

Antioxidant Characteristics of Hops and Hop Products

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ABSTRACT

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The method based on the reaction of stable DPPH radical proved to be the best for the determination of antioxidant characteristics of hops and hop products. Antioxidant activity is expressed as the rate of decline in absorbance of the reaction environment and assessed in relative percents. Differences in the values of antioxidant activity were determined in Czech and foreign hop varieties. The highest antioxidant activities in the scope of 70 to 80% rel. were measured in Saaz and Spalter Select. Antioxidant activity in most of the varieties moved in the scope of 40 to 60% rel. A part of antioxidant activity of hops is irreversibly lost in the course of drying. The loss does not usually exceed 5% of the original RA_{DPPH} value. Drying also resulted in a decrease of polyphenol compound contents. Drying in belt and chamber kilns is comparable from the point of view of preserving hop antioxidant properties. Results of determination of antioxidant activity in hot water extracts of raw hops and ground hops were comparable and statistically non-significant. The same held true for pelletizing of ground hops. The antioxidant activity of raw hops declined in the course of long-term storage in dependence on storage temperature. Storage temperature had no effect on the antioxidant activity of the hop pellets packed in a multi-layer foil without air access.

Key words: Antioxidants, DPPH, hop ageing, hop processing, hops, polyphenols.

INTRODUCTION

Antioxidants of plant origin are an important component of food with a positive effect on human health. They are able to eliminate from the organism reactive oxygen and nitrogen radicals that irreversibly damage live tissues and induce serious diseases. Oxidative damage is considered to be the main cause of ageing and of several degenerative diseases, such as cardiovascular disease and cancer. Hop is not a direct food material, but the antioxidants present in hops are of considerable importance in the brewing industry. They act in the course of beer production and storage as protection against the origin of undesirable sensorial active substances of stale flavor and have a favorable health effect on beer consumers.

Polyphenols play a key role in hop antioxidant activity as they have antioxidant, antimutagenic, anticarcinogenic, antimicrobial, antithrombotic, and anti-inflammatory effects and in addition they regulate blood pressure and blood glucose levels¹¹.

Today the brewing industry uses dozens of market hop varieties differing in content and composition of secondary metabolites, first of all resins and essential oils; therefore differences in their antioxidant properties can be rightfully supposed. Optimum moisture of dry hops is 8 to 12% by weight. Drying of hop is conducted in two types of kilns, chamber and belt. Drying in both types of dryers proceeds at temperatures of 50 to 60°C for 6 to 10 h. Changes in the antioxidant characteristics of hops in the course of drying have not yet been investigated. Pelletizing of hops is the most widespread form of processing of aromatic and bitter hop varieties today. In the first phase of production of pellets, raw hops are ground to fine powder with a prevailing particle size to 0.5 mm. After homogenization the powdery semi-product is forced through circular slots of the metal matrix and is pressed into pellets. Both grinding and pelletizing are operations during which hops are heated. The temperature of the hops at processing to pellets does not generally exceed the limit of 55°C. With regard to thermal loading of hops, the question arises to what extent this factor can affect the antioxidant state of this brewing raw material. Hop composition also does not remain stable after drying and pelletizing. Depending on storage conditions, the content and composition of hop resins, oils and other substances also change. Up to now we have not known how the antioxidant characteristics of hops are changed during long-term storage.

Many analytical methods for the determination of antioxidant activity in brewing raw materials and beer have been published over the last ten years. A number of analytical methods have been modified and adapted from other food industries (oils, fats, meat and milk) or from pharmaceutical research. Brewing materials and intermediaries of brewing technology, such as malts, sweet wort, hops and hop products, hopped wort and young beer are complex matrices, which contain many substances that oxidize more or less. A detailed summary concerning analytical methods for the determination of the antioxidant activity of brewing materials, intermediaries of brewing technology and beers was published by Moll¹⁰.

Analytical methods can be divided into two groups, chemical and physical ones. Many chemical and biochemical methods are based on the photometric determination of dynamics and on the intensity of the origin of color or

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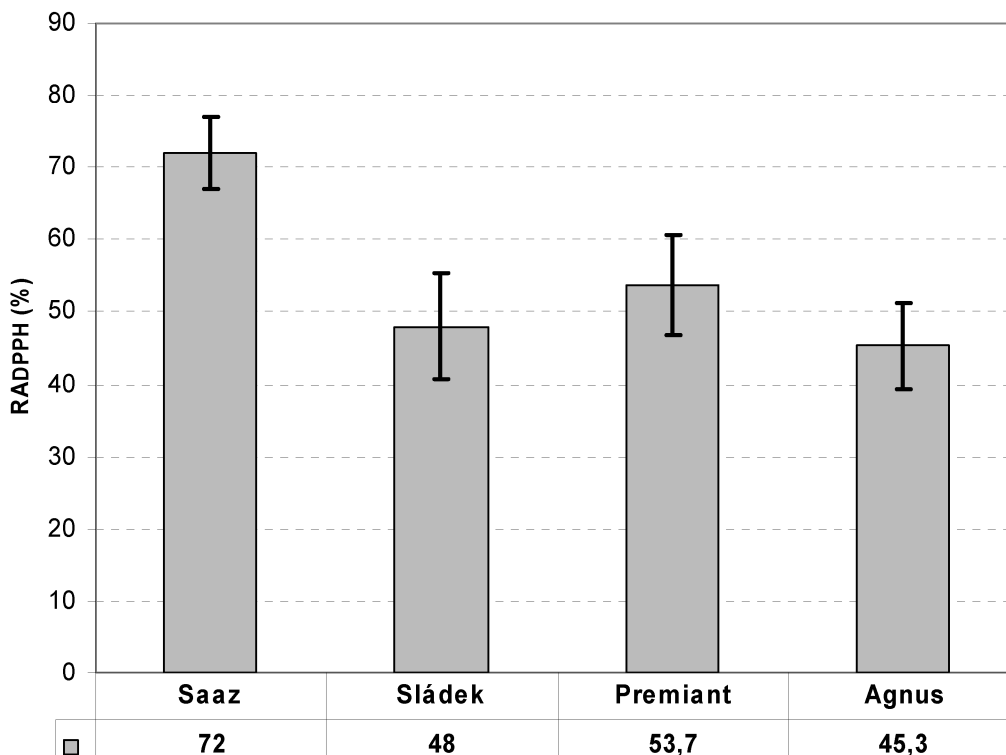


Fig. 1. Antioxidative activities of Czech hop varieties (crops 2004, 2005, 2006, minimum of five samples of each variety yearly).

discoloration of the studied environment as a result of changes in the stable radical concentrations. For the measurement of beer sensorial stability, Kaneda et al.⁶ used a stable radical 1,1-dipyridyl-2-pikryl hydrazyl (DPPH) and Araki et al.³ used a stable radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate), (ABTS).

Physical methods are based, for example, on the measurement of the oxido-reduction potential of solutions. Quantity of antioxidants is also determined by liquid chromatography with electrochemical or coulometric detection. Azo-compounds are appropriate for the study of the antioxidant activity of various substances in the process of lipid peroxidation under "in vitro" conditions that generate free radicals. As a source of alkylperoxyl free radicals, Liégeois et al.⁸ used water-soluble 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) to study the antioxidant properties of wort, malt and hops by the oxidation of linoleic acid in aqueous dispersions. Hops and some hop products are able to inhibit the auto-oxidation of lipids⁴. Lermusieau et al.⁷ demonstrated big differences in reduction activity between hop varieties. Hopping with pellets increased wort reduction activity, hopping with hop extract on the basis of carbon dioxide did not appear to affect the quality of the wort due to a very low content of polyphenols.

Over the last ten years the food industry has used the method of determination of free radicals or antiradical activity of various matrices using electron paramagnetic resonance spectrometry (EPR). The principle of this method is the measurement of energetic changes of free electron radicals by changing the electron orientation (spin) induced by a magnetic field. Antioxidant activity is

assessed on the basis of the reduction in free radicals (generated in a sample by warming or chemically or by the addition of a model stable radical) with the help of antioxidants present in the analysed matrix.

Each of the published methods for the determination of antioxidant activity covers a different spectrum of antioxidants. In the present study, three methods for the determination of antioxidant activity in hops were compared: assessment after Chapon and Louis⁵, Kaneda et al.⁶ and Araki et al.³ Further, results of the assessment of antioxidant characteristics of different hop varieties, fresh and dried hops, processed and non-processed hops are given.

METHODS

Three methods were used for the examination of antioxidant characteristics in raw hops and hop products. Determination of the antioxidant activity as described by Chapon and Louis⁵ is based on the reduction of iron complexes. Determination of antioxidant activity as described by Kaneda et al.⁶ is based on the reaction of the stable color free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) with reducing substances. The actual value of the free radical concentration was measured in the Research Institute of Brewing and Malting with the technique of electron paramagnetic resonance spectrometry (EPR). In the Hop Research Institute we determined these data spectrophotometrically. The antioxidant activity of the RA_{DPPH} samples given in the experimental part is expressed as relative decline in DPPH concentration under the conditions of the determination. The third tested method was the determination of TRAP (Total Reactive Antioxidant

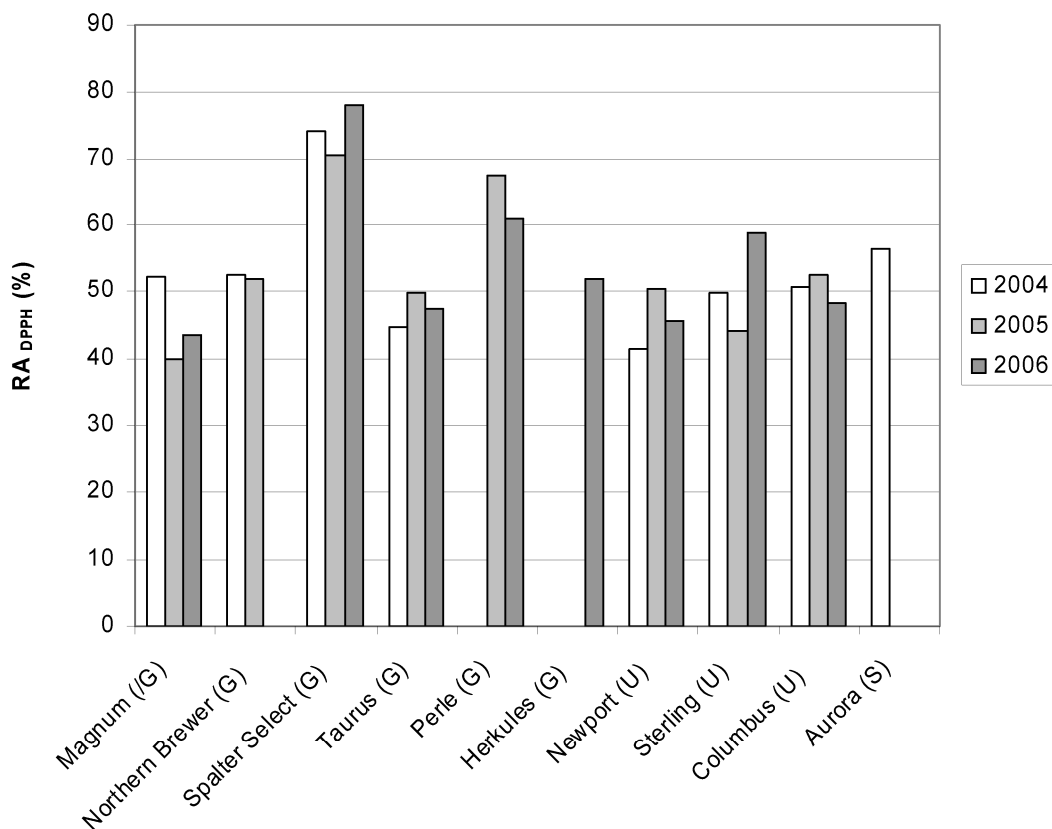


Fig. 2. Antioxidative activities of world hop varieties (crops 2004, 2005, 2006, one sample of each variety yearly). G – Germany, U – USA, S – Slovenia.

Table I. Correlation between antioxidative activity and polyphenols.

n = 15	Polyphenols ^a (mg/L)		
	TP	ANT	FLA
RA _{DPPH}	0.947	0.818	0.956
RA _{Chapon}	0.912	0.752	0.966

^aTP: Total polyphenols, ANT: anthocyanogens, FLA: flavanoids.

Potential) after Araki et al.⁴ based on the free radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate), ABTS.

Total polyphenol and flavanoid content was assessed according to Analytica EBC¹ and anthocyanogens pursuant to the Czech Brewing and Malting Analytica¹². The content of alpha bitter acids was determined with the EBC method 7.7¹ liquid chromatography method and the hop storage index was measured with the spectrophotometric method according to the methods of the ASBC².

First, a hot water extract was prepared from hop samples. A hot water extract was selected because the experimental conditions simulated hop processing in the brewing process. Determination of water content in freshly harvested hops showed the values of 75% ± 1.0% by weight. For determination of antioxidant activity, 5 g of dry matrix was weighed. This amount corresponded to ~20 g of green cones and ~5.5 g of dried cones. Before the analysis, dried cones were ground in a centrifugal mill to a particle size of 1.5 mm. The content of the boiling flask was brought to boil under a backflow condenser. Time of boiling was 30 min. Subsequently, the contents of the

flask was cooled and transferred quantitatively to a 1000 mL volumetric flask and distilled water was added to the gauge line. The prepared extract was filtered through a filtration paper and then through a cellulose membrane filter with a pore size of 0.45 µm. Pure filtrate was used for the measurement of reduction activity.

The effect of drying fresh green hops on antioxidant activity was studied in green and dried cones of the varieties Saaz, Sládek, Premiant, and Agnus in the course of harvests 2005 and 2006. Samples of green and dried hops were taken from hop growers who ran chamber and belt kilns. Samples were processed immediately after acquisition. In October 2005, a long-term storage experiment with the varieties Premiant and Saaz was established with the aim to determine the effect of hop ageing on reduction activity. From the technological line for the production of hop pellets of a hop processing Company in Žatec, a certain quantity of raw hops and pellets of both varieties were taken. Weight of the samples was ca. 100 g. Hop pellets were vacuum packed into a multilayer foil, raw hops were pressed using a laboratory press to small blocks and wrapped in paper. This treatment corresponded with the common commercial packaging of hops for breweries. Samples were stored at a room temperature of 20–24°C and a temperature of 2–3°C, in a number that enabled us to take a new sample at each sampling. In the course of the following period, hop samples were taken for the determination of reduction activity, alpha acid content, hop storage index and moisture content.

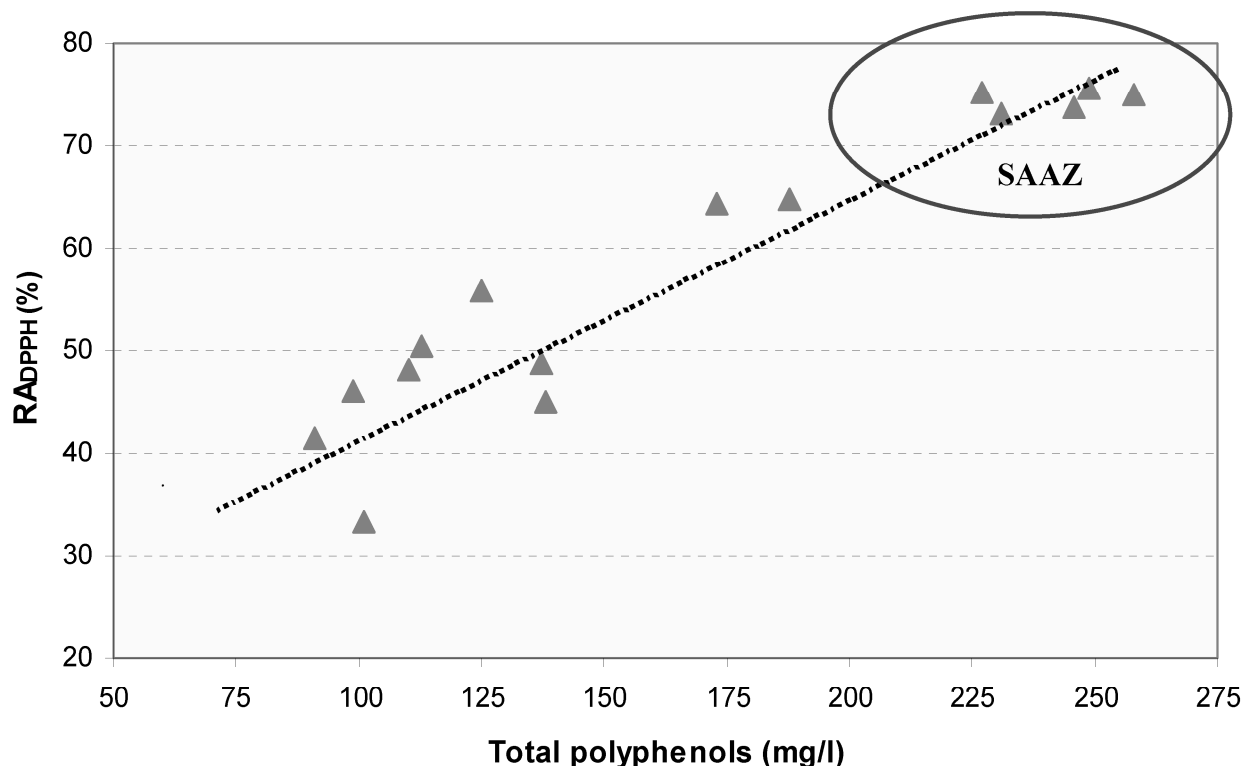


Fig. 3. Correlation between total polyphenols and antioxidative activity (Czech hop varieties, crop 2005).

Table II. Results of determination of antioxidative activity of green and dried hops from harvests 2005 and 2006.

Variety	Locality	Type of a dryer	RA _{DPPH} (% rel.)		Difference of RA _{DPPH} (dried-green %)
			Green	Dried	
Harvest 2005					
Saaz	Stekník	Belt	78.7	75.8	-2.9
Saaz	Vrbičany	Belt	78.5	75.3	-3.2
Sládek	Stekník	Belt	54.6	50.9	-3.7
Premiant	Stekník	Belt	56.3	53.5	-2.8
Agnus	Stekník	Belt	33.0	32.0	-1.0
Harvest 2006					
Saaz	Sedčice	Belt	63.4	62.7	-0.7
Sládek	Milostín	Belt	59.8	55.4	-4.4
Sládek	Očihov	Chamber	47.6	44.2	-3.4
Premiant	Stekník	Belt	50.1	48.0	-2.1
Sládek	Stekník	Belt	47.9	45.1	-2.8
Agnus	Stekník	Chamber	48.2	43.2	-5.0

RESULTS AND DISCUSSION

Antioxidant activity of Czech and foreign hop varieties

The antioxidant activity of Czech and foreign hop varieties was studied in the samples of raw hops from harvests 2004 to 2006. Foreign hops originating from Germany, USA and Slovenia were sampled in the amount of one sample from each variety. The number of samples of the Czech hop varieties was higher, each year at least five samples from each variety from different growing localities were analyzed. Hop antioxidant activity was assessed in hot water extracts with the methods using a free stable radical (Kaneda et al.⁶ and Araki et al.³ and the method according to Chapon and Louis⁵). Of the tested

methods, the determination with the stable DPPH radical with photometric or EPR detection proved to be the best. A good correlation between the spectrophotometric determination and EPR technique ($r = 0.878$, $n = 28$) was found. The Chapon method was applicable, however, it was sensitive for the given matrix. The assessment with stable ABTS radical according to Araki et al.³ was also less suitable as this radical reacted much faster compared to the DPPH and it limited possibilities to cover low differences in antioxidant activities between varieties. Results of the tested methods correlated well and a stronger correlation was found for the relationship of the methods of determination with DPPH and after Chapon and Louis ($r = 0.973$, $n = 18$) than for the methods of determination with DPPH and ABTS ($r = 0.743$, $n = 18$).

Table III. Results of a study on the effects of hop drying on polyphenol substances.

Variety/ Locality	Cones	Polyphenols ^a (mg/L)		
		TP	ANT	FLA
Saaz	Green	289	98.2	29.3
Vrbičany	Dried	229	98.0	20.6
Sládek	Green	185	82.9	19.6
Milostín	Dried	162	78.3	15.5
Sládek	Green	149	69.2	10.4
Očihov	Dried	141	62.5	8.9
Premiant	Green	140	49.2	8.0
Stekník	Dried	107	51.7	9.6
Agnus	Green	110	57.7	6.5
Stekník	Dried	105	49.4	4.4

^aTP: Total polyphenols, ANT: anthocyanogens, FLA: flavanoids.

Table IV. Results of the determination of the antioxidative activity of whole and ground hops from harvests 2005 and 2006.

Variety	Locality	RA _{DPPH} (% rel.)		Difference RA _{DPPH} (ground-raw %)
		Raw hops	Ground hops	
Harvest 2005				
Saaz	Lhota u Rak.	59.8	56.0	-3.8
Saaz	Polepy	66.2	67.2	1.0
Saaz	Račice	59.3	65.2	5.9
Saaz	Kokory	68.0	72.3	4.3
Sládek	Očihov	34.5	35.8	1.3
Sládek	Stekník	36.8	33.5	-3.3
Sládek	Oploty	46.5	45.2	-1.3
Sládek	Kryry	35.1	34.1	-1.0
Premiant	Stekník	38.3	40.7	2.4
Premiant	Staňkovice	41.8	41.7	-0.1
Agnus	Stekník	33.6	33.3	-0.3
Harvest 2006				
Saaz	Přlepy	77.7	80.5	2.8
Saaz	Počedělice	69.4	65.2	-4.2
Saaz	Tršice	58.6	57.3	-1.3
Sládek	Očihov	43.8	43.1	-0.7
Premiant	Hředle	57.1	59.6	2.5
Agnus	Mradice	45.1	49.0	3.9
Agnus	Stekník	41.2	36.8	-4.3

Fig. 1 shows the values of the antioxidant activities of Czech hop varieties in the period of 2004 to 2006 determined with the DPPH method. Fig. 2 summarizes the results of the antioxidant activities determined in the foreign hop varieties in the same period. It is evident from the results that the highest antioxidant activity within 70 to 80% rel. is exhibited by the varieties Saaz and Spalter Select. Most of other varieties followed at a relatively considerable distance, their antioxidant activity within 40 to 60% rel.

The reduction activity of the hops correlated with the content of polyphenol substances. For the set of hops evaluated simultaneously with the methods after Kaneda et al.⁶ and Chapon and Louis⁵, a strong correlation between the values of RA_{DPPH} and RA_{Chapon} and all the assessed groups of polyphenol substances was determined (Table I). The dependence of antioxidant activity on the total polyphenol content is evident from Fig. 3. The polyphenol content in hops relates to the variety Saaz, with high content of these substances and high value of antioxidant activity.

Table V. Results of determination of antioxidative activity of ground and pelleted hops from harvests 2005 and 2006.

Variety	Type/ specification	RA _{DPPH} (% rel.)		Difference RA _{DPPH} (pellets- ground %)
		Ground hops	Pellets	
Harvest 2005				
Saaz	G 45 – NB	72.9	71.6	-1.3
Saaz	G 45 – NB	70.9	71.9	1.0
Saaz	G 45 – NB	65.2	65.9	0.7
Premiant	G 90 – NB	45.6	45.7	0.1
Saaz	Úštěk, G 90	69.8	66.1	-3.7
Saaz	Úštěk, G 90	67.7	67.2	-0.5
Saaz	G 90- NB	62.1	63.7	1.6
Harvest 2006				
Saaz	G 90 – NB	76.5	75.0	-1.5
Saaz	G 90 – NB	72.0	73.1	1.1
Saaz	G 90 – NB	70.2	70.9	0.7
Saaz	G 90 – NB	76.9	78.0	1.1
Saaz	G 90 – NB	74.5	76.0	1.5
Saaz	G 90 – SB	72.6	77.1	4.5

Table VI. Results of determination of RA-DPPH antioxidative activity of hops during long-term storage.

Month	Raw hops		Pellets	
	Warm	Cold	Warm	Cold
Premiant				
October 2005	57.8	57.8	57.5	57.5
September 2006	53.2	45.6	50.5	50.2
Saaz				
October 2005	67.1	67.1	64.7	64.7
September 2006	64.2	58.7	62.2	62.2

The effect of drying on hop antioxidant activity

Table II presents summary results of the determination of antioxidant activities in green and dried hops from harvests 2005 and 2006 for all Czech hop varieties including specification of kiln type and sample origin. It is evident from the results that the antioxidant activities of the hot water extracts in dried and fresh green cones were different. Drying causes loss of some antioxidant activity of hops, but the loss is very low and it does not usually exceed 5% of the original value of RA_{DPPH}. Reduction activity of dried cones is systematically lower, nevertheless the differences are within the limits of the experimental error of repeatability⁹. Comparison of the set of data with the paired t-test proved that the differences were statistically significant. Although the number of data from the chamber kilns was much lower than from the belt ones, in terms of maintaining antioxidant characteristics of hops there was not any significant difference. In practice, high attention is paid to maintaining the drying temperatures, as at temperatures of drying above 60°C changes in color and sensory properties of the dried hops generally occur and the hops consequently lose value.

Some hop samples were also analyzed for their content of polyphenol substances (Table III). Some polyphenol substances were lost during drying. The total polyphenol content in the extract of the dried cones was approximately 5 to 30% rel.

Table VII. Alpha acids content, moisture and hop storage index (HSI) during the storage test.

Month	Cold						Warm					
	Raw hops			Pellets			Raw hops			Pellets		
	Alpha % weight	/moisture % weight	HSI	Alpha % weight	/moisture % weight	HSI	Alpha % weight	/moisture % weight	HSI	Alpha % weight	/moisture % weight	HSI
Premiant												
October 2005	8.45	9.3	0.28	8.70	6.7	0.29	8.45	9.3	0.29	8.70	6.7	0.29
September 2006	6.17	12.6	0.48	8.25	6.6	0.34	4.09	6.2	1.19	7.12	6.4	0.43
Saaz hop												
October 2005	3.65	9.1	0.31	3.51	6.5	0.31	3.65	9.1	0.31	3.51	6.5	0.31
September 2006	2.52	15.2	0.65	3.28	6.4	0.39	1.58	7.4	1.40	2.71	6.6	0.46

The effect of milling and pelletization on hop antioxidant activity

To determine the effect of hop pelletization on antioxidant activity, the pelletization process was divided into two parts. A preliminary test proved to be very difficult to meet technological concurrence of samples under production conditions (in the production line of the Hop Processing Company Žatec), i.e. raw hops from filling, ground hops from the homogenization silo and finished pellets. In the first part, the effect of milling of hops under the laboratory conditions was studied. Correctness of this method resulted from the distribution of particles of hops ground in a laboratory mill (Retsch ZM1- sieve 1.5 mm, hop moisture 7 to 9%) and hop powder that was created after dissolving the pellets in hot water and subsequent drying. The results of a metrical analysis of the pellets and cones milled in the laboratory showed that the size distribution of particles was practically identical and therefore comparable extractability of particles during extract preparation could also be expected. The effect of pelletization of the milled intermediates on antioxidant activity was examined by analyzing series of couples of ground hop operational samples from the homogenization silo and produced pellets taken behind a pelleting head from the technological line in the Hop Processing Company Žatec. In this case there was a very high probability of taking technologically concurrent samples.

The results of the determination of the antioxidant activity in the hot water extract of raw hops and ground cones in the laboratory mill Retsch ZM1 from harvests 2005 and 2006 are summarized in Table IV. Differences in the antioxidant activities of raw and ground hops were very small. This was also confirmed by statistical evaluation with a paired t-test, which evaluated the differences as statistically non-significant. Therefore, it can be stated that the milling of whole cones to powder as a first phase at production of hop pellets does not significantly change the reduction activity.

The results of the measurement of antioxidant activity in the ground hop samples and pellets in Saaz and Premiant in the period of 2005 and 2006 are given in Table V. Samples of Saaz prevailed markedly, given by the processing volume of this variety on the production line. Samples of hops were from harvests 2005 and 2006. Samples of ground hops were collected from the homogeniza-

tion silo before pelletization and samples of hop pellets were collected directly behind the pelletization head, so that technological concurrence of the taken samples was maintained. The hop pellets were weighed before determination of antioxidant activity without further treatment, as after adding to water they split into a powdery intermediary shortly after boiling. Weighed amounts of milled cones and pellets, taking into account moisture content, were adjusted so that the quantity of dry substance for the analysis was 5 g. The results of analyses in Table V show that the differences in antioxidant activities of ground hops and pellets were very small and statistically non-significant (paired t-test).

The effect of ageing and long-term storage on antioxidant activity of hops

Table VI presents the results of antioxidant activity measurements in all experimental variants at the beginning and at the end of the experiment (September 2006). Table VII summarizes the results of the determination in further quality parameters of hops that characterize ageing hops in terms of hop resin composition (content of alpha acids, moisture and hop storage index).

Results of the determination in antioxidant activities in Table VI show that during storage antioxidant activity declines at different rates, depending on the storage temperature and hop form. It was found that storage temperature had no detectable effect on the antioxidant activity of hop pellets packed in multi-layer aluminum foil without air access. Nevertheless, hop quality apparently deteriorated as the alpha acid content in Premiant declined by 18.2% rel. (warm) and 5.2% (cold) or by 22.8 and 6.5% rel. in Saaz. Deterioration in quality was also documented by the values of the hop storage index, which in Premiant rose to 0.43 (pellets-warm) and 0.34 (pellets-cold), in Saaz to 0.46 (pellets-warm) and 0.39 (pellets-cold). These values confirmed that a low storage temperature affected the state of the hop resins favourably.

Completely different results of antioxidant activity were observed in the pressed hops. The antioxidant activity of the hops stored in the cold was at the end of the experiment substantially lower than those stored in the warm. But the brewing value of the hops stored in the warm was markedly worse than that of the hops kept in the cold. This is documented by a 50–60% loss of alpha acid content (warm) or 27–31% (cold) and a value of the

hop storage index of higher than 1.00 (Table VII). The reason for this apparent paradox may arise from the high moisture in raw hops stored in the cold at a high relative humidity. Moisture content in raw hops stored in the warm declined from 9.3% to 6.2% (Premiant) and from 9.1% to 7.4% (Saaz). Moisture content in raw hops stored in the cold increased from 9.3% to 12.6% (Premiant) and 9.1% to 15.2% (Saaz) (Table VII). Since the experimental packets of $10 \times 10 \times 4$ cm were wrapped in paper, moisture may have had a longer time to penetrate the whole volume and accelerate reactions of hop polyphenol compounds, which as a result led to the deterioration of antioxidant properties. In practice, since hops are usually pressed into 50 kg blocks, of $50 \times 60 \times 50$ cm, this feature would be probably restricted to the outer layers of the package.

CONCLUSIONS

The highest antioxidant activity in the range of 70–80% was measured in Saaz and Spalter Select. The antioxidant activity in the other varieties varied mostly in the range of 40–60% rel.

The antioxidant activities of green and dried hop cones in Saaz, Sladek and Premiant differed significantly. Drying caused a loss of some antioxidant activity. The loss usually did not exceed 5% of the original value of RA_{DPPH} . No significant differences were found, between drying in the chamber and with belt kilns, in terms of maintaining the antioxidant characteristics of the hops.

Hop milling and pelletization did not affect hop antioxidant activity or the content of the polyphenol substances.

During storage, hop antioxidant activity declined at a different rate depending on the storage temperature and form of the hops. Storage temperature did not significantly affect the RA_{DPPH} antioxidant activity of the pelleted hops packed in a multi-layer foil without air access.

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