EVALUATION OF ANTIOXIDANT ACTIVITIES OF CEREALS AND THEIR MALTS

MIROSLAV ONDREJOVIČ[1], DANIELA CHMELOVÁ[1], EVA IVANIŠOVÁ[2], ŠTEFAN DRÁB[2], VRATISLAV PSOTA[3]

[1]*Department of Biotechnology, University of SS. Cyril and Methodius, J. Herdu 2, Trnava, SK-917 01, Slovak Republic (miroslav.ondrejovic@ucm.sk)*
[2]*Department of Storing and Processing of Plant Products, Slovak University of Agriculture, Tr. A. Hlinku 2, Nitra, SK-949 76, Slovak Republic*
[3]*Research Institute of Brewing and Malting, Plc., Mostecká 7, Brno, CZ-614 00, Czech Republic*

Abstract: The aim of this study was to determine the influence of malting on the antioxidant content in cereals such as wheat (PS Sunanka, Zaira, PS 57/11 and Vanda), oat (Dunajec) and barley (Laudis 550) harvested in 2013. Antioxidant and polyphenol contents of these cereals and malts were investigated. Secondary, technological parameters of prepared malts were evaluated and compared with malt from barley Laudis 550 used as reference material. Malting of selected cereals had an impact on antioxidant and polyphenol content and allowed a better extraction of these compounds from cereal matrix, except of barley malt, whose antioxidant and total polyphenol content remained comparable. For other cereal malts, antioxidant contents were 2.0, 1.8, 2.6, 2.9 and 3.2-fold higher and total polyphenol content were 1.8, 1.9, 1.9, 3.1 and 3.4-fold higher than in wheat (PS Sunanka, Zaira, PS 57/11, Vanda) and oat (Dunajec), respectively. From correlation analysis, the results showed that not all polyphenols released by malting have antioxidant activity. Technological parameters (friability, haze of wort, saccharification rate, filtration rate, extract and diastatic power) also indicated that good malt quality had oat Dunajec and wheat PS Sunanka and Zaira in comparison with reference material (barley Laudis 550).

Key words: oat, wheat, barley, antioxidant activity, malt, malting quality

1. Introduction

Cereals such as oat, wheat or barley belong to important components of the human nutrition which contain saccharides, proteins, lipids, fiber, polyphenols, vitamins, and minerals. Cereals are also a source of bioactive compounds with significant antioxidant activities (SHAHIDI and WANASUNDARA, 1992). Antioxidants may contribute to the protective effects against cardiovascular diseases and cancer and are believed to be the major factors to their health benefits (SERPEN *et al.*, 2007). Antioxidant content depends on the cereal types, varieties of cereals, environmental conditions and treatment type (ADOM *et al.*, 2005).

Oats contain large amount of phenolic compounds, tocols, phytic acid, flavonoids, sterols, avenanthramides and β-glucans (CHEN *et al.*, 2007). Some of these compounds are unevenly distributed within the kernel or hulls (EMMONS *et al.*, 1999; EMMONS and PETERSON, 1999). The major components with antioxidant activity in wheat belong to the group of phenolic acids (MPOFU *et al.*, 2006; ANSON *et al.*, 2008). The majority of phenolic compounds are located in the wheat bran. Barley is excellent source of phenolic compounds such as phenolic acids (benzoic and cinnamic

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acid derivates), flavonoids, tannins, proanthocyanidins and amino phenolic compounds (HERNANZ *et al.*, 2001; DVOŘÁKOVÁ *et al.*, 2010) which are concentrated in outer layer of barley grains (GOUPY *et al.*, 1999).

Phenolic compounds in cereals are present as free, soluble conjugated (glycosides) or insoluble (NARDINI and GHISELLI, 2004; DVOŘÁKOVÁ *et al.*, 2010). Soluble (free) polyphenols are compartmentalized within the plant cell vacuoles and insoluble (bound) polyphenols are distributed in the cell walls (BENGOECHEA *et al.*, 1997; WINK, 1997). Bound polyphenols may be released by various methods such as chemical (acid/base hydrolysis) and enzymatic methods. Enzymatic methods are proposed as a milder alternative to acid/base hydrolysis in which isolated enzymes, microorganisms and their enzymes or enzymes produced by plants are used to degrade linkages between polyphenols and saccharides (KIM *et al.*, 2006). Malting is the example of method which uses enzymes produced by plant material for release of bound polyphenols (e.g. ferulic, coumaric or caffeic acid) from matrix. Therefore, antioxidant activities may be increased during the later stages of germination (LU *et al.*, 2007).

The aim of this study was to compare antioxidant and polyphenol contents in selected cereals (wheat, oat and barley) with content of these compounds in their malts and to compare the quality of selected non-traditional malts such as oat and wheat malts to barley malt.

2. Material and methods

2.1 Plant material

Winter wheat (PS Sunanka, Zaira, PS 57/11 and Vanda) and oat (Dunajec) were obtained from The Plant Production Research Institute in Piešťany (SK) and spring barley (Laudis 550) was obtained from The Research Institute of Brewing and Malting (RIBM) in Brno (CZ). All cereals were cropped in 2013 and were sealed in polyethylene bags, and stored in a freezer at -20 °C until ready for extraction.

2.2 Malting of cereal samples

Cereal samples (0.5 kg) were malted in the micromalting plant of the company KVM (CR) by method traditionally used in the RIBM and it corresponds to the Mitteleuropäische Brautechnische Analysenkommision (MEBAK, 2011). Steeping was conducted in the steeping box. Temperature of water and air during the air rests was kept at 14.5 °C. Steeping time during the first day was 5 hours; during the second day 4 hours and on the third day water content in germinating grains was adjusted by steeping or spraying to the value of 45 %. Temperature in the course of germination was 14.5 °C. Total time of steeping and germination was 144 hours. Kilning was performed in a one-floored electrically heated kiln. Total kilning time was 22 hours, prekilning at 55 °C, kilning temperature was 80 °C for 4 hours. Changes of cereal grain mass during malting are described in Table 1.

Table 1. Malting yields of selected cereal varieties.

Parameter	PS Sunanka (wheat)	Zaira (wheat)	PS 57/11 (wheat)	Vanda (wheat)	Dunajec (oat)	Laudis 550 (barley)
Moisture of cereal grain [%]	11.2	11.2	10.7	11.4	11.2	13.1
Respiration losses [%]	3.3	3.4	5.4	4.4	4.3	3.8
Rootlet losses [%]	3.9	3.2	6.0	4.6	4.5	4.9
Total yield [%]	92.9	93.4	88.6	90.9	91.2	91.2
Moisture of malt [%]	4.3	4.5	3.6	4.5	4.6	4.3

2.3 Analysis of malting quality

Malting quality expressed by extract in malt dry matter, saccharification rate, filtration rate (EBC 4.5.1), extract of malt (congress mash), final attenuation of laboratory wort from malt (EBC 4.11), diastatic power (EBC 4.12), friability (EBC 4.15), haze of wort (EBC 9.29)) were determined according to the methods presented in publications EBC (2010).

2.4 Analysis of biologically active compounds

2.4.1 Extraction procedure

The samples (wheat, oat and barley) and their malts were dried at 50 °C during 3 hours. Cereal grains and malts were milled to a particle size of less than 0.5 cm and used for extraction immediately. The extraction was carried out with methanol as extraction solvent (solid-liquid ratio – 1:100; w/v) during 24 hours at laboratory temperature on the orbital shaker at 160 RPM. After them, the mixture was centrifuged at 4,000 RPM for 10 min. Supernatant was evaporated by rotary vacuum evaporator at 50 °C. Crude extract residue was weighed and converted by the following formula:

Crude extractive yield = (extract residue weight/cereal weight)×100%

Subsequently the residue was solubilized in methanol (0.5 ml). This extract was used for analyses.

2.4.2 Trolox equivalent antioxidant capacity

TEAC was determined using the method proposed by YEN and CHEN (1995) with modification to microplate form. The DPPH solution (0.012 %; w/v) (100 μl) was added to 25 μl of extract. The mixture was shaken at 100 RPM and allowed to stand at room temperature for 10 minutes. The decreasing of absorbance was monitored at 540 nm. The results were corrected for dilution and expressed in mg TEAC (Trolox equivalent antioxidant capacity) per gram of plant material.

2.4.3 Total polyphenol content (TPC)

Total polyphenol content was measured using Folin–Ciocalteu colorimetric method (SINGLETON *et al.* 1965). Plant extract (20 µl) was mixed with 20 µl of Folin-Ciocalteu reagent and incubated at room temperature for 5 minutes. Following the addition of 200 µl 20 % (w/v) water solution of Na_2CO_3 to the mixture and incubation for 5 minutes, total polyphenols were calculated on the base absorbance of mixture measured at 600 nm. The results were expressed in mg GAE (gallic acid equivalents) per gram of plant material.

2.5 Statistical analyses

All experiments (extraction, determination antioxidants and polyphenols) were realized in triplicate. The obtained data were evaluated by Excel (Microsoft, 2010).

3. Results and discussion

Malting is controlled germination in which the enzymes in cereal grains are activated or synthetized and leading to high nutrient bioavailability and releasing bound polyphenols with potential antioxidant activities (LU *et al.*, 2007).

In our work, we focused on the comparison and quantification of antioxidant and polyphenol contents in five cereal varieties (winter wheat – PS Sunanka, Zaira, PS 57/11 and Vanda and oat - Dunajec) with their malts. These cereals have been selected from previous results of MALIAR (2013) because of these showed significant levels of antioxidants among different varieties of wheat and oats. Spring barley variety Laudis 550 was used as reference material. This variety exhibits good malting quality (PSOTA *et al*., 2013) and it used for comparison of malt quality to other cereals.

3.1 Determination of malt quality

The traditional methods by the EBC Analysis (2010) were used to determine quality of malts. For evaluation of malting quality, some physical and chemical parameters are used which include grain, malt and wort analysis (NIELSEN and MUNCK, 2003). Malt is characterized by quality parameters such as friability, haze of wort, saccharification rate, filtration rate, extract, final attenuation of laboratory wort from malt and diastatic power. These parameters play significant role in the applications in food industry. Malting quality data of selected cereal varieties are shown in Table 2.

Considerable differences can be seen at friability of cereal varieties (Table 2). Winter wheat varieties such as PS Sunanka, Zaira, Vanda and PS 57/11 showed low friability (24.6 – 54.4 %) because endosperm was insufficiently broken during malting process. These results are corresponding to character of cereal grains because wheat has vitreous endosperm. On the other hand, oat variety Dunajec showed high friability (100 %). It means that endosperm is very broken and this material used in brewing process can cause insufficient foaming of beer. Malt friability is good indicator of enduse value. Samples with high friability (Dunajec and Laudis 550) had the potential to produce greater amounts of end product, while samples (all varieties of wheat) with low friability had the potential to cause problems with mashing, lautering and beer filtration (EDNEY and MATHER, 2004). Although oat variety Dunajec has a high friability, it has a high value of wort haze (22 EBC) and filtration rate (120 min.) in comparison to other cereal malts.

Table 2. Malting quality of selected cereal varieties.

Parameter	PS Sunanka (winter wheat)	Zaira (winter wheat)	PS 57/11 (winter wheat)	Vanda (winter wheat)	Dunajec (oat)	Laudis 550 (spring barley)
Friability [%]	51.0	54.4	24.6	32.4	100.0	69.2
Haze of wort (90 °) [EBC]	4.4	3.8	0.7	0.8	22.0	4.0
Saccharification rate [min]	10	10	10	10	10	10
Filtration rate [min]	45	60	45	30	120	30
Extract [%]	81.4	83.8	76.7	76.0	78.2	76.7
Final attenuation of laboratory wort from malt [%]	72.9	76.0	77.6	76.0	70.5	72.5
Diastatic power [WK]	200	220	220	220	200	200

All cereal varieties showed the average extract value from 76 to 78.2 %, except of PS Sunanka and Zaira (wheat) whose higher extract value (81.4 and 83.8 %, respectively) was caused by high content of starch in grain.

Enzymatic activity (β-amylase activity) is expressed using the diastatic power in Windisch-Kolbach units (WK) (KUNZE, 1996) and expresses amount of starch which can be saccharificated by amylases from 100 g of starch. Diastatic power is affected by drying and storage conditions. This parameter was comparable among selected cereal varieties (wheat, oat and barley) and varied from 200 to 220 WK. Final attenuation of laboratory wort from prepared cereal malts correlates with measured diastatic power.

Based on the results of these technological parameters, it can be assumed that oat Dunajec can be used for spirit production due to its high value of friability, haze of worth and filtration rate compared to our reference material (Laudis 550). Malting quality results of winter wheat varieties PS Sunanka and Zaira were comparable to spring barley Laudis 550 and therefore they are potentially useful for beer production.

3.2 Determination of biological activity of cereals and their malts

Free polyphenols are easily extracted to various solvents such as methanol, ethanol, acetone, diethyl ether etc. (STALIKAS, 2007). Our previous studies reported that antioxidants and polyphenols from cereals (wheat, oat) can be effectively extracted by methanol at laboratory temperature (IVANIŠOVÁ *et al.*, 2014; CHMELOVÁ *et al.*, 2015). Therefore, these extraction parameters were used for evaluation of antioxidants and polyphenols present in cereals and their malts.

In the first step, it was necessary to determine the amount of extractive compounds obtained by methanol extraction of cereals and their malts. Results are shown in Table 3.

Table 3. Crude extractive yields obtained by methanol extraction of cereals and their malts.

	Crude extractive yields [%]	
	Cereals	**Malts**
PS Sunanka (winter wheat)	2.9 ± 0.1	3.3 ± 0.6
Zaira (winter wheat)	3.7 ± 0.0	3.8 ± 0.1
PS 57/11 (winter wheat)	5.2 ± 0.5	6.1 ± 0.4
Vanda (winter wheat)	2.8 ± 0.1	4.4 ± 0.7
Dunajec (oat)	7.6 ± 0.3	10.7 ± 0.3
Laudis 550 (spring barley)	2.3 ± 0.6	6.6 ± 0.7

The results indicate that crude extractive yields are higher in malt (3.3 – 10.7 %) than in non-germinating cereals (2.3 – 7.6 %). This could partially be explained by the enzymatic release of bound polyphenols and other compounds during malting (QINGMING *et al.*, 2010). The influence of malting on releasing of bound biological active compounds was investigated. Therefore, antioxidant and polyphenol contents were determined in cereals and their malts. Antioxidant content in different cereal varieties was determined by DPPH method which is based on scavenging of radicals. This method is cheap, cost effective, easily interpreted and useful for screening and comparing large numbers of samples and has become routine practice in evaluating plant materials (RYAN *et al.*, 2011). Results are shown in Fig. 1.

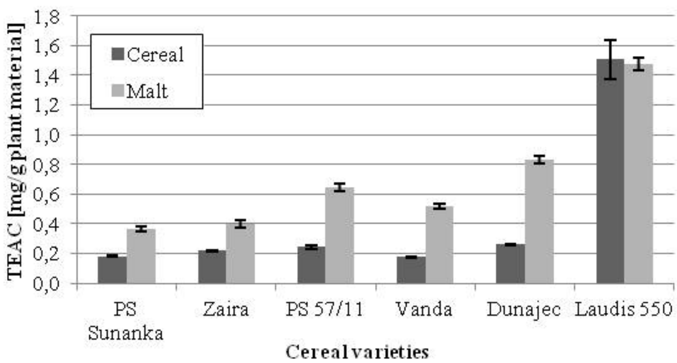

Fig. 1. Antioxidant contents in cereal varieties such as PS Sunanka (winter wheat), Zaira (winter wheat), PS 57/11 (winter wheat), Vanda (winter wheat), Dunajec (oat), Laudis 550 (spring barley) and their malts.

Antioxidant content varied significantly according to the cereal varieties (Fig. 1). The highest antioxidant content was observed in extracts from spring barley Laudis

550 with 1.4 mg TEAC per g while extracts from winter wheat variety Vanda showed the lowest antioxidant content (0.2 mg TEAC per g of plant material). Obviously, antioxidant activity increased during malting. Compared to cereals, antioxidant content of malts prepared from Dunajec, Vanda, PS 57/11, PS Sunanka and Zaira was 3.2, 2.9, 2.6, 2.0 and 1.8-fold higher, respectively. Spring barley Laudis 550 had comparable antioxidant content in cereal (1.51 ± 0.13 mg TEAC per g of plant material) and malt (1.48 ± 0.04 mg TEAC per g of plant material). In this case, malting process did not contribute to the increase of scavenging activity like in case of wheat and oat. These results may be explained by production of various enzymes during germination or antioxidant compositions in barley malt. Some of these antioxidants can be eliminated during barley malting, mainly at steeping phase. Therefore, it may seem that malting process does not releasing of bound compounds (GOUPY *et al.*, 1999).

Polyphenols are considered as a major group of compounds that contribute to antioxidant activities of different cereals (MPOFU *et al.*, 2006; ANSON *et al.*, 2008). Content of these compounds is often correlated with antioxidants in various plant materials. TPC of selected cereals and malts are shown in Fig. 2.

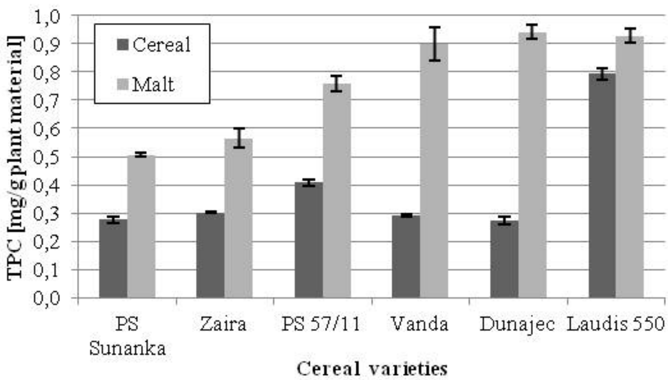

Fig. 2. Total polyphenol contents in cereal varieties such as PS Sunanka (winter wheat), Zaira (winter wheat), PS 57/11 (winter wheat), Vanda (winter wheat), Dunajec (oat), Laudis 550 (spring barley) and their malts.

Fig. 2 shows total polyphenol content (TPC) of cereals and their malts. TPC varied in range from 0.27 to 0.79 mg per g of plant material for cereals and from 0.51 to 0.94 mg per g of plant material for malts. The variation of the differences of the cereal samples increased during malting process when TPC was 1.2 – 3.4-fold higher than TPC in non-germinated cereals. TPC for referent cereal variety Laudis 550 (0.93 mg per g of plant material) was comparable to Dunajec (0.94 mg per g of plant material) and Vanda (0.90 mg per g of plant material). Laudis 550 with high amount of phenols also showed the highest antioxidant activity. However, Laudis 550 showed a small increase in antioxidant activity and polyphenol content in the course of the malting process.

By comparison results of antioxidant and polyphenol content, there was found a positive linear correlation (R^2 = 0.95) for cereals, but correlation coefficient between

TPC and antioxidant content for malts was weak (R^2 = 0.47). During malting process, the hydrolytic enzymes modify structure of germinated grain and may release some of bound polyphenols with potential antioxidant activities (DOBLADO *et al.*, 2007). These results indicate that not all release polyphenols have antioxidant activities.

4. Conclusion

Technological parameters of malts, antioxidant and total polyphenol contents of six cereal varieties PS Sunanka (winter wheat), Zaira (winter wheat), PS 57/11 (winter wheat), Vanda (winter wheat), Dunajec (oat), Laudis 550 (spring barley) and their malts were evaluated. From malting quality parameters, it was examined that oat Dunajec has potential to be used for spirit making and wheat PS Sunanka and Zaira have potential to be used for beer making. Malting process contributed to the increase of scavenging activity in case of wheat and oat, but antioxidant content in malt of Laudis 550 was comparable to its cereal. Total polyphenol content for Laudis 550 was comparable with Dunajec and Vanda. There was a positive linear correlation (R^2 = 0.95) for cereals, but correlation coefficient between total polyphenol content and antioxidant content for malts was weak (R^2 = 0.47).

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