Effect of the Antioxidant 2″-*O*-Glycosylisovitexin from Young Green Barley Leaves on Acetaldehyde Formaton in Beer Stored at 50 °C for 90 Days

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Beer samples were stored at 50 °C for 90 days, and the amount of acetaldehyde in the samples was measured periodically by gas chromatography. Acetaldehyde in the beer sample was derivatized to 2-methylthiazolidine with cysteamine and quantitatively analyzed with a gas chromatograph equipped with a fused silica capillary column and a nitrogen−phosphorus detector. All beer samples contained acetaldehyde ranging from 30 to 80 μM before storage. The concentration of acetaldehyde in beer samples increased by 873% after 90 days of storage. Significant formation of off-flavor was observed in these samples. When a beer sample was stored at 50 °C for 10 days with 1 1 μg/mL of 2″-*O*-glycosylisovitexin and butylated hydroxytoluene, the acetaldehyde content was reduced by 60 and 15%, respectively. On the other hand, addition of 1 μg/mL of α-tocopherol increased the acetaldehyde content by 18%.

Keywords: *Acetaldehyde; antioxidants; beer deterioration; off-flavor*

INTRODUCTION

Beer is one of the most popular alcoholic beverages in the world. It is consumed in almost every country in large amounts. For example, the U. S. production of beer was nearly 200 million barrels (31 gallons/barrel) in 1996. Commonly, beer is consumed cold, and its stability is prolonged if it is stored at refrigeration temperatures. However, beer is sometimes stored at elevated temperatures, such as during transportation, particularly in underdeveloped countries where refrigeration resources are limited. Oxidation occurs readily in beer at elevated temperatures (above room temperature), and subsequently its flavor quality decreases (Kaneda et al., 1990a). Among oxidation products, carbonyl compounds such as acetaldehyde and diacetyl have been considered to be responsible for off-flavors caused during the production and storage of beer (Harayama et al., 1991). Many carbonyl compounds, in addition to acetaldehyde and diacetyl formed during storage, have been reported as oxidation products, causing a stale flavor (Markl and Palamand, 1973). These compounds are also well-known as products of lipid peroxidation, which cause foods and beverages to become rancid. Therefore, the formation of carbonyl compounds has been widely used to monitor the degradation in the quality of foods and beverages, including beer (Clapperton, 1978).

Measuring the formation of certain aldehydes, such as malonaldehyde, is a commonly used method to investigate the degree of food deterioration. For example, malonaldehyde was used to monitor the inhibitory effect of rosemary extract toward the oxidation of beef samples (Wong, 1995). The details of the mechanisms of carbonyl formation in beer upon oxidation are not yet known. However, there is evidence that some free radicals, such as the hydroxy radical, initiate the oxidation reaction in beer (Kaneda et al., 1994). In fact, >80% of oxidative conversion from ethanol to acetaldehyde is caused by the hydroxy radical (Buxton et al., 1988). In the present study, the formation and inhibition of acetaldehyde in beer were determined using a newly developed gas chromatographic (GC) method to monitor the oxidative deterioration of beer at elevated temperatures.

EXPERIMENTAL PROCEDURES

Chemicals. Butylated hydroxytoluene (BHT) and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Cysteamine hydrochloride and 2,4,5-trimethylthiazole were obtained from Aldrich Chemical Co. (Milwaukee, WI). The standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 10 mg of 2,4,5-trimethylthiazole to 1 mL of dichloromethane and was stored at 5 °C.

2″-*O*-Glycosylisovitexin (2″-O-GIV) was isolated from young green barley leaves *(Hordium vulgare* L. var. *nudum Hook)* harvested 2 weeks after germination by a method previously reported (Osawa et al., 1992). The freeze-dried barley leaves were extracted with 80% ethanol, and the extract was then fractionated using column chromatography with Amberlite XAD-2 nonionic polymeric absorbent. The fraction containing the most 2″-O-GIV (60% methanol eluate) was recrystallized with methanol. A light yellow powder of 2″-O-GIV was obtained. Further purification of this powder was conducted with a preparative HPLC using a 25 cm × 1 cm i.d. Develosil ODS-5 column. Over 99% purity was obtained.

Sample Preparation. A beer containing no additives was obtained from a local brewery. Beer (150 mL) was placed in brown beer bottles, and each bottle top was sealed with a cork after the air in the headspace was purged with nitrogen gas. Samples were stored at either 5, 25, or 50 °C in the dark for 90 days. One sample, of which the headspace was not purged with nitrogen gas, was stored at 50 °C in the dark for 90 days. One set of samples was stored under a home fluorescent light at 25 °C for 90 days. The amount of acetaldehyde in the beer was measured at specific time intervals.

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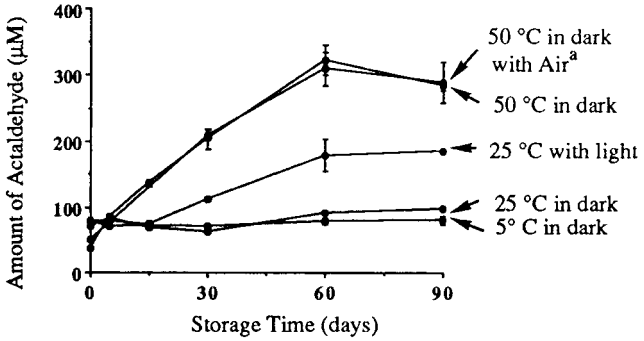

Figure 1. Amounts of acetaldehyde found in beer stored under different conditions for up to 90 days. [a] The headspace of the samples was not purged with nitrogen gas.

In a separate experiment, a 150 mL sample of beer containing α-tocopherol (1 μg/mL), BHT (1, μg/mL), or 2″-O-GIV (0.5, 1, 5, 10, or 20 μg/mL) was stored at 50 °C for 10 days, and then the amount of acetaldehyde was measured. One beer sample containing no antioxidant was stored with the other samples as a control.

Analysis of Acetaldehyde in Beer. Quantitative analysis of acetaldehyde was conducted using a GC method previously reported (Hayashi et al., 1985; Miyake and Shibamoto, 1993). Fifty milliliters of aqueous cysteamine solution (15 mg/mL) was added to a beer sample, and the pH of each sample was adjusted to 8 with a 2 N sodium hydroxide solution. The sample was stirred with a magnetic stirrer for 1 h at 20 °C and subsequently extracted with 75 mL of dichloromethane for 6 h using a liquid−liquid continuous extractor. The extract was dried over anhydrous sodium sulfate for 12 h. After removal of the sodium sulfate, the volume of each sample was brought up to exactly 100 mL with dichloromethane; 2,4,5-trimethylthiazole (100 μM) was added as a GC internal standard.

GC Conditions. A Hewlett-Packard model 5890 series II gas chromatograph equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 bonded phase fused silica capillary column (J&W Scientific, Folsom, CA) and a nitrogen−phosphorus detector (NPD) was used for quantitative analysis of 2-methylthiazolidine derived from acetaldehyde. The oven temperature was programmed from 70 to 180 °C at 4 °C/min and held for 10 min. The injector and detector temperatures were 250 and 275 °C, respectively. The helium carrier gas flow rate was 1 mL/min at a split ratio of 1:16.

RESULTS AND DISCUSSION

Analysis of acetaldehyde is difficult because it is highly volatile and reactive. Also, it is difficult to recover acetaldehyde from beverages such as beer because of its miscibility with water. Although ~900 volatile compounds have been reported in beer (Harayama et al., 1991), there has been virtually no specific report on the quantitative analysis of acetaldehyde. For example, Harayama et al. (1994) who used Tenax TA to trap beer headspace reported 39 components in beer, but acetaldehyde was not included.

A GC method with an NPD for trace analysis of acetaldehyde using a cysteamine derivative (2-methylthiazolidine) was developed in 1988 (Umano and Shibamoto, 1987). Later, the acetaldehyde contents of several beverages, including beer, were measured using this GC method (Miyake and Shibamoto, 1993). Because of the high sensitivity of an NPD, removal of the extracting solvent (dichloromethane) is not necessary for this method.

Figure 1 shows the results of acetaldehyde analysis in beer samples stored under different conditions for up to 90 days. The values are mean ± standard deviation (n = 3). All beer samples contained acetaldehyde ranging from 30 to 80 μM at the beginning of storage. Beer samples stored at 5 and 25 °C in the dark did not show any appreciable increase of acetaldehyde content over 90 days. Acetaldehyde content increased by 235% in a beer sample allowed to stand under a home fluorescent light at 25 °C. It is obvious that light promoted oxidation of ethanol to acetaldehyde. In aqueous solutions, a hydroxy radical formed by UV irradiation oxidizes ethanol to acetaldehyde (Buxton et al., 1988). However, the amount of acetaldehyde produced under light was much less than that produced by heat. Therefore, a further study was conducted using the samples heated at 50 °C in the dark.

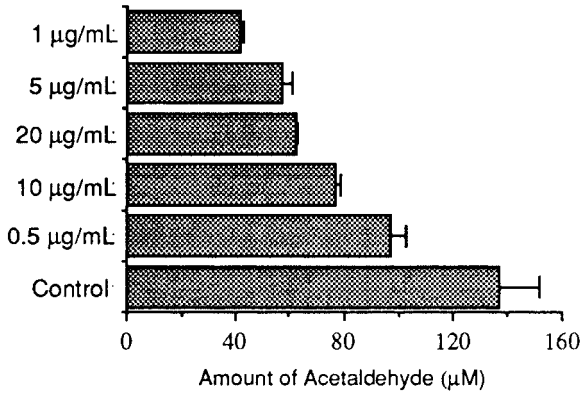

Figure 2. Inhibitory effect of 2″-O-GIV toward acetaldehyde formation in beer stored at 50 °C for 10 days.

When a beer sample was stored at 50 °C for 90 days in the dark, the amount of acetaldehyde increased by 873%. The oxygen content in the headspace of the samples did not influence acetaldehyde formation (Figure 1). This sample was not subjected to rigorous sensory panel evaluation; however, off-flavor was noticed. Meilgaard (1992) found that off-flavors, specifically the so-called cardboard flavor, were significantly generated in beer after it was stored for 4 weeks at 33 °C. Many carbonyl compounds including acetaldehyde, some of which cause off-flavor in beer (Harayama et al., 1991), are formed from lipids in foods and beverages with oxidative degradation (Franker, 1985). The proposed formation mechanisms of these carbonyl compounds were well established, indicating that monitoring one carbonyl compound such as formaldehyde, acetaldehyde, or malonaldehyde can assess the degree of oxidation (Esterbauer, 1982). The quality of beer is considerably damaged by oxidative deterioration. Therefore, it is necessary to prevent oxidation in beer during long-term storage. Certain antioxidants such as vitamin C have already been used for this purpose (Kaneda et al., 1990b).

Figure 2 shows the inhibitory effect of 2″-O-GIV toward acetaldehyde formation. The values are mean ± standard deviation (n = 3). 2″-O-GIV was used because its antioxidative activity and ability to scavenge radicals have been previously reported (Osawa et al., 1992; Kitta et al., 1992; Nishiyama et al., 1993, 1994). Nearly 60% of the acetaldehyde content was reduced by the addition of 1 μg/mL of 2″-O-GIV. When 0.5 μg/mL of 2″-O-GIV was used, there was approximately half of the activity of that obtained from 1 μg/mL of 2″-O-GIV. When the dose of 2″-O-GIV was increased to over 1 μg/mL, the activity reduced. It is difficult to explain this phenomenon, but it may be due to the water

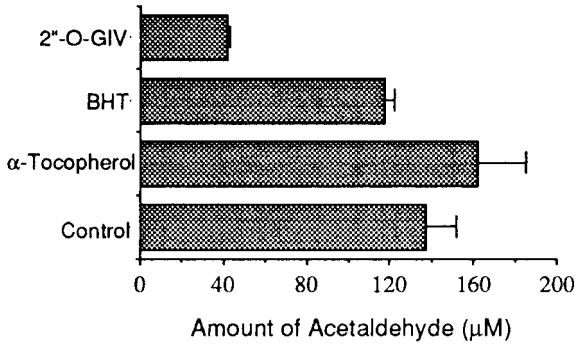

Figure 3. Inhibitory effect of 2″-O-GIV, BHT, and α-tocopherol toward acetaldehyde formation in beer stored at 50 °C for 10 days.

solubility of 2″-O-GIV. The water solubility of 2″-O-GIV is 0.1%. A dose relationship was observed when the amount of 2″-O-GIV was <1 μg/mL. Therefore, further experiments were performed using a 1 μg/mL dose.

Figure 3 shows the inhibitory effects of 2″-O-GIV, BHT, and α-tocopherol on acetaldehyde formation. The values are mean ± standard deviation (n = 3). 2″-O-GIV reduced acetaldehyde content more than either BHT or α-tocopherol did. It is interesting that the amount of acetaldehyde increased by 18% in a beer sample containing 1 μg of α-tocopherol after 10 days of storage at 50 °C. It is difficult to rationalize the prooxidative effect of α-tocopherol. The same phenomenon was observed when squalene was irradiated by UV light (Nishiyama et al., 1993) and when cod liver oil was oxidized by Fenton's reagent (Nishiyama et al., 1994). α-Tocopherol might be degraded into many products including acetaldehyde over a prolonged time period. However, further investigation is necessary to clarify this phenomenon. The low antioxidative effect obtained from BHT may be due to its low water solubility.

Deterioration of beer quality due to the formation of off-flavors such as staleness and haze is one of the most serious problems in the brewing industry. Among many factors causing deterioration in beer, formation of volatile aldehydes, including acetaldehyde upon heat treatment such as pasteurization, may play an important role in the formation of off-flavors. The degree of oxidation during pasteurization generally corresponded to that of nonpasteurized beer stored at room temperature (Kaneda et al., 1994). Also, acetaldehyde concentrations in beer reportedly correlate with mean panel scores for stale flavor (Schmitt and Hoff, 1979). According to these results, monitoring the formation and inhibition of acetaldehyde in beer stored under elevated temperatures is one avenue to understanding the mechanism of its deterioration.

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