Effects of Sulfur Dioxide on Formation of Fishy Off-Odor and Undesirable Taste in Wine Consumed with Seafood

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In order to evaluate sensory compatibility of alcoholic beverages with food, beverages and dried squid, namely, "surume", a common Japanese accompaniment, were consumed together. White wine and dried squid pairings had a more undesirable taste and more fishy off-odor than sake and dried squid pairings. The undesirable taste and fishy off-odor appeared to be caused by degradation of unsaturated fatty acids (e.g., docosahexaenoic acid (DHA)), which are found in fish and squid. Upon addition of DHA to the beverage, bitterness intensity, measured by instrumental taste sensor analysis, and the concentration of certain aldehydes reported to contribute to fishy flavors, increased in white wines, whereas they remained largely the same in sake. Among the major chemical constituents that distinguish wine from sake, only wine-specific sulfite markedly increased bitterness intensity and aldehyde levels upon addition of DHA. These results suggest that sulfur dioxide in wine participated in degradation of unsaturated fatty acids, causing an increase in undesirable taste and fishy off-odor in wine and seafood pairings.

KEYWORDS: Wine; seafood; fishy off-odor; undesirable taste; unsaturated fatty acids; sulfur dioxide

INTRODUCTION

Moderate drinking of alcoholic beverages with meals has a long tradition. In Japan, consumption of alcoholic beverages is widespread, particularly with supper. Because alcoholic beverages are often consumed with meals, their compatibility with food is important. Specific desirable combinations of alcoholic beverages and foods have been well-established empirically, e.g., "red wine with red meat and white wine with poultry and fish". Traditional alcoholic beverages often match a specific food of the country of origin. For example, Belgian fruit beers pair well with chocolate desserts. Nonetheless, the scientific basis for desirable and undesirable pairings has not been subjected to thorough analysis, although red table wines were recently shown to be a better accompaniment with cheese than dry white table wine on the basis of human perceptions (*1*). Mizuma et al. (*2*) reported that quantitative taste changes in a beverage taken separately or with food had a major influence on compatibility. With reference to recognized compatible pairings such as red wine and Camembert cheese, the sweetness and umami of the beverages were enhanced, whereas sourness, bitterness, and astringency were reduced, when taken with food (*2*). On the other hand, the opposite changes were observed in incompatible pairings, such as red wine and Japanese radish stewed with soy sauce (*2*). Parker (*3*) reported on changes in key flavor notes of a beer before and after consumption of dry-roasted peanuts. After consumption of the peanuts, the flavor of the beer was perceived differently; hoppy and malty notes were softened, and the beer was perceived as sweeter and less bitter, with a shorter lingering aftertaste.

For Japanese consumers, the pairing of foods and alcoholic beverages is a topic of great interest and importance (*4*). Sake, a Japanese traditional rice wine, is also known to pair especially well with Japanese foods (e.g., sliced raw fish and shellfish, sushi, and vegetables stewed with soy sauce) that typically comprise fish and shellfish as ingredients and stock components (*2, 5*). According to a 2003 report of the United Nations Food and Agriculture Organization, fish (seafood) consumption was 66 kg per capita per year in Japan, greatly exceeding the worldwide average of 16 kg per capita per year.

The taste sensor system developed by the Toko group (*6−8*) in cooperation with Intelligent Sensor Technology Inc. detects taste information as changes in electrical potential with several sensor probes corresponding to human taste cells and has been applied to the sensory evaluation of various beverages. Recent reports have found the taste sensor system to be extremely useful for objectively evaluating the astringent and umami taste intensities of tea (*9, 10*) and the bitterness taste intensities of pediatric drug formulations (*11*).

In the present study, we compared sake and white wine pairings with seafood. Initially, changes in taste and smell caused by pairing alcoholic beverages with seafood or individual chemical constituents were evaluated by sensory analysis, the taste sensor system, and gas chromatography. Subsequently, chemical changes influencing compatibility of the pairings were investigated.

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Table 1. Profiles of White Wine and Sake Samples

sample	alcohol[a]	specific gravity[b]	glucose[c]	fructose[c]	pH	titratable acidity[d]	total phenolics[e]	free SO$_2$[f]	bound SO$_2$[f]
wine A	13.7	0.9920	0.8	3.2	3.5	6.0	272	15	60
wine B	11.2	0.9988	8.0	11.7	3.0	5.1	158	14	92
wine C	10.2	1.0235	32.0	38.2	3.4	7.0	375	30	124
wine D	11.5	1.0033	5.6	19.0	3.2	6.4	113	nd	2
wine E	9.7	1.0159	9.0	38.5	3.3	9.9	123	nd	2
sake A	15.9	0.9994	27.3	nd	4.4	1.1	166	nd	nd
sake B	15.8	1.0007	26.4	nd	4.2	1.0	168	nd	nd
sake C	14.4	0.9939	18.8	nd	4.4	1.0	190	nd	nd

[a] Alcohol (%, v/v). [b] Specific gravity (15/4 °C). [c] Glucose and fructose (g/L). [d] Titratable acidity (g of tartaric acid/L). [e] Total phenolics (gallic acid equivalents mg/L). [f] Free and bound SO$_2$ (mg/L).

MATERIALS AND METHODS

Reagents. Xanthan gum was purchased from Junsei Chemical (Tokyo, Japan). 4,7,10,13,16,19-Docosahexaenoic acid (DHA) was from Nacalai Tesque (Kyoto, Japan). Propanal and (*E,E*)-2,4-heptadienal were purchased from Tokyo Chemical Industry (Tokyo, Japan). (*Z*)-4-Heptenal was from Fluka Chemie (Buchs, Switzerland), and (*E,Z*)-2,6-nonadienal was from Sigma-Aldrich (St. Louis, MO). Potassium metabisulfite (K$_2$S$_2$O$_5$), *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA), pentanal, 2-thiobarbituric acid (TBA), and butylhydroxytoluene were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Alcoholic Beverages and Seafood Samples. White wine and sake samples were purchased from liquor stores. All are popular and commercially available in Japan. Profiles of the coded samples are shown in **Table 1**. Each sample was chemically analyzed for its specific gravity using the DA-510 density/specific gravity meter (Kyoto Electronics Manufacturing, Kyoto, Japan), glucose and fructose content using F-kit D-glucose/D-fructose (R-Biopharm, Darmstadt, Germany), pH, and titratable acidity using titration to pH 8.2. Alcohol content was determined according to National Tax Administration methods (*12*), and total phenolics were measured using the Folin−Ciocalteu method and expressed as gallic acid equivalents (*13*). Sulfur dioxide (SO$_2$) concentrations in these samples were determined by a modification of the method of Rankine (*14*), as described by Goto et al. (*15*). Each sample analysis was carried out in duplicate with means presented in **Table 1**.

Dried squid (*Photololigo edulis*), "surume", and baked horse mackerel (*Trachurus japonicus*), which are common accompaniments to alcoholic beverages in Japan, were purchased from markets. The concentrations of DHA and eicosapentaenoic acid (EPA) (unsaturated fatty acids) in these two products were determined according to the Nutrition Labeling Standards of the Ministry of Health, Labour and Welfare in the Japan Food Research Laboratories (Osaka, Japan). Peroxide values of fats and oils in these two products were also measured according to the JOCS Standard Methods for the Analysis of Fats, Oils and Related Materials of the Japan Oil Chemists' Society.

Addition of DHA to Alcoholic Samples. Thirty milligrams of xanthan gum was added as a stabilizer to 100 mL of each alcoholic sample. Following dissolution of the xanthan gum, 5.7 μL (0.16 mM) of DHA was added and suspended in the solution. The amount of added DHA was determined based on the basal content in the dried squid used in the sensory analysis described below. The xanthan gum solutions lacking added DHA for each sample served as controls.

SO$_2$ Addition Test. Potassium metabisulfite was dissolved in a 0.4% tartaric acid solution containing 15% (v/v) ethanol. The solution was adjusted to pH 3.2 with sodium hydroxide. Additions were calculated based on the fact that 50% of potassium metabisulfite by weight is SO$_2$ (*16*).

Sensory Analysis. The sensory compatibility between beverage and dried squid was evaluated by 18 persons working at our institute. Half were experienced in sensory evaluation of alcoholic beverages, and the others were inexperienced university students. As alcoholic beverage samples, three white wines (wines A, B, and C) and three sakes (sakes A, B, and C) were used (**Table 1**). The six beverage samples were coded using three-digit random numbers. To avoid bias caused by the order of presentation, each panelist evaluated the samples in a different order. Panelists were instructed to drink 15 mL of each sample while chewing 0.5 g of surume and to evaluate the beverage sample on the basis of two parameters:
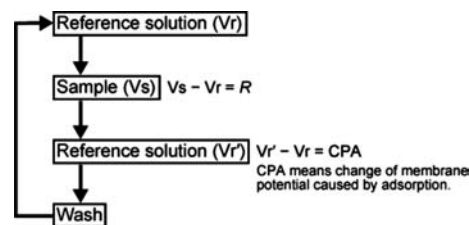
Figure 1. Taste sensor measuring procedure in this study.

intensity of undesirable taste and intensity of the fishy odor using the labeled magnitude scales (barely detectable, 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; strongest imaginable, 100) (*17, 18*). Data are expressed as means of the logarithms of the labeled magnitude scale values. Statistical analysis was performed by one-way analysis of variance (ANOVA). Statistical differences were evaluated by the Tukey−Kramer HSD test when the *F*-value was significant. A *p*-value of 0.05 or less was considered significant.

The sensory compatibility of white wine with baked horse mackerel was also evaluated by 26 persons working at our institute experienced in sensory evaluation of alcoholic beverages. Wines made without sulfite addition (D and E, **Table 1**) were used as "no sulfite" wine samples. Sulfite-spiked wines were samples D and E to which 200 mg/L potassium metabisulfite was added, wines D′ and E′, respectively. These four beverage samples were coded using three-digit random numbers. Paired comparison tests were conducted for two pairs of wine samples (wines D and D′; wines E and E′). Panelists were instructed to drink 15 mL of each sample while chewing 1.0−1.5 g of baked horse mackerel and to select the most incompatible wine sample according to the method described in ISO 5495:2005. To avoid order effects, half the panelists evaluated the untreated sample (wine D or E) first, followed by the SO$_2$-spiked sample (wine D′ or E′), and the other half evaluated samples in reverse order.

Both sensory analyses were performed in the sensory evaluation training room at the National Research Institute of Brewing.

Measurement of the Taste Intensity by the Taste Sensor System. The electrical potential corresponding to the taste intensity of a sample solution was measured by the SA402B taste sensor system (Intelligent Sensor Technology, Kanagawa, Japan), fitted with five sensor probes (SB2AAE, SB2CT0, SB2CA0, SB2C00, and SB2AE1) and two reference probes (*6−8*). Each sensor probe consists of a lipid/polymer membrane, an Ag/AgCl electrode, and an internal cavity filled with a 3.3 M KCl aqueous solution saturated with AgCl. The membrane of each sensor probe is made of different lipids or lipid mixtures. Each reference probe consists of a ceramic liquid junction, an Ag/AgCl electrode, and an internal cavity filled with a 3.3 M KCl aqueous solution saturated with AgCl. The difference between the electric potential of the working electrode and the reference electrode was measured by means of a high-input impedance amplifier connected to a computer.

A fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference sample (Vr) and also for rinsing electrodes. The method used to measure the sensitivity and the selectivity of adsorption of the samples is summarized in **Figure 1**. The electrode was first dipped into the reference solution (Vr) and then into the sample solution or suspension (Vs). The relative sensor output (*R*) is represented as the difference (Vs − Vr) between the potentials of the
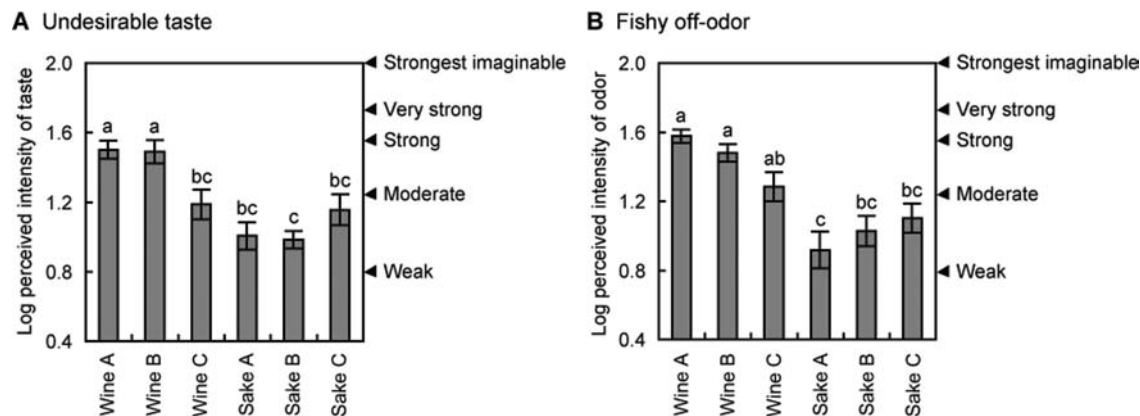
A Undesirable taste

B Fishy off-odor

Figure 2. Intensity of undesirable taste (**A**) and fishy off-odor (**B**) perceived in each sample together with dried squid assessed by sensory analysis. Each value is the mean $\pm$ SEM (n = 18). Values with different letters are significantly different at $p < 0.05$ by the Tukey–Kramer HSD test.

sample and reference solution. When the electrode is dipped into the reference solution again, the new potential of the reference solution is defined as Vr′. The difference (Vr′ − Vr) between the potentials of the reference solution before and after sample measurement is defined as CPA (*c*hange of membrane *p*otential caused by *a*dsorption) and corresponds to aftertaste.

Automatic sensor measurements were performed at room temperature. Measuring times were set for 30 s, and electrodes were rinsed after each measurement. The measurements were performed in the following order: the alcoholic beverage sample followed by the DHA-spiked sample. Each sample measurement was carried out in triplicate. In the present study, the differences (ΔR and ΔCPA) between the values of R and CPA, respectively, before and after DHA suspension, were used to predict the taste change by addition of DHA to the alcoholic samples.

Aldehyde Analysis. Aldehydes were analyzed using solid-phase microextraction (SPME) with on-fiber derivatization and gas chromatography/mass spectrometry (GC/MS) as descrived by Vesely et al. (*19*).

One hundred microliters of PFBOA solution (6 g/L) and 10 mL of deionized water were placed in a 20 mL glass vial and sealed with a PTFE/silicone septum and a crimp cap. A 65 μm poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) SPME fiber (57310-U; Supelco, Bellefonte, PA) was then placed in the headspace of the PFBOA solution for 10 min at 50 °C. The SPME fiber loaded with PFBOA was then exposed to the headspace of 10 mL of sample placed in a 20 mL glass vial for 60 min at 50 °C.

Aldehyde derivatives were analyzed using a HP6890 gas chromatograph equipped with a 5975C mass-selective detector (Agilent Technologies, Palo Alto, CA) and fitted with a HP-5MS capillary column, 30 m × 0.25 mm × 0.25 μm (J&W Scientific, Folsom, CA). Helium was used as carrier gas at a flow rate of 1 mL/min. The front inlet temperature was 250 °C. The injection was in splitless mode. The oven temperature program used was 40 °C for 2 min, followed by an increase of 10 °C/min to 140 °C and 7 °C/min to 250 °C. The final temperature was held for 3 min. Identification of the carbonyl PFBOA derivatives was performed by MS using electron impact ionization running in the scan mode. Quantification of aldehydes was carried out in the single-ion monitoring mode with monitoring for *m/z* 181.

Quantification was performed using the ratio of the peak areas of the internal standard (pentanal) and the identified compounds. SPME with on-fiber derivatization was carried out as described above except that 50 μg/L pentanal was added to each sample. Standard curves were prepared with propanal and (*E,E*)-2,4-heptadienal dissolved in each beverage. Each sample analysis was carried out in triplicate.

TBA Reaction. A TBA reaction for beverage and other samples was performed as described by Nomura and Kiso (*20*). The TBA solution was prepared by adding 0.5% of TBA to a 50% (v/v) aqueous ethanol solution and mixing for 30 min. The reaction mixtures comprised 0.1 mL of 1.2% butylhydroxytoluene in acetic acid, 4.9 mL of the sample solution or suspension, and 1 mL of the TBA solution. The mixture was heated to 70 °C for 40 min and cooled in water, after which 3 mL of chloroform was added, and the mixture was shaken and centrifuged at 2000 rpm for
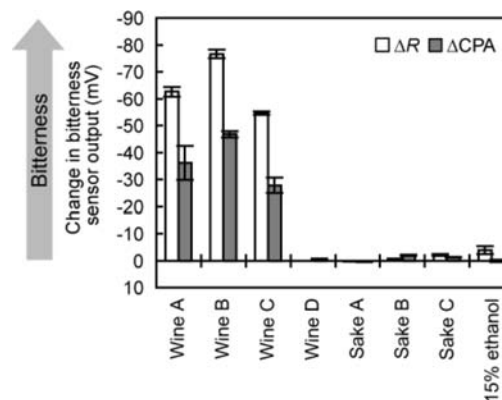
Figure 3. Change in taste sensor output values for each sample by addition of DHA. The vertical bars represent SD values (n = 3).

10 min. Absorbance at 530 nm of the upper (aqueous) phase was then determined. Each TBA reaction was carried out in triplicate.

RESULTS AND DISCUSSION

Sensory Compatibility between Beverage and Dried Squid. In order to compare sake and white wine pairings with seafood, changes in taste and smell caused by pairing beverages with dried squid were evaluated by sensory analysis. White wine and dried squid pairings had a more intense undesirable taste and a more intense fishy off-odor than the sake and dried squid pairings (**Figure 2**). Significant differences between all sakes and wine A or B were detected in the intensities of the undesirable taste and fishy off-odor.

It has been reported that carbonyl compounds are produced from oxidation of polyunsaturated fatty acids in fish by lipoxygenase or by autoxidation (*21*) and contribute to fishy flavor (*22*). Because the dried squid contained high levels of polyunsaturated fatty acids (DHA, 0.94 g/100 g; EPA, 0.25 g/100 g; peroxide value of fats and oils, ≤1 mequiv/kg), it is likely that the undesirable taste and fishy off-odor were caused by oxidation of the polyunsaturated fatty acids. Changes in taste and smell that occurred following addition of polyunsaturated fatty acids to the white wines and sakes were then analyzed.

Addition of Polyunsaturated Fatty Acids to White Wines and Sakes. Changes in beverage taste before and after addition of DHA were determined using the taste sensor system. The R (relative sensor output) and CPA (change of membrane potential caused by adsorption) values of the bitterness sensor (SB2C00) decreased markedly after addition of DHA to wines A, B, and C,
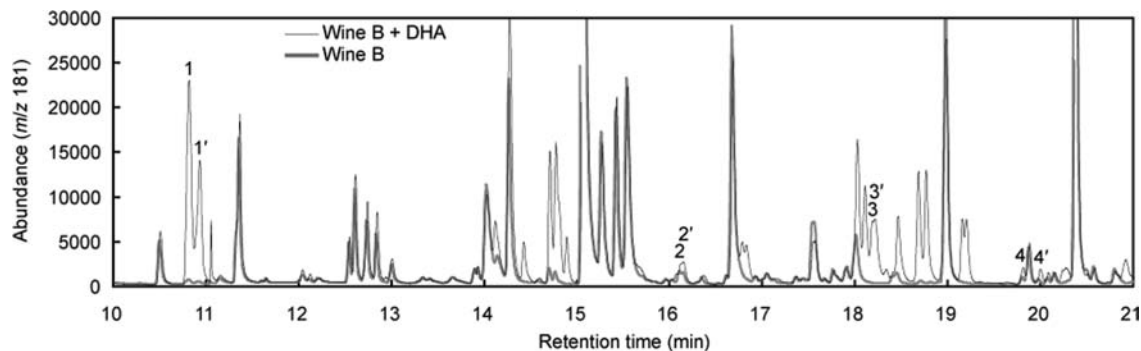
Figure 4. Gas chromatogram of PFBOA derivatives in white wine B before and after addition of DHA. Peaks: 1, 1′, propanal; 2, 2′, (*Z*)-4-heptenal; 3, 3′, (*E*,*E*)-2,4-heptadienal; 4, 4′, (*E*,*Z*)-2,6-nonadienal.

whereas these values barely changed in wine D, in the sakes (sakes A, B, and C), or in aqueous ethanol (**Figure 3**). These results indicate that bitterness increased markedly and persisted as an aftertaste as a result of adding DHA to wines A, B, and C. Similar results were also obtained by addition of EPA and linoleic acid instead of DHA (data not shown). Certain oxidized lipids have been reported to taste bitter (*23−25*). Therefore, we speculate that oxidized polyunsaturated fatty acids were formed and tasted bitter in wines A, B, and C. The responses of the other sensors, umami (SB2AAE), saltiness (SB2CT0), sourness (SB2CA0), and astringency (SB2AE1), barely changed in any of the beverage samples (data not shown). Although we have not investigated the relationship between the bitterness sensor response and the undesirable taste perceived by sensory analysis, the undesirable taste, which was sensed more strongly in the white wine and dried squid pairings (**Figure 2**), appeared to consist partly of bitterness produced by exposure of polyunsaturated fatty acids contained in the dried squid to the wines.

GC/MS analysis of the alcoholic beverages before and after addition of DHA showed that DHA addition to wines A, B, and C resulted in an increase in peaks corresponding to carbonyl PFBOA derivatives in the chromatograms, whereas no obvious changes were observed in the chromatograms for wine D, the sakes, or aqueous ethanol. The chromatograms for wine B and that containing DHA are shown in **Figure 4**. Most aldehydes formed two geometrical isomers of the derivatives that are represented by two peaks in the chromatogram. Among the peaks found to increase after DHA addition to wines A, B, and C, those corresponding to propanal (peak nos. 1 and 1′ in **Figure 4**), (*Z*)-4-heptenal (2 and 2′), (*E*,*E*)-2,4-heptadienal (3 and 3′), and (*E*,*Z*)-2,6-nonadienal (4 and 4′) were identified by comparison of the retention times and mass spectra with those of authentic standards. These aldehydes were previously reported to have flavors characteristic of alcoholic-like, seashore-like, oxidized oil-like, and cucumber-like/insect-like, respectively, and to contribute toward fishy flavors together with other aldehydes and ketones (*22*). They are also produced from oxidation of polyunsaturated fatty acids during fish storage (*22, 26, 27*). The concentrations of propanal and (*E*,*E*)-2,4-heptadienal were determined, as these two compounds of the four identified compounds showed comparatively large peaks in the chromatograms of DHA-spiked wines. Standard curves showed excellent linearity with $R^2 = 0.99$. It was difficult to determine the concentrations of (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal. Propanal and (*E*,*E*)-2,4-heptadienal levels increased markedly to about 300 and 10−20 μg/L, respectively, by addition of DHA to wines A, B, and C, while levels in the other samples increased to less than 21 and 1.3 μg/L, respectively (**Figure 5**). Based on the taste sensor system, the four identified aldehydes did not taste bitter in wines
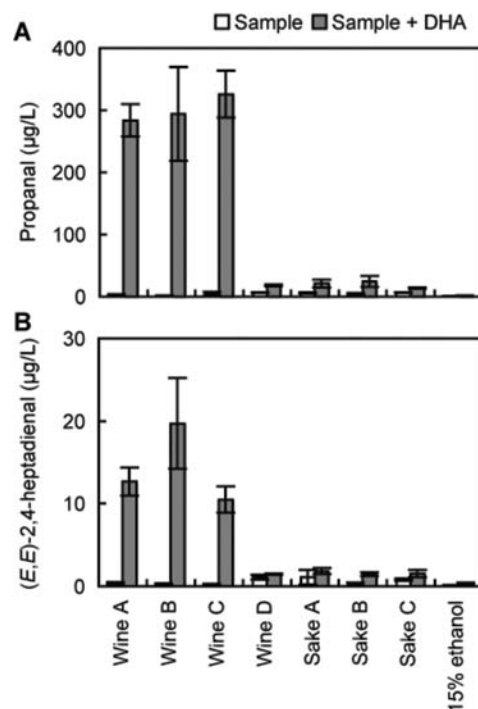

Figure 5. Concentrations of propanal (**A**) and (*E*,*E*)-2,4-heptadienal (**B**) in each sample before and after addition of DHA. The vertical bars represent SD values ($n = 3$).

A, B, and C (data not shown). We speculate that other products formed by oxidization of polyunsaturated fatty acids tasted bitter in these wines.

The peroxidic degrees of DHA added to the beverage samples were also measured by TBA reaction (A_{530}). A_{530} values for the TBA reaction mixture increased significantly after addition of DHA to wines A, B, and C (**Figure 6**). This result is consistent with oxidation and decomposition of DHA occurring, leading to aldehyde production in these wines. The aldehydes thus produced, combined with TBA and produced the red pigments.

Effects of Wine Constituents. Wine is a more complex medium than sake, containing organic acids, metals, polyphenols, and SO_2, among other compounds. In order to investigate the effects of wine constituents on the observed increase in aldehydes and bitterness (as assessed in the taste sensor system) caused by DHA addition, individual constituents were added to sake B, a regular type of sake, and aldehydes and bitterness were then analyzed before and after addition of DHA. Concentrations of the added substances were set to approximate the concentrations in sake B to those in wine B (**Table 2**). Potassium metabisulfite was found to
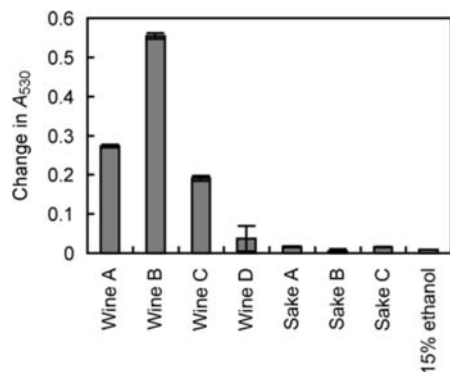
Figure 6. Change in A_{530} values for each sample by addition of DHA in the TBA reaction. The vertical bars represent SD values ($n = 3$).

Table 2. Effects of Wine Constituents on Aldehyde Production and Change in Taste Sensor Output Values by Addition of DHA

substance added to sake B	concn (mg/L)	aldehyde production	change in bitterness sensor output	
			ΔR (mV)[a]	ΔCPA (mV)[a]
phosphoric acid	666	−	−1 ± 0	−2 ± 1
citric acid	109	−	−1 ± 0	−1 ± 1
malic acid	900	−	−1 ± 0	−1 ± 1
succinic acid	232	−	−1 ± 0	−1 ± 0
acetic acid	70	−	−11 ± 1	−11 ± 1
tartaric acid	1320	−	−1 ± 0	0 ± 1
Fe (FeCl$_2$)	3	−	−1 ± 1	−3 ± 2
Fe (FeCl$_3$)	3	−	−1 ± 0	−1 ± 0
Mg (MgCl$_2$)	50	−	−1 ± 0	−1 ± 0
(+)-catechin	30	−	−1 ± 0	−2 ± 1
potassium metabisulfite	200	+	−75 ± 2	−32 ± 3
hydrochloric acid to pH 3.1		−	0 ± 0	−2 ± 1
combination of all above substances		+	−81 ± 8	−47 ± 14
none		−	−1 ± 0	−2 ± 0

[a] Values are means ± SD ($n = 3$).

be the only added constituent that caused an increase both in aldehyde levels and in bitterness responses (**Table 2**). In this case, all of the four identified aldehydes increased. The free and bound SO$_2$ concentrations in sake B to which 200 mg/L potassium metabisulfite had been added were 58 and 43 mg/L, respectively. A fraction of added SO$_2$ appeared to bind to constituents such as aldehydes and form addition compounds in sake B, as in wines. While SO$_2$ is a normal but minor yeast metabolite found in wine, it is also commonly added by winemakers, often in the form of potassium metabisulfite as a disinfectant and antioxidant. At the same time, 200 mg/L potassium metabisulfite contains 70 mg/L potassium. In order to investigate the effect of potassium on the increase in aldehydes and bitterness caused by DHA addition to sake B containing potassium metabisulfite, 245 mg/L potassium dihydrogen phosphate (KH$_2$PO$_4$) (70 mg/L potassium) was also added to sake B. Potassium dihydrogen phosphate did not cause an increase in aldehyde levels after addition of DHA to the sake B mixture. The changes in bitterness sensor output values by addition of DHA were −1 ± 0 mV (ΔR) and −4 ± 4 mV (ΔCPA), which were very small relative to the changes in potassium metabisulfite content (**Table 2**). The results described above suggest that SO$_2$ addition accelerated DHA oxidation and decomposition in wines A, B, and C and that an undesirable taste and smell were generated as a result. In contrast, DHA addition to wine D, containing only trace amounts of SO$_2$, barely altered bitterness sensor responses, aldehyde concentrations, or A_{530} values for the TBA reaction (**Figures 3**, **5**, and **6**).

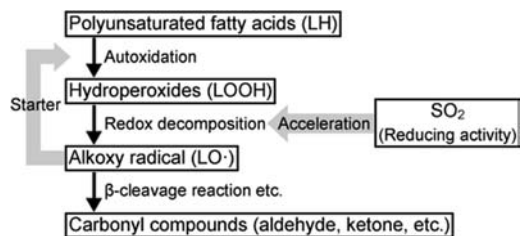**Figure 7.** Presumed effect of SO$_2$ on oxidation and decomposition of polyunsaturated fatty acids. LH is lipid, LOOH is lipid hydroperoxide, and LO· is lipid alkoxy radical.
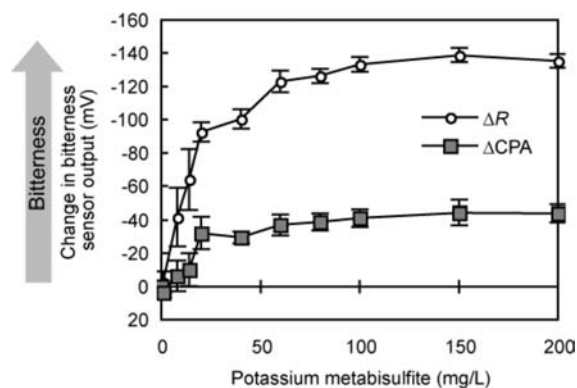

Figure 8. Effects of potassium metabisulfite concentrations on change in taste sensor output values by DHA addition to 0.4% tartaric buffer (15% ethanol, pH 3.2). The vertical bars represent SD values ($n = 3$).

Polyunsaturated fatty acids (LH) such as linoleic acid, DHA, and EPA, having two or more double bonds, are easily oxidized and changed to hydroperoxides (LOOH) by enzyme action or by nonenzymatic autoxidation during preservation, manufacturing, and cooking. Nishiike et al. (*28*) reported that the decomposition of linoleic acid hydroperoxide was accelerated by red and white wines and by 0.5 mM sodium sulfite. Because sodium sulfite is a strong reductant, the authors presumed that it would be likely to reduce a hydroperoxide to an alkoxy radical (LO·). As an alkoxy radical is reactive, it can initiate lipid oxidation by abstracting a hydrogen atom from another polyunsaturated fatty acid. Alkoxy radicals also form various secondary products such as aldehydes through β-cleavage reactions, for example (**Figure 7**). In the present study, lipid hydroperoxides (the primary products of DHA oxidation) appeared to form before exposure to SO$_2$, because A_{530} values for the TBA reaction mixture increased slightly even after addition of DHA to 15% ethanol (**Figure 6**). The lipid hydroperoxides may have reacted with SO$_2$ in the wine to form alkoxy radicals, which, in turn, generated a variety of secondary products including aldehydes through cleavage and other reactions. However, further study is required to confirm this supposition.

Effect of SO$_2$ Concentration on Increase in Bitterness and Aldehydes. In order to determine the effect of SO$_2$ concentration, potassium metabisulfite was added at various concentrations to a 0.4% tartaric acid solution (pH 3.2) containing 15% (v/v) ethanol. Even a concentration as low as 20 mg/L potassium metabisulfite (about 10 mg/L SO$_2$) caused a decrease in R and CPA bitterness sensor values after DHA addition (**Figure 8**). The response reached a maximum at about 100 mg/L potassium metabisulfite but changed little upon further additions up to 200 mg/L. Changes in aldehyde concentrations (propanal and (*E,E*)-2,4-heptadienal) exhibited a pattern similar to that of the bitter taste sensor response (**Figure 9**). Falcone and Maxwell
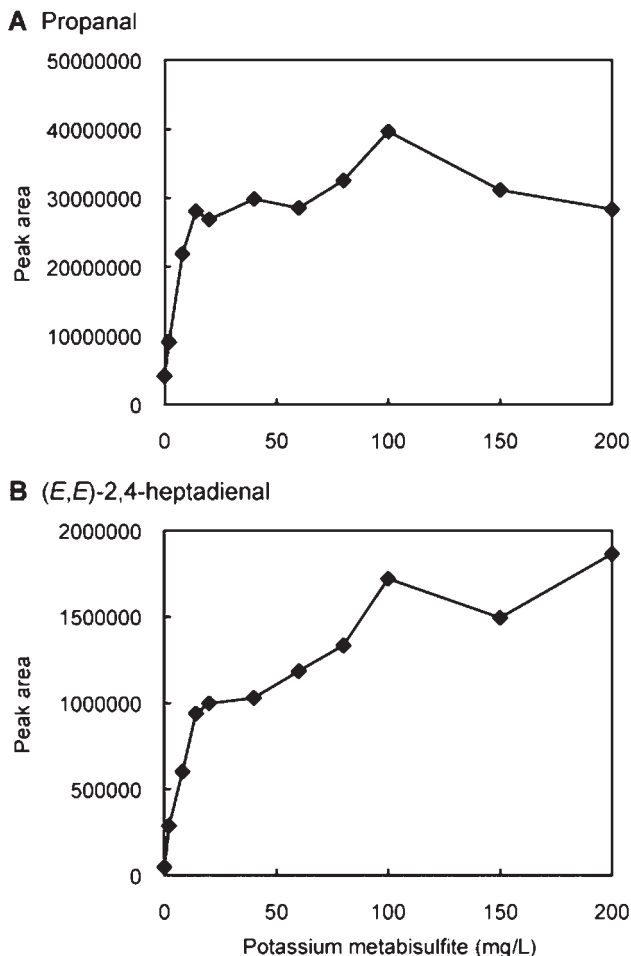
A Propanal

B (*E,E*)-2,4-heptadienal

Figure 9. Effect of potassium metabisulfite concentrations on propanal (**A**) and (*E,E*)-2,4-heptadienal (**B**) production by DHA addition to 0.4% tartaric buffer (15% ethanol, pH 3.2). Peak area of PFBOA derivative of each aldehyde is shown.

Table 3. Sensory Evaluation of Compatibility between White Wine and Baked Fish Using a Paired Comparison Test

wine sample	free SO_2 (mg/L)	bound SO_2 (mg/L)	no. of panelists who scored pairing incompatible	significance level (%) by two-sided paired test
D	nd	2	9	
D′ (sulfite dissolved)	59	46	17	20
E	nd	2	5	
E′ (sulfite dissolved)	24	78	21	1

reported that the concentrations of free and total SO_2 in 10 white wines ranged from 10 to 30 and from 40 to 140 mg/L, respectively (*29*). If these values are representative, many wines may contain enough free SO_2 to produce undesirable taste and odor compounds from reaction with polyunsaturated fatty acids. In this study, although wine C contained the largest amount of SO_2 in wines A, B, and C (**Table 1**), it scored lower than wines A and B in **Figures 2**, **3**, **5B**, and **6**. These results suggest that reaction of SO_2 with polyunsaturated fatty acids is not absolutely dependent on SO_2 concentrations in real wines. Other wine constituents may influence the reaction.

Sensory Compatibility between White Wine SO_2 and Fish. Using white wines with and without SO_2 as beverage samples, the sensory compatibility between each wine sample and baked horse mackerel (DHA, 0.94 g/100 g; EPA, 0.59 g/100 g; peroxide value of fats and oils, 33.3 mequiv/kg) was evaluated by a paired comparison test in order to investigate the influence of SO_2 on pairing white wine with fish. When 200 mg/L potassium metabisulfite (about 100 mg/L SO_2) was added to the no-sulfite wine samples, a fraction of the SO_2 was present as bound SO_2 (46 mg/L in wine D′ and 78 mg/L in wine E′) (**Table 3**). The SO_2 appeared to bind to wine constituents such as aldehydes and form addition compounds in wines D′ and E′. For both pairs of wine samples (D and D′; E and E′), more panelists selected the wine spiked with potassium metabisulfite (D′ or E′) as the more incompatible sample (**Table 3**). The significance of the difference between wine E and E′ was at the 1% threshold using a two-sided paired test.

Although the consumption of wines made without sulfite addition is increasing to a certain degree, SO_2 is still routinely added as an antioxidant and antimicrobial agent in most commercial wines. We speculate that the tradition of "white wine with fish" refers to consumption of white wine with whitefish such as Pacific cod (polyunsaturated fatty acids, 0.07 g/100 g (*30*)), oriental shrimp (0.06 g/100 g (*30*)), and king crab (0.08 g/100 g (*30*)) that contain low levels of polyunsaturated fatty acids. The results obtained in the present study suggest that fishy off-odor and undesirable taste are produced from the combination of wine containing SO_2 and seafood such as horse mackerel (polyunsaturated fatty acids, 0.95 g/100 g (*30*)), chum salmon (0.91 g/100 g (*30*)), Pacific saury (4.58 g/100 g (*30*)), firefly squids (0.94 g/100 g (*30*)), fatty meat of bluefin tuna (6.41 g/100 g (*30*)), and other fish which contain high levels of polyunsaturated fatty acids. Consumer tests will be required to confirm our findings.

ABBREVIATIONS USED

DHA, 4,7,10,13,16,19-docosahexaenoic acid; EPA, 5,8,11,14, 17-eicosapentaenoic acid; PFBOA, *O*-(2,3,4,5,6-pentafluoro-benzyl)hydroxylamine; TBA, 2-thiobarbituric acid.

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