

Behavior and Role of Iron Ions in Beer Deterioration

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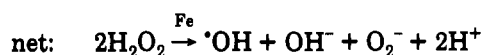
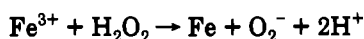
The behavior of iron ions during beer oxidation was studied, using an electron spin resonance (ESR) technique at 77 K, to clarify the role of metal ions in beer deterioration. An ESR signal due to non-heme Fe^{3+} ($g = 4.3$) was detected in beer, and its peak height increased during beer oxidation. The increase during beer storage was accelerated by the addition of H_2O_2 , Fe^{3+} , or Fe^{2+} . The signal for free radicals ($g = 2.004$) was detected in beer by the addition of KOH just before the analysis. It was increased by the storage of beer with or without the addition of iron ions and/or H_2O_2 . The addition of Fe^{2+} , Fe^{3+} , H_2O_2 , $\text{Fe}^{2+}-\text{H}_2\text{O}_2$, or $\text{Fe}^{3+}-\text{H}_2\text{O}_2$ accelerated the oxidative degradation of the main bitter components, isohumulones, during the storage of beer, while the addition of DETAPAC or EDTA inhibited the oxidative degradation. It was thought that Fe^{2+} ions dominant in fresh beer are oxidized to Fe^{3+} ions during the beer oxidation and that the strong complexes of Fe^{3+} with beer components (non-heme Fe^{3+}) increase, leading to haze formation. It seems that the changes in iron ions following the metal-catalyzed free-radical reactions lead to the deterioration of beer quality.

INTRODUCTION

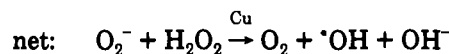
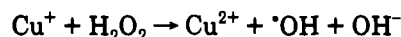
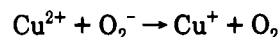
Flavor staling, browning, and haze formation take place in packaged beer during long storage on the shelf. It is well-known that the deterioration of beer quality results from oxidative changes in beer components (Hashimoto, 1988; Narzias, 1986). The Maillard reaction and oxidation of polyphenols are responsible for browning. Haze is caused by the polymerization reactions of polyphenols and protein, and the haze formation is accelerated by an increase in the oxygen level in the packaged beer. It has been thought that unsaturated aldehydes, especially *trans*-2-nonenal, are responsible for the "cardboard flavor", an aging off-flavor, and that these aldehydes are produced by the oxidation of beer components such as alcohols, isohumulones, and unsaturated fatty acids. However, the oxidative reactions of beer components, especially the mechanisms of the flavor staling, have not been fully elucidated.

In previous papers (Kaneda et al., 1988, 1989, 1990, 1991) it was indicated that free-radical reactions are responsible for beer deterioration; several active oxygens are produced during the storage of beer to initiate free-radical reactions, leading to flavor staling and haze formation. Transition-metal ions such as Fe and Cu in beer seem to play an important role in these radical reactions; they participate in the metal-catalyzed reactions such as Fenton and Haber-Weiss reactions to produce the active oxygens:

Fenton reaction



Haber-Weiss reaction



Beer has trace levels of heavy metals such as iron, copper, and nickel. It has been thought that these metal ions have important roles in foam formation and stability, haze formation, and gushing in beer (Bishop et al., 1974; Gardner, 1972; Gramshaw, 1970). The contents of the iron, cobalt, nickel, and zinc ions in beer are concentrated in the foam and haze of beer. The addition of iron and nickel to beer induces gushing in beer.

It seems that the existing forms of the metal ions in beer significantly affect these phenomena, including flavor staling. However, the behavior of metal ions in beer has not been fully elucidated. It has not been clarified whether the metal ions are in a free or complexed state in beer or in what states ions exist in beer. It has been difficult to study them because they are only at trace levels in beer.

The purpose of this paper is to clarify the behavior of metal ions during beer oxidation using an electron spin resonance (ESR) technique and to discuss the role of metal ions in beer staling.

EXPERIMENTAL PROCEDURES

Beer. Bottled beer, commercial Japanese lager beer, was used as the sample for the ESR measurements.

Reagents. Diethylenetriaminepentaacetic acid (DETAPAC), ethylenediaminetetraacetic (EDTA) disodium salt, ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and iron(III) sulfate [$\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$] were obtained from Wako Pure Chemicals (Tokyo). Hydrogen peroxide (31% w/v) was purchased from Mitsubishi Gas Co. (Tokyo) and stored at 4 °C.

Incubation of Beer. Beer (620 mL) with ca. 30 mL of headspace air and with or without several reagents was bottled and incubated at 37 °C for several days.

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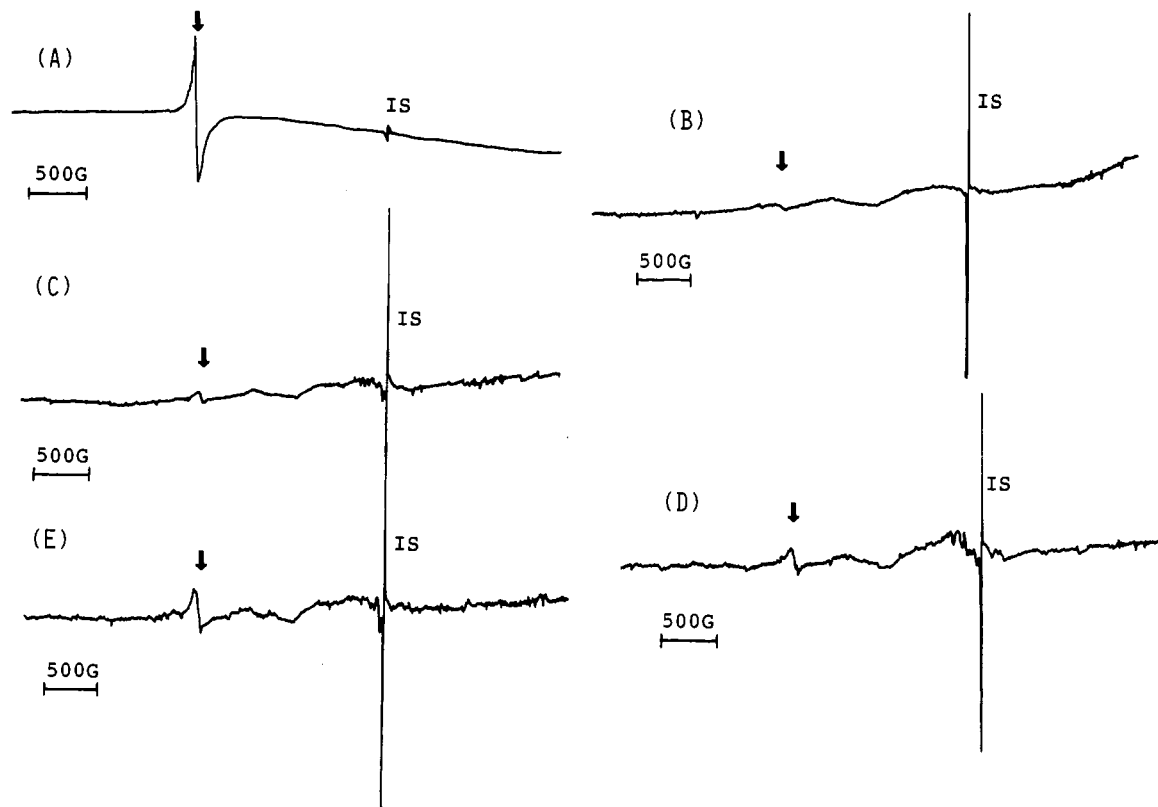


Figure 1. ESR spectra of beer at 77 K. (A) EDTA-Fe³⁺ alkaline solution as a reference of the spectra; (B) fresh beer (no treatment); (C) storage at 37 °C for 10 days; (D) addition of 0.03 M EDTA and 0.2 M KOH to beer stored at 37 °C for 10 days; (E) addition with 4.5 μM Fe³⁺ and storage at 37 °C for 10 days. IS, internal standard (Cr³⁺); downward solid arrow, $g = 4.3$.

Sample Preparation. Potassium hydroxide (KOH) and EDTA were added to 10 mL of the beer prepared as mentioned above (final concentrations: 0.2 M KOH and 0.03 M EDTA) just prior to the ESR analysis.

ESR Measurement. A 0.4-mL sample solution was transferred in an ESR quartz tube, and the ESR spectrum was measured at liquid N₂ temperature (77 K) with a JM-FE3 ESR spectrometer (JEOL Ltd. Japan). A reserver (ES-VCD 2X, JEOL Ltd.) was used for storage of the sample at 77 K during the ESR analysis. The g value was determined by that of Cr³⁺ ($g = 1.9800$) in MgO used as a standard. The relative peak height of each peak was calculated with respect to the signal intensity of the Cr³⁺ ions.

Beer Analysis. Isohumulones (isocohumulone, isohumulone, isoαhumulone) were determined according to the method of Ono et al. (1984) using HPLC, and their total value was calculated. Total iron and copper contents were determined by atomic absorption method using a polarized Zeeman atomic absorption spectrophotometer Z-8000 (Hitachi, Tokyo). 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).

RESULTS

When the ESR analysis of beer was carried out at 77 K, a signal was detected at $g = 4.3$, which was the same g value as that of Fe³⁺-EDTA (Figure 1). The addition of Fe³⁺ increased the signal intensity. The signal peak height was also increased when EDTA and KOH were added to the beer just before its ESR analysis. Moreover, it increased during the incubation of beer at 37 °C, and its increase was accelerated by the addition of H₂O₂, Fe²⁺, or Fe³⁺ (Figures 1 and 2). The beer used in this study had 100 ppb of iron ions (Fe²⁺ and Fe³⁺) and 30 ppb of copper

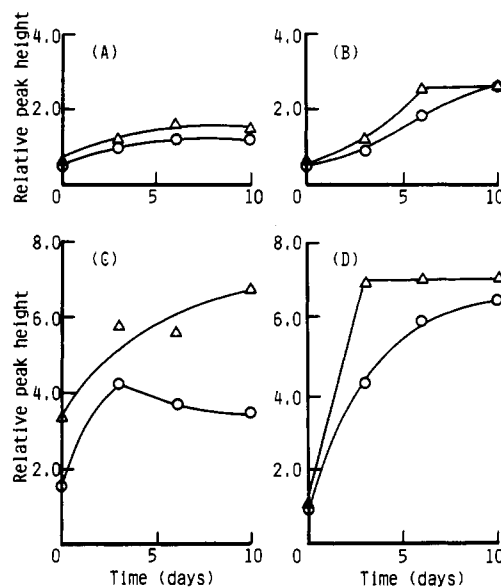


Figure 2. Changes in the signal intensity at $g = 4.3$ in beer incubated at 37 °C. Beer with (A) no additions, (B) 5 mM H₂O₂, (C) 4.5 μM Fe³⁺, or (D) 4.5 μM Fe²⁺ was incubated at 37 °C. (O) No treatment; (Δ) addition with 0.03 M EDTA and 0.2 M KOH just before the analysis.

ions (Cu⁺ and Cu²⁺). The Cu²⁺ signal could not be detected in this study.

When KOH was added to beer just before the ESR analysis, another signal was detected at $g = 2.004$ (Figure 3). The signal peak height was increased by the incubation of beer at 37 °C for 10 days. The increase in signal intensity was accelerated by the addition of Fe³⁺ or Fe³⁺-H₂O₂. The same tendency was observed for the case of Fe²⁺ addition with or without H₂O₂ (data not shown).

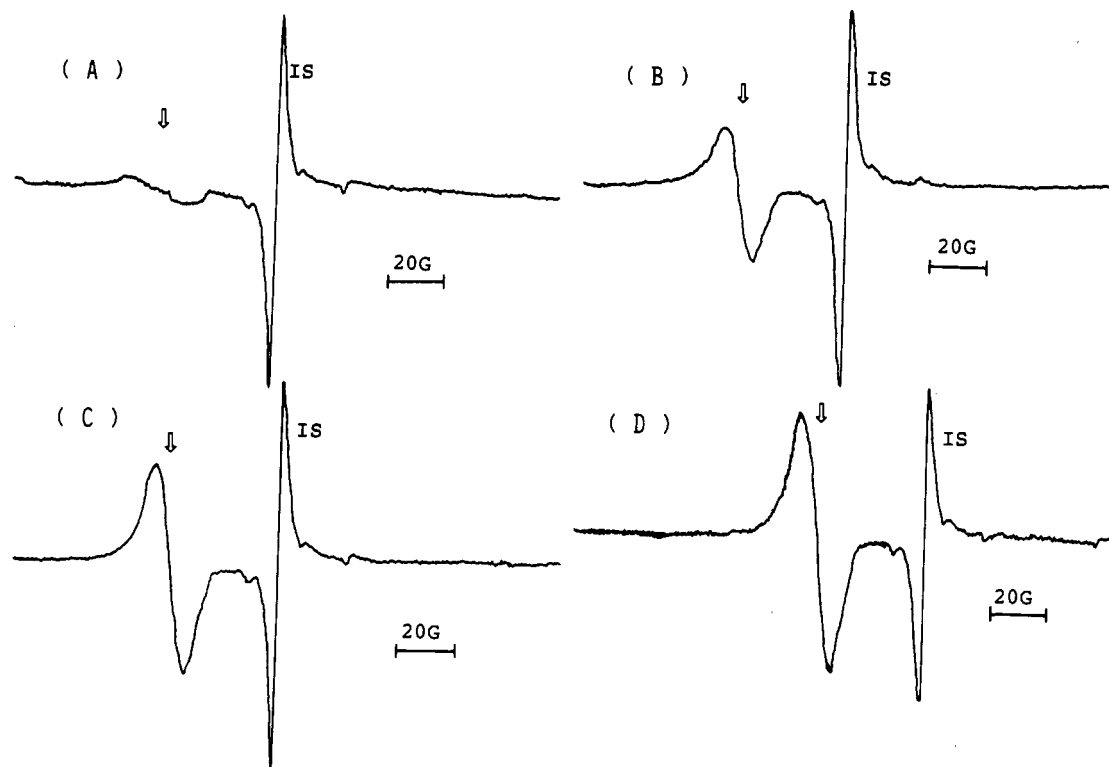


Figure 3. ESR spectra of beer with KOH at 77 K. KOH (0.2 M) was added to beer just before the analysis. (A) Fresh beer (no treatment); (B) storage at 37 °C for 10 days; (C) addition with 4.5 μM Fe^{3+} and storage at 37 °C for 10 days; (D) addition with 4.5 μM Fe^{3+} and 5 mM H_2O_2 and storage at 37 °C for 10 days. IS, internal standard (Cr^{3+}); downward open arrow, $g = 2.004$.

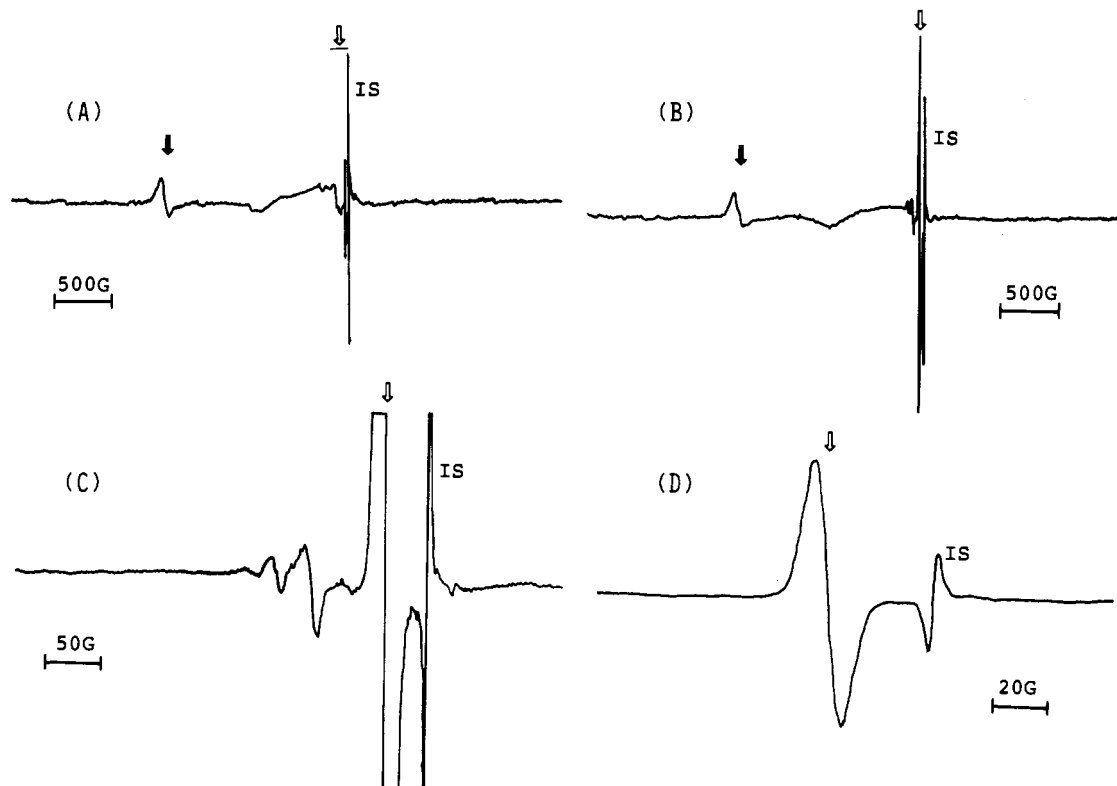


Figure 4. ESR spectra of beer haze at 77 K. (A) No treatment; (B) and (C) addition with 0.03 M EDTA and 0.2 M KOH just before the analysis; (D) addition with 0.2 M KOH just before the analysis. IS, internal standard (Cr^{3+}); downward solid arrow, $g = 4.3$; downward open arrows $g = 2.004$.

When beer was stored without opening at room temperature for 2 years, beer haze occurred. The precipitate formed in the aged beer was collected and dissolved in beer (3.9 mg/mL of beer), and its ESR spectrum was observed at 77 K (Figure 4). The higher signal was detected

at $g = 4.3$, but the peak height was not increased by the addition of EDTA and KOH. This signal was at trace levels in the original aged beer (data not shown). A sharp signal was also detected at $g = 2.004$, and the peak height was significantly increased by the addition of EDTA and

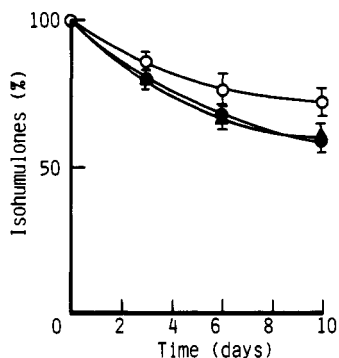


Figure 5. Effect of iron ions on degradation of isohumulones in beer during storage at 37 °C. Each value represents a percentage of isohumulones with respect to that of beer with an incubation time of 0 days, no additions. (O) No additions; (●) 4.5 μM Fe²⁺; (▲) 4.5 μM Fe³⁺.

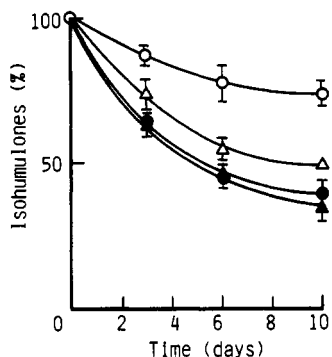


Figure 6. Effect of H₂O₂ or H₂O₂-iron ions on degradation of isohumulones in beer during storage at 37 °C. Each value represents a percentage of isohumulones with respect to that of beer with an incubation time of 0 days, no additions. (O) No additions; (Δ) 5 mM H₂O₂; (●) 5 mM H₂O₂ and 4.5 μM Fe²⁺; (▲) 5 mM H₂O₂ and 4.5 μM Fe³⁺.

KOH or only KOH. The other signals were also observed and became sharper with the addition of EDTA and KOH. Contents of iron and copper ions in the precipitate solution were 425 and 890 μg/L, respectively. The iron and copper contents in the original beer were 50 and 15 μg/L, respectively.

The content of isohumulones decreased during the incubation of beer at 37 °C (Figure 5). When Fe³⁺ or Fe²⁺ ions were added to beer, the decrease was accelerated. The addition of H₂O₂, H₂O₂-Fe²⁺, or H₂O₂-Fe³⁺ further accelerated the degradation during the storage of beer at 37 °C (Figure 6). The difference in the effects of Fe²⁺ and Fe³⁺ on the degradation of isohumulones could not be observed. However, when EDTA or DETAPAC was added to beer, it was inhibited (Figure 7).

When beer was incubated at 37 °C, chill haze increased (Table I). The haze formation was accelerated with the addition of H₂O₂, H₂O₂-Fe²⁺, or H₂O₂-Fe³⁺, while it was inhibited by EDTA or DETAPAC.

DISCUSSION

The ESR analysis can detect only the ions with unpaired electrons with high sensitivity, so that ferric ions (Fe³⁺) and cupric ions (Cu²⁺) can be detected, whereas ferrous ions (Fe²⁺) and cuprous ions (Cu⁺) cannot. In this study, the ESR spectrum of beer was measured at 77 K for higher sensitivity. The signal peak at $g = 4.3$, which was detected in the stored beer, was increased by the addition of Fe³⁺ and had the same g value as that of Fe³⁺-EDTA, indicating the existence of non-heme Fe³⁺ complex in beer (Figure 1). It has been thought that the heavy metals (aluminum,

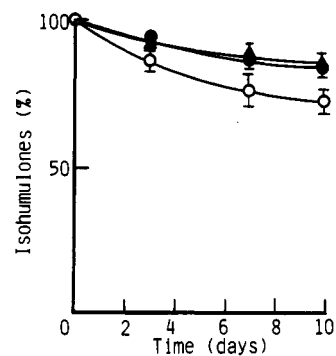


Figure 7. Effect of EDTA and DETAPAC on degradation of isohumulones in beer during storage at 37 °C. Each value represents a percentage of isohumulones with respect to that of beer with an incubation time of 0 days, no additions. (O) No additions; (●) 1 mM DETAPAC; (▲) 1 mM EDTA.

iron, copper, nickel, tin, and radium) form chelates with amino, carboxyl, sulfhydryl, and phenolic hydroxyl groups in beer. Such chelates are probably responsible for beer haze (Gramshaw, 1970). It seems that the complexes of iron ions with beer components exist in aged beer, and their structures are probably similar to that of Fe-EDTA.

The peak height of non-heme Fe³⁺ was increased by the addition of EDTA and KOH just before the analysis (Figures 1 and 2), indicating that free Fe³⁺ and/or weakly bound Fe³⁺ with beer components in beer form the complexes with EDTA in the alkaline condition during the analysis (Fe³⁺-EDTA). Therefore, the peak height at $g = 4.3$ in the beer with EDTA and KOH may show the total Fe³⁺ ions in beer, including free Fe³⁺, weakly bound Fe³⁺, and non-heme Fe³⁺ (strong complexes of Fe³⁺). The peak of non-heme Fe³⁺ was not detected in fresh beer with or without EDTA. Chapon et al. (1971) showed that beer can reduce a complex of Fe³⁺ with α,α' -dipyridyl to the Fe²⁺ complex. So, it seems that iron ions exist in the reduced state, Fe²⁺, in fresh beer. Therefore, it is conceivable that Fe²⁺ ions dominant in fresh beer are oxidized to Fe³⁺ ions during the process of beer oxidation. Although copper ions could not be analyzed in this study, they probably show the same behavior as iron ions in beer; Cu⁺ ions in fresh beer are oxidized into Cu²⁺ ions during the process of beer oxidation.

The signal at $g = 2.004$, which was detected in stored beer with KOH, shows the existence of free radicals in beer (Figure 3). It could not be detected in beer at pH 4.2-4.3. On the basis of the g value, it was speculated that the free radicals may be derived from semiquinone radicals which are produced in the alkaline condition. The free radicals increased with beer storage, and the increase was accelerated by the addition of H₂O₂ and/or iron ions. It seems that their phenolic precursors are produced in metal-catalyzed free-radical reactions during the storage of beer.

It is well-known that protein and polyphenolic material are the predominant constituents of typical chill and permanent haze of beer. These components, accompanied by smaller amounts of carbohydrate and inorganic constituents, yield a mineral ash upon combustion (Gramshaw, 1970). The heavy metals exist in hazes at concentrations of 1-80 × 10³ times greater than the concentrations remaining in the beer. These metals probably distribute themselves among the protein, polyphenols, and protein-polyphenol complexes in beer but form particularly strong complexes with polyphenols and their protein complexes. In this study, total iron ions occur in the precipitate solution at concentrations approximately 8.5 times greater

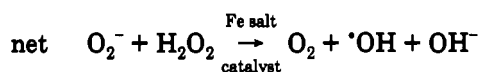
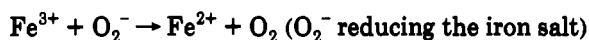
Table I. Effect of Several Reagents on Formation of Chill Haze (Milligrams per Liter) in Beer

incubation time, ^a days	no additions (control)	1 mM DETAPAC	1 mM EDTA	5 mM H ₂ O ₂	5 mM H ₂ O ₂ + 4.5 μM Fe ²⁺	5 mM H ₂ O ₂ + 4.5 μM Fe ³⁺
0	0.3					
10	1.4	0.8	0.6	3.6	8.6	7.4

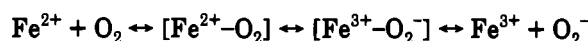
^a Beer was incubated at 37 °C.

than those remaining in the original beer. The non-heme Fe³⁺ was also significantly concentrated in beer haze which occurred in the aged beer with normal air contents (Figure 4). It was shown that the non-heme Fe³⁺ is produced in packaged beer under practical conditions. The addition of EDTA and KOH did not increase the signal peak height, permitting speculation that Fe³⁺ ions have already existed in the strongly chelated states in haze. Copper ions were more concentrated in the haze solution than iron ions (ca. 60 times). The precipitate solution also included a high level of free radicals ($g = 2.004$), and the radicals were significantly increased by the addition of KOH. Table I shows that Fenton and Haber–Weiss reactions play important roles for the haze formation in beer. Thus, it can be concluded that metal-catalyzed free-radical reactions may occur in the coordination of metal ions during beer storage and that stable radicals derived from phenolic radicals are finally produced from part of the polyphenols to form beer haze.

As cited in the Introduction, it has been shown that active oxygens such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and the hydroxyl radical (·OH) are produced during the process of beer oxidation (Kaneda et al., 1988, 1989, 1990, 1991). The active oxygens attack several beer components and initiate a series of free-radical reactions, leading to beer staling. A trace level of transition-metal ions plays an important role in these free-radical reactions. It is well-known that ·OH formation can be accounted for as follows, if the Haber–Weiss reaction is catalyzed by traces of transition-metal ions (Halliwell and Gutteridge, 1985):



It has been also shown that the mixed function oxidation (MFO) system (MFO system, O₂–Fe³⁺ or Cu²⁺–electron donors) also produces the active oxygens to mediate the oxidative inactivation of enzymes (Fucci et al., 1983; Stadtman and Oliver, 1991). It seems that these metal-catalyzed reactions, which produce the active oxygens, easily occur in the complexes of the metal ions with beer components; for example, polyphenols, which form the chelates with Fe³⁺ or Cu²⁺ in beer, can act as electron donors in the MFO system. Irwin et al. (1991) studied the role of cupric ions, oxygen, and polyphenols in beer flavor instability using model systems. They speculated that the Cu²⁺-catalyzed oxidations, which require prooxidants such as cysteine and polyphenols to recycle the copper through its reduced state, may produce unsaturated aldehyde such as aging off-flavor during aging. As previously cited, iron ions mainly exist as Fe²⁺ ions in fresh beer before storage. In the presence of molecular oxygen, ferrous ions are oxidized to ferric ions, the electron being accepted by oxygen during the formation of superoxide (Bamforth et al., 1988):



It seems that these metal-catalyzed reactions producing active oxygens may occur in beer during storage and lead to the staling of beer quality.

It has been shown that the oxidative degradation of isohumulones is partly responsible for the formation of the cardboard off-flavor (Hashimoto, 1988; Narziss, 1986). It has also been reported that isohumulones are degraded by active oxygens such as ·OH to produce several carbonyls during beer storage (Kaneda et al., 1989). Figures 5 and 7 indicate that metal ions such as iron play an important role for the degradation of isohumulones during the beer oxidation. Figure 6 showed that the Fenton and Haber–Weiss reactions accelerated the degradation during the storage of beer. There were no differences in the effect between Fe²⁺ and Fe³⁺ on the degradation of isohumulones. The metal-catalyzed Haber–Weiss reaction might be involved in the oxidative degradation of isohumulones in beer. It has also been reported that isohumulones form complexes with proteins in beer and that they are strongly bound by metals and have an important role in foam formation and stability or gushing of beer (Gardner, 1972). It seems that a “site-specific” degradation of isohumulones by metal-catalyzed free-radical reactions may easily occur in the complexes of metal–isohumulone or metal–isohumulone–protein when molecular oxygens come in contact with these complexes during the process of beer oxidation. The inhibition of the degradation of isohumulones by EDTA and DETAPAC is probably because metal ions may be removed from the complexes with isohumulones by the formation of complexes, EDTA–metal ions and DETAPAC–metal ions, so that the active oxygens produced by the metal-catalyzed reactions cannot reach the isohumulones.

On the basis of the results presented so far, the behavior and the role of iron ions in beer staling can be speculated; ferrous ions exist as the predominant iron species in fresh beer. The ferrous ions are oxidized into ferric ions such as free Fe³⁺, weakly bound Fe³⁺, and strong chelates of Fe³⁺ (non-heme Fe³⁺) during the process of beer oxidation. The non-heme Fe³⁺ ions increase with beer staling and are finally responsible for beer haze. The non-heme iron ions consist of iron complexes with proteins, isohumulones, and/or polyphenols. These changes in iron ions may occur during the producing reaction of ·OH, such as an iron-catalyzed Haber–Weiss reaction, and lead to the deterioration of beer quality. Especially when molecular oxygens come in contact with the iron–isohumulone complexes during the storage of beer, a site-specific degradation of isohumulones by the metal-catalyzed free-radical reactions takes part and accelerates the formation of the aging off-flavor. The metal-catalyzed site-specific oxidation also occurs in the complex of iron with polyphenols and leads to the haze formation. A trace level of copper ions probably has the same behavior and role as the iron ions. The free-radical reactions initiated by the metal catalysis proceed in beer and finally lead to the formation of stable phenolic radicals which are constituents of beer haze.

In conclusion, the complexes of transition-metal ions formed in beer during the process of brewing and/or beer

storage have important roles not only on foam and gushing but also on degradation of beer quality during beer storage.

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