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INFLUENCE OF pH ON FLAVOUR STALING DURING BEER STORAGE

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A decreasing pH accelerated an increase in the chemiluminescence production and degradation of isohumulones and procyanidins during the storage of beer and using a model system. The sensory test showed that the addition of HCl to fresh beer accelerated the flavour staling during beer storage but that the addition of HCl to stored beer did not significantly accelerate the flavour staling. Therefore, it was thought that the acceleration of beer flavour staling is not dependent on a decrease in pH such that the decreasing pH isolates stale flavour aldehydes by a dissociation from staling-flavour aldehyde adducts but based on the fact that the decreasing pH accelerates the flavour staling reactions, free radical reactions, during beer storage.

Key Words: *Beer, pH, flavour staling, free radical reactions.*

INTRODUCTION

The normal pH of bottom fermentation beers at the end of fermentation is 4.2–4.4, rarely 4.0 or less[2]. The importance of the control of finished beer pH is well accepted since the influence of pH on beer flavour, and physical and microbiological stabilities is clearly recognized. The pH of beer has a very marked influence on the development of lactic organisms; a low pH inhibits their growth[2]. On the other hand, colloidal haze is accelerated by a low pH. Taylor[16] showed that pH control during brewing has a relative significance by stimulating yeast growth and wort buffering capacity on beer pH, and the consequent influence on beer flavour, potential haze stability, and head formation ability.

Grigsby et al.[4,5] first showed that a correlation exists between the pH of beer and its oxidizability; the oxidized flavour decreased with increasing pH. When the pH of beer was reduced to below its normal level, oxidation was enhanced. But the detailed mechanism for the difference in the flavour staling rate of beer with pH have not been fully elucidated. Nordlöv and Winell[12] confirmed this tendency and explained that a nonenal-SO_2 adduct would tend to dissociate at low pH values, leading to isolation of stale flavour aldehydes such as *trans*-2-nonenal. However, our recent studies showed that the level of *trans*-2-nonenal-bisulfite adducts in beer is too low to be the main cause of the production of the cardboard flavour during beer storage[14].

The purpose of this study was to confirm the relationship between beer pH and beer deterioration rate and to clarify its detailed mechanism.

MATERIALS AND METHODS

Beer. Bottled Japanese lager beers were commercially obtained.

Reagents. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Catechin and epicatechin were purchased from the Sigma Chem. Co. Procyanidin B3 was obtained from Prof. P. Dondeyne of Katholieke Universiteit Leuven. *Trans*-Isohumulones were prepared and purified from hop extract according to Sharpe and Ormrod[8].

Model System. Isohumulones (25 mg/L) or catechin (50 mg/L) in 0.1 M acetate buffer (pH 3.8, 4.3, or 4.8) including 5.0% (v/v) ethanol were incubated with 1.0 mM H_2O_2 and 1.0 µM $FeSO_4$ at 20°C.

Beer analysis. Isohumulones, catechin, epicatechin, and procyanidin B3 were determined using HPLC[9,13]. The chemiluminescence production and the DPPH-reducing activity of beer was measured as previously described[10,11].

Sensory evaluation. The sensory test was carried out as previously described[10]. The staling degree of each beer was assigned using a scale of 1–5 based on the average values assessed by a panel of 16 trained tasters and the values were statistically evaluated using a *t*-test; 1 represented "not present" while 5 represented "very strong".

RESULTS AND DISCUSSION

When 0.2 ml or 0.5 ml of 5.0 N HCl was added to the bottled beer (633 ml), the beer pH was changed from 4.3 to 4.1 or 3.8, respectively. When the beers with and without HCl were stored at 37°C for 3 days, the beer flavour deteriorated (Table I). In this study, the oxidation reactions were more accelerated in the beer than the usual packaged beer, because air was involved into a bottle during addition of distilled water (control) or HCl. The staling degree of the beer flavour during storage increased with decreasing beer pH. The significant differences in the averages of the staling degree between the beers with pH 4.3 and pH 4.1 and between the beers with pH 4.3 and pH 3.8 were confirmed by a statistical t-examination. In terms of the relationship between beer pH and its flavour staling, two reactions, oxidation and dissociation reactions, have been thought. Grigsby et al.[4,5] speculated that reducing beer pH enhances flavour staling reaction (oxidation). While Nordlöv and Winell[12] explained

TABLE I. Effect of beer pH on flavour staling during storage at 37°C for 3 days

	pH Preparation before storage			pH Preparation after storage		
	pH 4.3 control	pH 4.1 addition of HCl	pH 3.8 addition of HCl	pH 4.3 control	pH 4.1 addition of HCl	pH 3.8 addition of HCl
Staling degree	3.1	3.4*	4.2	2.9	3.1	2.8

*Statistically significant (*t*-test, 0.05).

that reducing beer pH leads to isolation of stale flavour aldehydes from aldehyde-SO_2 adducts (dissociation). When HCl was added to the beer after the storage at 37°C for 3 days and the beer was stored at 2°C for 1 day, there was no significant difference in the flavour staling degree between the beers with and without HCl. The addition of these levels of HCl to fresh beer had no effect on its flavour and taste in the sensory evaluation (data not shown). The storage at 2°C for 1 day is a condition that cannot accelerate so much flavour staling reaction but can dissociate aldehydes from aldehyde-sulfite adducts by decreasing pH. Therefore, it was shown that the acceleration of beer flavour staling is not dependent on a decrease in pH such that the decreasing pH isolates stale flavour aldehydes by a dissociation from staling-flavour aldehyde adducts but based on the fact that the decreasing pH accelerates the flavour staling reactions during beer storage.

When HCl was added to beer, its chemiluminescence (CL) reached a maximum later than that of the beer with no additions, so that the total CL intensity of the beer with HCl for 90 min was lower than that of the beer without HCl (Fig. 1(A)). However, when the beers were stored at 37°C for 2 days, the maximum CL intensity of the beer with HCl was higher than that without HCl. Thus, the total CL intensity of the stored beer with HCl was higher than that without HCl. Therefore, it was thought that the CL producing reactions in beer during storage were accelerated by the decreasing pH of the beer. Previously, it has been shown that the increase in CL production during beer storage is dependent on free radical reactions which are started by the active oxygens produced in

FIG. 1. Effect of pH on chemiluminescence (CL) production of beer. (A) CL producing patterns of beers before (1, 2) and after (3, 4) storage at 37°C for 2 days. 1 and 3, pH 4.3; 2 and 4, pH 3.8. (B) Increase in CL production of beer during storage of 37°C for 2 days.

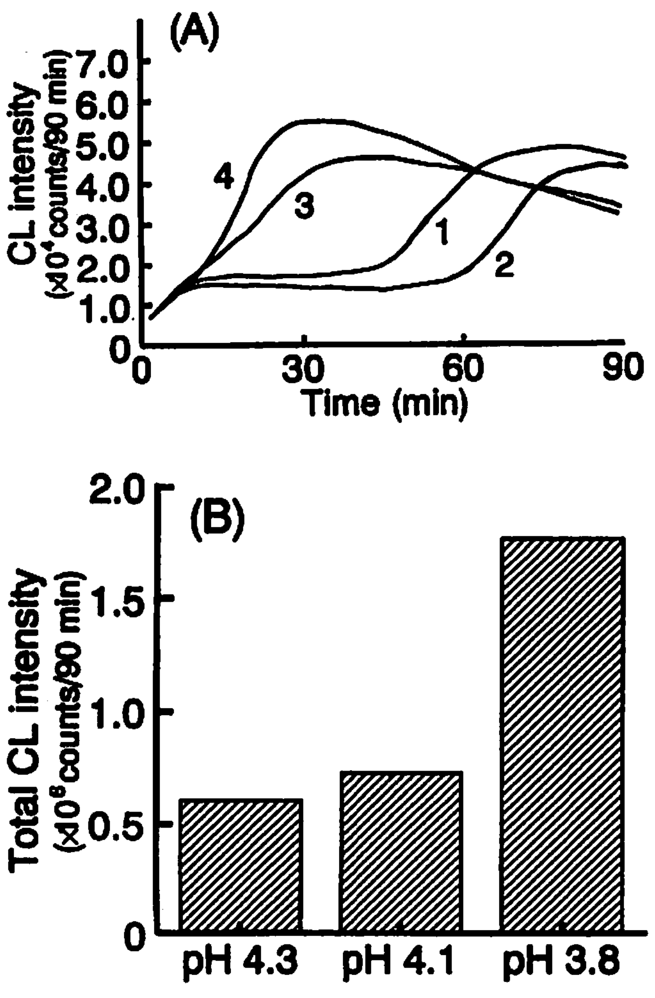


FIG. 2. Effect of pH on degradation of isohumulones in beer during storage at 37°C for 15 days. The value shows the residual percentage of isohumulone contents in beer after the storage. A and B, no additions; C and D, addition of 1 mM H_2O_2; A and C, pH 4.3; B and D, pH 3.8.

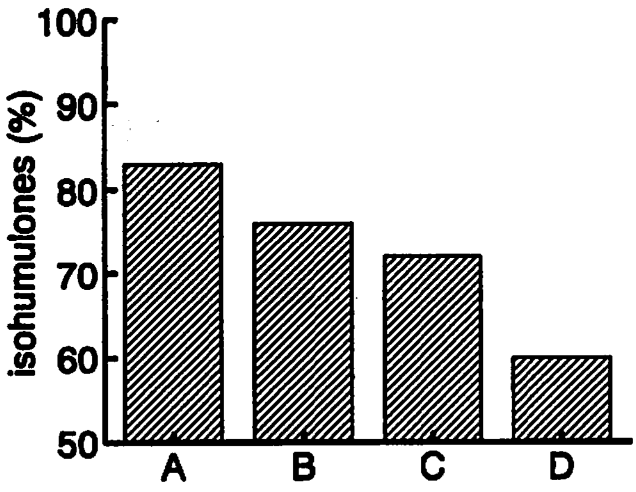


TABLE II. Effect of pH on DPPH-reducing activity of beer during storage*

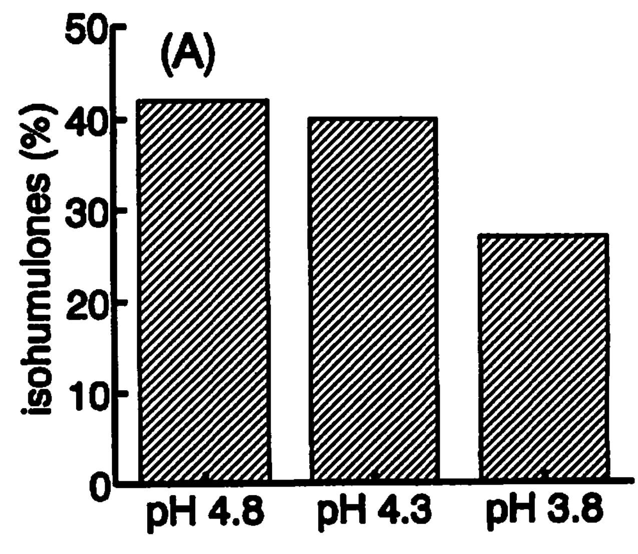
	Before storage	After storage
pH 4.3	1.07	0.98
pH 3.8	1.07	0.94

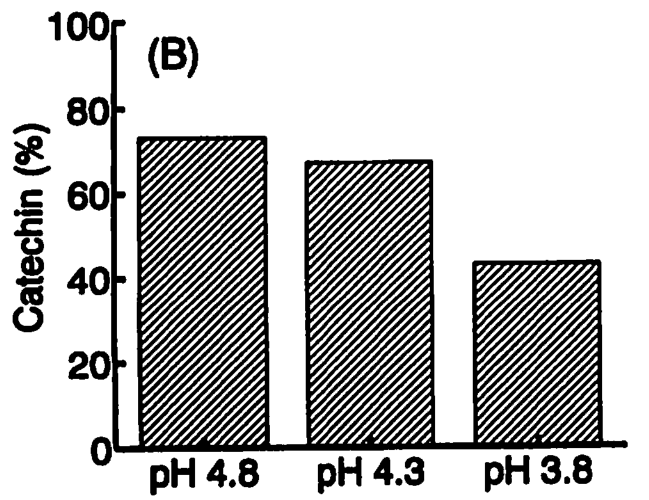
*Beer (583 ml) was stored with air (ca. 50 ml) at 37°C for 5 days.

beer. It seems that the decreasing pH accelerates the free radical reactions in beer during storage.

When beer was stored at 37°C, isohumulones were degraded (Fig. 2). A decrease in pH accelerated the degradation of the isohumulones during beer storage. The addition of hydrogen peroxide (H_2O_2) decreased the isohumulone contents during the beer storage. The degradation of isohumulones by H_2O_2 was accelerated by decreasing beer pH. When beer was stored with air at 37°C for 5 days, the reducing activity of the beer

FIG. 3. Effect of pH on degradation of *trans*-isohumulones (A) and catechin (B) by Fenton reaction in beer model system. *Trans*-isohumulones (25 mg/L) or catechin (50 mg/L) were incubated with 1.0 mM H_2O_2 and 1.0 μM $FeSO_4$ in 5% (v/v) ethanol-0.1 M acetate buffer (pH 3.8, 4.3 or 4.8) at 20°C for 4 hr. The values show the residual percentages.





toward 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was decreased (Table II). The decrease in DPPH-reducing activity during the storage was slightly accelerated by decreasing beer pH. It has been shown that the DPPH-reducing activity of beer is based on the reducing activity of polyphenols in beer, containing proanthocyanidins[11]. The decrease in DPPH-reducing activity of beer during storage is caused by the oxidation of polyphenols in beer. Therefore, it seems that a decrease in pH accelerates the oxidative degradation of beer components during storage.

Trans-Isohumulones and catechin were also decreased in a model system which causes a Fenton reaction (Fig. 3). Fenton reaction produces superoxide (O_2^-) and hydroxyl radical ($\cdot OH$) from H_2O_2 by the catalytic action of iron[6].

Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe + O_2^- + 2H^+$$

$$\text{Net: } 2H_2O_2 \xrightarrow{Fe} \cdot OH + OH^- + O_2^- + 2H^+$$

The degradations of isohumulones and catechin were accelerated by decreasing pH of the system. It is known that the concentration of perhydroxyl radical ($HO_2\cdot$) and O_2^- depends on pH, where the pK_a for the dissociation of $HO_2\cdot$ is 4.7–4.9[1,3,15]. Decreasing pH increases the amount of $HO_2\cdot$ in the system by the dissociation of O_2^-.

$$HO_2\cdot \Leftrightarrow H^+ + O_2^-$$

The reactivity of $HO_2\cdot$ is much higher than that of O_2^-. The $HO_2\cdot$ is the more potent oxidant of the $HO_2\cdot/O_2^-$ pair which is well known from the respective redox potentials ($O_2 + e \rightarrow O_2^-$, $E^\circ = -0.33$ V; $H^+ + O_2^- + e \rightarrow HO_2^-$, $E^\circ = 1.0$ V) as well as from the kinetic studies of their spontaneous decay. Bielski et al.[1,3] showed that while O_2^- does not react with linoleic acid, its conjugate acid, the $HO_2\cdot$ does react at a biologically significant rate. The rate constants of $HO_2\cdot$ with amino acids (alanine and cysteine), ascorbic acid, and catalase are also hundreds fold higher than those of O_2^- [1,3,15]. It seems that the decreasing pH accelerates the radical degradation of isohumulones and catechin, because of the increasing $HO_2\cdot$, which has a significantly higher reactivity in the system.

The staling mechanism during beer storage has already been proposed[7]; initially O_2^- is generated from molecular oxygen ($^3O_2$) due to autooxidation during the storage of packaged beer. Next, $\cdot OH$ is produced by metal catalysis reactions, such as the Haber-Weiss reaction and Fenton reaction from O_2^- and H_2O_2. These active oxygens attack the beer components such as isohumulones, sugars, alcohols, fatty acids, and polyphenols and initiate a series of radical reactions in the beer to produce flavour staling carbonyls. Some of the carbonyls are directly or *via* some condensation reactions responsible for the staling off-flavours of beer. Based on the results presented so far, it is thought that a decreasing pH increases $HO_2\cdot$ *via* a dissociation from O_2^- produced in the early stage of oxidation during beer storage. The $HO_2\cdot$ has an extremely high reactivity with beer components and accelerates the free radical reactions in beer. Therefore, the decreasing pH of beer accelerates the flavour staling during storage.

Taylor concluded that the key to consistent beer pH is the maintenance of consistent wort composition and fermentation conditions[16]. It is known that the addition of different amounts of raw grains, and especially copper sugars, which do not have the buffering action of wort, will reduce the pH of the final beer below 4.0[2]. The effective buffers in wort are carboxylic acid groups, related to glutamate and aspartate, peptides/ polypeptides containing glutamate and aspartate, and organic acids (e.g., citrate)[16]. During fermentation in brewing, free amino acids are absorbed by the yeast, leaving the peptides and polypeptides containing glutamate and aspartate, plus citrate, plus other organic acids (such as lactate, succinate, pyruvate) excreted from yeast as the main buffer system in beer. This study confirmed that maintaining beer pH through consistent brewing conditions, such as raw materials, wort producton and fermentation, are significant for maintaining the flavour stability of the finished beer.

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