JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Factors Influencing the Formation of Medicinal Off-Flavor from Ascorbic Acid and α,β-Unsaturated Aldehydes

Kensuke Sakamaki,* Susumu Ishizaki, Yasutaka Ohkubo, Hiroshi Fujimoto, and Akira Fujita

Technical Research Institute R&D Center, T. Hasegawa Co., Ltd., 29-7, Kariyado, Nakahara-ku, Kawasaki-shi, 211-0022, Japan

ABSTRACT: In a previous study, the formation and formation pathway of 6-propylbenzofuran-7-ol as an off-flavor compound formed from ascorbic acid and (*E*)-hex-2-enal in a test apple beverage were investigated. In the present study, elucidating the pH, temperature, and (*E*)-hex-2-enal and ascorbic acid concentrations that lead to the generation of 6-propylbenzofuran-7-ol in various model solutions was performed. The quantities of 6-propylbenzofuran-7-ol generated in model solutions increased when the initial concentrations of (*E*)-hex-2-enal and ascorbic acid increased, and linear correlations between the quantities and concentrations were observed. The quantity of 6-propylbenzofuran-7-ol steadily rose in step with pH from pH 2.24, peaked at pH 3.73, and steadily decreased in step with pH to pH 4.24. The quantity of 6-propylbenzofuran-7-ol increased sharply as the storage temperature rose. Meanwhile, no 6-propylbenzofuran-7-ol was produced when short-duration heating processes such as heat sterilization were applied. These results will allow to us prevent the occurrence of off-flavor by regulating the initial pH, storage temperature, and (*E*)-hex-2-enal and ascorbic acid concentrations. Reactions with ascorbic acid and other α,β-unsaturated aldehydes were also investigated, and it was confirmed that corresponding 6-alkylbenzofuran-7-ols were formed.

KEYWORDS: *apple beverage, off-flavor, ascorbic acid, (E)-hex-2-enal, 6-propylbenzofuran-7-ol, pH, storage temperature, initial concentration*

INTRODUCTION

In a previous work 6-propylbenzofuran-7-ol (**1**) was identified as a medicinal off-flavor compound observed after storage of a test apple beverage at 40 °C for 8 weeks.[1] The main constituents responsible for the formation of the off-flavor compound in the test apple beverage turned out to be ascorbic acid and (*E*)-hex-2-enal. Once this was determined, the possible reaction pathway of 6-propylbenzofuran-7-ol (**1**) was elucidated by investigating with isotope-labeled precursors and by isolating the reaction intermediates and byproducts.[2] The investigations revealed that the pathway involved two routes and gave two products other than 6-propylbenzofuran-7-ol (**1**), namely, 2,3-dihydro-6-propylbenzofuran-3,7-diol (**2**) and 3-(2-furoyl)hexanal (**3**) (Figure 1). Figure 2 shows the compounds identified as products and intermediates of the reaction of ascorbic acid and (*E*)-hex-2-enal.

(*E*)-Hex-2-enal has a fresh, green odor and is found in many natural foods as an odor constituent. (*E*)-Hex-2-enal is also added to processed foods as a flavor ingredient to confer freshness. The compound is regarded as an important flavor component in apple juices.[3,4] Ascorbic acid is a water-soluble vitamin with many biological functions. Ascorbic acid is present in all animal and plant cells and is particularly abundant in rose hips, black and red currants, strawberries, parsley, oranges, lemons, grapefruit, and various cabbages and potatoes.[5] Manufacturers of processed food often use ascorbic acid as an antioxidant or nutritional supplement. Now that ascorbic acid and (*E*)-hex-2-enal have been identified as constituents responsible for the formation of the off-flavor compound, it may be possible to reduce the formation of this compound by understanding the conditions under which it forms in the presence of ascorbic acid and (*E*)-hex-2-enal.

It is known that degradation of ascorbic acid causes problems such as nonenzymatic browning and off-flavor.[6−13] Huelin et al.[9] demonstrated the effect of pH on the decomposition of ascorbic acid and the yield of furfural by studying the anaerobic decomposition of ascorbic acid in the pH range of foods and in more acidic solutions. In a study by Yuan et al.[10] on the effects of pH on the decomposition of ascorbic acid, pH significantly influenced the degradation processes and products of ascorbic acid, and high temperature appeared to promote the degradation of ascorbic acid. Our group hypothesized that these parameters were also important for the formation of 6-propylbenzofuran-7-ol (**1**). The pH range of fruits is approximately 2.5−4.0,[14] and that of fruit beverages is almost the same.

The first objective of this study was to investigate how the initial pH value, storage temperature, and initial concentrations of ascorbic acid and (*E*)-hex-2-enal affected the amount of 6-propylbenzofuran-7-ol generated in model solutions during storage. The amounts of compounds in model solutions were quantitated by HPLC. α,β-Unsaturated aldehydes like (*E*)-hex-2-enal also have been identified in wide-ranging natural foods, and the following were identified in various foods: (*E*)-but-2-enal in yellow passion fruit[15] and green mate;[16] 2-methylpent-2-enal in black tea,[17] grape,[18] papaya,[19] and green and roasted mate;[16] (*E*)-oct-2-enal in grape,[18] apricot,[20] yellow passion fruit,[15] and guava fruit;[21] (*E*)-non-2-enal in grape,[18] apricot,[20] and grapefruit;[22] (*E*)-dec-2-enal in grape,[18] yellow passion fruit,[15] and guava fruit[21]. We therefore decided to additionally investigate whether benzofuranols were generated by the reaction of these α,β-unsaturated aldehydes and ascorbic acid. Identification of benzofuranols was performed by GC−MS.

Received: October 17, 2012
Revised: November 26, 2012
Accepted: December 1, 2012
Published: December 1, 2012

dx.doi.org/10.1021/jf3044499 | *J. Agric. Food Chem.* 2012, 60, 12428−12434

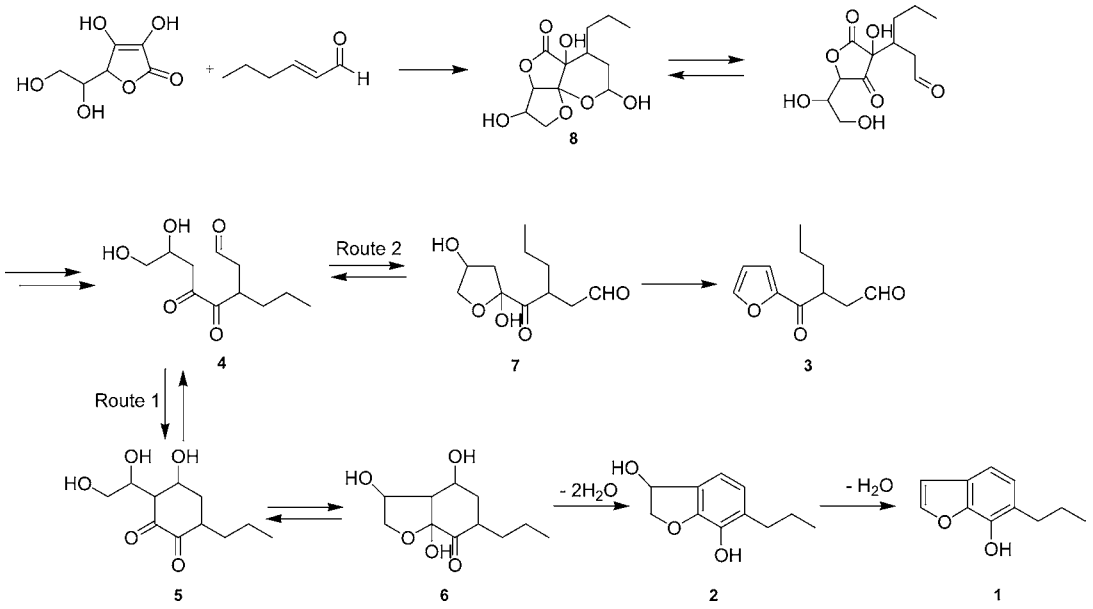

Figure 1. Possible reaction mechanism for the formation of 6-propylbenzofuran-7-ol (**1**) from ascorbic acid and (*E*)-hex-2-enal.[2]

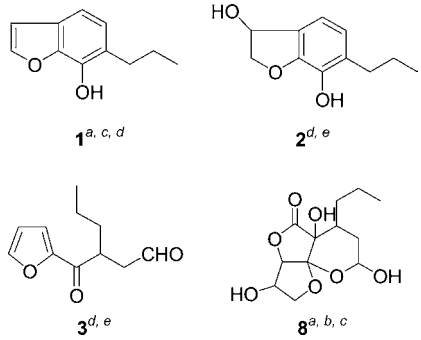

Figure 2. The compounds identified as products and intermediates of the reaction of ascorbic acid and (*E*)-hex-2-enal. 6-Propylbenzofuran-7-ol (**1**),[1] 2,3-dihydroxy-6-propylbenzofuran-3,7-diol (**2**),[2] 3-(2-furoyl)hexanal (**3**),[2] 1,3,7-trioxa-8-oxo-5,9,12-trihydroxy-10-propyltricyclo-[4.3.2.$0^{2,6}$.$0^{2,9}$]-dodecane (**8**).[2] Identification procedure: (a) NMR (^{1}H, ^{13}C, HMQC, and HMBC), (b) HRMS, (c) FT-IR, (d) NMR (^{1}H, ^{13}C), (e) GC–MS.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained commercially: (*E*)-hex-2-enal (PFW Aroma Chemicals B.V., Barneveld, The Netherlands); (*E*)-oct-2-enal, (*E*)-non-2-enal, and (*E*)-dec-2-enal (Inoue Perfumery Mfg. Co., Ltd., Tokyo, Japan); (*E*)-but-2-enal and (*E*)-2-methylpent-2-enal (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); ascorbic acid (DSM Nutrition Japan K.K., Tokyo, Japan); citric acid (Iwata Chemical Co., Ltd., Shizuoka, Japan); and trisodium citrate (Komatsuya Corporation, Osaka, Japan). All other reagents and solvents were of analytical grade.

Model Experiments. Three model experiments were performed to elucidate the influences of the following: (1) the initial concentrations of ascorbic acid and (*E*)-hex-2-enal, (2) the pH and temperature, and (3) heat sterilization. The model solutions used in the experiments were made up of ascorbic acid, (*E*)-hex-2-enal, citric acid, and trisodium citrate. Nine model solutions were prepared for the experiment to elucidate the influences of the initial concentrations of ascorbic acid and (*E*)-hex-2-enal (Table 1). The pH was adjusted to approximately 3.2 with citric acid and trisodium citrate (actual measurement value: pH 3.17–3.23). The model solutions (100 mL) were filled in 120 mL glass bottles, stored at 60 °C in darkness for 1 week, and then analyzed.

Five model solutions were prepared for the experiment to elucidate the influences of pH and temperature (Table 3). The model solutions

Table 1. Model Solutions Prepared To Elucidate the Influences of the Ascorbic Acid and (*E*)-Hex-2-enal Concentrations

		concn (g/L)			
expt no.	pH	ascorbic acid	(*E*)-hex-2-enal	citric acid[a]	trisodium citrate[a]
A-1	3.17	0.5	0.05	0.2	
A-2	3.17	0.5	0.10	0.2	
A-3	3.17	0.5	0.30	0.2	
B-1	3.20	1.0	0.05	0.1	
B-2	3.20	1.0	0.10	0.1	
B-3	3.20	1.0	0.30	0.1	
C-1	3.23	3.0	0.05		0.1
C-2	3.23	3.0	0.10		0.1
C-3	3.23	3.0	0.30		0.1

[a]Used for adjustment of pH.

Table 2. Quantitation Result of Three Products Generated in Model Solutions Prepared To Elucidate the Influences of the Ascorbic Acid and (*E*)-Hex-2-enal Concentrations after Storage at 60 °C for 1 Week

	concn[a] (mg/L)		
	benzofuranol **1**	diol **2**	aldehyde **3**
A-1	0.02	0.24	0.16
A-2	0.11	0.44	0.42
A-3	0.47	1.68	1.67
B-1	0.24	1.02	1.03
B-2	0.52	2.10	2.24
B-3	1.91	7.39	7.67
C-1	1.12	4.40	5.18
C-2	2.12	8.93	10.35
C-3	6.44	27.05	30.46

[a]Calculated as the mean value of triplicates.

dx.doi.org/10.1021/jf3044499 | J. Agric. Food Chem. 2012, 60, 12428–12434

Table 3. Model Solutions Prepared To Elucidate the Influences of pH and Temperature

	concn (g/L)			
pH	ascorbic acid	(*E*)-hex-2-enal	citric acid[a]	trisodium citrate[a]
2.24	1.0	0.1	11.0	
2.74	1.0	0.1	1.3	
3.20	1.0	0.1	0.1	
3.73	1.0	0.1		0.15
4.24	1.0	0.1		0.45

[a]Used for adjustment of pH.

(100 mL) were filled in 120 mL glass bottles, respectively stored at 50 °C, 60 °C, and 70 °C for 1 week, and then analyzed.

A single model solution identical to the pH 3.73 model solution used above was prepared to elucidate the influence of sterilization. This model solution was divided into two parts. One part was cold-filled into 190 mL steel cans (non sterilized sample). The other part was hot-filled (90 °C) into 190 mL steel cans and kept at 90 °C for 3 min (sterilized sample). The samples were then analyzed.

Reaction of Ascorbic Acid and α,β-Unsaturated Aldehydes. (*E*)-But-2-enal, (*E*)-2-methylpent-2-enal, (*E*)-oct-2-enal, (*E*)-non-2-enal, and (*E*)-dec-2-enal were used as α,β-unsaturated aldehyde. Solutions consisting of 200 mg of ascorbic acid, 20 mg of citric acid, 20 mg of α,β-unsaturated aldehyde, and 200 mL of deionized water were stored at 60 °C for 1 week. After storage, each solution was extracted with diethyl ether (3 × 40 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated with a Vigreux column under atmospheric pressure, and analyzed by GC−MS−O.

Quantitation of 6-Propylbenzofuran-7-ol (1) by HPLC. The HPLC analyses were performed with a Shimadzu HPLC system (Kyoto, Japan) consisting of a DGU-20A degasser, an LC-20AD pump, a SIL-30AC autoinjector, and a CTO-20AC column oven. An ED-723 electrochemical detector (GL Science Co., Tokyo, Japan) was used for the detection of benzofuranol **1**. Chromatographic analysis was performed on a CAPCELL PAK C18 MG column (150 mm × 3.0 mm i.d., 5 μm; Shiseido, Tokyo, Japan). Separation was achieved by a mobile phase containing EDTA·2Na (0.1 mM), sodium perchlorate (50 mM) in 50% methanol (v/v), and water at a flow rate of 0.5 mL/min. An electrochemical detector with a diamond working electrode combined with an Ag/AgCl reference electrode was operated at +0.5 V. A 30 μL sample or standard solution was injected into the column at a constant column temperature of 40 °C. The quantitation was performed via peak area by external calibration. A calibration curve was plotted from the standard solutions using quadratic fit for the relationship of the area sum versus the concentration for the peaks.

Quantitation of 2,3-Dihydro-6-propylbenzofuran-7-ol (2) and 3-(2-Furoyl)hexanal (3) by HPLC. The HPLC analyses were performed with a Shimadzu HPLC system (Kyoto, Japan) consisting of a DGU-20A degasser, an LC-20AD pump, a SIL-20AD autoinjector, a CTO-20AC column oven, and an SPD-M20A photodiode array detector. Chromatographic analysis was performed on an InertSustain C18 column (150 mm × 4.6 mm i.d., 3 μm; GL Sciences Co.). The mobile phase consisted of a combination of water/acetonitrile/phosphoric acid (90/10/0.1) (A) and water/acetonitrile/phosphoric acid (10/90/0.1) (B). The gradient was linearly increased from 0% to 100% B in 10 min, and held at 100% for 13 min. The flow rate was 1.2 mL/min. A 5 μL sample or standard solution was injected into the column at a constant column temperature of 40 °C. All chromatograms were recorded at 210 and 275 nm. The quantitation was performed via peak area by external calibration: diol **2** at 210 nm, and aldehyde **3** at 275 nm. A calibration curve was plotted from the standard solutions using quadratic fit for the relationship of the area sum versus the concentration for the peaks.

Gas Chromatography−Mass Spectrometry (GC−MS). The GC−MS analyses were performed with an Agilent 7890 gas chromatograph (GC) combined with an Agilent MSD5975 quadrupole mass spectrometer equipped with a TC-WAX capillary column (0.25 mm i.d. × 60 m, 0.25 μm film thickness; GL Sciences Co.). Each sample was injected in 0.2 μL volumes in a split mode (30: 1) at a constant temperature of 250 °C. The oven temperature was held at 40 °C for the initial 3 min and then increased to 230 °C at a rate of 3 °C/min, with a constant carrier helium gas flow of 1.8 mL/min. Mass spectra in the electron impact (EI) mode were recorded at 70 eV ionization energy. The linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes.

Gas Chromatography (GC). The GC analyses were performed with an Agilent 6890 GC equipped with a flame ionization detector (FID; 250 °C). The column, sample volume, split ratio, injection temperature, oven temperature program, carrier gas, and flow rate were all the same as those set for the GC−MS analysis described above. The purity of the compounds was calculated by integration of the chromatogram obtained by the FID.

Nuclear Magnetic Resonance (NMR) Spectra. ^{1}H, ^{13}C, HMQC, and HMBC experiments were performed on a JEOL JNM-ECX400 spectrometer, using $CDCl_3$ as solvent. The chemical shifts were measured from the signal of tetramethylsilane used as an internal standard. The chemical shifts (δ) and coupling constants (J) were expressed in parts per million (ppm) and hertz (Hz), respectively.

High Resolution Mass Spectra (HRMS). The HRMS were recorded on a JEOL JMS-700.

Isolation of 6-Methylbenzofuran-7-ol from Large-Scale Reaction of Ascorbic Acid and (*E*)-But-2-enal. Ascorbic acid (3.0 g, 17.0 mmol) was dissolved in deionized water (400 g) under nitrogen atmosphere, and (*E*)-but-2-enal (3.0 g, 42.8 mmol) was then added. The mixture was vigorously stirred and kept at 100 °C for 7 h. After cooling to room temperature, the reaction mixture was extracted with diethyl ether (3 × 50 mL). The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate, and concentrated under high vacuum. The residue (0.87 g) was purified by silica gel chromatography using *n*-hexane/ethyl acetate (20: 1, v/v) into a yellow oil of 6-methylbenzofuran-7-ol (80 mg; yield, 3.2%; purity, 96.7%). MS-EI (*m/z*: intensity in %): 148 (M^+, 92), 147 (100), 131 (11), 91 (30), 65 (13). HRMS (EI): found 148.0512, calculated for $C_9H_8O_2$ $[M]^+$ 148.0524.

Gas Chromatography−Mass Spectrometry−Olfactometry (GC−MS−O). The GC−MS−O analyses were performed with an Agilent 7890 GC combined with a 5975 mass selective detector and a sniffing port. The effluent of the column at the end of the capillary was divided into two branches and routed by deactivated fused silica capillaries to the mass detector and sniffing port, respectively. The column, sample volume, split ratio, injection temperature, oven temperature program, carrier gas, flow rate, and ionization mode were all the same as those set for the GC−MS analysis described above.

RESULTS AND DISCUSSION

This report describes a series of model experiments to elucidate the generation conditions of 6-propylbenzofuran-7-ol (**1**). In a previous work, 2,3-dihydro-6-propylbenzofuran-3,7-diol (**2**) and 3-(2-furoyl)hexanal (**3**) were identified other than 6-propylbenzofuran-7-ol (**1**) from the reaction of (*E*)-hex-2-enal with ascorbic acid. As it turned out, there were two routes by which these compounds formed (Figure 1).[2] Given the strong likelihood that different conditions would also change the amounts of all three products, that is, benzofuranol **1**, diol **2**, and aldehyde **3** via this multistep mechanism, we decided to quantitate all three compounds in the model solutions by HPLC. The compound 6-propylbenzofuran-7-ol (**1**) was first detected from a test apple beverage stored at 40 °C for 8 weeks.[1] For the present experiments the generation of the compounds was accelerated by increasing the temperature to 50−70 °C and shortening the storage period to 1 week.

Effects of the Initial Concentrations of (*E*)-Hex-2-enal and Ascorbic Acid on the Formation of 6-Propylbenzofuran-7-ol (1). To confirm whether the generation of benzofuranol **1** depends on the initial concentration of ascorbic

acid or (*E*)-hex-2-enal, nine (A1−3, B1−3, C1−3) model solutions with different ascorbic acid and (*E*)-hex-2-enal concentrations were prepared (Table 1). The pH of the solutions was adjusted to almost the same value with citric acid and trisodium citrate. After storage at 60 °C for 1 week, we quantitated benzofuranol **1**, diol **2**, and aldehyde **3** by HPLC (Table 2). Figure 3a

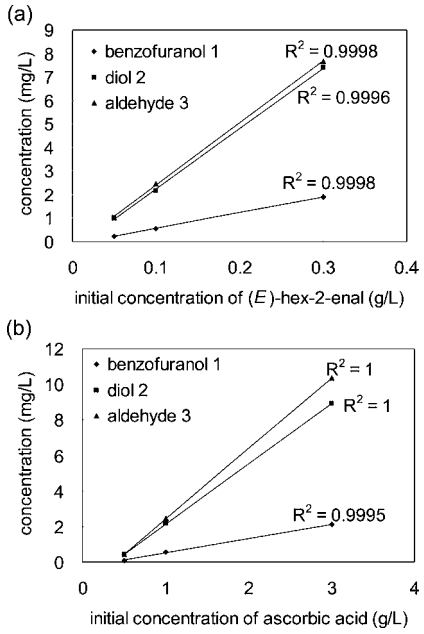

Figure 3. Correlation between the initial concentration of (a) (*E*)-hex-2-enal (initial concentration of ascorbic acid: 1.0 g/L, results of experiments B-1, B-2, and B-3) or (b) ascorbic acid (initial concentration of (*E*)-hex-2-enal: 0.1 g/L, results of experiments A-2, B-2, and C-2) and the quantities of 6-propylbenzofuran-7-ol (◆), 2,3-dihydroxy-6-propylbenzofuran-3,7-diol (■), and 3-(2-furoyl)hexanal (▲) generated in model solutions after storage at 60 °C for a week.

shows a typical example of the correlations between the initial (*E*)-hex-2-enal concentrations and quantitation results of the three compounds at the initial ascorbic acid concentration of 1.0 g/L. The amounts of the three compounds generated in the model solution increased with the increase of the initial (*E*)-hex-2-enal concentration and showed a linear correlation. A similar tendency was obtained at the other initial ascorbic acid concentrations. All products generated in the model solution increased in step with the increase of the initial concentration of ascorbic acid, and all showed linear correlations (Figure 3b). On this basis, it was confirmed that increasing initial concentrations of ascorbic acid or (*E*)-hex-2-enal left the mechanism of these reactions unchanged but increased the amount of the respective products by increasing the opportunity for reaction.

Effects of the pH and Storage Temperature on the Formation of 6-Propylbenzofuran-7-ol (1). Huelin et al.[9] investigated the pH dependency of the anaerobic decomposition of ascorbic acid. They reported that the decomposition was accelerated in the pH range of 3−4 and at pH value below 1. It was surmised that pH was another important parameter for the formation of 6-propylbenzofuran-7-ol (**1**). Five model solutions with different pH value were prepared, and every solution contained 1.0 g/L of ascorbic acid and 0.1 g/L of (*E*)-hex-2-enal (Table 3). The pH of the model solutions was adjusted in the range of 2.24−4.24, that is, close to the pH ranges of fruits (about 2.5−4.0[14]) and fruit beverages (close to that of fruits). Each model solution was stored at 50 °C, 60 °C, or 70 °C for 1 week and quantitated (Figure 4). Under all storage

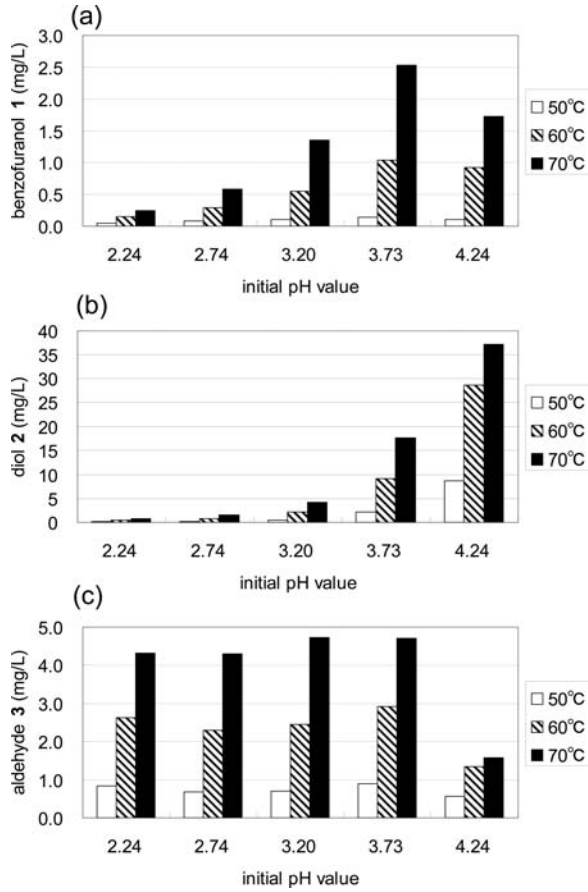

Figure 4. Influences of the initial pH values and the storage temperatures on the quantities of (a) 6-propylbenzofuran-7-ol, (b) 2,3-dihydroxy-6-propylbenzofuran-3,7-diol, and (c) 3-(2-furoyl)hexanal generated in model solutions after storage for 1 week.

temperatures, the amount of benzofuranol **1** increased with increasing pH from 2.24, peaked at pH 3.73, and thereafter fell until pH 4.24. The amount of diol **2**, on the other hand, increased throughout the whole pH range up to pH 4.24. The amount of aldehyde **3** changed little as the pH rose from 2.24 to 3.73, but steadily decreased from pH 3.73 to 4.24. We therefore surmised the following: that the increasing initial pH value from 2.24 to 4.24 accelerated the reaction of route 1; that the last dehydration from **2** to **1** could not easily proceed at pH 4.24; and that benzofuranol **1** and diol **2** were decreased and increased, respectively. The reaction of route 2 was less affected in the range of pH 2.24−3.73, but decelerated at pH 4.24 (Figure 1). The reaction to generate **6** via **5** from **4** (route 1) and the reaction to generate **7** from **4** (route 2) are estimated to be reversible reactions. The rate of each reaction seems to differ at different pH values, and the state of chemical equilibrium also seems to differ. The quantities of the products therefore changed.

Figure 4a also shows the temperature dependency of the formation of benzofuranol **1**. At pH 3.73, the quantity of benzofuranol **1** in the model solution stored at 50 °C was only 0.14 mg/L. At higher storage temperatures of 60 and 70 °C the quantity increased to 1.04 mg/L (about 7.4 times) and 2.53 mg/L (about 18 times), respectively. Similar trends were obtained at the other pH values although the ratio to the increase was different. These

dx.doi.org/10.1021/jf3044499 | J. Agric. Food Chem. 2012, 60, 12428−12434

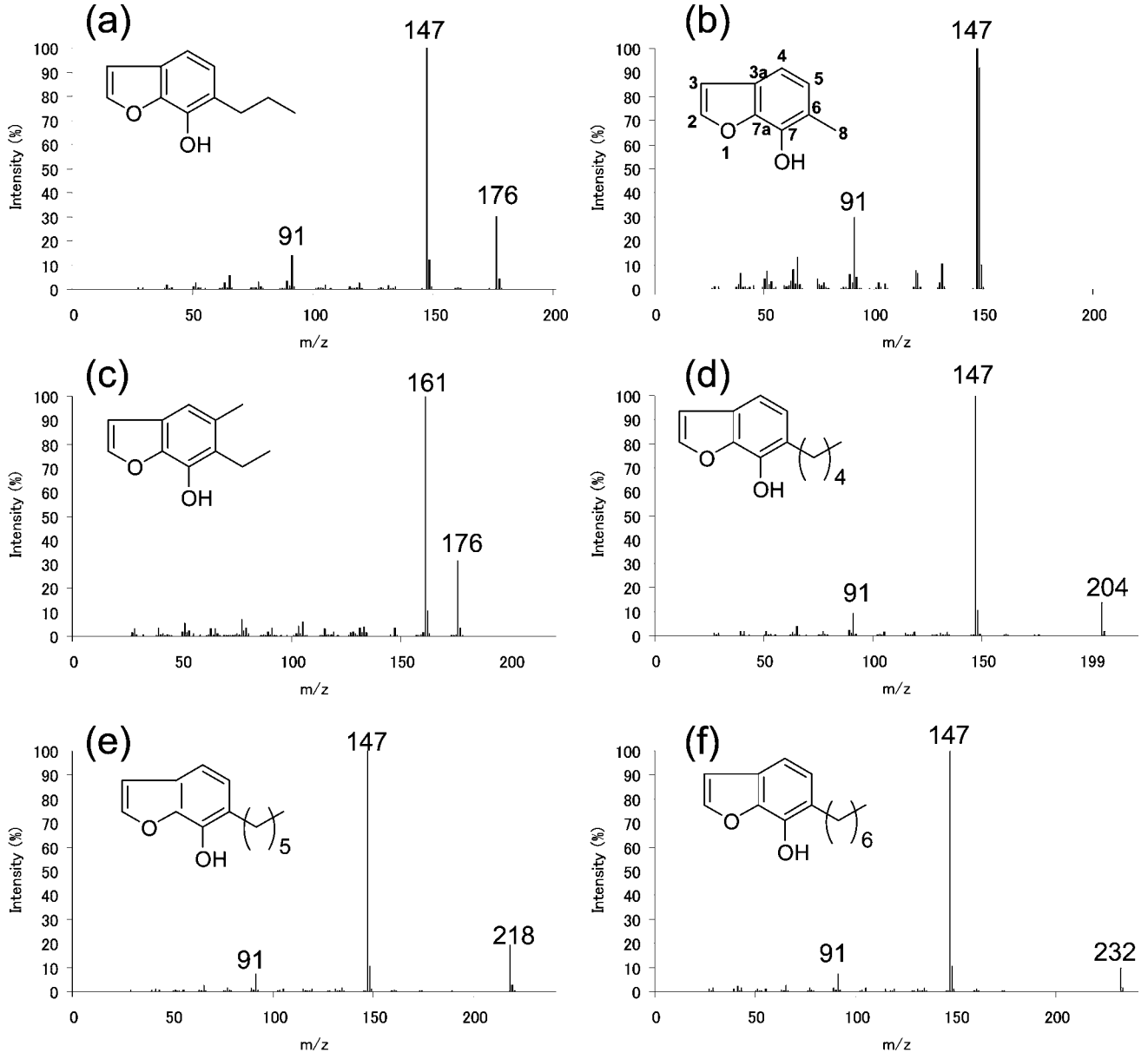

Figure 5. Mass spectra of (a) 6-propylbenzofuran-7-ol and benzofuranols formed from ascorbic acid with (b) (*E*)-but-2-enal, (c) 2-methylpent-2-enal, (d) (*E*)-oct-2-enal, (e) (*E*)-non-2-enal, and (f) (*E*)-dec-2-enal.

findings clearly reveal the temperature dependency of the formation of benzofuranol **1** which increases sharply at higher storage temperatures. The amounts of diol **2** and aldehyde **3** also increased as storage temperature rose (Figure 4b,c). The high storage temperature accelerated both reaction routes, and all products increased.

Effect of Sterilization on the Formation of 6-Propylbenzofuran-7-ol (1). Having observed the sharp increases in the reaction products at higher storage temperatures, we decided to compare the samples before and after heating sterilization. For this experiment, the model solution at pH 3.73 was used, the solution that generated the most benzofuranol **1**. In this case, none of the compounds were detected in the samples either before or after the heating sterilization. The experiment thus confirmed that short-duration heating such as heat sterilization does not lead to the formation of these compounds.

Formation of Benzofuranols from Other *α,β*-Unsaturated Aldehydes. Given the wide prevalence of *α,β*-unsaturated aldehydes such as (*E*)-hex-2-enal in natural foods, we decided to investigate whether these compounds and ascorbic acid in combination produce medicinal off-flavors. (*E*)-But-2-enal was added to an aqueous solution of ascorbic acid, and the solution was stored at 60 °C in darkness for a week. After storage a medicinal odor was detected from the solution organoleptically. Next, the volatile compounds of the solution were analyzed by mean of GC−MS−O, and strong medicinal odor was detected at RI 2405 (TC-WAX). From the mass spectrum this compound was assumed as 6-methylbenzofuran-7-ol (Figure 5b). To determine the chemical structure, this compound was isolated from a large-scale reaction of (*E*)-but-2-enal and ascorbic acid and analyzed by NMR. The NMR (^{1}H, ^{13}C, HMQC, and HMBC) analyses identified the compound as 6-methylbenzofuran-7-ol (Tables 4, 5). It was concluded that the 6-methylbenzofuran-7-ol was formed via the same reaction pathway as 6-propylbenzofuran-7-ol (**1**).

Next, (*E*)-2-methylpent-2-enal, (*E*)-oct-2-enal, (*E*)-non-2-enal, and (*E*)-dec-2-enal were respectively added to aqueous

dx.doi.org/10.1021/jf3044499 | *J. Agric. Food Chem.* 2012, 60, 12428−12434

Table 4. Assignment of ^{1}H NMR Signals (400 MHz, $CDCl_3$) of 6-Methylbenzofuran-7-ol

H at relevant C atom[a]	δ (ppm)	I	mult	J (Hz)
H−C(8)	2.40	3	s	
HO−C(7)	5.62	1	br	
H−C(3)	6.75	1	d	2.4
H−C(5)	7.03	1	d	8.0
H−C(4)	7.08	1	d	8.0
H−C(2)	7.55	1	d	2.4

[a]Arbitrary numbering of carbon atoms refers to structure (Figure 5b).

Table 5. Assignment of ^{13}C NMR Signals (100 MHz, $CDCl_3$) of 6-Methylbenzofuran-7-ol

		heteronuclear ^{1}H, ^{13}C connectivity[b]	
C atom[a]	δ (ppm)	via $^1J_{C,H}$	via $^{2,3}J_{C,H}$
C(8)	15.1	H−C(8)	H−C(5)
C(3)	107.3	H−C(3)	H−C(2), H−C(4)
C(4)	112.4	H−C(4)	
C(6)	119.6		H−C(8), H−C(5), H−C(4)
C(5)	126.0	H−C(5)	H−C(8)
C(3a)	126.8		H−C(2), H−C(3), H−C(5), H−C(4)
C(7)	139.0		H−C(8), H−C(5)
C(7a)	143.7		H−C(2), H−C(3), H−C(4)
C(2)	144.3	H−C(2)	H−C(3)

[a]Arbitrary numbering of carbon atoms refers to structure (Figure 5b). [b]Assignments based on HMQC ($^1J_{C,H}$) and HMBC ($^{2,3}J_{C,H}$) experiments.

solutions of ascorbic acid, and the solutions were stored in the same conditions. GC−MS−O analysis detected a medicinal odor from all solutions after storage. Based on mass spectra (Figure 5) and retention indices (Table 6), it was concluded

Table 6. Benzofuranols Formed from the Reaction of α,β-Unsaturated Aldehydes with Ascorbic Acid and RI on TC-WAX and TC-1 of the Benzofuranols

			RI on	
α,β-unsaturated aldehyde	benzofuranol	GC−O	TC-WAX	TC-1
(*E*)-hex-2-enal	6-propylbenzofuran-7-ol (**1**)	medicinal	2520	1473
(*E*)-but-2-enal	6-methylbenzofuran-7-ol	medicinal	2405	1301
(*E*)-2-methylpent-2-enal	5-methyl-6-ethylbenzofuran-7-ol	medicinal	2567	1480
(*E*)-oct-2-enal	6-pentylbenzofuran-7-ol	medicinal	2745	1669
(*E*)-non-2-enal	6-hexylbenzofuran-7-ol	medicinal	2850	1774
(*E*)-dec-2-enal	6-heptylbenzofuran-7-ol	medicinal	2957	1878

that the aldehydes went through the same reaction as the (*E*)-hex-2-enal and benzofuranols were formed (Figure 6). Thus it was confirmed that the reaction of α,β-unsaturated aldehydes with ascorbic acid generated benzofuranols with a medicinal odor. This means that there may be an off-flavor problem in foods including α,β-unsaturated aldehyde and ascorbic acid.

In conclusion, the amount of 6-propylbenzofuran-7-ol (**1**) generated in model solutions was affected by the pH, the storage temperature, and the initial concentrations of (*E*)-hex-2-enal and ascorbic acid. In the previous work, the orthonasal odor thresholds of benzofuranol **1** were determined in water and in apple beverage by means of the triangle test. The human

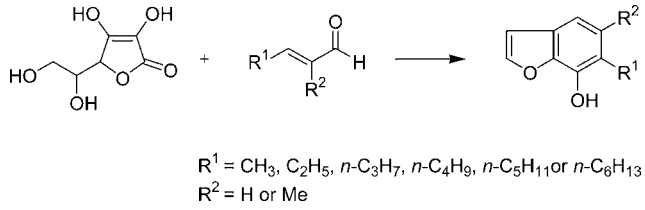

Figure 6. Formation of benzofuranols from the reaction of ascorbic acid with α,β-unsaturated aldehydes.

recognition thresholds for medicinal odor in that study were 31.4 μg/L in water and 24.0 μg/L in apple beverage, while the detection thresholds were 19.6 μg/L in water and 8.6 μg/L in apple beverage.[1] The concentrations of benzofuranol **1** were higher than the odor thresholds in all model solutions. Yet the amount of benzofuranol **1** can be reduced by regulating the conditions under which fruit beverage is manufactured and stored. The amount of benzofuranol **1** generated in a beverage can be reduced by reducing the initial concentration of (*E*)-hex-2-enal or ascorbic acid or keeping the pH value safely above or below 3.73. A lower storage temperature is also vital for preventing the occurrence of off-flavor.

The other α,β-unsaturated aldehydes also reacted with ascorbic acid, and formed 6-alkylbenzofuran-7-ols with a medicinal odor. Steps must be taken to prevent the occurrence of off-flavor whenever these aldehydes are used for beverages or foods.

AUTHOR INFORMATION

Corresponding Author

*Tel: +81-44-411-0298. Fax: +81-44-434-5257. E-mail: kensuke_sakamaki@t-hasegawa.co.jp.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Kikue Kubota and Dr. Yasujiro Morimitsu, Ochanomizu University, for HRMS measurements.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; GC−MS, gas chromatography−mass spectrometry; NMR, nuclear magnetic resonance; RI, retention index; HMQC, heteronuclear multiple quantum correlation; HMBC, heteronuclear multiple bond correlation; HRMS, high resolution mass spectra

REFERENCES

(1) Sakamaki, K.; Ishizaki, S.; Ohkubo, Y.; Tateno, Y.; Fujita, A. Identification of the medicinal off-flavor compound formed from ascorbic acid and (*E*)-hex-2-enal. *J. Agric. Food Chem.* **2011**, *59*, 6667−6671.

(2) Sakamaki, K.; Ishizaki, S.; Ohkubo, Y.; Tateno, Y.; Nakanishi, A.; Fujita, A. Elucidating the formation pathway of the off-flavor compound 6-propylbenzofuran-7-ol. *J. Agric. Food Chem.* **2012**, *60*, 9967−9973.

(3) Su, S. K.; Wiley, R. C. Changes in apple juice flavor compounds during processing. *J. Food Sci.* **1998**, *63*, 688−691.

(4) Steinhaus, M.; Bogen, J.; Schieberle, P. Key aroma compounds in apple juice changes during juice concentration. In *Flavour Science: Recent Advances and Trends*; Bredie, W. L. P., Peterson, M. A., Eds.; Elsevier: Amsterdam, The Netherlands, 2006; pp 189−192.

(5) Belitz, H.-D.; Grosch, W.; Schieberle, P. *Vitamins in Food Chemistry*; Belitz, H.-D., Grosch, W., Schieberle, P., Eds.; Springer-Verlag: Berlin, Heidelberg, 2009; Chapter 6, pp 305−319.

(6) Rouseff, R. L.; Nagy, S.; Naim, M.; Zahavi, U. Off-flavor development in citrus juice products. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, The Netherlands, 1992; pp 211−227.
(7) Kurata, T.; Sakurai, Y. Degradation of L-ascorbic acid and mechanism of nonenzymic browning reaction. *Agric. Biol. Chem.* **1967**, *31*, 170−176.
(8) Tatum, J. H.; Shaw, P. E.; Berry, R. E. Degradation products from ascorbic acid. *J. Agric. Food Chem.* **1969**, *17*, 38−40.
(9) Huelin, F. E.; Coggiola, G. S.; Sidhu, G. S.; Kennett, B. H. The anaerobic decomposition of ascorbic acid in the pH range of foods and in more acid solutions. *J. Sci. Food Agric.* **1971**, *22*, 540−542.
(10) Yuan, J. P.; Chen, F. Degradation of ascorbic acid in aqueous solution. *J. Agric. Food Chem.* **1998**, *46*, 5078−5082.
(11) Lee, H. S.; Nagy, S. Quality changes and nonenzymic browning intermediates in grapefruit juice during storage. *J. Food Sci.* **1988**, *53* (168−172), 180.
(12) Nagy, S.; Randall, V. Use of furfural content as an index of storage temperature abuse in commercially processed orange juice. *J. Agric. Food Chem.* **1973**, *21*, 272−275.
(13) Nagy, S.; Dinsmore, H. L. Relationship of furfural to temperature abuse and flavor change in commercially canned single-strength orange juice. *J. Food Sci.* **1974**, *39*, 1116−1119.
(14) Belitz, H.-D.; Grosch, W.; Schieberle, P. Fruits and fruit products. In *Food Chemistry*; Belitz, H.-D., Grosch, W., Schieberle, P., Eds.; Springer-Verlag: Berlin, Heidelberg, 2009; Chapter 18, pp 807−861.
(15) Werkhoff, P.; Güntert, M.; Krammer, H.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076−1093.
(16) Kawakami, M.; Kobayashi, A. Volatile constituents of green mate and roasted mate. *J. Agric. Food Chem.* **1991**, *39*, 1275−1279.
(17) Mick, W.; Schreier, P. Additional volatiles of black tea aroma. *J. Agric. Food Chem.* **1984**, *32*, 924−929.
(18) Schreier, P.; Drawert, F.; Junker, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976**, *24*, 331−336.
(19) Flath, R. A.; Forrey, R. R. Volatile components of papaya (*Carica papaya* L., Solo variety). *J. Agric. Food Chem.* **1977**, *25*, 103−108.
(20) Greger, V.; Schieberle, P. Characterization of key aroma compounds in apricot (*Orunus armeniaca*) by application of the molecular sensory science concept. *J. Agric. Food Chem.* **2007**, *55*, 5221−5228.
(21) Idstein, H.; Schreier, P. Volatile constituents from guava (*Psidium guajava*, L.) fruit. *J. Agric. Food Chem.* **1985**, *33*, 138−143.
(22) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189−5193.