH working solution was added to 100 μl of a sample. Similarly, the calibration curve was prepared using 100 μl of Trolox at the concentration range of 20–1000 $\mu\text{mol l}^{-1}$ in methanol and 1.9 ml of the DPPH working solution. In case of negative control, DPPH was mixed with the acetate buffer instead of Trolox. The absorbance was measured at 515 nm after 10 min.

The content of antioxidants in the sample is determined as a percentage decrease of colour intensity (% inhibition) related to the negative control sample (Equation (1)). The total antioxidant capacity of barley and malt samples was expressed as an equivalent of Trolox (TEAC) per 1 g of dry matter of a sample ($\mu\text{mol g}^{-1}$).

2.3. Statistical analysis

Statistical analysis was performed using general linear model, where factor significances were identified by analysis of variance (ANOVA) and pairwise comparisons were realized by the Least Significant Differences (LSDs). Linear dependence was judged by Pearson correlation coefficients (r). All statistical tests were realized on the level of significance 0.05. Principal components analysis (PCA) was performed for graphical illustration of relations among particular characteristics. This method allows reduction of multi-dimensional space of characteristics into space given by two principal components. Characteristics printed close to unit circle border mean high quality of representation of these characteristics using principal components. Angle of lines defined by origin and co-ordinates of characteristics relates to correlation of these characteristics: roughly, angle close to 0° means correlation close to 1, angle 90° or 270° means independency and angle 180° means correlation close to -1 .

3. Results and discussion

The TEAC of barley and malt water extracts determined with ABTS were in the range of $1.6\text{--}3.0 \mu\text{mol g}^{-1}$ in barley, and of $2.2\text{--}3.3 \mu\text{mol g}^{-1}$ in malt. TEAC obtained using DPPH method ranged from 0.9 to $2.0 \mu\text{mol g}^{-1}$, and from 1.2 to $3.2 \mu\text{mol g}^{-1}$ for barley and malt, respectively. Comparing ABTS and DPPH methods, TEAC levels obtained with ABTS method were by 24% higher in malt than in barley. However, using the DPPH the differences in TEAC between malt and barley were even more pronounced (59%). The differences in TEAC between malt and barley can be explained as a result of the antioxidant pattern change during malting, particularly during germination and kilning (Inns et al., 2011; Goupy et al., 1999; Maillard et al., 1996; Čechovská et al., 2012).

3.1. Impact of the factors on the antioxidant capacity of spring barley and malt

In order to identify the factors influencing the level of TEAC, ANOVA was applied. The results are summarized in Table 1. The TEAC of barley and malt was influenced by all considered factors in the following order of significance: the impact of the year, the impact of the variety and the impact of the Zn^{2+} fertilizer application.

3.1.1. Impact of the year

Generally, the weather conditions may have an impact on all parameters of agricultural crops. Thus, it was expected that also the antioxidant capacity could have been influenced by this factor. The

Table 1

ANOVA evaluation of TEAC of barley and malt.

Variable	Degrees of freedom	Mean sum of squares			
		Barley		Malt	
		ABTS	DPPH	ABTS	DPPH
Variety	5	0.9228***	0.3177***	0.4574***	1.6712***
Treatment	2	0.8534***	0.3215***	0.2523***	0.2727***
Year	2	2.7359***	2.6442***	1.4238***	9.8166***
Residue	152	0.0123	0.0063	0.0148	0.0243

***p < 0.001.

average TEAC of barley (calculated through all samples analyzed) ranged from 2.1 to $2.5 \mu\text{mol g}^{-1}$ (ABTS) and from 1.2 to $1.7 \mu\text{mol g}^{-1}$ (DPPH). The statistically highest TEAC were obtained in 2010 (the average TEAC levels are summarized in Table 2). These results correspond with the current knowledge about the solar radiation impact on overall antioxidant activity (Stratil et al., 2006) because the highest solar radiation recorded during the entire experiment was in the 2010's last two months of barley vegetation period when the grains were formed.

The antioxidant capacity can be influenced also by the protein content. In general, the higher the protein content is, the lower the antioxidant capacity is expected (Maillard et al., 1996), confirmed also in our study. The highest TNB (12%) in barley determined in 2009 corresponded with the lowest TEAC obtained from this harvest.

The average levels of TEAC in malt were in the range of $2.7\text{--}3.0 \mu\text{mol g}^{-1}$, and $1.8\text{--}2.6 \mu\text{mol g}^{-1}$ when ABTS and DPPH were used, respectively. Similarly, TEAC determined with ABTS were the highest in malt also in 2010. However, DPPH TEAC levels were significantly higher in 2011 (Table 2).

The dominant impact of the year on the TEAC of barley and malt was clearly confirmed by the results obtained with DPPH. Whereas, impact of the year was not so pronounced as concerns the TEAC determined with ABTS.

3.1.2. Impact of the variety

The impact of the variety was assessed to be the second most significant factor influencing the variability of TEAC of barley and malt. Average levels of TEAC in the individual varieties are listed in

Table 2

Average levels of TEAC of barley and malt, LSD pairwise comparison among the factors.

Factor		TEAC [$\mu\text{mol g}^{-1}$]			
		Barley		Malt	
		ABTS	DPPH	ABTS	DPPH
Variety	Aksamit	2.5 ^a	1.6 ^a	2.9 ^b	2.3 ^b
	Bojos	2.1 ^c	1.4 ^c	2.7 ^c	2.1 ^c
	Jersey	2.4 ^b	1.5 ^b	3.0 ^a	2.6 ^a
	Prestige	2.4 ^b	1.5 ^b	2.9 ^{ab}	2.5 ^a
	Radegast	2.1 ^d	1.3 ^c	2.6 ^d	2.0 ^d
	Sebastian	2.1 ^{cd}	1.4 ^d	2.7 ^c	2.2 ^{bc}
	LSD (0.05)	0.060	0.043	0.065	0.084
Treatment	Control	2.4 ^a	1.5 ^a	2.9 ^a	2.3 ^a
	Zn1	2.3 ^b	1.4 ^b	2.8 ^a	2.3 ^b
	Zn2	2.1 ^c	1.4 ^c	2.7 ^b	2.2 ^c
	LSD (0.05)	0.042	0.030	0.046	0.059
Year	2009	2.1 ^b	1.2 ^c	2.7 ^b	1.8 ^c
	2010	2.5 ^a	1.7 ^a	3.0 ^a	2.4 ^b
	2011	2.2 ^b	1.4 ^b	2.7 ^b	2.6 ^a
	LSD (0.05)	0.042	0.030	0.046	0.059

a, b, c, d, e – significant difference among the levels in the same column on $\alpha < 0.05$; LSD – least significant difference; Zn1 – foliar application of Zn^{2+} fertilizer at DC 31; Zn2 – foliar application of Zn^{2+} fertilizer at DC 55.

Table 2. In summary, they laid in the range of 1.3–2.5 $\mu\text{mol g}^{-1}$ and of 2.0–3.0 $\mu\text{mol g}^{-1}$ in barley and malt, respectively. The use of both analytical methods resulted in the similar trend in results. The highest levels of TEAC of barley were found in Aksamit, the lowest levels in Radegast. Concerning malt, the highest TEAC were determined in Jersey followed by Prestige and Aksamit. The lowest TEAC of malt corresponded with the raw material because the lowest levels were found in Radegast as well.

The malt samples produced from Jersey and Prestige possessed the highest level of saccharolytic and proteolytic modification (RE 45). Similar published studies have described the significant impact of the variety on the antioxidant properties. The significant differences in the genotypes have been attributed to the content of phenolic compounds in general (Dvořáková et al., 2008; Zhao and Zhao, 2012). The phenolic compounds are released from proteins and polysaccharides with hydrolytic enzymes during the germination stage. The lowest content of polyphenolic compounds and RE 45 was found in variety Radegast by another study carried out on a similar spectrum of varieties (Mikyška et al., 2011).

3.1.3. Impact of the Zn^{2+} fertilizer

ANOVA results show also significant impact of the Zn^{2+} fertilizer on the TEAC levels of barley and malt (Table 1). However, its percentual impact (data not shown) is the lowest from all considered factors. The Zn^{2+} application resulted in decreased level of TEAC in barley and malt. The decreasing trend in TEAC is shown in Table 2. This phenomenon could be explained by the increasing formation of phenolic compounds under the stress conditions caused by Zn deficiency (Cakmak, 2008).

3.2. Comparison of ABTS and DPPH methods

ABTS and DPPH methods are based on different mechanisms. Furthermore, the reactivity of the phenolic compounds is dependent on their chemical structures (Zhao et al., 2008). Therefore, the results obtained by different methods can be biased. In general, the DPPH method provides lower values related to Trolox than the ABTS method due to higher stability (and thus lower reactivity) of the DPPH radical. The reduction potential of DPPH radical is -1.2 V , whereas the one for ABTS radical -0.67 V is the same as for another radical used in beer analysis, the DCPI (2,6-dichloro-phenol-indophenol) radical, which is known to react primarily with sugar reductones and melanoidins (Maillard reaction products), in wort, and beer. It is known that DPPH radical reacts with polyphenols

(catechins, proanthocyanidins), but not with the phenolic acids and sugars (Kaneda et al., 1995). Therefore, spectra of antioxidants determined by DPPH and ABTS are partly different. The advantage of the ABTS radical is its high reactivity, and thus likely the ability to react with a broader range of antioxidants. On the other hand, the preparation of the ABTS reagent is more difficult and its stability is lower compared to DPPH. This can lead to the unbiased results (Stratil et al., 2007).

The linear dependence of TEAC levels obtained with ABTS and DPPH was evaluated. The Pearson correlation coefficients are summarized in Table 3.

The strong correlation between both two method results for barley was confirmed ($r = 0.820$). However, TEAC were on average by 58% higher for ABTS compared to DPPH. Noteworthy, both methods are useful for assessment of the differences in TEAC among the individual barley samples.

The correlation between the malt TEAC obtained with ABTS and DPPH was lower ($r = 0.367$). The higher variability in malt TEAC of both methods is obvious on Fig. 2. The high variability in malt results is likely caused by malting and the year of harvest. Fig. 3 shows the average TEAC obtained in the years of 2009–2011 for both methods. The average TEAC provided with ABTS were by 23% higher than the results of DPPH but the ratio of ABTS/DPPH varied within the year (51%, 25% and 3%). The average increase of TEAC between barley and malt was as follows: 28%, 19% and 25% for ABTS, and 48%, 45% and 82% for DPPH.

3.3. The relation between the antioxidant capacity and barley qualitative parameters

PCA was used for the evaluation of relations between TEAC and qualitative parameters of barley. First two principal components (factors) explained almost 85% of variability in the multivariate data set consisting of values for TEAC (data obtained with ABTS and DPPH), TGW, TNB, STA, and GER. The inspection of the PCA loadings plot (Fig. 1A) revealed an inverse relationship between TEAC and both TGW and TNB. Based on these observations it means that the larger grains with the high amount of endosperm contain less antioxidants than the varieties with lower amount of endosperm. This is in good agreement with the previously published studies dealing with impact of Zn^{2+} fertilizers on TGW (European Commission, 2008).

Furthermore, TEAC obtained with ABTS and DPPH methods were correlated to the grain qualitative parameters. The correlation

Table 3

Pearson correlation coefficients (r) of some variables ($n = 160$).

	B ABTS	B DPPH	M ABTS	M DPPH	TGW	STA	TNB	EXT	RE 45	FER	TNM	FRI	BG
MQI	0.333***	0.434***	0.536***	0.410***	−0.471***	0.471***	−0.596***	0.633***	0.669***	0.430***	−0.622***	0.788***	−0.527***
BG	0.111	0.104	−0.073	0.335***	0.188*	−0.214**	0.015	−0.164*	−0.477***	0.035	0.032	−0.682***	
FRI	0.228**	0.373***	0.375***	0.262**	−0.494***	0.654***	−0.622***	0.643***	0.426***	0.207**	−0.633***		
TNM	−0.37***	−0.612***	−0.353***	−0.700***	0.468***	−0.837***	0.991***	−0.924***	−0.083	−0.336***			
FER	0.339***	0.281***	0.443***	0.474***	−0.019	0.103	−0.296***	0.174*	0.541***				
RE 45	0.170*	0.047	0.336***	0.166*	−0.096	0.014	−0.04	0.086					
EXT	0.182*	0.448***	0.153	0.620***	−0.347***	0.857***	−0.928***						
TNB	−0.368***	−0.611***	−0.327***	−0.700***	0.464***	−0.850***							
STA	0.148	0.355***	0.073	0.494***	−0.306***								
TGW	−0.588***	−0.578***	−0.525***	−0.13									
M DPPH	0.419***	0.563***	0.367***										
M ABTS	0.704***	0.737***											
B DPPH	0.820***												

B ABTS – TEAC of barley determined with ABTS method; B DPPH – TEAC of barley determined with DPPH method; M ABTS – TEAC of malt determined with ABTS method; M DPPH – TEAC of malt determined with DPPH method; TGW – Thousand grain weight; STA – Starch content; TNB – Total protein content in barley; EXT – Extract yield; RE 45 – Relative extract at 45 °C; FER – Apparent Final Attenuation; TNM – Total protein content in malt; FRI – Friability; BG – content of β -glucans in wort; MQI – Malting quality index. ***significant at $\alpha = 0.001$; **significant at $\alpha = 0.01$; *significant at $\alpha = 0.05$.

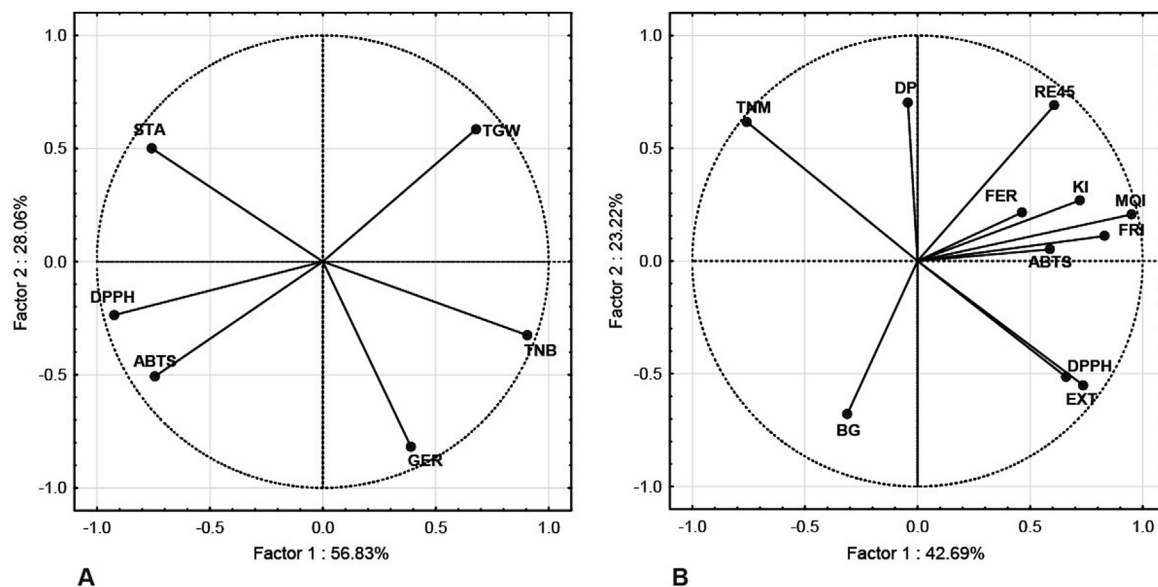


Fig. 1. The PCA loadings plot: (A) visualization of the relations among the evaluated parameters of barley; (B) visualization of the relations among the evaluated parameters of malt. Note: ABTS – TEAC obtained by ABTS; DPPH – TEAC obtained by DPPH; GER – Germinative energy; STA – Starch content; TGW – Thousand grain weight; TNB – Total protein content in barley; BG – content of β -glucans in wort; DP – Diastatic power; EXT – Extract yield; FER – Apparent final attenuation at 72 °C; FRI – Malt friability; KI – Kolbach index; MQI – Malting quality index; RE 45 – Relative extract at 45 °C; TMN – Total protein content in malt.

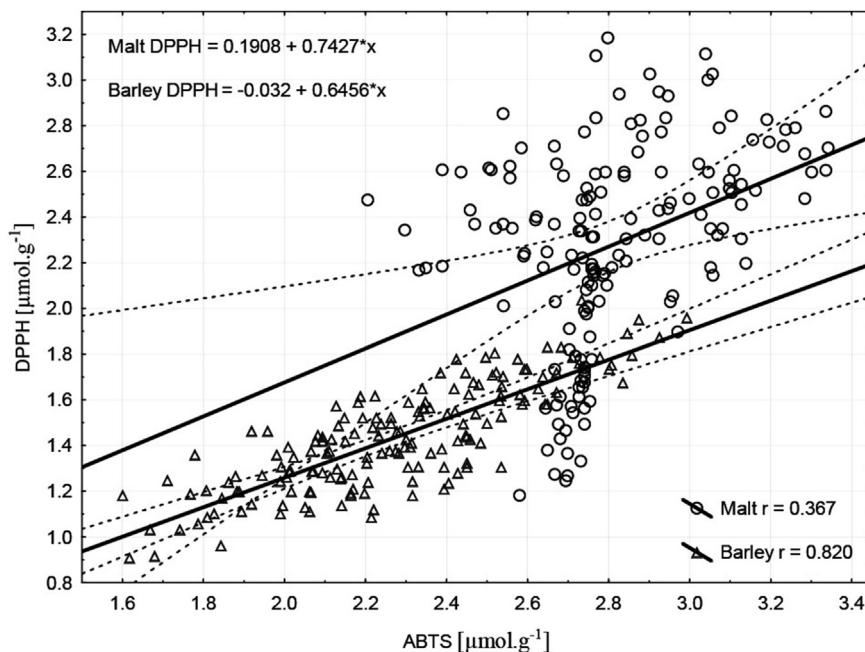


Fig. 2. Linear correlation of ABTS and DPPH methods in analysis of barley and malt.

coefficients are summarized in Table 3. The results correspond to those obtained by PCA. The statistically significant ($p < 0.001$) inverse correlations were obtained between TEAC (ABTS data) and TGW ($r = -0.588$), and between TEAC (ABTS) and TBN ($r = -0.368$). Similarly, correlation of TEAC based on DPPH to both TGW and TBN resulted in statistically significant ($p < 0.001$) negative correlation with r values of -0.578 and -0.612 , respectively. As for relation between TEAC and STA, statistically significant positive correlation was observed only for TEAC values obtained with DPPH ($r = 0.355$). No statistically significant positive correlation was observed between ABTS TEAC and STA.

3.4. The relation between the antioxidant capacity and malt qualitative parameters

In the next step, PCA was applied to study relation between the antioxidant capacity and malt qualitative parameters. TEAC, EXT, RE 45, TMN, KI, DP, FRI, BG, FER and MQI were used as input variables. In this case, first two principal components explained 66% of the data variability. The increasing trend in TEAC obtained with ABTS was observed in relation to the parameters expressing saccharolytic, proteolytic and cytolytic modification, i.e. RE 45, KI, FER, FRI (Fig. 1B).

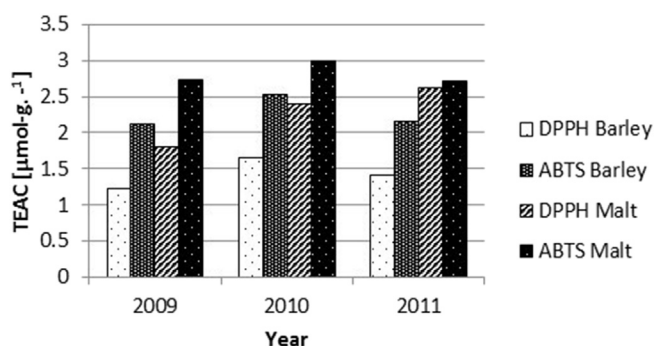


Fig. 3. Comparison of barley and malt TEAC in period of 2009–2011.

The relation between TEAC (DPPH) and malt qualitative parameters was lower. The most obvious positive and negative relations were between TEAC and EXT, and TEAC and TNM, respectively. In general, the malt samples of higher quality (higher grain modification) contain more antioxidants than less modified malts.

The outcomes from the evaluation of linear dependence of TEAC and malt qualitative parameters showed a similar trend in correlation for values obtained with both analytical methods (Table 3). Statistically significant positive correlation ($p < 0.001$) of TEAC (ABTS) to MQI ($r = 0.536$), FER ($r = 0.443$), FRI ($r = 0.375$) and RE 45 ($r = 0.336$) was observed. Similarly, TEAC (DPPH) were positively correlated to MQI ($r = 0.410$), FER ($r = 0.474$), FRI ($r = 0.262$) as well. In addition, EXT and BG were found to have a positive correlation to TEAC (DPPH) on the level of significance of $p < 0.001$.

Negative correlation coefficients were calculated for the linear dependence of TEAC obtained with both methods, and TNM (Table 3), $r = -0.353$ and -0.700 for ABTS and DPPH, respectively.

4. Conclusions

Both used methods, ABTS and DPPH, represent an effective tool for the assessment of antioxidant capacity of spring barley and malt. Although, significant differences in TEAC of both data sets were observed, both methods enabled to show the TEAC variability among the individual barley varieties, and between barley and malt samples.

Application of the methods on barley samples revealed that ABTS determination reflected more the impact of the variety on the TEAC whereas statistical evaluation of the DPPH results showed the impact of the year. The considered factors (year, variety and application of the Zn^{2+} fertilizer) were responsible for 87% and 89% of TEAC variability of barley when ABTS and DPPH were used, respectively. It can be concluded that the bigger grains desirable for malting possessed lower TEAC.

Concerning the malt TEAC, strong impact of the variety was observed for data obtained with both methods (29% and 26% for ABTS and DPPH, respectively). Variability of 61% was caused by the year impact (DPPH). For the ABTS methods, only 72% variability of the results could have been explained by the considered factors. Its TEAC were more affected by other unidentified factors to the larger extent than the DPPH TEAC. TEAC obtained with both methods were dependent on the malt qualitative parameters. It was observed that TEAC have been increased with higher grain modification.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jcs.2016.11.004>.

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