Development and Correlation Between the Organic Radical Concentration in Different Malt Types and Oxidative Beer Stability

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ABSTRACT

An optimized version of the patented electron spin resonance spectroscopy method, using a novel internal standard (^{52}Cr:MgO), was used to monitor the development of organic radicals during the malting process and the radical content in different commercial specialty malts. The temperature during the withering and kilning steps in malt production had a direct influence on the generation of stable organic radicals in the finished malt, whereby higher temperatures resulted in greater radical concentrations. The majority of organic radicals in pilsner malt were bound in the husk and, therefore, did not significantly transfer into the wort and subsequent beer. In roasted malts, the organic radicals were distributed more uniformly throughout the kernel. Furthermore, depending on the organic radical content and Maillard reaction products present in the endosperm of crystal and roasted malts, more oxidation reactions took place during the mashing, and there was higher radical formation during the wort boiling process. Consequently, the endogenous antioxidant potential value and sulfur dioxide content in the final beer were reduced. These results indicate that there is a direct link between organic radicals in the endosperm generated by the kilning temperature during the malting process and color as an indicator of Maillard reaction products and the oxidative stability of the resultant beer.

Keywords: Electron spin resonance spectroscopy, Flavor stability, Maillard reaction products, Malting process, Organic radicals, Specialty malts

RESUMEN

Una versión optimizada del método patentado de espectroscopia de la resonancia de spin electrónico, utilizando un nuevo estándar interno (^{52}Cr:MgO), fue utilizada para supervisar el desarrollo de los radicales orgánicos durante el proceso de malteado y el contenido de radicales en diferentes tipos de malta comercial. La temperatura durante los pasos de marchitez y secado en la producción de malta tuvo una influencia directa en la generación de radicales orgánicos estables en la malta terminada, que el aumento de las temperaturas como resultado una mayor concentración de radicales. La mayoría de los radicales orgánicos en malta pilsner fueron atados con la cáscara y, por tanto, no afectaron significativamente la transferencia en el mosto y la cerveza posteriores. En maltas tostadas, los radicales orgánicos se distribuyeron de manera más uniforme en todo el grano. Por otra parte, según el contenido radical orgánico y productos de la reacción de Maillard presente en el endospermo de malta cristal y de malta tostada, más las reacciones de oxidación se llevó a cabo durante la maceración, y fue mayor formación de radicales durante el proceso de cocción del mosto. En consecuencia, el valor antioxidante endógeno potencial y contenido de dióxido de azufre en la cerveza final se redujeron. Estos resultados indican que existe una relación directa entre los radicales orgánicos en el endospermo generado por la temperatura de secado durante el proceso de malteado y el color como un indicador de productos de la reacción de Maillard y la estabilidad oxidativa de la cerveza resultante.

Palabras claves: Espectroscopia por resonancia del espín del electrón, Estabilidad del sabor, Maltas especiales, Maltería proceso, Productos de la reacción de Maillard, Radicales orgánicos

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The application of electron spin resonance (ESR) spectroscopy to the analysis of malt solids has been limited until now. Kaneda et al (11) and Takoi et al (22) patented a method to measure the organic radical content in barley (9), green malt, and malt. Recently this method was optimized using a new internal standard (15,17) that allows rapid quantification of organic radicals in malt using a simplified method.

The first part of this research encompassed the development and fate of organic radicals during malting of different malt types, which were determined using ESR solid measurements. Due to varied opinions from different scientists concerning the influence of colored specialty malt on radical generation and oxidative processes during the brewing process, the effect of colored malts on oxidative beer stability was investigated. In addition, some beers were brewed with a percentage of crystal and roasted malt types. Finally, the investigation focused on the influence of malt type on the radical generation and endogenous antioxidative potential (EAP) of the beer (measured by ESR spectroscopy) as an indicator of oxidative beer flavor stability (13,16,18).

Until recently, the oxidative stability of dark beers was believed to be higher than that of beers brewed with 100% pilsner malt (25), mainly due to the presence of Maillard products such as melanoidins, which can have an antioxidative effect, based on the high reduction power of beers brewed with dark malts (3,5). Bright et al (4) reported a high antioxidant potential in dark malts, which inhibits the formation of linoleic acid hydroperoxides in a Fenton-type reaction. According to Inns et al (10), the temperature during kilning reduces the final content of phenolic acids, as well as the antioxidant activity, but their study showed that at 80–100°C there was an increase in antioxidant activity and level of phenolic acids (9). According to Cantrell and Griggs (5), reductones have a central role in the production of flavor and color compounds in beer and are also responsible for the antioxidant properties of specialty malts. Beers brewed with roasted malts or roasted barley showed the highest reducing powers. When the reducing power per unit of color was calculated, pale malts had the highest values, and it was concluded that there is a relationship between reducing power and color (5).

On the other hand, some researchers have reported a negative effect of roasted malts on beer flavor stability. Forster et al (7) found that dark beers brewed with a percentage of dark malts resulted in a high concentration of the carbonyls 3-methylbutanal, 2-methylbutanal, 2-phenylethanal, and isobutanal in the final beer, which contribute to stale flavor in pale lager beers. Coghe et al (6) reported that malts roasted at >150°C showed less antiradical activity compared with malts of the same color roasted at lower temperatures for a longer period of time. They concluded that the antioxidative activity of malt is absolutely dependent on the time–temperature roasting program. Comparing different types of specialty malts, Preuß et al (20) reported differences in flavor stability between beers brewed with dark crystal malt type 1 and roasted malts. Dark crystal malt type 1 gave a better head retention and flavor stability than roasted malts. Flavor stability also increased with the

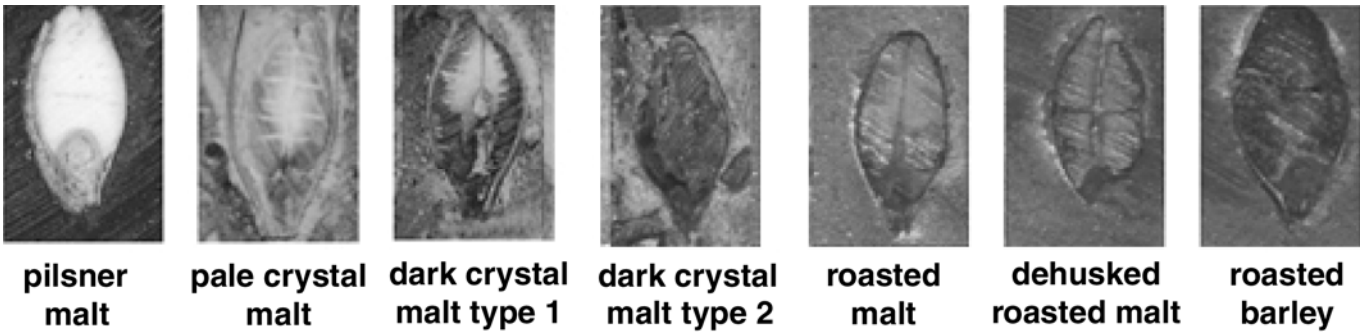

Fig. 1. Sectioned malt types used in the study. Electron spin resonance spectroscopy was performed on intact grains and respective malt fractions.

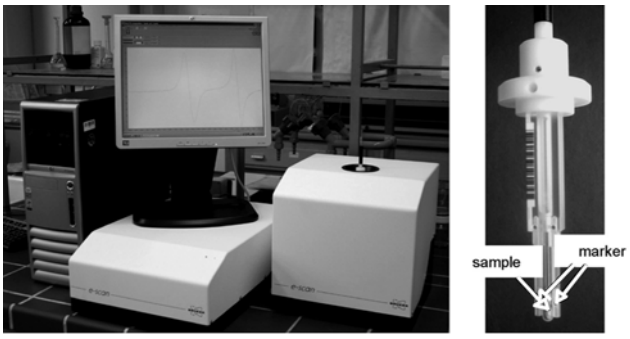

Fig. 2. Left, Electron spin resonance (ESR) spectrometer with cavity (e-scan, Bruker BioSpin). Right, ESR sample holder where marker substance is measured simultaneously with solid sample (malt or barley kernel, husk, or spent grain).

kilning time. Sovrano et al (21) demonstrated that the antioxidative potential of specialty malts does not increase with browning degree. In fact, there was more antiradical activity for crystal malts with intermediate browning degree compared with black malts.

Concerning Maillard reaction products found in greater quantities in roasted malts, Nøddekær and Andersen (19) reported a prooxidative effect of these products on the oxidative stability of caramel and stout beers based on an acceleration of the Fenton reaction system through these compounds. Recently, Furukawa Suárez et al (8) investigated the effect by adjusting beers to the same color using different crystal and roasted malts and coloring agents and, using sensory analysis, GC-MS, and ESR spectroscopy, found that this process has a negative effect on the oxidative stability of finished beers. The smallest effect on oxidative beer stability was achieved when using melanoidin malt to adjust the color of a beer (8).

Methner et al (17) showed that the temperature during the malting process, especially during withering and kilning, affects the development of organic radicals in the final malt. They also demonstrated that the distribution of these organic radicals in pilsner malt fractions is very different. In pilsner malt, the maximum organic radical concentration is in the husk fraction, whereas only low concentrations are detectable in the flour fraction. Because the majority of these very stable radicals is located in the husks, a large percentage is removed during the brewing process through the spent grain after mashing. Consequently, the influence on oxidation reactions during wort boiling and on oxidative beer stability results from the reaction of the remaining organic radicals in the brewing process (17).

Given this, a thorough investigation of the development of these radicals during the withering and kilning process was conducted, and the influence of organic radicals in different malt types on oxidative beer stability was investigated. In particular, the role of the organic radicals and Maillard reaction products found in higher concentrations in color malts treated for longer times at higher temperatures on the radical generation in wort and subsequent oxidative beer stability compared with pilsner malt was studied.

EXPERIMENTAL

Malting Process and Malt Types

Malting was performed in a pilot-scale malting plant (type A1-2008, Seeger) with a capacity of 800 g × 8 = 6,400 g. The malting parameters were steeping time of 27-hr total (2-hr first wet steep, 0.5-hr first air rest, 2-hr second wet steep, 19.5-hr second air rest, and 3-hr third wet steep) at 14°C; germination time of 6 days at 15°C; steeping degree correction of 43% by manual spraying; turning 3 times per day for 10–20 min; withering for 17 hr at 50°C; and kilning for 4 hr at 80°C. "Marthe" spring barley was used in the malting process.

Commercial malts such as pilsner malt and six different crystal and roasted malts, including pale crystal malt, two types of dark crystal malts, roasted malt, dehusked roasted malt, and roasted barley, were used (Fig. 1).

Mashing and Fermentation Trials

Beers were produced on a small scale (1 L) using 10% specialty malt and 90% pilsner malt. The control beer had a grist load of 100% pilsner malt. The mashing program was 55°C for 10 min; from 55 to 62°C at 1 degree Celsius/min; 62°C for 60 min; from 62 to 72°C at 1 degree Celsius/min; 72°C for 20 min; and from 72 to 78°C at 1 degree Celsius/min. The total time was 113 min. The mash-out temperature was 78°C, and the mashing temperature tolerance was ±0.3°C. The mash was fermented for 5 days at 12°C (±0.3°C) using bottom-fermenting yeast (spiked with 15×10^6 CFU/mL).

Solid ESR Measurements

The organic radical content of samples taken during the malting process was quantified using a version of the method patented by Kaneda et al (11) and Takoi et al (22) that was optimized using a novel internal standard (^{52}Cr:MgO) for solids measurement (15,17) (Figs. 2 and 3). This optimized method considerably reduced dispersion and improved quantification of the organic radical concentration in the intact malt kernel (Fig. 3) and its respective fractions (15,17). Use of the newly introduced marker substance simplified quantification of organic radicals.

The ESR spectra were obtained with an X-band spectrometer (e-scan, Bruker BioSpin) using dry, unmilled raw barley, green malt, and kilned malt kernels. Milled aliquots (0.2 g) of a desired malt fraction were also measured. Settings were center field: 3,505 G; attenuation: 12.0 dB; sweep width: 120 G; receiver gain: 2.52×10^3; resolution: 512; modulation amplitude: 2.621 G; modulation frequency: 86 kHz; conversion time: 10 msec; time constant: 5 msec; scans: 18; harmonic: 1; and phase: 1.538 degrees. Calibration was performed using tempol in solution.

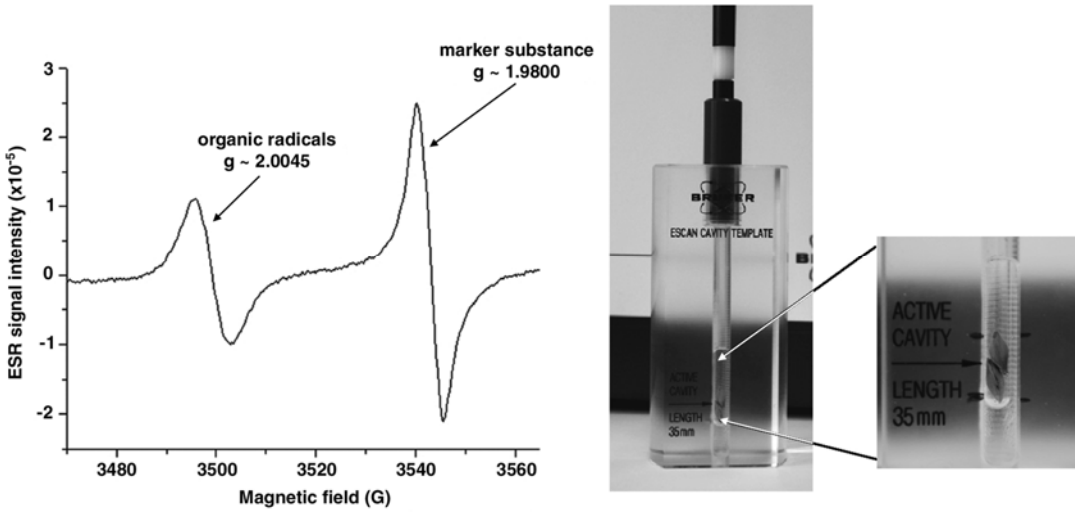

Fig. 3. Electron spin resonance (ESR) spectroscopy for solids. Left, Spectrum showing sample and marker signals. Right, ESR cavity where the marker substance is measured simultaneously with the solid sample (malt or barley kernel, husk, or spent grain).

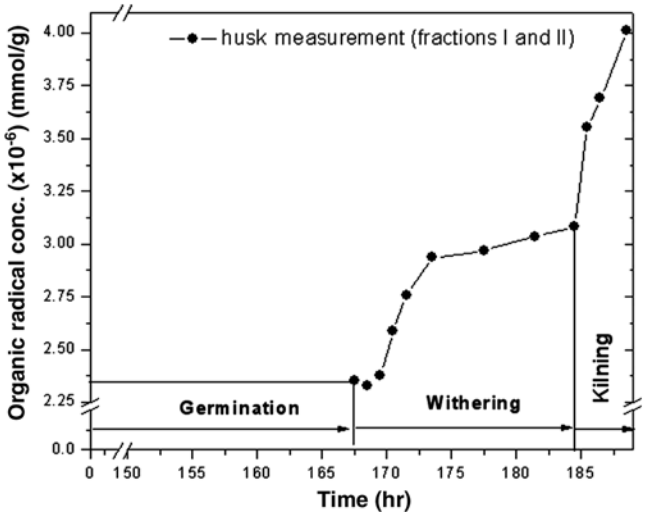

Fig. 4. Evolution of organic radical concentration in husk fractions during malting (duplicate malting process; husk measurement was determined in triplicate).

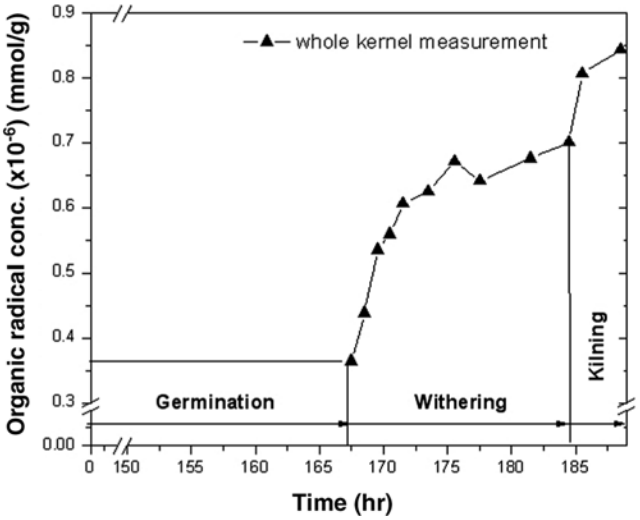

Fig. 5. Evolution of organic radical concentration in whole-kernel fractions during malting (duplicate malting process; kernel measurement was determined in triplicate).

EAP Determination of Wort and Beer Samples

The EAP value of wort and beer was measured, according to Kunz et al (13,16) and Methner et al (18), based on the lag-ime measurement proposed by Uchida and Ono (23) and Kaneda et al (12). The method of Kunz et al (13,16) and Methner et al (18) features α-(4-pyridyl-1-oxide)-*N*-tert-butylnitrone (POBN) as the spin-trap reagent to avoid the distortion caused by the pH effect on the velocity of radical generation and oxidative processes when the standard spin-trap reagent *N*-tert-butyl-α-phenylnitron is used. The EAP value indicates the time point in the beer sample when radical generation increases rapidly with time during accelerated beer aging at raised temperatures (60°C). The extent of oxidative processes and radical formation during wort boiling is indicated by the t_{450} value (t_{600} value for beer), which represents the ESR signal value at this time under forcing conditions at 60°C.

The procedure used was 16.2 or 8.4 mg of 99% POBN (Sigma-Aldrich) (for wort or beer, respectively) diluted in 100 µL of wort or 50 µL of bidistilled water (final concentration in sample was 6.8–6.9 m*M* POBN). Amber vials were used for preparation of ESR samples. Next, 700 or 250 µL of ethanol (for wort or beer, respectively) and 10 mL of wort or 12 mL of beer sample were added to the vials. At the start of the measurement, the diluted POBN was added directly to each sample and held at 60°C. ESR spectra were obtained using an X-band spectrometer (e-scan, Bruker BioSpin). Spectrometer settings were center field: 3,484 G; attenuation: 0 dB; sweep width: 14 G; receiver gain: 2.0×10^3; resolution: 512; modulation amplitude: 1.49 G; modulation frequency: 86 kHz; conversion time: 10 msec; time constant: 40 msec; and scans: 30 (wort) or 20 (beer).

Sulfur Dioxide Determination

Sulfur dioxide content in beer was determined by continuous flow analysis (1,2). Beer samples were acidified with 2*M* sulfuric acid (H_2SO_4) and heated to 95°C to release the bound sulfur dioxide. The gaseous sulfur dioxide formed was dialyzed through a Teflon membrane into a formaldehyde solution. Thereafter, *p*-rosaniline solution was added. The *p*-rosaniline molecule bonded with the sulfur dioxide and formaldehyde at 45°C, forming a red-colored complex compound. Absorption was measured using a UV/VIS photometer at 560 nm. Measurements were made using an optimized procedure with a Teflon membrane according to the Skalar method (1,2,14).

RESULTS AND DISCUSSION

The first part of the investigation examined a pilot malting process for pilsner malt. The development of the organic radical concentration was monitored during the steeping, germination, withering, and kilning processes. The organic radical concentration was measured during the malting process, and the content was quantified for the whole kernel and husk using ESR spectroscopy. The development of organic radical content in the husk and whole-kernel fractions of green malt during the malting process is shown in Figures 4 and 5, respectively. The results indicate that withering and kilning had a major influence on radical generation. A significant increase in radical concentration during these two processes indicated that intensive oxidation reactions occurred as a result of high temperatures. During the steeping and germination processes, the concentration of organic radicals was at a minimum. When the withering process started (50°C for 17 hr), during the first 4 hr the gradient of the concentration of organic radicals was at a maximum; after this, the concentration continued to increase but at a slower rate. When the 80°C hold began, a much greater gradient was observed for 1 hr, followed by a slower increase in radical concentration (Figs. 4 and 5). These results clearly show that radical generation under high temperature mainly governed organic radical formation during the malting process and also support previous studies that identified temperature as having a greater influence than oxygen content and air flow on the formation of radicals during malting (17).

After milling and sieving of malt samples, the organic radical content was quantified for different malt fractions (husk: I; coarse grits: II and III; fine grits: IV and V; and flour: VI). Depending on the malt type, the organic radical concentrations were located in different fractions (Figs. 6 and 7). In pilsner malt, the highest concentrations were located in the husk, whereas the lowest concentrations were found in the flour fraction of the endosperm. In contrast, organic radicals were more evenly distributed throughout the various fractions of crystal and roasted malts. Pilsner malt contained more organic radicals per gram in the husk and coarse grist fractions than did the crystal malts. However, the crystal malts, with the exception of pale crystal malt, had a higher overall organic radical content than did pilsner malt (Fig. 8, whole-kernel measurement). This high concentration can be explained by the high organic radical content in the flour fraction of crystal malts (Fig. 6). Roasted malts (Fig. 7) had a much higher organic radical concentration throughout the malt fractions, all the way to the endosperm, compared with crystal and pilsner malts. The differences between the organic radical content in roasted barley compared with roasted malt were due to the fact that the barley is roasted without a previous malting process. Therefore, radical generation during malting did not take place. The dehusked version of the roasted malt had a lower radical content than the roasted malt, which was due to the fact that the husk contains the majority of the radicals present in the kernel, and this fraction was removed before malting.

After mashing, a quasi-mass balance was performed by assessing the radical content in the malt and subsequent spent grain. Previous studies (17) had already shown that the stable organic radicals

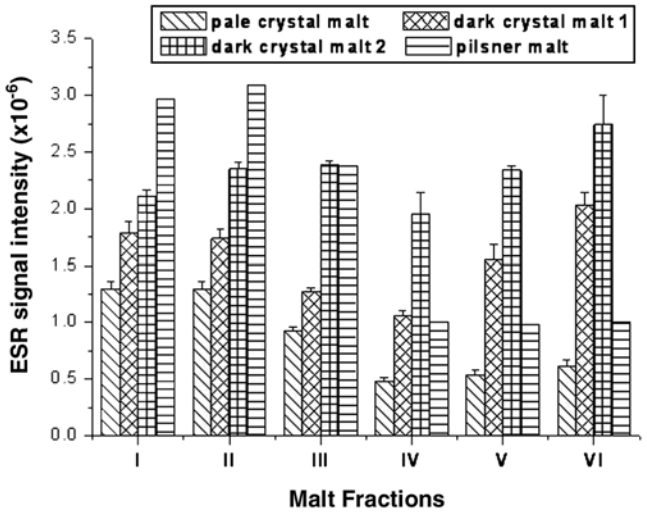

Fig. 6. Distribution of organic radicals in malt fractions of pilsner malt and some commercial crystal malts. Malt fractions: I, husk; II, coarse grits I; III, coarse grits II; IV, fine grits; V, fine grits II; and VI, flour (determined in triplicate).

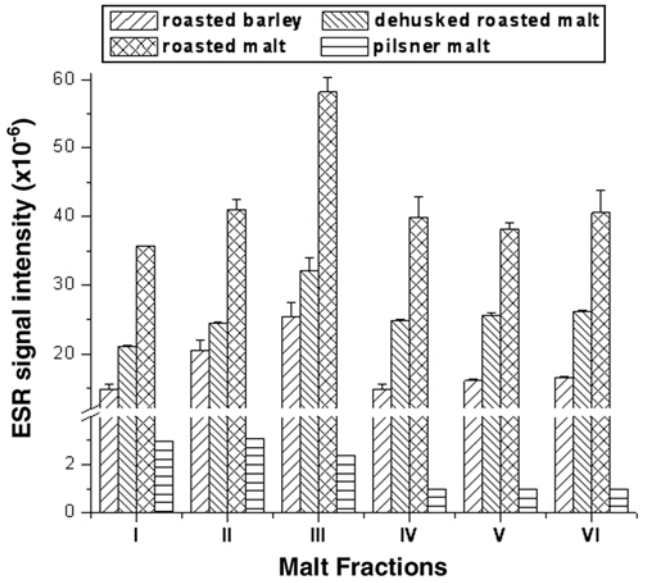

Fig. 7. Distribution of organic radicals in malt fractions of some commercial roasted malts and barley. Malt fractions: I, husk; II, coarse grits I; III, coarse grits II; IV, fine grits; V, fine grits II; and VI, flour (determined in triplicate).

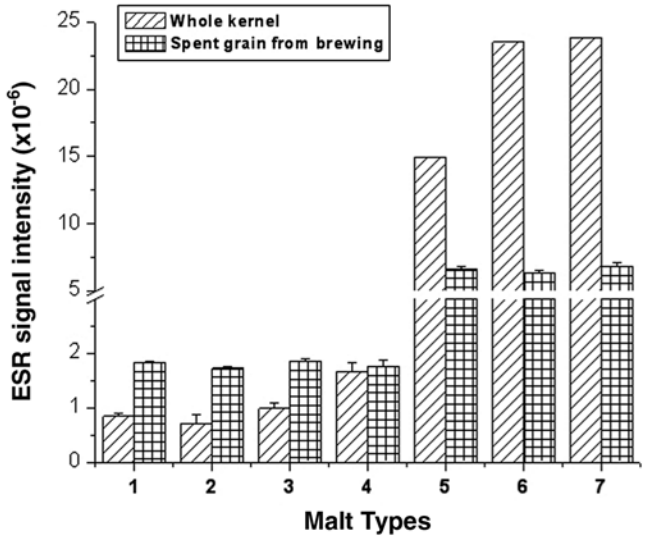

Fig. 8. Organic radical content of whole kernel and spent grain in different malt types: 1, pilsner malt; 2, pale crystal malt; 3, dark crystal malt 1; 4, dark crystal malt 2; 5, roasted barley; 6, roasted malt; and 7, dehusked roasted malt (determined in triplicate).

contained in pilsner malt are mostly removed from the brewing process by the spent grain after mashing. This behavior differs depending on the type of specialty malt used (Fig. 8).

Pilsner and crystal malts had a higher residual level of organic radicals in spent grain compared with the average content in the malt kernel. According to previous studies, a high percentage of the available stable organic radicals is removed during the brewing process through the spent grain after mashing. By comparison, the roasted malts and roasted barley have a much higher organic radical content in the malt kernel and spent grain. Additionally, the average organic radical content in the malt kernel of roasted malts and roasted barley is much higher in relation to the spent grain. Based on these results, it is reasonable to assume that a greater percentage of the available organic radicals were solubilized in the mash and played a role in oxidative reaction processes during mashing when using roasted malts and roasted barley. Given this, and the very high radical content in the malt kernel of roasted malts, the entry of the organic radicals in the brewing process is much higher compared with pilsner or crystal malts and can negatively influence oxidative beer stability.

The wort that was produced with 10% specialty malt or barley and 90% pilsner malt was assessed by ESR spectroscopy to investigate the influence of different roasted malts on radical formation in wort and oxidative beer stability. In the wort samples, ESR measurements did not display any lag-time behavior in radical generation. However, the radical content rose in a hyperbolic fashion to a final level at approx. 450 min into the assay. The t_{450} value showed the extent of oxidative processes and radical formation during mashing and wort boiling. The results shown in Figure 9 reflect higher organic radical generation caused by the use of roasted malt and barley (t_{450} value) than by dark or pale crystal and 100% pilsner malt. The lowest t_{450} value was observed in wort produced from 100% pilsner malt, and the highest value was observed for the wort produced with 10% roasted malt. With the exception of dark crystal malt, the increase in radical generation was directly correlated with the measured average content (whole kernel) of organic radicals in the specialty malt used in the brewing process (Fig. 8). The temperature treatment during kilning and roasting also clearly had a significant influence on organic radical generation during the mashing or boiling process.

The same worts, after fermentation, were measured by ESR spectroscopy, as in Kunz et al (13), Methner and Kunz (16), and Methner et al (18), and a similar trend in radical generation was apparent. Compared with the wort measurement, the beer made from malts kilned and roasted at higher temperatures showed a greater propensity to produce free radicals (t_{600}) and resulted in a finished beer with a lower EAP value (Fig. 10). The oxidative flavor stability, as indicated by the EAP value, was negatively correlated with the resulting organic radical concentration in the flour fraction in direct correlation to the color (Fig. 11) as an indicator of Maillard reaction products. Higher kilning temperatures yielded malts that produced beer with lower EAP values (Fig. 10).

The sulfur dioxide content was also measured after fermentation (Fig. 12). Fermentation was monitored, and the trials showed a similar trend in yeast growth and extract consumption, as well as pH development. However, final sulfur dioxide content was very different between the seven green beers, showing a clear effect of the crystal and roasted malts on sulfur dioxide formation or consumption during the fermentation process. The results generally agree with the EAP value and radical formation in the beer samples measured by ESR spectroscopy. With the exception of the pale crystal malt, the dark crystal and roasted malts produced much lower sulfur dioxide contents than did pilsner malt.

In accordance with previous studies (17), monitoring organic radical development during the malting process provided clear in-

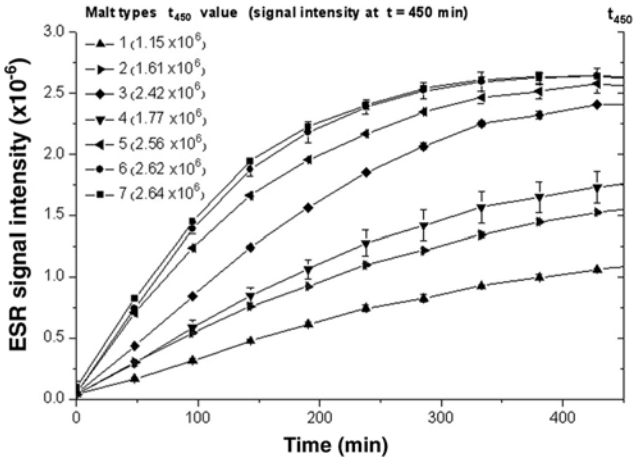

Fig. 9. Electron spin resonance (ESR) measurements for boiled wort. Wort was produced using 90% pilsner and 10% specialty malts: 1, pilsner malt; 2, pale crystal malt; 3, dark crystal malt 1; 4, dark crystal malt 2; 5, roasted barley; 6, roasted malt; and 7, dehusked roasted malt (determined in duplicate).

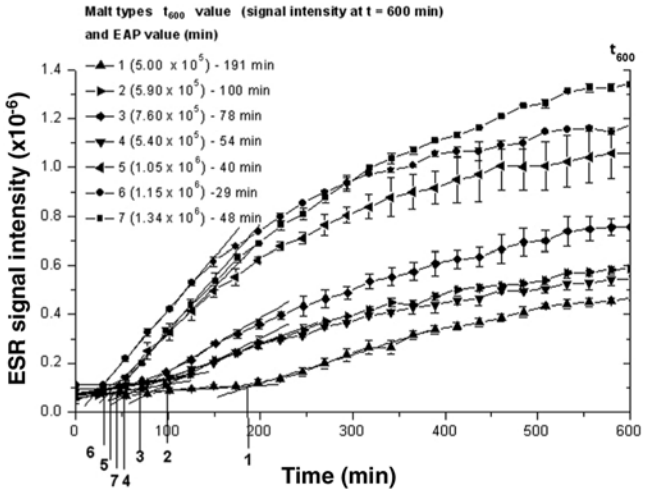

Fig. 10. Endogenous antioxidative potential of beer determined by electron spin resonance (ESR) spectroscopy. Wort was produced using 90% pilsner and 10% specialty malts: 1, pilsner malt; 2, pale crystal malt; 3, dark crystal malt 1; 4, dark crystal malt 2; 5, roasted barley; 6, roasted malt; and 7, dehusked roasted malt (determined in duplicate).

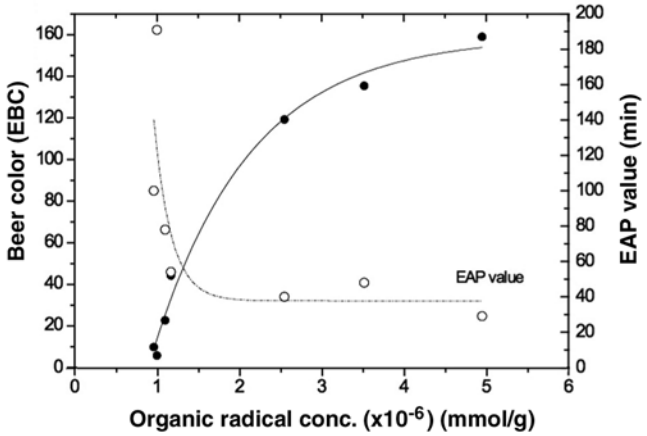

Fig. 11. Beer color and endogenous antioxidative potential (EAP) value versus organic radical content in the flour fraction (determined in duplicate).

sights into the effect of temperature on the development of these stable compounds in the final malt. Whereas the majority of organic radicals in pilsner malt are located in the husk, in roasted malts they are more uniformly distributed throughout the kernel. The temperature during the withering, kilning, and roasting steps in malt production had a direct influence on the generation of stable organic radicals, and the results were similar to those from the investigation by Coghe et al (6) on color formation as an indicator of the Maillard reaction compounds found in the finished malt. Consequently, there is an apparent relationship between the concentration of Maillard reaction products in the malt endosperm and the organic radical content. Higher temperatures during the malting process resulted in greater radical and Maillard reaction product contents.

The demonstrated quasimass balance of the organic radicals in whole kernels versus spent grain shows much higher solubilization of organic radicals in the brewing process when using roasted malts. Given this, and due to the very high radical content in the malt kernel of roasted malts, the entry of organic radicals into the brewing process is much higher compared with pilsner or crystal malts. This leads to the higher participation of these radicals in the oxidative reaction processes during mashing compared with crystal and pilsner malts and can negatively influence oxidative beer stability.

Supporting the results from Furukawa Suárez et al (8), in the current study the Maillard reaction products and organic radicals in the malt intensified the oxidative reactions in the brewing process and subsequent beer, where large amounts of antioxidants were consumed. In turn, this reduced the EAP value in the final beer. Additionally, Wedzicha reported in 1984 (24) that SO_2 can easily bind to melanoidins. The results presented in this study indicate a direct relationship between the organic radicals in the endosperm generated by kilning temperatures during malting and the oxidative stability of the resultant beer. The pro-oxidative effect of the malts used in this study cause an oxidized flavor in dark beers sooner than in pale beers. However, from a sensory viewpoint, this flavor note can sometimes appear later in dark beers, because it is masked by the strong, sweet, candy flavor derived from the Maillard reaction products (19).

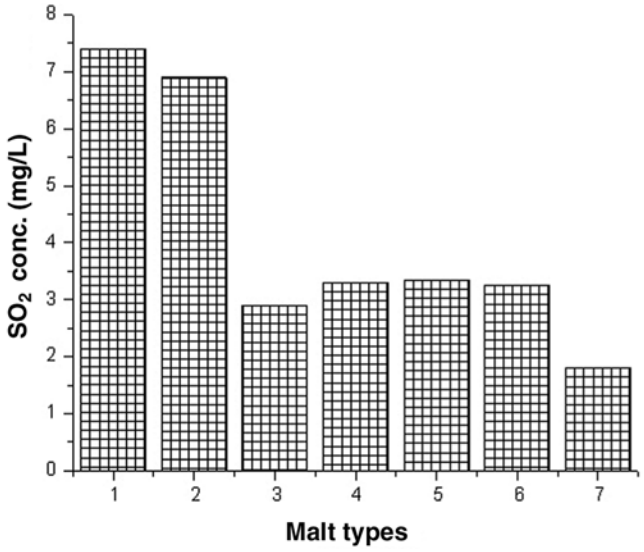

Fig. 12. Sulfur dioxide content of beer samples. Malt type: 1, pilsner malt; 2, pale crystal malt; 3, dark crystal malt 1; 4, dark crystal malt 2; 5, roasted barley; 6, roasted malt; and 7, dehusked roasted malt. Results are from one trial.

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LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Beer-10A, Spectrophotometric color method, -21, Total sulfur dioxide; Statistical Analysis-4, Youden unit block collaborative testing procedure, -5, Comparison of test methods. The Society, St. Paul, MN, 2008.
2. American Society of Brewing Chemists. Report of the Subcommittee on Total Sulfur Dioxide Analysis: Total sulfur dioxide analysis using a segmented flow analyzer (International Method). *J. Am. Soc. Brew. Chem.* 67:245-246, 2009.
3. Bamforth, C. W., Muller, R. E., and Walker, M. D. Oxygen and oxygen radicals in malting and brewing. *J. Am. Soc. Brew. Chem.* 51:79-88, 1993.
4. Bright, D., Stewart, G., and Patino, H. A novel assay for antioxidant potential of specialty malts. *J. Am Soc. Brew. Chem.* 57:133-137, 1999.
5. Cantrell, I. C., and Griggs, D. I. Malt: Its role in oxidation. *Tech. Q. Master Brew. Assoc. Am.* 33:82-86, 1996.
6. Coghe, S., Gheeraert, B., Michiels, A., and Delvaux, F. R. Development of Maillard reaction related characteristics during malting and roasting. *J. Inst. Brew.* 112:148-156, 2006.
7. Forster, C., Narziss, L., and Back, W. Investigations of flavor and flavor stability of dark beers brewed with different kinds of special malts. *Tech. Q. Master Brew. Assoc. Am.* 35:73-77, 1998.
8. Furukawa Suárez, A., Kunz, T., Cortés, N., MacKinlay, J., Hughes, P. S., and Methner, F.-J. Impact of colour adjustment on flavour stability of pale lager beers with a range of distinct colouring agents. In: *Proceedings of the 32nd Congress of the European Brewery Convention.* Fachverlag Hans Carl, Nürnberg, Germany, 2009.
9. Goupy, P., Hugues, M., Boivin, P., and Amiot, M. J. Antioxidant compounds of barley. *Proc. Congr. Eur. Brew. Conv.* 27:445-451, 1999.
10. Inns, E. L., Buggey, L. A., Nursten, H. E., and Ames, J. M. Effect of a simulated kilning regime on the profile and antioxidant activity of the free phenolic acids extracted from green malt. *Tech. Q. Master Brew. Assoc. Am.* 42:204-208, 2005.
11. Kaneda, H. Method of evaluation of green malt qualities by electron spin resonance spectroscopy and method of evaluating malt qualities. U.S. Patent 6952098 B2, 2005.
12. Kaneda, H., Kano, Y., Osawa, T., Kawakishi, S., and Kamada, K. The role of free radicals in beer oxidation. *J. Am. Soc. Brew. Chem.* 47:49-53, 1989.
13. Kunz, T., Methner, F. J., Kappl, R., and Hüttermann, J. Verfahren zur Bestimmung des endogenen antioxidativen Potenzials von Getränken mittels ESR-Spektroskopie. Deutsche Patentanmeldung U30121 and Offenlegungsschrift Deutsches Patent- und Markenamt DE 10 2005 043 113 A1, 2005. Method for determining the endogenous antioxidative potential of beverages by means of ESR spectroscopy. U.S. patent 20080248580, 2005.
14. Kunz, T., Schiwek, V., Harms, D., and Methner, F.-J. Optimized analysis methods for the determination of SO_2 in beer and malt. *Brauwelt Int.* 27:216-220, 2009.
15. Maier, D., Schmalbein, D., Jiang, J. J., Weber, R., Kamlowski, A., and Schmidt, T. Probehead and a sample substance for an electron spin resonance dosimeter. Germany patent DE 10207723, 2002.
16. Methner, F.-J., and Kunz, T. A new "EAP-determination" method for beer and other beverages using ESR spectroscopy. *Brauwelt Int.* 24: 210-211, 2006.
17. Methner, F.-J., Kunz, T., and Kobayashi, N. Investigations on the behavior of organic radicals in barley and malt during the malting and mashing process by electron-spin-resonance spectroscopy. In: *World Brewing Congress 2008 Proceedings* (CD). American Society of Brewing Chemists and Master Brewers Association of the Americas, St. Paul, MN. Presentation O-12, 2008.
18. Methner, F.-J., Kunz, T., and Schön, T. Application of optimized methods to determine the endogenous anti-oxidative potential of beer and other beverages. (CD) *Proc. Congr. Eur. Brew. Conv*. 31:755-764, 2007.
19. Nøddekær, T. V., and Andersen, M. L. Effect of Maillard and cara-

melization products on oxidative reactions in lager beer. *J. Am. Soc. Brew. Chem.* 65:15-20, 2007.
20. Preuß, T., Forster, C., Thum, B., and Back, W. Dark malt: The key to distinct flavour attributes and high flavour stability in dark beer. *European Brewery Convention Monograph 31: Symposium Flavour and Flavour Stability, Nancy* (CD). Fachverlag Hans Carl, Nürnberg, Germany. Pp. 1-13, 2001.
21. Sovrano, S., Buiatti, S., and Anese, M. Influence of malt browning degree on lipoxygenase activity. *Food Chem.* 99:711-717, 2006.
22. Takoi, K., Kaneda, H., Toru, K., Watari, J., and Takashio, M. Application of compact high performance electron spin resonance for malt quality estimation. *J. Am. Soc. Brew. Chem.* 61:146-151, 2003.
23. Uchida, M., and Ono, M. Improvement for the oxidative flavor stability of beer—Role of OH-radicals in beer oxidation. *J. Am. Soc. Brew. Chem.* 54:198-204, 1996.
24. Wedzicha, B. L. Control of non-enzymic browning. In: *Chemistry of Sulphur Dioxide in Foods*. Elsevier, London. Pp. 183-229, 1984.
25. Woffenden, H. M., Ames, J., Chandra, S., Anese, M., and Nicoli, C. Effect of kilning on the antioxidant and pro-oxidant activities of pale malts. *J. Agric. Food Chem.* 50:4925-4933, 2002.