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Reducing power of hop cultivars and beer ageing

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Abstract

Since lipid auto-oxidation during wort boiling is a determining for the appearance of staling flavour in aged beers, we have investigated the reducing power of hops added in the boiling kettle. An assay based on the inhibition of linoleic acid oxidation in the presence of an initiator [2,2′-azobis(2-amidino-propane) dihydrochloride = AAPH] enabled us to distinguish hop varieties and conditionings. Large differences in hop flavanoid contents explained the higher antioxidant activity of low-α-acid samples versus bitter varieties and CO_2 hop extracts. As expected, adding hop pellets to the kettle effectively increased the overall reducing activity of wort. Supercritical CO_2 hop extracts had no significant effect due to their extremely low level of polyphenols. The concentration of the very well-known marker of beer ageing, *trans*-2-nonenal, was lower in boiled wort exhibiting a better reducing power. The AAPH reducing power test applied to hops or worts was thus efficient to predict the nonenal synthesis during boiling. Hop varieties and conditionings emerged from this work as key-parameters for improving the reducing power of wort and the flavour stability of the final product. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hop; Reducing power; Lipid oxidation; Polyphenols; Beer staling; *trans*-2-nonenal

1. Introduction

Flavour changes in packaged beer constitute one of the most serious problems in brewing. Among the carbonyls responsible for these changes, *trans*-2-nonenal is very important, imparting to the product a cardboard off-flavour at a concentration as low as 0.035 ppb (μg kg^{-1}) (Meilgaard, 1993). Despite the high reducing activity of yeast, oxidation during mashing and boiling appears as the key step in development of this stale flavour during beer storage (Lermusieau, Noël, Liégeois & Collin, 1999). According to Collin et al. (1997), Noël, Liégeois, Lermusieau, Bodart, Badot and Collin (1999) and Noël, Metais et al. (1999), *trans*-2-nonenal is derived from linoleic acid oxidation at the end of the boiling stage and can be protected from yeast activity by binding to nitrogenous compounds such as amino acids and proteins. *trans*-2-Nonenal would be further released by acidic hydrolysis, mainly when the beer pH is low or the storage temperature inadequate.

Most efforts in breweries now focus on inhibiting lipid auto-oxidation reactions in the brewhouse. Adding sulphites to the kettle proves an interesting way to limit the cardboard flavour in aged beers when too highly oxidized green worts are used (Lermusieau et al., 1999). Optimising the natural reducing substances in raw materials seems, however, more attractive. A new assay, recently developed by Liégeois, Lermusieau and Collin (1999, 2000) for assessing the relative antioxidant properties of raw materials, has revealed the exceptional intrinsic reducing activity of hops (30 times more efficient than malt).

The aim of the present work was therefore to investigate various hop cultivars and conditionings. We have compared how effectively six hop varieties (harvest 1998, T90 pellets) and two supercritical CO_2 hop extracts inhibit linoleic acid oxidation in an aqueous dispersion, relating the results to the α-acid and polyphenol contents. The reducing power was also assessed for six worts boiled in the presence of different hop varieties and conditionings. Nonenal potential measurements on boiled wort (defined by Drost, Van den Berg, Freijee, van der Velde & Hollemans, 1990 as the potential of wort to release *trans*-2-nonenal when cooked 2 h at 100°C and pH 4 under Argon), were used

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to assess the wort reducing power influence on the nonenal synthesis during this step. Eventually, we wanted to accredit the AAPH reducing power test developed by Liégeois et al. (1999, 2000) in foreseeing the nonenal potential of pitching wort by measuring its antioxidant activity.

2. Materials and methods

2.1. Extraction of antioxidants from hop

Saaz, Hallertau, Tettnang, Styrian Goldings, Challenger and Nugget varieties, all pellets T90 from the harvest 1998, were received from Van Mollem- Carty & Cie (Opwijk, Belgium). Two supercritical CO_2 hop extracts (Saaz and Target) were also purchased. All samples were stored at −75°C until needed. The α-acids and iso-α-acids extracts were from Labor Veritas (Zurich, Switzerland).

Hop pellets were crushed in a mortar. Ground samples (1 g) were extracted four times under nitrogen with 7 ml methanol 205 super purity (Romil, Cambridge, England) by shaking for 15 min and sonicating for 5 min. After centrifugation (3500 *g*, 10 min), the supernatant (25 ml) was collected and diluted ten times with methanol. For CO_2 hop extracts, an aliquot (1 g) was dissolved under nitrogen in 25 ml methanol by sonicating for 10 min and diluted ten times in order to obtain a 4 mg hop equivalent per ml of solution.

2.2. Antioxidant activity assay

An aqueous solution of linoleic acid (99%, Sigma Chemical Co., St. Louis, USA) was prepared weekly according to Surrey (1964) with minor modifications : 0.25 ml of linoleic acid was added under stirring to 5.0 ml of 0.05 M borate buffer solution (boric acid, Merck, Darmstadt, Germany), pH 9, containing 0.25 ml of tween 20® (Merck, Darmstadt, Germany). The resulting dispersion was clarified by adding 1 ml of 1 N sodium hydroxide (Vel, Leuven, Belgium). The volume was adjusted to 50 ml with additional borate buffer. This solution was stored at 4°C under argon until needed.

2,2′-Azobis(2-amidino-propane) dihydrochloride (AAPH, Aldrich Chem., Milwaukee, USA) (40 mM) was daily prepared in 0.05 M phosphate buffer, pH 7.4 (Merck, Darmstadt, Germany).

The antioxidant activity was determined as the inhibition times (T_{inh}) of linoleic acid oxidation induced in an aqueous solution by the free radical initiator AAPH (Liégeois et al., 1999, 2000). In a 3 ml quartz cell, 0.16 mM linoleic acid (= 30 μl of the above solution) was oxidized by 2 mM AAPH (= 150 μl of the above solution) in 50 mM phosphate buffer (pH 7.4) under air at 37°C. Oxidation was completed in the presence of either 10 μl of methanolic hop extract [initially at 4 mg hop equivalent per ml; final concentration in the assay = 13.3 ppm (mg kg^{-1})] or 7.5 μl of wort (dilution factor in the assay = 400). In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of methanol (0.33%). The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. A Shimadzu UV-240 spectrophotometer equipped with an automatic sample positioner allowed analyses of six samples every minute.

2.3. Polyphenol and flavanoid quantification in methanolic hop extracts

In order to remove humulones, 1 g of ground hop pellets was first extracted three times with 7 ml diethyl ether (super purity solvent, Romil, Loughborough, England) by shaking for 15 min and sonicating for 5 min (McMurrough & Hennigan, 1984). After centrifugation (3500 ***g***, 10 min), the supernatant was removed. The residual hop was then extracted with methanol as just described. Residual diethyl ether was removed by concentrating the solution to 15 ml (Rotavapor, Buchi, Switzerland). The volume was finally adjusted to 25 ml with methanol.

Total polyphenols were determined according to Bishop (1972). Flavonoids were determined by the method of Delcour and Jansens de Varebeke (1985; results expressed in catechin equivalents).

2.4. Wort boiling

Either 1.275 g CO_2 Saaz extract (28.5% α-acids) were dissolved in 5 ml methanol or 2.7 g ground hop pellets of Saaz (2.9% α-acids) or Challenger (6.3% α-acids) were added to 1.5 l of an industrial wort (12°P, 90% malt, 10% corn, pH 5.4) as it began to boil or 7 min before the end of boiling (total boiling time: 75 minutes). After a 20-min clarification, the hot trub was removed and the wort quickly cooled. Boiling experiments were done in duplicate, using 2-l flasks heated in the same 113°C glycerol bath. The inhibition time of the wort was measured before the boiling step and in the pitching wort after cooling.

2.5. Nonenal potential experiment applied to worts (based on the method of Drost et al., 1990)

The pH of 1.5 l of wort was adjusted to 4 with 85% phosphoric acid (Merck, Darmstadt, Germany). After being purged for 15 min with argon (Air Liquide, Liège, Belgium) to reduce the oxygen level, the wort was heated at 100°C for 2 h in a 2 l closed vessel and then cooled to 4°C and kept at that temperature overnight

prior to *trans*-2-nonenal analysis. *trans*-2-Nonenal was extracted by vacuum-distillation, transferred to dichloromethane (grade HPLC, Romil, Cambridge, England), and concentrated to 0.5 ml before analysis by gas chromatography as described by Lermusieau et al. (1999). The variation coefficient of the method was under 10% (Noël, Liégeois et al., 1999).

3. Results and discussion

3.1. Reducing power of various hop cultivars

The reducing powers of six types of hop pellets (T90) from the 1998 harvest and of two CO_2 hop extracts were determined by means of the AAPH-induced oxidation assay proposed by Liégeois et al. (1999, 2000). As already mentioned (Liégeois et al., 1999), hops displayed a very high intrinsic antioxidant activity (Table 1). Methanol was chosen here to recover most antioxidants soluble in hot aqueous solutions, such as boiling worts (McMurrough & Madigan, 1996; Stevens, Taylor & Deinzer, 1999). As the hopping rate actually depends, in brewery, on the α-acid content (bitter substances), the results were also calculated in ppm α-acid equivalents. As shown by Liégeois et al. (2000), this was made possible since a linear relationship exists between reducing power and hop concentration.

Surprisingly, very high T_{inh} differences appeared between hop varieties, mainly when given for 1 ppm α-acid equivalents in the assay. The Saaz variety, usually recognized as the best one for flavour (Perpète, Mélotte, Dupire & Collin, 1998), also displayed the best reducing power with an eight-times-longer inhibition time than the Nugget sample.

The higher the α-acid concentration in hop pellets, the shorter the inhibition time, indicating that α-acids do not contribute significantly to the reducing power of the hop. When the antioxidant assay was applied to 1 ppm α-acids (in the 3-ml test), the calculated inhibition time was as low as 4.8 min. Hop extracts with a high level of bitter substances and almost no polyphenols displayed the least ability to confer reducing power to the boiling wort and, in this case only, α-acids can explain a great part of the antioxidant activity.

Table 1
Reducing power of six hops pellets (harvest 1998, T90) and two CO_2 hop extracts

Variety	T_{inh} for the 13.3 ppm of hop used in the assay[a] (min.)	α-Acid value[b] (g/100g)	T_{inh} for 1 ppm α-acid equivalent in the assay[c] (min.)
Pellets			
Saaz	62.9	2.9	162.8
Hallertau	55.4	3.3	126.0
Tettnang	52.8	4.8	82.6
Styrian Goldings	42.9	4.4	73.2
Challenger	40.3	6.3	48.0
Nugget	38.0	11.8	24.2
CO_2 extracts			
Target	53.8	46.3	8.7
Saaz	50.6	28.7	13.2

[a] Mean of extraction and assay duplicates; variation coefficient under 2% according to Liégeois et al. (1999)
[b] According to the analytica EBC (1987)
[c] Deduced from the hop α-acid content.

3.2. Contributors to the reducing power of hop

To identify other contributors to the reducing power of hop, we subtracted the contribution of α-acids to the global hop inhibition time (Table 2). For this purpose, the linear relationship between the reducing power of α-acids solutions and concentration was checked (data not shown).

Phenolic compounds emerge from many works as the most efficient natural antioxidants (Liégeois et al., 2000; Maillard & Berset, 1995; Saint-Cricq de Gaulejac, Provost & Vivas, 1999; Sawa, Nakao, Akaike, Ono & Maeda, 1999). Since Asano, Kowaka and Hashimoto (1981) and Erdal, Outtrup and Ahrenst-Larsen (1985) revealed an inverse correlation between α-acid and polyphenol contents in hops, we logically suspected that this might partially explain our experimental reducing power values. Total polyphenol and flavanoid contents were therefore experimentally determined in all our samples. As depicted in Table 2, the higher the polyphenol or flavanoid content, the longer the inhibition time.

When total polyphenols or flavanoids were plotted versus the inhibition time, good correlations were obtained (Fig. 1), confirming the key role of the latter in hop antioxidant activity.

Table 2
Hop reducing power calculated after α-acid inhibition time subtraction (relationship with polyphenol and flavanoid contents)

Variety	T_{inh} after subtraction of the α-acid contribution (min.)	Total polyphenols in hop (g/100g)	Flavanoids in hop (g/100g, catechin equivalent)
Saaz	61.1	3.79	0.92
Hallertau	53.3	2.71	0.74
Tettnang	49.7	2.23	0.58
Styrian Goldings	40.1	2.13	0.44
Challenger	36.3	1.04	0.35
Nugget	30.5	0.52	0.20

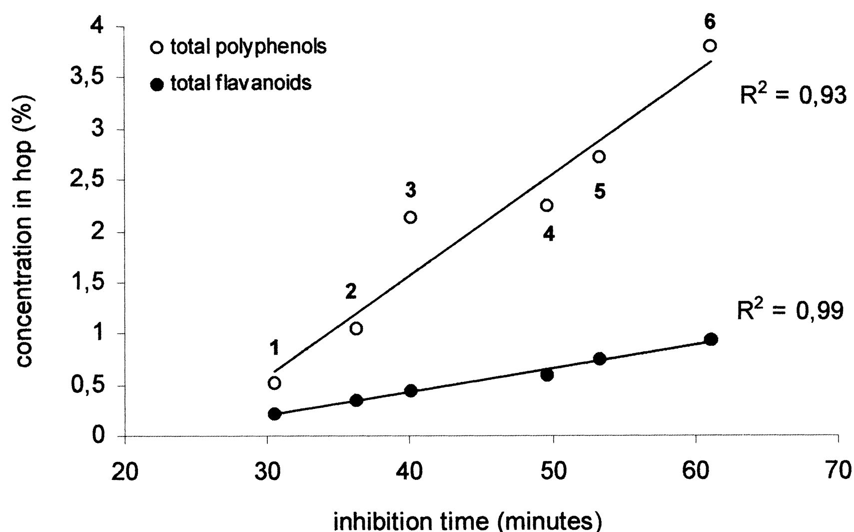

Fig. 1. Relationship between total polyphenols or flavanoids and the inhibition times measured for the six hop pellet varieties: 1, Nugget; 2, Challenger; 3, Styrian Goldings; 4, Tettnang; 5, Hallertau; 6, Saaz.

3.3. Relationship to beer ageing

trans-2-Nonenal, synthesized by linoleic acid autooxidation during boiling, can persist in the fresh beer because it binds to nitrogenous compounds of wort (Noël, Liégeois et al., 1999). This yeast-resistant *trans*-2-nonenal fraction, released as free *trans*-2-nonenal during aging, can be estimated by measuring the nonenal potential (Drost et al., 1990). If the wort's reducing power is optimised by a good choice of hop, the nonenal potential should thereby logically be decreased.

Two pellet varieties, Saaz and Challenger, were chosen for their very different intrinsic reducing powers. The hopping rate was 1.8 g/l in both cases. To produce wort with the CO_2 Saaz extract, a lower hopping rate was chosen (0.85 g/l) because of the very high α-acid content of the extract. Hops were added to the wort, either at the beginning or 7 min before the end of the boiling stage.

During this stage, part of the wort polyphenols from the malt precipitated, as expected. As shown in Table 3, reducing compounds derived from Maillard reactions (melanoidins, reductones; Pflugfelder, 1992) were unable to balance these losses in the absence of hop. This led to shorter inhibition times for the pitching wort than for the unboiled green wort.

When Saaz or Challenger hop pellets were added, the reducing power of the pitching wort increased by 29–36% (Table 3), confirming that hop contributes to the polyphenols content of beer. After boiling, differences between antioxidant activities of Saaz and Challenger worts could again be observed, as expected by measurements on fresh hop samples. The higher the reducing power of hop sample, the higher the reducing power of wort. On the other hand, CO_2 hop extracts had almost no effect on this reducing power and this is readily explained by the quasi absence of polyphenols in such extracts and by the precipitation or isomerization of α-acids (Tinh = 4.8 min ppm^{-1}) into iso-α-acids (Tinh = 0 min ppm^{-1}) during this hot step.

Table 3
Experimental reducing power values (dilution factor in the assay = 400, results reported for a 12°P wort) for the unboiled wort and for pitching worts resulting from various kinds of hopping either at the beginning or at the end of the boiling stage (1.8 g/l Saaz or Challenger pellets or 0.85 g/l CO_2 Saaz extract)

	T_{inh} (min.)		T_{inh} increase against the blank (%)
	Boiling duplicates	Average	
Wort before boiling	–	39.3[a]	–
BLANK = Boiling without hop	36.9[a] 36.7[a]	36.8	–
Saaz pellets[b,d]	49.8[a] 50.5[a]	50.2	+36
Challenger pellets[b,d]	48.3[a] 46.5[a]	47.4	+29
CO_2 Saaz extract[c,d]	38.9[a] 37.4[a]	38.2	+4
Saaz pellets[b,e]	51.3[a] 48.0[a]	49.7	+35
Challenger pellets[b,e]	48.4[a] 49.1[a]	48.8	+33
CO_2 Saaz extract[c,e]	39.1[a] 38.8[a]	39.0	+6

[a] Four assays were realized for the unboiled wort and two assays were realized for each boiling duplicate-variation coefficient of the assay = 2%.
[b] 4.5 ppm hop in the assay.
[c] 2.125 ppm hop in the assay.
[d] Added at the beginning of the boiling.
[e] Added 7 min before the end of the boiling.

Table 4
Nonenal potential (NP) of pitching worts hopped at the beginning of the boiling stage (results calculated for a 12°P wort) with various kinds of hops (1.8 g/l Saaz or Challenger pellets or 0.85 g/l of CO_2 Saaz extract)

	NP[a] (ppb)		T_{inh} (min.)
	Boiling duplicates	Average	Average
BLANK = boiling without hop	3.0 3.4	3.2	36.8
Boiling with Saaz pellets	2.5	2.5	50.2
Boiling with Challenger pellets	2.4 2.6	2.5	47.4
Boiling with CO2 Saaz extract	3.2 2.8	3.0	38.2

[a] Variation coefficient of the assay under 10% (Noël, Liégeois et al., 1999).

Surprisingly, hopping at the end of the boiling step leads to similar values as hopping at the beginning. This means that soluble hop reducing compounds were quickly transferred into aqueous solution at 100°C and that the most unstable antioxidants had disappeared after 27 min (7 min of boiling and 20 min of clarification).

Nonenal potential measurements on pitching worts (hopping at the beginning of boiling) again demonstrated the benefit of using polyphenol-rich conditionings, such as pellets, for improving the organoleptic stability of beer (Table 4). In agreement with the opinions of most brewers, CO_2 hop extracts emerged as the least effective at preventing staling. By the global comparison of the four brews (reducing power and nonenal potential), it seems that the method used for assessing the hop and the wort reducing power is useful in estimating the susceptibility of wort to *trans*-2-nonenal synthesis. The higher the reducing power brought to the wort by hop, the lower the nonenal synthesized and linked to nitrogenous compounds.

4. Conclusion

Although hop is added in small amounts in the kettle, our results confirm that it contributes greatly to the reducing power of wort. Yet the antioxidant activity of hop depends on the variety and on its quality and processing. Polyphenols are the main contributors to the reducing power of hop. Thus, as revealed by nonenal potential measurements, pellet samples can prevent linoleic acid oxidation in the kettle, source of the cardboard flavour in aged beer. In all cases, the measurement of the wort reducing power with the AAPH test proved effective in foreseeing the quality of the protection against *trans*-2-nonenal synthesis during boiling.

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References

Asano, K., Kowaka, M., & Hashimoto, N. (1981). Haze index of hops: an attempt to predict the effect of hop polyphenols on colloïdal stability of beer. *Rept. Res. Lab. Kirin Brewery Co., Ltd, 24*, 1–6.

Bishop, L. R. (1972). Analysis committee of the European Brewery Convention: the measurement of total polyphenols in worts and beers. *J. Inst. Brew, 78*, 37–38.

Collin, S., Noël, S., Bonte, S., Metais, N., Bodart, E., Peladan, F., & Dupire, S. (1997). The use of $^{18}O_2$ in appraising the impact of oxidation processes during mashing and beer storage. In *Proceedings of the 26th European Brewery Convention Congress* (pp. 535–544). Oxford: IRL Press.

Delcour, J. A., & Janssens de Varebeke, D. A. (1985). New colourimetric assay for flavanoïds in pilsner beers. *J. Inst. Brew, 91*, 37–40.

Drost, B. W., Van den Berg, R., Freijee, F. J. M., van der Velde, E. G., & Hollemans, M. (1990). Flavor stability. *J. Am. Soc. Brew. Chem., 48*, 124–131.

Erdal, K., Outtrup, H., & Ahrenst-Larsen, B. (1985). Barley and hop proanthocyanidins and the colloïdal stability of beer. In *Proceedings of the 20th European Brewery Convention Congress* (pp. 459–466). Oxford: IRL Press.

European Brewery Convention (1987). *Analytica EBC, Brauerei-und-getränke-rundschau*, (4th ed.) E113.

Lermusieau, G., Noël, S., Liégeois, C., & Collin, S. (1999). A non-oxidative mechanism for the development of trans-2-nonenal in beer. *J. Am. Soc. Brew. Chem., 57*, 29–33.

Liégeois, C., Lermusieau, G., & Collin, S. (1999). A global reduction power test instead of monitoring malt lipoxygenasic activity. In *Proceedings of the 26th European Brewery Convention Congress* (pp. 461–468). Oxford: IRL Press.

Liégeois, C., Lermusieau, G., & Collin, S. (2000). Measuring antioxidant efficiency of wort, malt, and hops against the 2,2′-azobis(2-amidinopropane) dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid. *J. Agric. Food Chem., 48*(4).

Maillard, M. N., & Berset, C. (1995). Evolution of antioxidant activity during kilning: role of insoluble bound phenolic acids of barley and malt. *J. Agric. Food Chem., 43*, 1789–1793.

McMurrough, I., & Hennigan, G. P. (1984). Tanning properties of flavanols in barley and hops measured by reaction with cinchonine sulphate in relation to haze formation in beer. *J. Inst. Brew, 90*, 24–32.

McMurrough, I., & Madigan, D. (1996). Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthocyanidins fom barley. *J. Agric. Food Chem., 44*, 1731–1735.

Meilgaard, M. C. (1993). Individual differences in sensory threshold for aroma chemicals added to beer. *Food Quality and Preference, 4*, 153–167.

Noël, S., Liégeois, C., Lermusieau, G., Bodart, E., Badot, C., & Collin, S. (1999). Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *J. Agric. Food Chem., 47*, 4323–4326.

Noël, S., Metais, N., Bonte, S., Bodart, E., Peladan, F., Dupire, S., & Collin, S. (1999). The use of oxygen 18 in appraising the impact of oxidation processes during beer storage. *J. Inst. Brew, 105*, 269–274.

Perpète, P., Mélotte, L., Dupire, S., & Collin, S. (1998). Varietal discrimination of hop pellets by essential oil analysis. I. Comparison of fresh samples. *J. Am. Soc. Brew. Chem., 56*, 104–108.

Pflugfelder, R. L. (1992). Wort reducing power — sources, methods of analysis and influence on beer quality. In *Proceedings of the 5th J. De Clerck Chair: Leuven*.

Saint-Cricq de Gaulejac, N., Provost, C., & Vivas, N. (1999). Comparative study of polyphenol scavenging activities assessed by different methods. *J. Agric. Food Chem., 47*, 425–431.

Sawa, T., Nakao, M., Akaike, T., Ono, K., & Maeda, H. (1999). Alkylperoxyl radical-scavenging activity of various flavanoids and other phenolic compounds: implications for the anti-tumor promoter effects of vegetables. *J. Agric. Food Chem., 47*, 397–402.

Stevens, J. F., Taylor, A. W., & Deinzer, M. L. (1999). Quantitative analysis of xanthohumol and related prenylflavonoids in hop and beer by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 832*, 97–107.

Surrey, K. (1964). Spectrophotometric method for determination of lipoxidase activity. *Plant Physiology, 39*, 65–70.