New Procedures to Improve the Flavor Stability of Beer

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ABSTRACT

Palliatives for enhancing the stability of beer flavor have been evaluated by organoleptic tests, but primarily by use of an accelerated aging technique, followed by estimation of staling substances using thiobarbituric acid (TBA). TBA has also been used to trace the quantities of carbonyl compounds and their precursors through malting and brewing and in beer treated with palliatives and substances that cause flavor deterioration. Beer staling is reduced by scavengers of the hydroxyl radical (mannitol, ascorbic acid) and is increased by agents which elevate hydroxyl levels (e.g., peroxide/metals). Staling substances develop during malt kilning and wort boiling, but primarily during fermentation. There is evidence that soybean extracts enhance the shelf life of beer flavor if included in the fermenting wort; we suggest that this action is caused by superoxide dismutase. Procedures for making soybean preparations devoid of lipoxidase include heating and soaking beans in ethanol. However, doubts are cast upon the role of lipoxidase in staling.

Key words: Flavor stability, Hydroxyl, Hydroperoxide measurement, Metal ions, Soybean, Superoxide dismutase

Even before beers are packaged they start to alter in flavor; Dalgliesh has reviewed the changes that occur (10). Several types of reaction have been invoked as being responsible for the development of the various aged characters during the storage of beer (21). Most authors appear to have decided on the development of so-called cardboard notes in beer as being of key importance to the perception of staling. It is accepted that such flavors are caused by various unsaturated carbonyl compounds (notably 2-*trans*-nonenal), materials with very low flavor thresholds (20). What is not generally agreed is the nature of the precursors of such substances (11). Possibly the favored route is by the peroxidation of unsaturated fatty acids to hydroxy fatty acids, which subsequently decompose nonenzymically to the corresponding aldehyde (32). The crucial role which oxygen has to play in causing these aging reactions is undisputed, however. As a consequence, it is well-established brewing dogma to minimize oxygen levels in processing, at least down-stream of the fermenter, and preferably during wort preparation as well (12).

Molecular oxygen, O_2, is itself relatively unreactive—e.g., the activation energy for its reaction with lipid is between 35 and 65 kcal/mol (26). In recent years, numerous studies of lipid peroxidation in animal membranes, as well as in foodstuffs, have focused upon the participation of reduced, "activated" forms of oxygen (Fig. 1) (25). Indeed there is some controversy regarding which of these radicals is the main causative agent of oxygen damage (35). Nevertheless, superoxide radical occupies a key position in this regard, as it is the first intermediate from which the other radicals can be produced. Thus, prevention of the accumulation of superoxide would be expected to provide protection against oxidative damage caused by any of the radicals. Accordingly, all aerobic organisms so far examined have been found to produce superoxide dismutase (SOD) and catalase as protective enzyme (14) catalyzing the reactions:

$$2O_2^{\cdot-} + 2O_2^{\cdot-} + 4H^+ \xrightarrow{SOD} 2H_2O_2 + 2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$\text{Net: } 4O_2^{\cdot-} + 4H^+ \rightarrow 2H_2O + 3O_2$$

The only example of the use of such enzymology in preventing oxidative deterioration in foodstuffs has been the successful application of SOD for retarding the aging of sliced apples, potatoes, and mushrooms (27). Little research seems to have been devoted to elucidating the role which radical forms of oxygen, or the procedures which eliminate them, have on the stability of beers. In this work we have approached the study of beer flavor stability from this standpoint.

EXPERIMENTAL

Addition of Reagents to Beer

Test substances (palliatives and causative agents) were added to bottled lager from a commercial filling line either before pasteurization or, for an initial assessment, to packaged beer.

The concentration of all additive solutions was such that in each case 1 cm^3 of additive was included per 275 cm^3 beer. Except in cases where high headspace air was being evaluated, dearation by fobbing to the top of the bottle before crowning was induced by tapping. Where required, bottles were pasteurized in a commercial tunnel pasteurizer.

Estimation of Flavor Stability

Beers were analyzed for susceptibility to aging by the method of Parsons and Cope (29).

Estimation of Peroxides

The method for estimating peroxides was based on that of Asakawa and Matsushita (4). First, 25 cm^3 of aqueous malt extract (25 g/100 cm^3 H_2O), wort, or beer was extacted with 2×50 cm^3 of chloroform. The combined chloroform extracts were filtered and concentrated to dryness by rotary evaporation. Each extraction was performed in duplicate. To one tube was added 35% aqueous trichloroacetic acid (1 cm^3); 0.36% aqueous thiobarbituric acid, TBA (2 cm^3); and 0.22% ethanolic butylated hydroxytoluene (BHT; 0.1 cm^3). The other tube contained 0.36% aqueous TBA (2 cm^3), 0.22% ethanolic BHT (0.1 cm^3), and 2.78% aqueous $FeSO_4 \cdot 7H_2O$ (0.1 cm^3).

The tubes were then stoppered and mixed thoroughly before incubating at 60°C for exactly 1 hr. After cooling and mixing, glacial acetic acid (1 cm^3) and chloroform (2 cm^3) were added to each tube. After vigorous shaking and centrifugation, the A_{530} of the upper aqueous layer was measured. The difference in A_{530} between the solutions containing and lacking iron is due to peroxide material. The A_{530} of the solution produced in the absence of iron is a measure of preformed carbonyl compounds. An A_{530} value of 1 corresponds to approximately 0.02 μmol of carbonyl compound.

Isolation of Superoxide Dismutase from Soybeans

Soybeans were ground in a domestic coffee mill and extracted for 1 hr at 20°C with five volumes of 50 m*M*-potassium phosphate buffer, pH 7.8. Extracts were clarified by passing through glass wool. Wolf (34) reports that soaking of soybeans in ethanolic solution inactivates lipoxidase. Accordingly, some beans were soaked in 50% (v/v) ethanol for 17 hr before washing in deionized water and grinding. Commercial samples of grits and flour derivatives of soybeans were extracted without milling. For bulk extraction of soybean derivatives, separation of residual solids from supernatant was effected using a wine press.

Enzyme Assays and Protein Measurement

SOD was assayed by a xanthine oxidase-cytochrome *c* assay (5). Lipoxidase was measured by the method of Baxter (6), whereas

catalase was measured by following the degradation of H_2O_2 (8). Protein was estimated by ultraviolet absorbance (23).

Potassium superoxide purchased from Sigma-London Chemical Co. was dissolved in dicyclohexyl-18-crown-6 (31). The same company also supplied Tiron (4,5-dihydroxy-1,3-benzene disulphonic acid, disodium salt).

RESULTS

Prediction of Flavor Stability for use in Assessment of Palliative Measures

One of the difficulties in flavor stability trials has been the greatly extended period over which such experiments need to be performed. Thus, it was timely that Parsons and Cope produced a method for predicting flavor stability of beers within 48 hr (29). The technique involves accelerated aging of beers at 60° C, followed by reaction of the aged beer with TBA, which forms colored complexes with the carbonyl substances responsible for stale flavors. These authors reported a distinct inverse relationship between the rate of development of material absorbing at 530 nm and the time at which stale characters first become noticeable in the beer. Although the precise relationship differed between breweries, within a single production unit the method is a reliable guide to shelf life. Hence, it was used in a series of experiments designed to assess the efficiency of existing and new palliative measures for enhancing flavor stability.

Palliative Procedures for Improving Flavor Stability

Using the TBA procedure, it was possible to screen a number of potential additives selected for their ability to eradicate radical forms of oxygen. Such scavengers, and the radicals with which they interact, are listed in Table I. The efficiency of each in combating flavor deterioration was assessed by adding the agent to freshly packaged lager beers, capping, accelerated aging, and TBA analysis, with results for SOD/catalase, ascorbic acid, and mannitol as follows:

Ascorbic acid. This widely used scavenger of the superoxide radical (17) when added at 12 mg/L increased the resistance-to-staling value (RSV) of the lager from 219 to 318. (Ascorbic acid may also scavenge hydroxyl ion [2]).

Mannitol. At 5 m*M* mannitol there was an approximate doubling in RSV, although the benefits are much less pronounced at higher incorporation rates (Fig. 2). A number of anti-oxidative agents are believed to shift their action to one of pro-oxidation at increased levels (13). It is noteworthy that ethanol is also a hydroxyl scavenger.

SOD/catalase. Inclusion in the lager of 525 units of SOD (bovine liver) and 3,000 units of catalase caused a minimal increase in RSV from 264 to 275. As the superoxide and peroxide radicals are intermediaries in the production of hydroxyl, it is surprising that SOD/catalase was not more effective. (Indeed, addition of 75 μM-potassium superoxide to bottled lager beers caused an increase in A_{530} of 0.036 over control bottles when carbonyl levels were estimated by TBA analysis.) There are several possible explanations for this. It is reasonable to expect that inhibitors of the enzymes are present in beer, preventing their action, although a greater possibility is that the enzymes are inactivated during the accelerated aging stage at 60° C and that the continual presence of SOD/catalase throughout beer storage is necessary, rather than the initial depletion of superoxide radicals. Most enzymic palliatives are not suited for use in accelerated aging techniques involving high temperatures. A third probability for the relative inefficiency of the SOD/catalase couple is that hydroxyl can be formed in reactions not demanding the intermediacy of superoxide (18). For example, hydroxyl ions are generated from peroxide in the presence of metal ions such as iron and copper (18). Thus it might be anticipated that if hydroxyl radicals were the primary cause of flavor deterioration, the removal of metal ions would be beneficial. The chelating agent diethylenetriaminepentaacetic acid, pentasodium salt (DPTA), at 20 mg/L increased RSV from 241 to 296.

Development of Precursors of Aged Flavors During the Malting and Brewing Processes

Although oxidative damage can clearly occur in the final package, it is recognized that the purported precursors of cardboard flavors, i.e., hydroxy fatty acids, can develop much earlier in beer production. Thus Anness and Baxter (3) imply that oxidation of unsaturated fatty acids in the mash tun, and even earlier during malting by lipoxidase, will have a major bearing on the shelf life of the final product. Nonenzymic peroxidation of such lipids by activated forms of oxygen could also take place: indeed it has been suggested that the superoxide radical is involved at the active site of lipoxidase (15).

Two approaches were made to investigate this possibility: addition of a heat-stable SOD, plus catalase, to mashes (or to fermenter) with subsequent organoleptic and TBA analysis of flavor stability; and measurement of lipid peroxides through processing.

Use of Heat-Stable SOD

Although barley and malt contain three SOD species (5), they are quite heat-labile and are rapidly destroyed at mash conversion

TABLE I
Radicals of Oxygen and Their Scavengers

Potential Oxidant	Palliative
Superoxide	Superoxide dismutase Tiron Ascorbic acid
Peroxide	Catalase
Hydroxyl	Mannitol Ethanol

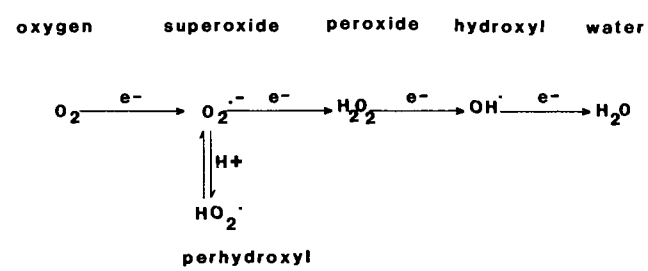

Fig. 1. The reduction of oxygen.

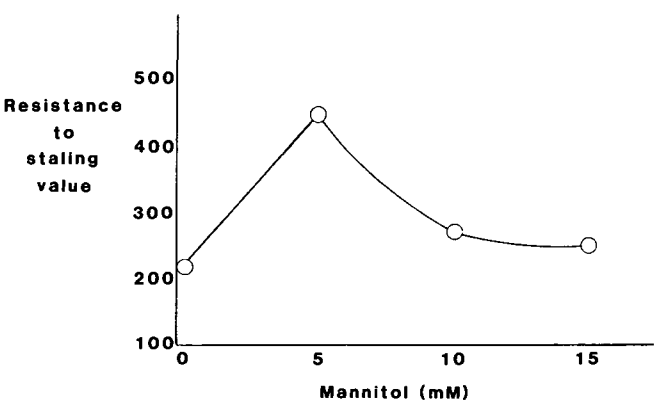

Fig. 2. The effect of varying mannitol concentrations on flavor stability of beer. (Each value on this plot is the mean of four separate measurements.)

temperatures, e.g., 65°C. Conversely, soybeans were shown to constitute an extremely rich source of this activity, which was apparently much more resistant to thermal inactivation (5). There is considerable literature on antioxidant properties of soybean flour and its derivatives in many foodstuffs, but not beer (22). However, only recently has SOD been considered as an active ingredient of bean curd (tofu) (1).

As no bulk commercial source of SOD exists, soybeans constitute a most promising material in this regard. Unfortunately, in addition to SOD, soybeans also produce copious quantities of lipoxidase. Clearly it is important to eliminate the latter, while maintaining SOD.

Heating of soy extracts at 50°C causes partial destruction of both SOD and lipoxidase (Fig. 3). However, significant amounts of lipoxidase survive—indeed there is a slight increase in its activity upon prolonged heating. At 72°C, though, lipoxidase is rapidly and irreversibly inactivated within 5 min, while 20% of SOD survives over 1 hr heating at this temperature. Lesser quantities of SOD survive 1 hr at 91°C. In a separate experiment it was shown that lipoxidase is totally destroyed in less than 20 min at 65°C.

A range of commercial soybean preparations, flours, and grits that are toasted during their preparation were found to be devoid of lipoxidase, whereas they retain low levels of SOD (Table II). Of special interest, however, is the retention of 75% of SOD in soybeans soaked in ethanolic solution, a process that inactivates lipoxidase. Clearly such a treatment is attractive for the preparation of high SOD/low lipoxidase beans.

Nevertheless, in the short term, commercial soy grits were used as the source of SOD. A beer was brewed in which soy grits were included as 3% of an otherwise all-malt grist, with 1.6×10^6 units of catalase also added. A separate beer was produced using an all-malt grist but including an aqueous extract of soy grits (2.5×10^4 units SOD) in the fermenter. The soy extract was supplemented with 8.3×10^5 units of catalase. A third (control) beer was produced with no soy preparation. The bottled beers were tasted immediately and again after seven weeks storage at 15°C, then held for 16 hr at 60°C before chilling to 4°C.

Initial flavor profile analysis of the beers revealed only slight differences between control and trial beers. Indeed, the trial brews were initially considered to possess a slight degree of stale character (Table III). Analyses for each beer were similar (Table IV), although the beer brewed with soy extract in fermenter had a significantly higher content of vicinal diketones and a lower bitterness, whereas that brewed with soy in the mash had a lower foam stability and higher free amino nitrogen level.

After aging the beers, all nine tasters could distinguish the control beer from that to which soy extract had been added in the fermenter (Table V). Four of the tasters described the control beer as being the more stale. Two-thirds of the tasters preferred the trial beer.

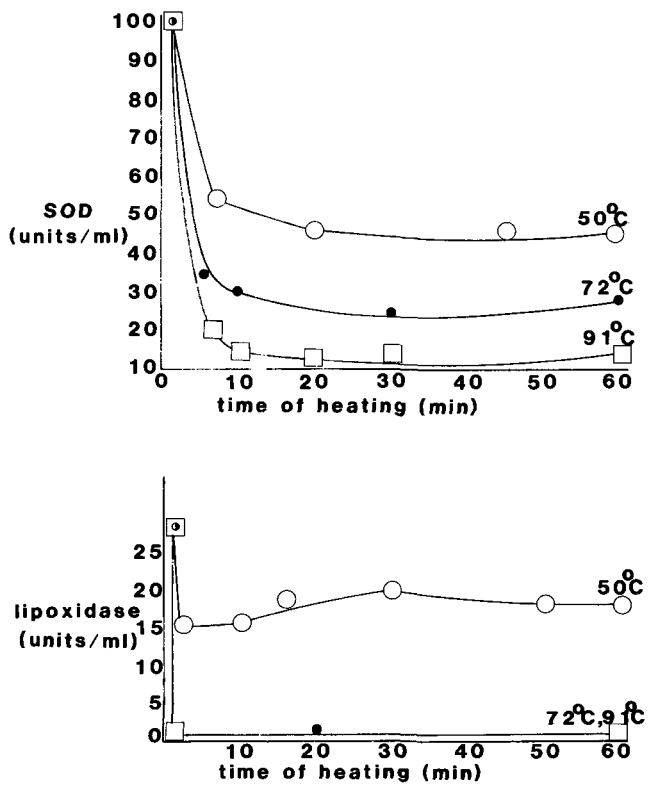

Fig. 3. The influence of heat on superoxide dismutase and lipoxidase from soy.

TABLE II
Superoxide Dismutase and Lipoxidase in Commercial Soy Preparations

	Superoxide Dismutase (units/mg protein)	Lipoxidase (units/mg protein)
Soybeans	7.2	1.8
Soy granular concentrate	2.1	n.d.[a]
Defatted grits	1.8	n.d.
Defatted flour	1.1	n.d.
Soy protein isolate 1	1.8	n.d.
Soy protein isolate 2	1.5	n.d.
Ethanol-treated soybeans	5.4	n.d.

[a] n.d., not detectable.

TABLE III
Extract from Flavor Profile of Beers Including Soy Preparations (On a Scale of 0–8)

		Control	Soy Added to Mash	Soy Added to Fermenter
Catty/ribes	Aroma	0	0	0.3
	Flavor	0	0	0.4
Stale/cardboard	Aroma	0	1.1	0.6
	Flavor	0.3	0.7	0.9
Meaty	Aroma	0	0.6	0
	Flavor	0	0.6	0
Toffee-like	Aroma	0.4	0.4	0.7
	Flavor	0.7	0.6	0.9
Aldehydic	Aroma	0	0	0.7
	Flavor	0	0	0.9
Soapy/fatty	Aroma	0	0	0.4
	Flavor	0	0	0.7

TABLE IV
Analysis of Beers Produced with Soy Inclusions

	Control	Soy Added to Mash	Soy to Fermenter
Original gravity (° Plato)	9.3	9.3	9.5
Present gravity (° Plato)	0.8	0.8	0.8
Final gravity (° Plato)	0.2	0.5	0.4
Alcohol (% v/v)	4.36	4.36	4.51
Free amino nitrogen (mg/L)	35.5	49.1	31.1
Color (° EBC)[a]	5.5	6.5	6.0
Haze (EBC)	0.4	0.5	0.45
pH	4.11	4.17	4.29
Bitterness (EBC)	23.2	25.3	21.5
Vicinal diketones (ppm)	0.17	0.18	0.32
Head retention value(s)	124	115	122

[a] European Brewing Convention.

By contrast, while again all tasters could distinguish the beers, that brewed with soy grits in the mash was judged more stale than the control from three of the tasters' comments (Table V).

The findings of these trials hold promise for addition of soy extract to fermenter as a possible means of enhancing shelf life. However, larger trials with more detailed flavor analysis are required. Meanwhile it is noted that RSVs of 303 and 439, respectively, were measured for beers produced with soy grits to mash and soy extract to fermenter, respectively, supporting the promise of the second approach.

Measurement of Lipid Peroxides

The Asakawa and Matsushita (4) method for measuring lipid peroxides depends on the ability of iron to catalyze the decomposition of hydroperoxides with measurement of the aldehydes released using TBA. The inclusion of butylated hydroxytoluene prevents further oxidation of unsaturated fatty acids by the iron.

Table VI presents the values for such hydroperoxides throughout processing. In view of the lack of availability of authentic material with which to calibrate the method, results are quoted as differential absorption values in the presence or absence of iron. Also given are the values obtained in the absence of iron, which are proportional to the quantities of already-formed carbonyl substances.

Significant levels of both classes are present in untreated barley, decreasing slightly during germination, whereas both increase slightly in kilning.

Although there is no inverse relationship between hydroperoxides and carbonyls in malting or mashing, a major decline in hydroperoxide occurs during wort boiling, and this is accompanied by a large increase in the level of carbonyl compounds. A slight increase in each category occurs between the end of the boil and the completion of cooling.

Lipoxidase apparently did not effect significant lipid oxidation during germination, although it may have acted during kilning. Probably the SOD native to germinating barley(s) is sufficient to prevent nonenzymic oxidation of lipids.

Wort boiling has a major influence on levels of carbonyl compounds, which are produced at the expense of hydroperoxides. Of particular importance, however, is the major increase in carbonyl content during fermentation. This cannot be ascribed to an increase in acetaldehyde, which is lost in the rotary evaporation stage of the analytical procedure. Clearly the efficacy of SOD added to fermenter in enhancing flavor stability may be by preventing development of such carbonyl compounds.

TABLE V
Two-Glass Taste-Test Comments on Beers Brewed or Fermented with Soy

Taster No.	Flavor Difference Detected?[a]	Description of Difference	Preference[b]
a) Beer containing soy extract added to fermenter (A) versus no soy control (B).			
1	yes	...	A
2	yes	A, diacetyl B, stale, very cardboard	A
3	yes	A, oxidized B, badly oxidized	A
4	yes	A, oxidized	B
5	yes	A, cleaner, slightly sweet, fresh B, dirty, cardboard, oxidized, very stale	A
6	yes	...	B
7	yes	A, smoother and fuller	A
8	yes	A, slightly stale B, stale aroma, more stale	A
9	yes	slight difference	B
b) Beer mashed with soy (C) versus no soy control (B).			
1	yes	C, harsh, mouthcoating after-taste	B
2	yes	C, slightly smoother	C
3	yes	B, grainy, astringent C, more sulfury	C
4	yes	B, stale, musty C, fresh	C
5	yes	B, slightly oxidized C, extremely oxidized, unpleasant	B
6	yes	B, fresher	B
7	yes	C, more bitter, unpleasant after-taste	B
8	yes	C, metallic, bitter	C
9	yes	...	C
10	yes	B, mouthcoating, more lager character C, rubbery, bland, soft, satiating, stale	B

[a]Significant difference.
[b]No significant preference.

The Role of Pasteurization

Of all the stages involved in producing beers, pasteurization is traditionally regarded as potentiating most problems with regard to subsequent flavor stability. Accordingly, a number of agents of relevance to flavor stability were added to beer, and their effect on the level of carbonyl compounds before and after pasteurization was assessed (Table VII).

The quantity of carbonyl compounds in unpasteurized beer was increased by the addition of hydrogen peroxide and copper, and, to a much lesser extent, by iron and linoleic acid. The derogatory effect of iron and copper can be entirely negated by inclusion with the metal of DPTA. A high air content in the bottle is only slightly detrimental, compared to peroxide, although pasteurization in the presence of high air did cause a significant increase in TBA value, especially in the presence of metal ions, irrespective of whether glucose oxidase/glucose was added as a scavenger of ground-state oxygen. Carbonyl compounds increased upon pasteurization in the presence of H_2O_2.

SO_2, ascorbic acid, and DPTA inhibited the development of carbonyl compounds, whereas the superoxide scavenger Tiron had no protective influence.

Role of Unsaturated Fatty Acids

In view of the purported role for linoleic acid as the ultimate precursor of carbonyl staling compounds in beer, it is remarkable

TABLE VI
Hydroperoxides and Carbonyl Substances during Malting and Brewing[a]

	Hydroperoxides as A_{530}[b]	Carbonyls as A_{530}[c]
Barley	0.056	0.060
Green malt	0.051	0.056
Kilned malt	0.075	0.084
Wort first runnings	0.076	0.098
Copper cast	0.024	0.145
Paraflow (cold)	0.034	0.160
Racking	0.037	0.255
Ex-centrifuge	0.035	0.251
Ex-bottle	0.035	0.254

[a]All values are quoted after correction to a specific gravity of 9.5° Plato.
[b]The reported values have been calculated by subtracting without-iron values from total iron values.
[c]Without iron.

that when this substance was added at 10 mg/L to lager before carbonation and bottling, the resultant beer had an RSV of 381, as compared to 250 for the control, an observation repeatedly made and supported by organoleptic analysis. It should be recognized that the role of linoleic acid in flavor deterioration has been demonstrated with the acid added early on in processing. Even so, there are reports in the literature that high-lipid grists fail to shorten flavor life unduly (19) and that beers brewed from worts supplemented with spent-grain lipids had marginally better flavor life than did controls (33).

The present observations are in keeping with the theory that excess linoleic acid neutralizes activated forms of oxygen, primarily hydroxyl, whose major effect in beer is to degrade hydroxy fatty acids, producing carbonyl substances.

DISCUSSION

It is concluded that the damaging effects that oxygen can have on flavor stability are entirely accountable in terms of the action of activated forms of oxygen. Thus, the addition of superoxide to freshly packaged beer has a negative influence equivalent to that of high air levels (ground-state oxygen), whereas peroxide is even more damaging to flavor life. These data, together with the dramatic influence of copper and the palliative effect of mannitol, are consistent with the concept that hydroxyl is the main causative agent of oxidative damage.

The hydroxyl ion, $OH^{\cdot}$, is extremely reactive, combining with almost every type of substance (18). It is much more reactive than ground-state oxygen, superoxide or peroxide, and can be produced, for example, in both the Fenton reaction and the Haber-Weiss reaction.

Fenton reaction:

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

Haber-Weiss reaction:

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

Copper (I) salts react with H_2O_2 to form hydroxyl radicals much more rapidly than does iron (II) (18). The remarkable effect of copper sulphate in causing such a large increase in TBA value is probably due to its catalysis of the Haber-Weiss reaction.

Clearly the elimination of superoxide, but especially peroxide and metals, will be of singular benefit to the shelf life of small-pack beers. Only when ground-state oxygen is activated to superoxide and beyond is it able to cause damage. Thus, flavor deterioration and other oxidative changes in beer are dependent on the degree to which this oxygen activation occurs, rather than on the absolute level of ground-state oxygen. Indeed, beers produced with especial attention to preventing access of air during production—e.g., use of deaerated liquor, static mashes, gentle run-off, absence of centrifugation, etc.—showed no particular improvement in flavor stability over those produced under less rigorous conditions.

Above all, traces of metals such as copper and iron should be eliminated at all stages. Whereas the binding of copper by protein prevents its ability to form hydroxyl (30), such is not the case for iron (18). DNA strand breakage is exacerbated by hydroxyl, produced by the intermediacy of a range of metal ions in decreasing order of potency Cu (II) > Zn > Fe (II) > Co (II) > Mn (28). These are believed to cause the oxidation of unsaturated fatty acids, which are the true malefic agents. From this it can be inferred that a broad spectrum of metal ions may be to the detriment of flavor stability, even when bound to protein or other materials.

Interestingly, chelated or nonchelated metal ions, such as copper and iron, degrade preformed lipid hydroperoxides as well as catalyzing the initial oxidation (18).

Although hydroxyl is probably the main causative agent of oxidative damage, it is relevant that the protonated form of superoxide, perhydroxyl, is more reactive than is $O_2^{\cdot-}$ in aqueous solution (7). The pKa of superoxide is 4.88, so that at beer pH $O_2H^{\cdot}$ will predominate, to potentiate damaging effects. Clearly, relatively small variations in beer pH may have a significant influence on the degree of oxidative damage.

If the presence of superoxide is prevented in the first instance, e.g., by using SOD plus catalase, then pH is of secondary relevance in this context. The SOD/catalase couple may be efficacious when added in the fermenter. Soy appears to be a sufficient source of SOD for commercial purposes, albeit a source depleted in catalase, which is essential to obviate the accumulation of peroxide. It is possible that the carbonyl substances responsible for staling are already produced in green beer and that their appearance during storage is in reality a result of variation in the level of other beer components.

The use of soybeans in brewing is not novel, for they have been used to achieve increased attenuation and yeast crops, accelerated fermentation rates, and reduced yeast degeneration (24). Elevated levels of H_2S were reported from the use of soy preparations (24), but in the present work only slightly higher scores for H_2S were reported for beers brewed with an inclusion of soy. Effects on attenuation and H_2S production were nullified by heat treatment of soy (24), so such effects were not expected.

TABLE VII
Influence of Additions on Levels of Carbonyl Compounds in Beer Pre- and Post-Pasteurization

	A_{530}	
Addition	**Unpasteurized**	**Pasteurized**
Series A		
Nil (Control)	0.089	0.083
SO_2 (10 ppm)	0.066	0.065
Ascorbic acid (20 ppm)	0.074	0.075
DPTA (20 ppm)[a]	0.074	0.074
Cu^{++} (1 ppm)	0.150	0.153
Cu^{++} (1 ppm) + DPTA (20 ppm)	0.076	0.083
Linoleic acid (10 ppm)	0.094	0.093
Tiron (250 ppm)	0.087	0.093
Series B		
Nil (Control 2)	0.086	0.094
Glucose oxidase (50 units/L)	0.087	0.098
Glucose oxidase[b] + glucose (0.25%)	0.099	0.098
H_2O_2 (4 ppm)	0.142	0.165
H_2O_2 + glucose oxidase + glucose	0.134	0.164
High air (10 ml)	0.089	0.104
High air (10 ml) + glucose oxidase + glucose	0.084	0.103
Fe^{++} (1 ppm)	0.093	0.090
Fe^{++} (1 ppm) + DPTA (20 ppm)	0.084	0.095
Series C		
Nil (Control 3)	0.087	0.104
Low air	0.087	0.105
High air (10 ml)	0.098	0.122
Cu^{++} (1 ppm)	0.190	0.204
Cu^{++} (1 ppm) + high air	0.212	0.248
Fe^{++} (1 ppm)	0.087	0.107
Fe^{++} (1 ppm) + high air	0.091	0.122
Fe^{+++} (1 ppm)	0.087	0.110
Fe^{+++} (1 ppm) + high air	0.087	0.123

[a] Diethylenetriaminepentaacetic acid.
[b] Glucose oxidase includes catalase (50 units/L).

The protective effect of radical scavengers depends upon the nature of the palliative-radical complex (9). If this product is unreactive, protection is achieved; however, if it remains reactive, this may also have (lesser) damaging effects. This may explain observations of protection afforded by low concentrations of additive, with less protection at high concentrations, e.g., with mannitol. Thus, although hydroxyl is the causative agent of lipid peroxidation, under certain conditions mannitol can promote the damage (16).

CONCLUSIONS

The main causative agent of flavor staling is the hydroxyl radical. A reduced rate of flavor deterioration would be achieved more effectively by preventing hydroxyl radical development through processing than by over emphasizing the direct uptake of oxygen. Elimination of metal ions, superoxide, and peroxide will favor enhanced shelf life. Soybean extract added to fermenter may constitute one means for enhancing the flavor stability of beer, probably through its being a source of superoxide dismutase. Soy may not enhance shelf life when added during mashing. The presence of SOD in barley and malt is probably sufficient to prevent hydroxyl development during germination and to minimize its occurrence during kilning and mashing.

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