Voltammetric Assay for the Aging of Beer

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A new method for the evaluation of beer aging, based on a voltammetric analysis of beer distillates, is described. By measuring the current of both acetaldehyde and sulfite voltammetric peaks it is possible to distinguish between fresh, naturally aged, and artificially aged beers. The results obtained for the ratio of acetaldehyde and SO_2 currents correlate well with those given by an expert sensory panel. The kinetics of the combining reaction of sulfite with acetaldehyde was followed for different acetaldehyde/SO_2 molar ratios by using a programmed voltammetric procedure. The formation of an acetaldehyde−sulfite adduct is rapid, and the reaction equilibrium is reached after 30 min, which is in accordance with the results previously obtained by other methods. This voltammetric-based approach seems to be a new attractive tool for detecting chemical equilibria of the addition of sulfite to carbonyls in beer model systems.

KEYWORDS: Beer aging; voltammetry; acetaldehyde; sulfite

INTRODUCTION

After the use of polarographic methods nearly ceased in the early 1960s, voltammetric methods gained a renewed interest in the 1980s with the advent of the application of anodic stripping voltammetry in the analysis of trace metal ions (*1*). More recently, with the appearance of new and more versatile instrumentation on the market, voltammetry has been recovering part of its lost popularity, and the possibility of its application in the analysis of some constituents of beer has been referred (*2−4*). Here we report a voltammetric application for the evaluation of beer aging.

Flavor stability, an important quality criterion for beer, has long been a concern to the brewing industry. During storage, beer quality is gradually decreased and the production of stale flavor, the formation of haze, and browning occur. The off-flavors that typically develop in aged beer include cardboard, sweet, and toffee notes (*5*). Although these flavors are easily identifiable to sensory analysts trained in the assessment of beer flavor, there is a definite requirement for analytical methods that may detect the specific compounds responsible for these flavors and thereby enable quantitative determination of beer staling. However, many of these compounds are either unknown or are present at such low concentrations that their analysis is difficult and inaccurate. Although the stale flavor of beer has commonly been evaluated by sensory analysis, an objective evaluation using only this method is difficult. As an alternative to sensory analysis, many researchers have tried to develop sensitive biochemical analysis for beer staling. Color increase, sometimes advocated as a measurement of oxidation or brown-

ing, is known to be an index of deterioration (*6*). Grigsby (*7*) and later Parsons and Cope (*8*) reported that thiobarbituric acid (TBA) values could be used to measure carbonyl species produced on forced aging. Lynch and Seo (*9*) showed that the measurement of ethylene in the headspace of beer was useful as an index for following beer staling. Schmitt and Hoff (*10*) also reported that the acetaldehyde concentration in beer was correlated with the mean panel scores for stale flavor. There have been numerous studies indicating that volatile long-chain unsaturated aldehydes are the main compounds responsible of the initial stale flavor of beer (*11−13*). *E*-2-Nonenal is believed to be the major source of the cardboard character appearing in stale beer (*11−13*), but as has been found at very low levels (0.1 ppb), its analysis is laborious, time-consuming, and expensive. The concept of "nonenal potential", developed by Drost (*13*) for the assessment of oxidation during wort production, is useful in predicting susceptibility of beer to staling. Nevertheless, the significance of the autoxidation of unsaturated fatty acids in brewhouse operations or in the finished beer is not considered by measuring the "nonenal potential" (*14*). Generally, all investigators agree that *E*-2-nonenal is an important component in the complex mixture of oxidized beer flavors, although certainly many other components contribute as well. An increase of *β*-damascenone concentration during the artificial aging of a variety of commercial Belgian beers has been recently reported, indicating that it can be used as a good analytical marker of beer aging (*15*). Other species are sometimes measured directly as indices of staling. Among these are furanic aldehydes, for example, furfural and 5-(hydroxy-methyl)furfural. Although the concentrations of furfural and 5-(hydroxymethyl)furfural typically found in beer are not

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thought to be significant in terms of overall beer flavor, the analysis of these compounds may provide a useful device for the detection of high-temperature stress of packaged beer (*16*). A rapid method to monitor the taste stability of beer based on the "absorption integral" (AI), a value that correlates well with furanic aldehydes, was recently presented by Klein (*17*). In the past few years, it was shown that EPR spin trapping provides a useful technique for measuring the free radicals that form during the oxidation of beer, thus predicting beer shelf life (*18*). The application of chemiluminescence analysis for the evaluation of beer staling using the single photoelectron counting system has been studied by Kaneda (*19*).

The work reported here describes a new method for the assessment of beer aging, based on a voltammetric analysis of beer distillates. Fresh beers and naturally and artificially aged beers are analyzed according to the proposed method, and the results obtained are compared with those given by an expert sensory panel. The influence of sulfur dioxide level and storage temperature on beer deterioration is discussed as well as the kinetics of the combining reaction of sulfite with acetaldehyde.

MATERIALS AND METHODS

Instrumentation. Voltammetric work was performed using an Autolab PGSTAT 10 voltammetric system (EcoChimie) controlled with a PC equipped with GPES for Windows, version 4.4 software. A Metrohm 663 VA voltammetric stand was used in the HMDE mode. The three-electrode potentiostatic system was completed with a glassy carbon auxiliary electrode and an AgCl/Ag (3 M KCl) reference electrode. Distillation was performed using a Kjeltec 1026 distilling unit (Tecator). Total oxygen content was measured using an Orbisphere oxygen meter.

Reagents and Solutions. All chemicals were of analytical reagent grade, and purified water from a Millipore system (Simplicity 185) was used in solution preparation.

Sodium disulfite (Riedel de Haen), $Na_2S_2O_5$, was used as a source of SO_2. A 1000 mg L^{-1} stock solution of SO_2 was prepared and calibrated using an excess of standard iodine solution, which was back-titrated with standard sodium thiosulfate solution.

A commercial acetaldehyde solution (Aldrich) was diluted to prepare a stock solution; 1 M derivatizing agent [hydrazine dihydrochloride (Aldrich)] for acetaldehyde determination was prepared freshly every day by dissolving in 6 M NaOH and adjusting the pH value to 5.

Beers. One brand of a commercial lager beer, produced at industrial scale, was used. A total of 37 bottled beers with a total O_2 content of <0.7 mg L^{-1} under different storage conditions were tested. Beers were set into three trials: (A) 13 fresh beers (maintained at 4 °C); (B) 10 artificially aged beers (7 days at 37 °C in the dark); (C) 14 naturally aged beers (6 months at 20 °C in the dark). Groups A and C reflect natural conditions of storage, so greater numbers of samples was analyzed.

Procedures. A sample of 150 mL of carbonated beer was subjected to steam distillation until 100 mL of distillate was collected for further analysis.

In the voltammetric analysis, 5.00 mL of beer distillate was added to 20.00 mL of 0.1 M acetate buffer, pH 3.8, followed by 0.5 mL of 1 M hydrazine dihydrochloride. The voltammogram was run after 5 min of derivatization reaction.

The square wave voltammetric determinations were performed in deoxygenated solutions (by purging nitrogen for 5 min), using a frequency of 25 Hz; the potential was scanned between −0.5 and −1.3 V, with a pulse amplitude of −25 mV and a step of −2.5 mV.

Sensory Evaluation. Eight tasters of the UNICER's (the main portuguese Brewery Group) internal sensory panel with at least 1 year of sensory experience were recruited on the basis of their good sensory ability to identify the stale off-flavors. The panel was contracted for three 1 h sessions per week, which took place in the morning at an adequately isolated taste room. Panelists were asked to comment on the general quality (discrimination test) as well as to describe the flavor
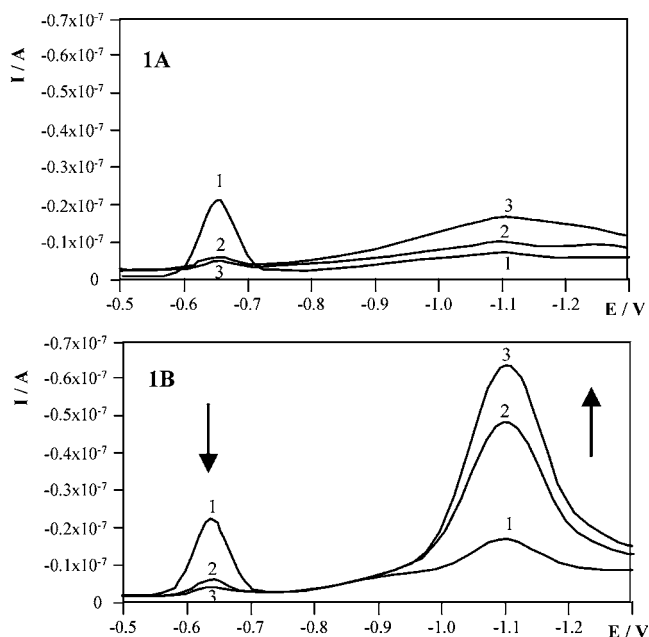

Figure 1. Voltammograms of beer distillates in 0.1 M acetate buffer, pH 3.8: (A) direct analysis of beer distillates; (B) analysis of beer distillates after derivatization with hydrazine dihydrochloride. Storage conditions: 1, fresh beer; 2, artificially aged beer (1 week, 37 °C); 3, naturally aged beer (6 months, 20 °C).

profile according to a special form (description test). The beers were tasted in a paired test in which a beer stored at 4 °C was the reference. All beers were tasted at 4 °C and evaluated on a scale from −3 to +1.

RESULTS AND DISCUSSION

Voltammetric Analysis of SO_2 and Acetaldehyde. A clearly defined wave obtained by differential pulse polarography or square wave voltammetry has been applied in the determination of sulfur dioxide in foods (*20*). Several investigations on the electrochemical reduction of sulfur dioxide in aqueous solution at a dropping mercury electrode (DME) have reported two waves in acidic medium and no waves in neutral or slightly alkaline media. Although there has been much disagreement in the interpretation of polarographic reduction of aqueous sulfur dioxide, a one-electron reduction of HSO_3^- was suggested by Reynolds and Yuan (*21*):

$$HSO_3^-{}_{(aq)} + H^+ + e^- \rightarrow SO_2^-{}_{(aq)} + H_2O$$
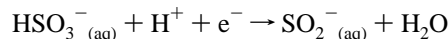
Aldehydes can be determined voltammetrically in either acidic or basic media. The derivatives of carbonyl compounds are more easily determined than the free carbonyl compounds themselves, with which complicating side reactions such as hydration, acetal formation, or enolization tend to lower the concentration of the free carbonyl group (*22*). In the presented procedure, the beer distillates are mixed with the supporting electrolyte (0.1 M acetate buffer, pH 3.8, containing 0.02 M hydrazine dihydrochloride) and allowed to stand for at least 5 min before the voltammogram is run (**Figure 1B**). By reacting with hydrazine under acidic conditions, acetaldehyde can be quantitatively converted into a hydrazone:

$$N_2H_4 + R-\overset{O}{\underset{H}{C}} \longrightarrow R-\overset{N-NH_2}{\underset{H}{C}} + H_2O$$
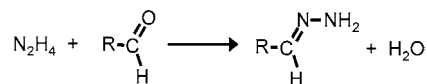
Once formed, this electroactive adduct is stable. No deterioration of the voltammetric signal was observed after 1 h of
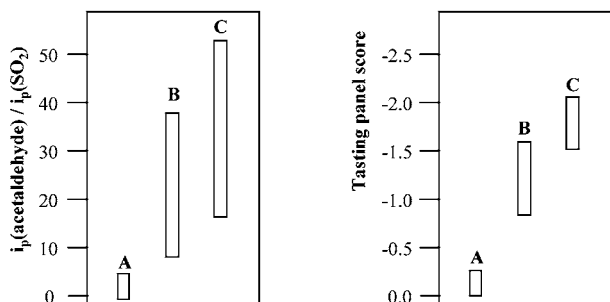
Figure 2. Comparison between the ratio of the voltammetric peaks of acetaldehyde and SO$_2$ and the taste panel score in the analysis of 37 samples of beer: (A) fresh; (B) artificially aged beer (1 week, 37 °C); (C) naturally aged beer (6 months, 20 °C). The bars in the figure represent the 95% confidence limits of the mean of each group.

standing. The reaction of hydrazine with the acetaldehyde is not immediate, and a reaction time of 5 min was observed to be sufficient. The reduction of the hydrazone at the DME is a two-electron process, and the current is proportional to acetaldehyde concentration.
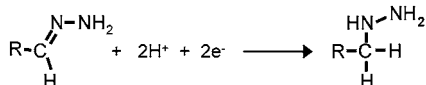
Voltammetric Analysis of Beer Distillates. Beers stored at different temperatures were subjected to steam distillation. In the direct analysis of beer distillates only one peak was found due to the presence of SO$_2$ (**Figure 1A**). After derivatization with hydrazine dihydrochloride, a second peak was observed corresponding to the hydrazone derivative of acetaldehyde (**Figure 1B**).

The peaks showed opposite tendencies with beer storage. The first compound (SO$_2$) gradually decreases and almost disappears after 6 months at 20 °C, whereas the acetaldehyde content (right peak) becomes higher with storage time. Each beer of each group previously described was analyzed by voltammetry, and the chemical data were correlated with sensorial changes followed by an expert sensory panel. The average of the results obtained and the intervals of variation of each group for statistical 95% confidence limits are presented in **Figure 2**. To define the predictive correlation describing the relationship between the sensory evaluation and the presented method, experimental data are correlated in **Figure 3**. Because of the limited scale of the sensory analysis (panelists often exclude the highest and lowest scores), it was decided to plot the total taste score against the mean of the ip(acetaldehyde)/ip(SO$_2$) ratio

obtained for each sensory score. The results obtained for the 37 beers are considered, even though no more than 11 values are presented in accordance with the scores provided by the sensory evaluation. As shown in **Figure 3**, a linear relationship was observed between the values provided by the sensory panel and the mean of the values obtained by the proposed method for the 37 beers. Taking into consideration the low number of sensory data, as well as the subjectivity and limited accuracy from the sensory results, even if carried out by a trained sensory panel, the linear correlation coefficient ($r = 0.84$) must be regarded as satisfactory. This assay also showed a higher rate of flavor staling of beer stored for 6 months at 20 °C (natural aging) when compared to the beer stored at 37 °C for 1 week (artificial aging), which is in conformity with the results from sensory analysis. On the basis of the results obtained, the predictive correlation for the analyzed beer is indicated by the equation $y = -0.0398x - 0.2816$. Because acetaldehyde and sulfur dioxide contents may be influenced by the raw materials, as well as by technological factors, it is strongly suggested that every brewery should investigate the ratio of acetaldehyde/SO$_2$ currents for different degrees of staling of its own beer.

As previously mentioned, the phenomenon of beer flavor instability is so complex that to attribute the origin of the off-flavors to a specific compound would be imprudent. It is therefore important to emphasize that, although acetaldehyde and SO$_2$ certainly play a key role in the staling mechanisms, their importance in these mechanisms depends on the beer.

Reaction of Sulfite with Acetaldehyde. To understand the equilibrium reaction between sulfite and acetaldehyde held in the voltammetric cell, the kinetics of the combining of sulfite with acetaldehyde became the focus of our next investigation.

As shown in **Figure 4** the height of the wave of sulfite, observed close to -0.65 V (pH 3.8), was found to decrease markedly with acetaldehyde addition, suggesting that the well-known reaction between HSO$_3^-$ and acetaldehyde is occurring in the voltammetric cell.

This allows the investigation of the formation of an acetaldehyde–sulfite adduct by measuring the decrease of the height of the wave attributed to sulfite. Different acetaldehyde/SO$_2$ molar ratios were studied for a constant SO$_2$ initial concentration. A programmed voltammetric procedure was used that allows the acquisition of the voltammogram every 2 min, during a reaction time of 2 h. The addition of sulfite to acetaldehyde is quite rapid in the conditions used, as shown in **Figure 5**. After 30 min, the reaction equilibrium is practically reached, which is in accordance with what was previously described by other methods (*23, 24*).
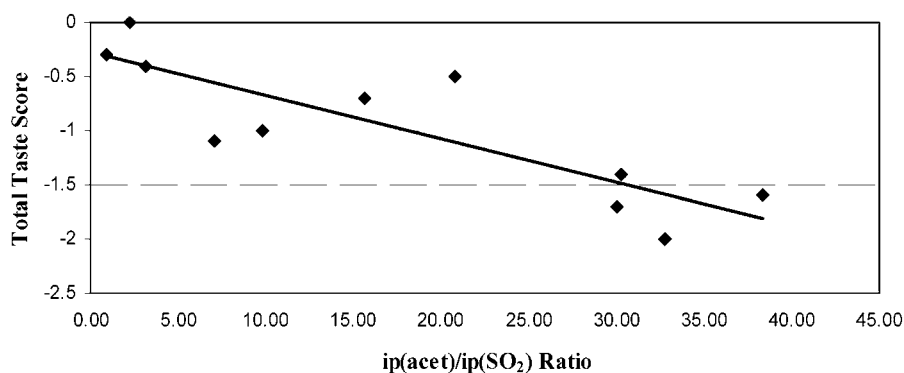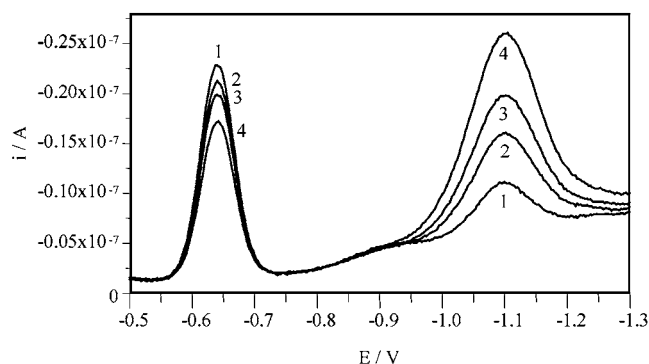

Figure 3. Correlation adequacy of the proposed method compared to the sensory evaluation. The total taste score is plotted against the mean of the ip(acetaldehyde)/ip(SO$_2$) ratio obtained for each sensory score. A linear correlation coefficient $r = 0.843$ was obtained for the predictive relationship described by the equation $y = -0.0398x - 0.2816$.

Figure 4. Voltammograms of a fresh beer distillate derivatized with hydrazine dihydrochloride in 0.1 M acetate buffer, pH 3.8: (left peak) SO_2; (right peak) derivatized acetaldehyde. Standard additions of acetaldehyde: 1, 0 ppm; 2, 2 ppm; 3, 4 ppm; 4, 8 ppm.
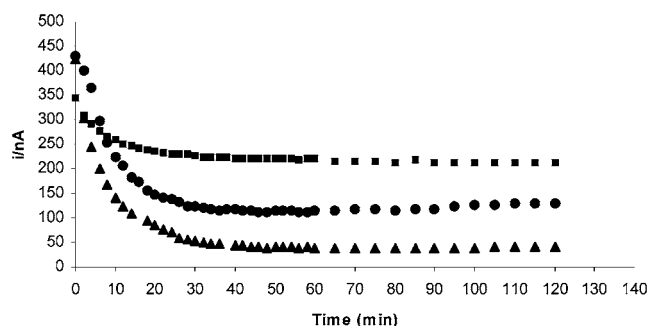

Figure 5. Kinetics of the combining of sulfite with acetaldehyde. Peak currents for SO_2 (10^{-4} M) decrease with the addition of different concentrations of acetaldehyde in 0.1 M acetate buffer, pH 4.0. Concentrations of acetaldehyde: ■, 5×10^{-5} M; ●, 10^{-4} M; ▲, 1.5×10^{-4} M.

For SO_2 and acetaldehyde concentrations of 10^{-4} M, approximately the amounts found in beer, the percentage of bound SO_2 is roughly 90% considering the stoichiometry of 1:1. Furthermore, Chapon et al. (*23*) demonstrated that 98% of the SO_2 is bound for an equimolar concentration of 10^{-3} M. The extension of sulfite binding of acetaldehyde is, obviously, related with the correspondent equilibrium constant of adduct formation ($K = 6.6 \times 10^5$), which is identical in a pH range of 2−6 (*25*). Because, in a multicomponent system containing different concentrations of carbonyls with various equilibrium constants, sulfite is preferentially bound to those carbonyl compounds with the highest values of equilibrium constant, the binding power of acetaldehyde is larger than that of the other carbonyl compounds, such as α-ketoacids and carbohydrates (*25*).

Because acetaldehyde is the main component of the carbonyl compounds in beer, making up about 60% of the total aldehydes (*26*) or 97% of volatile carbonyl compounds in Japanese beers (*27*), it as a key factor influencing the beer aging process. The protective effect of SO_2 against beer staling is due to interaction with carbonyls, responsible for the stale flavor that develops in stored beer, rendering complexes that are not flavor active (*26, 28*). A recent study indicates that unsaturated aldehydes, including *E*-2-nonenal, can form a variety of irreversible adducts with sulfur dioxide (*28*). Due to the great affinity of acetaldehyde for SO_2, the formation of such adducts is highly dependent on the acetaldehyde level. Accordingly, the ratio of acetaldehyde and free SO_2 determined by the proposed assay can be used as a relevant indicator for the assessment of the deterioration level of beer.

In conclusion, because SO_2 and acetaldehyde play a key role in the staling reaction mechanisms leading to the flavor changes that occur in beer, a fast and simple voltammetric method, which combines the measurements of SO_2 and acetaldehyde levels in beer distillates, was developed to evaluate beer flavor stability. It was shown that those compounds can therefore be used as good chemical indicators, reflecting sensorial changes during beer storage. The interaction of sulfite with carbonyl compounds has been intensively studied not only in beer but also in a vast variety of food systems. Therefore, the new voltammetric-based approach presented for the study of the combining reaction of sulfite with carbonyls seems to be a plausible alternative to other existing methods. Work is in progress to detect the addition reaction of sulfite to other carbonyl compounds in beer model systems.

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