Contribution of Carbonyl–Bisulfite Adducts to Beer Stability

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The mechanism of bisulfite adduct contribution to stability of beer quality was studied. Formaldehyde– and acetaldehyde–bisulfite adducts had only a poor oxidation potential around 0.93 V, which is the oxidation potential of free sulfite so that they lost their reducing activity for iodine. However, they inhibited the chemiluminescence production of beer and *Cypridina* lucifern analog dependent luminescence in beer, indicating that they inhibit the free radical reactions in beer. The acetaldehyde–bisulfite adduct inhibited haze formation and flavor staling of beer during storage. Therefore, it could be thought that aldehyde–bisulfite adducts have a radical scavenging activity and protect the free radical chain reactions during beer storage, leading to the stability of beer quality.

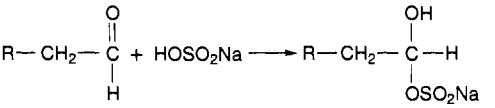
Keywords: *Beer; stability; bisulfite adducts; radical scavenger*

INTRODUCTION

Sulfite is usually considered to prevent the flavor staling of beer, and some breweries have used it for the stabilization of beer quality (Brewer and Fenton, 1980). It is also known that sulfite is produced by yeasts during fermentation and survives in the finished beer (Nordiöv, 1985; Ryder et al., 1989). Many researchers have studied the factors that influence sulfite formation by yeast during fermentation. Kaneda et al. (1991c, 1992) showed that fermentation conditions play an important role in the flavor stability of beer. Wort fermentation conditions could control the sulfite level and flavor stability of the finished beer. Narziss et al. (1993) also showed that sulfite produced during fermentation contributes to the flavor staling of finished beer and concluded that a sulfite content in packaged beer from 8 to 9 ppm leads to the most stable beer flavor on the shelf.

In terms of the contribution of sulfite to beer stabilization, two main mechanisms have been proposed: First, sulfite inhibits beer oxidation during storage, acting as an antioxidant. The second mechanism is the masking action of sulfite for staling flavor. Kaneda et al (1991b) showed that sulfite inhibited the production of active oxygen and the progress of free radical reactions in beer which lead to flavor stability in beer. However, there is an opinion that sulfite produced by yeast during wort fermentation combines with several carbonyls to produce sulfite adducts in beer and that the sulfite loses its stabilizing power by forming these adducts (Chapon et al., 1981).

Sulfite easily combines with beer components, especially acetaldehyde, and forms stable bisulfite adducts (Brenner and Stern, 1970; Delcour et al., 1982; Nordiöv, 1985). It is well-known that acetaldehyde equilibrium is rapidly reached and nearly complete conversion is obtained even in the absence of excess bisulfite (Fieser and Fieser, 1952). Higher aldehydes behave in much the same manner, regardless of the size of the lone alkyl



group, presumably because the substances all have in common the formyl group, –CHO. Acetaldehyde is the main aldehyde component of beers. Geiger et al. (1976) found it to be 60% of the aldehydes in American beers, while Hashimoto considers 97% of the aldehydes in Japanese beers to be acetaldehyde. Delcour et al. (1982) investigated enzymatic and gas chromatographic (GC) methods for acetaldehyde determination in beer and concluded that enzymatic assay determined both free and sulfite-bound acetaldehyde, while the GC procedure determines only free acetaldehyde. The average acetaldehyde content for 11 types of Pilsner beer was 5.1 ppm when determined using the enzymatic assay and 4.7 ppm using the GC analysis. It has been believed that this difference is due to sulfite-bound acetaldehyde and that most of the bisulfite adducts in beer are the acetaldehyde–bisulfite adduct. It is still a common question for brewers whether sulfite naturally produced by yeast during wort fermentation can contribute to the flavor stability of finished beer.

Recently, a new theory that stale-flavor carbonyls such as *trans*-2-nonenal can be masked by adduct formation with hydrogen sulfite has received particular attention (Nordiöv, 1985; Nordiöv and Winell, 1983). Drost et al. (1990) demonstrated that the oxidation products of malt lipids such as *trans*-2-nonenal are enzymatically and nonenzymatically produced during wort making. These carbonyls are masked by the adduct formation with bisulfite, which is produced by yeast during fermentation. These stale-flavor carbonyl adducts are more easily dissolved in young beer and survive in the finished beer and are responsible for flavor staling. However, the evidence to confirm this speculation has not been reported; that is, the *trans*-2-nonenal–bisulfite adduct has not been detected in beer.

Thus, the mechanism of sulfite contribution to beer stability has not yet been conclusively determined. The purpose of this paper is to study the antioxidative activity of bisulfite adducts in beer to clarify the stabilizing mechanism of sulfite toward beer quality.

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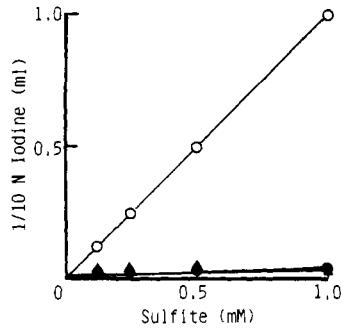

Figure 1. Iodine titration of sulfites in 5% (v/v) ethanol–0.1 M acetate buffer (pH 4.3): ○, Na_2SO_3; ●, formaldehyde–sodium bisulfite; ▲, acetaldehyde–sodium bisulfite.

MATERIALS AND METHODS

Apparatus. The chemiluminescence (CL) detector was a single photoelectron counting system, CLD-100 and CLC-10, manufactured by Tohoku Electronic Industries Co. Ltd., equipped with a personal computer, PC-9801 UM(NEC), for integration and a digital plotter, UP-6803A (National). Polarographic analysis was carried out using a P-1100 polarographic analyzer (Yanagimoto Manufacturing Co., Ltd.).

Beer. Bottled lager beer, brewed with malt, hop, and adjuncts such as rice and cornstarch and obtained before pasteurization and without the addition of L-ascorbic acid, was used.

Reagents. 2-Methyl-6-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one, *Cypridina* luciferin analog (CLA), and hydroxymethanesulfonic acid (formaldehyde–sodium bisulfite) were purchased from Tokyo Kasei Kogyo Co. Ltd. Acetaldehyde–sodium sulfite was synthesized by the addition reaction of sodium hydrogen bisulfite (Wako Pure Chemicals Ltd.) and acetaldehyde (over 99%, Merck–Schuchardt). It has a purity of over 90% based on the HPLC analysis.

Determination of Sulfites. Sulfite was determined by the HPLC–electrochemical detection (ECD) method as previously described (Kaneda et al., 1991a). The determination limit in beer is 1 μmol/L.

Polarographic and Iodine Titration Analyses of Sulfites. Reducing activities of sodium sulfite, formaldehyde–sodium bisulfite, and acetaldehyde–sodium bisulfite were measured using polarographic and iodine titration analysis methods. Differential pulse polarographic analysis for 50 μM sulfites in 5% (v/v) ethanol–0.1 M acetate buffer (pH 4.3) was carried out: scan rate, 5 mV/s; pulse interval, 0.5 s; modulation amplitude (ΔE), 50 mV. Sulfites (0–1 mM) in 5% (v/v) ethanol–0.1 M acetate buffer (pH 4.3) were titrated with 0.1 N iodine using 0.2% starch as an indicator.

Measuring the CL of Beer and CLA-Dependent Luminescence. Beer, after being degassed by supersonication, was placed at 20 °C for 20 min, and then 12 mL of beer was placed in a stainless steel dish (50 × 10 mm) and its CL measured at 60 °C. Three milliliters of beer was added to 0.1 M acetate buffer (pH 4.3) with 0.1 μM CLA, and the CLA-dependent luminescence was measured at 60 °C.

Beer Analysis. Isohumulones (isocohumulone, isohumulone, isoadhumulone) were determined according to the method of Ono et al. (1984) using HPLC, and their total value was calculated. The incubated beer was held at 0 °C for 1 day, and chill haze was measured with a NDH-1001 DP haze meter (Nippon Denshoku Kogyo Co. Ltd., Japan). The turbidity of the beer shows kaolin concentration (milligrams per liter).

Sensory Evaluation. The sensory test was carried out using the slightly modified descriptive analysis method of the European Brewing Convention (1987). The staling degree of each beer was assigned using a scale from 1 to 5 based on the average values assessed by a panel of 16 trained tasters; 1 represented "not present" and 5 represented "very strong".

RESULTS AND DISCUSSION

Figure 1 shows the titration curves of free, formaldehyde–, and acetaldehyde–bisulfites with iodine in 5% (v/v) ethanol–acetate buffer (pH 4.3), which was the beer model system. Formaldehyde– and acetaldehyde–bisulfite adducts had no reducing activity for iodine. Figure 2 shows the differential pulse polarogram for these sulfites in the model system. Free and combined sulfites had a peak around + 0.85 V, indicating that these sulfites have an oxidation potential from 0.90 to 0.95 V. However, the peaks of formaldehyde– and acetaldehyde–bisulfites were significantly lower, and their peak heights were about one-fifth that of the free sulfite. It is known that the oxidation potential of sulfite is 0.93 V. An oxidizing reagent like iodine has been generally used for determining the free sulfite in a natural product, which is based on the fact that this reagent instantaneously reacts with free sulfite. It seems that bisulfite adducts combined with carbonyls have only a weak oxidation potential at 0.93 V, so they lose their reducing activity toward several oxidizing reagents such as iodine.

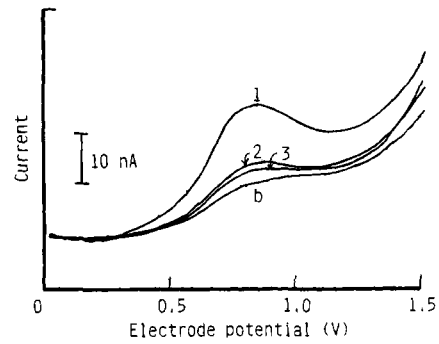

Figure 2. Differential pulse polarogram for sulfites in 5% (v/v) ethanol–0.1 M acetate buffer (pH 4.3): b, blank (no additions); 1, 50 μM Na_2SO_3; 2, 50 μM formaldehyde–sodium bisulfite; 3, 50 μM acetaldehyde–sodium bisulfite. Conditions: scan rate, 5 mV/s; pulse interval, 0.5 s; modulation amplitude (ΔE), 50 mV.

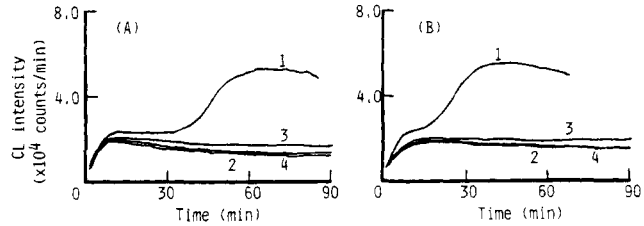

Figure 3. Effect of sulfites on chemiluminescence production of beer before (A) or after (B) storage at 37 °C for 2 days: 1, control (no additions); 2, 100 μM Na_2SO_3; 3, 100 μM formaldehyde–sodium bisulfite; 4, 100 μM acetaldehyde–sodium bisulfite.

When fresh beer with no additions was placed at 60 °C, the chemiluminescences (CL) were immediately produced (Figure 3). The emission intensity was found to remain constant for 30 min. After that time, it increased and reached a maximum after approximately 60 min. When the beer was stored at 37 °C for 2 days, the production of CL was accelerated. The CL intensity reached a maximum at an earlier stage, and the maximum CL intensity increased. When free sulfite, the formaldehyde–bisulfite adduct, or the acetaldehyde–bisulfite adduct was added to beer, the CL production was significantly inhibited. The CL intensities of beers with sulfites remained constant (did not increase), and the maximum CL intensities were not observed. Even though the beers with these sulfites were stored at 37 °C for 2 days, an acceleration of the CL production could not be observed. During the

Table 1. Changes in Sulfite Contents in Beer during Storage at 37 °C for 2 Days

addition	before storage		after storage	
	free	total	free	total
control (no additions)	nd[a]	nd	nd	nd
100 μM free sulfite	100	100	9	46
100 μM formaldehyde–bisulfite	nd	100	nd	54
100 μM acetaldehyde–bisulfite	nd	100	nd	52

[a] Not detected.

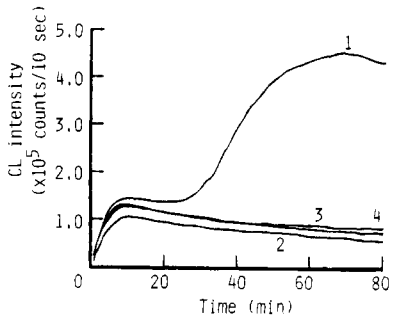

Figure 4. Effect of sulfites on production of CLA-dependent luminescence in beer: 1, control (no additions); 2, 100 μM Na_2SO_3; 3, 100 μM formaldehyde–sodium bisulfite; 4, 100 μM acetaldehyde–sodium bisulfite.

storage of the beer with added sulfites, about 50% of the added sulfites disappeared (Table 1). No difference in the residual total sulfite after the storage between free sulfite and carbonyl–bisulfite adducts was observed, indicating that the same level of carbonyl–bisulfite adducts with free sulfite is oxidized during beer storage. In previous papers (Kaneda et al., 1990a–c), it was shown that the CL is produced via the free radical reactions of beer components during beer storage and that the oxidation process in beer and the staling degree of beer flavor can be evaluated from the CL producing pattern. Therefore, it was shown that the carbonyl–bisulfite adducts inhibited the free radical reactions in beer as well as the free sulfite due to autoxidation.

When beer was incubated with a *Cypridina* luciferin analog (CLA) at 60 °C, CLA-dependent luminescence was immediately produced and the emission intensity was found to remain constant for 30 min (Figure 4). After that time, it increased and reached a maximum in 60 min. The addition of free sulfite, the formaldehyde–bisulfite adduct, or the acetaldehyde–bisulfite adduct inhibited the production of the CLA-dependent luminescence. No difference in the inhibition level of the CLA-dependent luminescence by the addition of these three kinds of sulfites was observed. The CLA can react only with singlet oxygen ($^1O_2$) and superoxide (O_2^-) and shows a marked luminescence. It was also shown that the CLA-dependent luminescence is produced during beer oxidation and that active oxygens such as $^1O_2$ and O_2^- are produced during beer storage (Kaneda et al., 1991b). The staling mechanism during beer storage has been proposed; At first, O_2^- is generated from molecular oxygen ($^3O_2$) due to autoxidation during the storage of packaged beer. Next, a hydroxyl radical ($^{\bullet}OH$) is produced by metal catalysis reactions, such as the Haber–Weiss reaction, from O_2^- and H_2O_2. These active oxygens attack beer components such as isohumulones, sugars, alcohols, fatty acids, and polyphenols and initiate a series of radical reactions in beer to produce flavor-staling carbonyls and haze. Some of the carbonyls are directly responsible for or are responsible via some condensation reactions for the staling off-flavors of beer. It is also well-known that free sulfite is a scavenger of active oxygen such as O_2^- and H_2O_2. It was indicated that carbonyl–bisulfite adducts have a radical scavenging activity and can trap active oxygen produced during the process of beer oxidation, as well as free sulfite. Chapon et al. (1981) showed that free sulfite rapidly combines with the volatile and nonvolatile fractions in beer and that bound sulfite can react with H_2O_2 in the model system. They thought that a portion of the sulfite engaged in the formation of addition compounds with carbonyl substances can be oxidized in coupled reactions and, accordingly, can contribute to the elimination of O_2 present in the complex medium. Chapon's speculation was confirmed in this study.

When beer was incubated at 37 °C for 8 days, the amounts of isohumulones (isocohumulone, *n*-isohumulone, and isoadhumulone) decreased and the formation of chill haze occurred (Table 2). When free sulfite, the formaldehyde–bisulfite adduct, or the acetaldehyde–bisulfite adduct was added to beer, the amounts of isohumulones decreased and chill haze formation was inhibited. Previously, it was shown that free radicals generated by the addition of hydrogen peroxide (H_2O_2) in the presence of metals and by the γ-ray irradiation attacked isohumulones and proanthocyanidins in beer and directly or indirectly degraded them. Radical reactions are responsible for the oxidative degradation of isohumulones and polyphenols during the storage of beer and lead to flavor staling and haze formation. Therefore, it was indicated that both free sulfite and the aldehyde–bisulfite adducts inhibit free radical reactions in beer and delay the degradation of beer components such as isohumulones and polyphenols during the storage of beer. The inhibitions of the degradation of isohumulones were different among the sulfite species. The addition of the acetaldehyde–bisulfite adduct more significantly inhibited the degradation of isohumulones than the free sulfite. It seems that the acetaldehyde–bisulfite adduct is more hydrophobic and can more easily contact hydrophobic compounds such as isohumulnes than free sulfite, so it can more significantly inhibit the free radical reactions of isohumulones.

When the beer with the formaldehyde–bisulfite adduct was stored at 37 °C, the formation of chill haze was significantly accelerated. It is well-known that the

Table 2. Effect of Sulfites on Degradation of Isohumulones and Haze Formation in Beer during Storage at 37 °C

addition	storage time (days)	isohumulones (%)				haze (mg of kaolin/L)
		isocohumulone	*n*-isohumulone	isoadhumulone	total	
control (no additions)	0	100	100	100	100	0.1
	10	70	60	61	63	24.5
100 μM free sulfite	10	79	69	71	73	15.0
100 μM formaldehyde–bisulfite	10	82	69	65	73	51.0
100 μM acetaldehyde–bisulfite	10	92	81	99	88	14.0

Table 3. Effect of Sulfites on Flavor Staling of Beer Stored at 37 °C for 8 Days

addition	before storage	after storage
control (no additions)	1.2[a]	4.1
100 μM free sulfite		3.3
100 μM acetaldehyde–bisulfite		3.6

[a] Each value shows the staling degree of the beer flavor which was evaluated in a sensory test: scale of 1–5, 1 being freshest.

addition of formaldehyde to beer or wort immediately induces haze formation. It is also well-known that formaldehyde combines selectively with beer polyphenols such as proanthocyanidins and the complex is insoluble in beer, leading to the occurrence of precipitates. These formaldehyde–tannin complexes are thought to be analogous to the well-known phenol–formaldehyde bakelite polymers, and the minor insoluble complexes might be analogous to urea–formaldehyde and amine–formaldehyde resins. These reactions have been tried in the mashing process for the selective removal of proanthocyanidins in beer (Macey et al., 1968; Withey and Briggs, 1966). The other aldehydes such as acetaldehyde, butylaldehyde, and propionic aldehyde do not have the removal action. When the formaldehyde–bisulfite adduct was added to beer and the beer was stored at 0 °C for 1 day, the haze increase was not observed (0.1 mg of kaolin/L). It was shown that the formaldehyde–bisulfite adduct does not have condensation activity. It seems that the acceleration of chill haze formation during the storage of beer with the formaldehyde–bisulfite adduct is caused by the release of formaldehyde from the adduct. It can be easily thought that the sulfite group in the adduct is oxidized to sulfate during the storage of beer and, consequently, the adduct is separated into sulfate and formaldehyde.

The flavor-staling degree of the stored beer with free sulfite or the acetaldehyde–bisulfite adduct was studied using a sensory test. When beers were stored at 37 °C for 8 days, a stale flavor appeared (Table 3). The addition of free sulfite or the acetaldehyde–bisulfite adduct inhibited the flavor staling of beer during storage. The significant difference in the average staling degree between beers with and without sulfites in the sensory test was confirmed by the statistic t examination. Flavor staling of packaged beer is caused by the oxidation reactions of the beer components. Higher molecular aldehydes such as *trans*-2-alkenals have extremely low thresholds and have been recognized as an aging off-flavor. It has been thought that the oxidative degradations of isohumulones, higher molecular alcohols, and unsaturated fatty acids are responsible for the production of flavor-staling aldehydes such as *trans*-2-nonenal. As previously described, it has recently been shown that the flavor staling occurs via free radical reactions in beer and that the addition of sulfite inhibits the free radical reactions and flavor staling in beer (Kaneda et al., 1991a,b). Therefore, it was thought that the acetaldehyde–bisulfite adducts can inhibit the free radical reactions in beer as well as free sulfite, leading to increased flavor stability of beer during storage.

In conclusion, one contributing mechanism for beer stabilization on the shelf can be proposed: bisulfite adducts, which are complexes of sulfite produced by yeast during fermentation with aldehydes in beer, do not have an oxidation potential around 0.93 V, so they lose their reducing activity for several oxidizing reagents such as iodine. However, they do not lose their reactivity with free radicals. They retain their scavenging activity of free radicals such as active oxygen and can trap free radicals produced in beer during storage, so that they inhibit the free radical reactions of beer components such as isohumulones and polyphenols, leading to protection from the deterioration of beer quality such as flavor staling and haze formation.

LITERATURE CITED

Brenner, M. W.; Stern, H. Dissolved oxygen and antioxidants. *Tech. Q., Master Brew. Assoc. Am.* **1970**, 150–157.

Brewer, J. D.; Fenton, M. S. The formation of sulphur dioxide during fermentation. *Proceedings of the 16th Convention, The Institute of Brewing (Australia and New Zealand Section)*; 1980, pp 155–164.

Chapon, L.; Chapon, S.; Djeuga, N. Free sulfite in beers—Kinetic studies. *J. Am. Soc. Brew. Chem.* **1981**, *40*, 31–39.

Delcour, J. A.; Caers, J. M.; Dondeyne, P.; Delvaux, F.; Robberechts, E. An enzymatic assay for the determination of acetaldehyde in beers. *J. Inst. Brew.* **1982**, *88*, 384–386.

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

European Brewing Convention. Descriptive analysis (International method). *Analytica–EBC*, 4th ed.; 1987; E–299.

Fieser, L. F.; Fieser, M. Aldehydes and Ketones. In *Textbook of Organic Chemistry*; Maruzen: 1952; pp 197–198.

Geiger, E.; Piendl, A. Technological Factors in the formation of acetaldehyde during fermentation. *Tech. Q. Master Brew. Assoc. Am.* **1976**, *13*, 51–63.

Hashimoto, N. Studies on volatile carbonyl compounds in beer. *Rep. Res. Lab. Kirin Brew.* **1966**, *9*, 1–10.

Kaneda, H.; Kano, Y.; Osawa, T.; Ramarathnam, N.; Kawakishi, S.; Kamada, K. Detection of free radicals in beer oxidation. *J. Food Sci.* **1988**, *53*, 885–888.

Kaneda, H.; Kano, Y.; Kamimura, M.; Osawa, T.; Kawakishi, S. Detection of chemiluminescence produced during beer oxidation. *J. Food Sci.* **1990a**, *55*, 881–882.

Kaneda, H.; Kano, Y.; Osawa, T.; Kamimura, M.; Kawakishi, S. Evaluation of beer deterioration by chemiluminescence. *J. Food Sci.* **1990b**, *55*, 1361–1364.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Kamimura, M. Role of Free Radicals in Chemiluminescence Generation during the Beer oxidation. *Agric. Biol. Chem.* **1990c**, *54* (8), 2165–2166.

Kaneda, H.; Kano, Y.; Kamimura, M.; Kawakishi, S.; Osawa, T. A study of beer staling using chemiluminescence analysis. *J. Inst. Brew.* **1991a**, *97*, 105–109.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Koshino, S. Role of active oxygens on deterioration of beer flavour. *Proceedings of the 23rd Congress, European Brewery Convention (Lisbon)*; 1991b; pp 433–440.

Kaneda, H.; Kimura, T.; Kano, Y.; Koshino, S.; Osawa, T.; Kawakishi, S.; Koshino, S. Role of fermentation conditions on flavor stability of beer. *J. Ferment. Bioeng.* **1991c**, *72*, 26–30.

Kaneda, H.; Kano, Y.; Sekine, T.; Ishii, S.; Takahashi, K.; Koshino, S. Effect of pitching yeast and wort preparation on flavor stability of beer. *J. Ferment. Bioeng.* **1992**, *73*, 456–460.

Macey, A.; Stowell, K. C.; White, H. B. Use of formaldehyde for the reduction of the anthocyanogen content of wort. *Proceedings of the American Society of Brewing Chemists*; 1968; pp 22–28.

Narziss, L.; Miedaner, H.; Graf, H.; Eichhorn, P.; Lustig, S. Technological approach to improve flavor stability. *Tech. Q. Master Brew. Assoc. Am.* **1993**, *30*, 48–53.

Nordiöv, H. Formation of sulphur dioxide during beer fermentation. *Proceedings of the 20th European Brewery Convention (Helsinki)*; 1985; pp 291–298.

Nordiöv, H.; Winell, B. Beer flavor stabilization by interaction between bisulfite and trans-2-nonenal. *Proceedings of the 19th European Brewery Convention (London)*; 1993; pp 271–278.

Ryder, D. S.; Power, J.; Siebel, R. E. A question of flavor stability and instability. *Proceedings of the Convention, The Institute of Brewing*; **1989**; pp 366–391.

Withey, J. S.; Briggs, D. E. The use of formaldehyde in resteeping. *J. Inst. Brew.* **1966**, *72*, 474–479.

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