Determination of Hydrogen Peroxide in Fish Products and Noodles (Japanese) by Gas–Liquid Chromatography with Electron-Capture Detection

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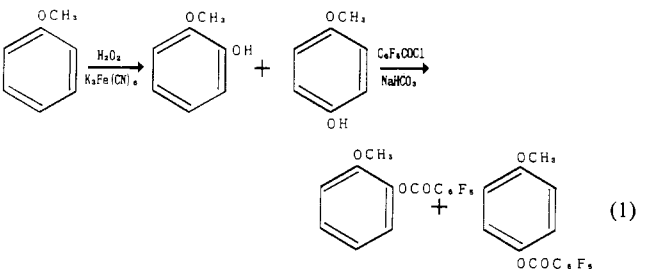
A sensitive and practical method for the determination of hydrogen peroxide (H_2O_2) in various fish products and noodles (Japanese) is described. The method is based on the gas chromatographic measurement of 2-hydroxyanisole, as its pentafluorobenzoyl (PFB) derivative, which is a product of the hydroxylation of anisole by reaction with H_2O_2 and potassium hexacyanoferrate(III) [$K_3Fe(CN)_6$] in the acid solution. The PFB derivative of 2-hydroxyanisole is determined by gas–liquid chromatographic separation on a 5% OV-17, 5% OV-210 mixed-phased column using an electron-capture detector (GLC-ECD). The calibration curve for H_2O_2 in the range 0.1–1.0 μg/mL is linear and sufficiently reproducible for quantitative determination; the detection limit for H_2O_2 is 0.06 μg/mL of sample extract. The coefficient of variation (n = 5) of the values determined is below 4.9%, and the average recovery rate throughout the procedure, including derivatization and gas–liquid chromatography, was above 87.1% in the fish products and noodles investigated. Although various possible metal ions and organic substances in the samples to be analyzed were tested at relatively high levels, all of them did not interfere with the production of 2-hydroxyanisole. The derivatization process was simple and rapid, requiring about 60 min, and the resulting derivative was stable for at least 1 week if stored under appropriate conditions.

INTRODUCTION

H_2O_2 is often the focus of clinical, environmental, and biological studies, and it is used in many industrial and related applications as an oxidizing, bleaching, and sterilizing agent. To avoid excessive use, the Ministry of Health and Welfare of Japan has set a tolerance limit which H_2O_2 in treated foods such as fish products and noodles must not exceed. The development of simple, sensitive, and accurate methods for the determination of trace amounts of H_2O_2 in various samples is therefore of great interest. There are numerous methods for determining H_2O_2, including color and absorption measurement, titration, fluorometry, electrochemical methods, chemiluminescence, and gas chromatography. However, the main techniques are based on enzymatic colorimetric methods (Guilbault et al., 1967; Guilbault and Brignac, 1968) using a combination of peroxidase or catalase as a catalyst and various indicator substrates, which are oxidized by H_2O_2 to yield a chromogen or fluorophore. One of the advantages of each of the methods described above is the specificity of the reaction because of the use of the enzyme as an analytical reagent. However, the oxidation of an indicator substrate by H_2O_2 catalyzed with peroxidase is easily influenced by the presence of reducing substances such as ascorbic acid (Nicholls and Shonbaum, 1963). Furthermore, in the case of the use of catalase the determinable amount of H_2O_2 is also influenced by the water content in the sample to be analyzed (Toyoda, 1982). On the other hand, the determination of H_2O_2 by gas chromatography was reported by Kobayashi and Kawai (1982). This highly sensitive method is based on the formation of *o*-pentafluorobenzyloxime after reaction with pentafluorobenzyloxylamine (PFBOA) and formaldehyde derived from the oxidation of methanol by H_2O_2 in the presence of catalase, followed by GLC-ECD. However, this method may be not suitable for the analysis of trace amounts of H_2O_2 because many investigators have reported that formaldehyde in samples such as fish meat (Amano et al., 1963; Amano and Yamada, 1964; Fish et al., 1956; Fujimaki et al., 1965; Kido et al., 1980; Soudan, 1961; Yamada and Amano, 1965; Yamada et al., 1969; Yoshida and Imaida, 1980; Vaisey, 1956) and fish products (Ishiwata and Tanimura, 1973; Yamanaka et al., 1979) is produced by the enzymic action or the oxidation of H_2O_2. In addition, another problem with their procedure is that it is somewhat troublesome to employ because the PFBOA derivatizing agent must be synthesized.

We have found that 2- and 4-hydroxyanisole can quantitatively be prepared by a reaction of H_2O_2 and anisole in the presence of $K_3Fe(CN)_6$ in acidic medium, followed by GLC-ECD after derivatization of both hydroxylated anisoles with pentafluorobenzoyl chloride (PFB-Cl) in a weak alkaline solution. These reaction steps can be summarized as



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However, both the quantitative production of 4-hydroxyanisole and the stability of its PFB derivative, compared with those of 2-hydroxyanisole, were somewhat poor. Therefore, H_2O_2 amounts in the sample were determined by measurement of the PFB derivative of 2-hydroxyanisole in this method.

This GLC method is a simple, highly sensitive, and practical means of determining H_2O_2 residues in the samples. The recovery of H_2O_2 added to various fish products and noodles was satisfactory. Furthermore, by use of the procedure, H_2O_2 residues were confirmed in commercial fish products and noodles.

MATERIALS AND METHODS

Reagents. A 0.3% solution of H_2O_2 was prepared by diluting a 30% stock solution (Tokyo Kasei Kogyo Co., Tokyo) with

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distilled water. This solution was further diluted to give working solutions with a concentration of 1.0 μg/mL and was prepared daily as a precaution for this study and was used within 30 min after preparation. The standards of anisole, 2-hydroxyanisole, 3-hydroxyanisole, and 4-hydroxyanisole (Tokyo Kasei Kogyo), were of analytical reagent grade. The PFB-Cl (99% pure) was obtained from Aldrich Chemical Co. (Milwaukee, WI) and was used without further purification. 1,2,3,4,5,6-Hexachlorocyclohexane (β-BHC; Wako Pure Chemical Co., Osaka, Japan), used as an internal standard in hexane, was prepared at a concentration of 1.0 μg/mL. $K_3Fe(CN)_6$ and other chemicals were of reagent grade or better, and all organic solvents used were of pesticide grade (Wako).

Equipment and Conditions. A Shimadzu GC-4BM gas chromatograph fitted with an ECD (^{63}Ni) was used to determine the PFB derivative of hydroxyanisole. A 1.5 m × 3 mm (i.d.) glass column was packed with the mixed-phase of 5% OV-17 and 5% OV-210 (13:10) on 80–100-mesh Chromosorb W (HP) (Nihon Chromato Kogyo, Tokyo). Column temperature was held at 150 °C; inlet and detector temperatures were 280 °C. Carrier flow (N_2) was 75 mL/min. The identification of the hydroxy derivative of anisole was achieved through gas chromatography–mass spectrometric analysis (GC–MS) performed on a Shimadzu GC-MS QP 1000A combined gas chromatograph–mass spectrometer. The gas chromatographic analyses were accomplished with 18% DC-550 on 80–100-mesh Chromosorb W (HP) with a column oven temperature of 170 °C. The operating parameters were as follows: helium flow, 30 mL/min; ion source temperature, 290 °C; separator temperature, 260 °C; trap current, 60 μA; ionizing voltage, 70 eV; accelerating voltage, 3.5 kV. In addition, identification of its PFB derivative was achieved by the same GC–MS operating parameters and the above mixed column at 150 °C.

Sample Preparation. An accurately weighed sample (generally about 10 g) of finely ground sample was placed in the 100-mL stainless steel container of a homogenizer (Ace type, Nihon Seiki Kaishia Co., Ltd.), 30 mL of buffer solution (0.2 M sodium acetate–acetic acid, pH 4.0) was added, and the mixture was homogenized at high speed for 5 min in an ice bath. The contents of the vessel, with rinsings, were transferred to a 50-mL centrifuge tube and centrifuged at 1400*g* for 10 min.

Analytical Procedure. A 4-mL aliquot of the supernatant solution was placed in a 10-mL test tube fitted with a ground-glass stopper, and subsequently 40 μL of anisole, 0.3 mL of 2% $K_3Fe(CN)_6$ solution, and 0.5 mL of acetic acid were added. After reaction at 60 °C with occasional shaking for 40 min in a water bath and cooling to room temperature, the reaction mixture was transferred into a 50-mL separating funnel, and then 5 mL of dichloromethane and 20 mL of distilled water were added. The mixture was shaken for 5 min, and the operations were further repeated. The combined dichloromethane layer (if necessary, it was centrifuged each time after extraction) was re-extracted with 5 mL of 0.1 M NaOH solution; the alkaline solution was washed with 10 mL of benzene. (This step was taken to eliminate the slight amount of dichloromethane that remained in the alkaline extract prior to taking the PFB derivative up in hexane after pentafluorobenzoylation for GLC-ECD.) The alkaline solution was further neutralized with 1 N HCl solution (about 5 drops), and then 5 mL of 8% $NaHCO_3$ solution and 40 μL of PFB-Cl were added. The mixture was shaken for 2 min and allowed to stand for 10 min and then extracted with 5 mL of the internal standard solution, separated, and dried with 1 g of anhydrous Na_2SO_4. A 2-μL volume of the solution was injected into the GLC-ECD. The contents of H_2O_2 in fish products and noodles were determined from the peak height ratio of the PFB derivative of 2-hydroxyanisole to β-BHC on the gas chromatograph and comparison with the calibration curve.

Calibration Curve. A series of standard H_2O_2 solutions were prepared by diluting the stock solution. Aliquots were placed in a test tube to give amounts of 0.1, 0.25, 0.5, 0.75, and 1.0 μg/mL of H_2O_2. According to the procedure described above, 10 mL of dichloromethane extracts was obtained in each instance and then re-extracted with 0.1 M NaOH solution. After pentafluorobenzoylation of addition of PFB-Cl and extraction, a 2-μL aliquot of the mixture was injected into the GLC column at 150 °C. As shown in Figure 1, the retention time of the PFB derivative of 2-hydroxyanisole relative to that of β-BHC was 0.57. The corresponding peak height ratios of the PFB derivative to β-BHC were plotted against the amounts of H_2O_2 analyzed.

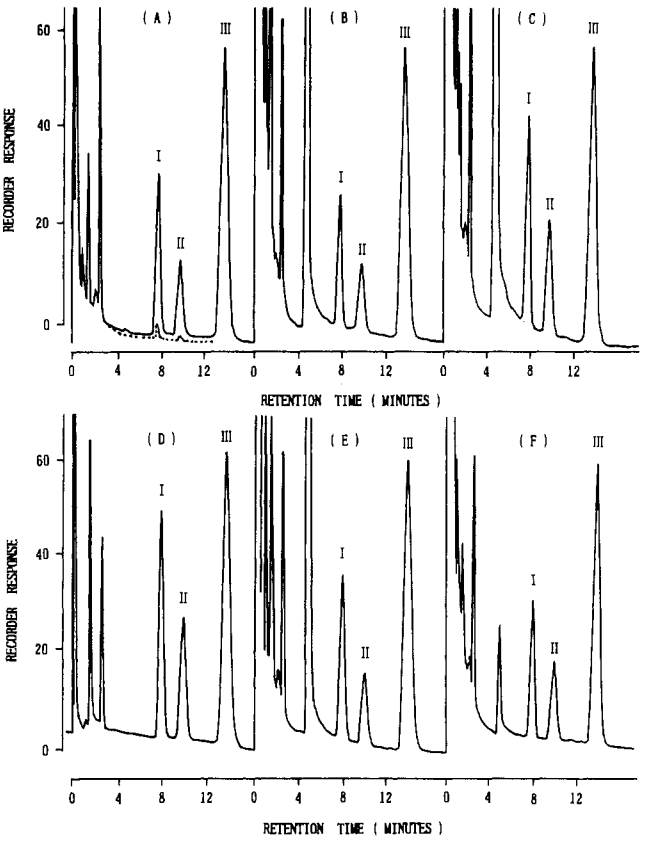

Figure 1. Gas chromatograms of derivatized extracts of a standard reaction mixture (A) to which H_2O_2 was added at level of 0.5 ppm, Japanese noodles (B), and various fish products (hanpen, C; narutomaki, D; Kamaboko, E; and chikuwa, F). Peaks: I, PFB derivative of 2-hydroxyanisole; II, PFB derivative of 4-hydroxyanisole; III, β-BHC. The dotted line in (A) shows the shape of the chromatogram obtained from H_2O_2-free sample solution. Reaction and GLC-ECD conditions are as in the text.

RESULTS AND DISCUSSION

To identify the product derived from a reaction of H_2O_2 and anisole, the dichloromethane extract obtained according to the described procedure was subjected to GC–MS. This resulting solution gave three single peaks with retention times of 1.2, 3.3, and 5.6 min on the gas chromatogram. Of these peaks, the peak with retention time of 1.2 min was in fair agreement with that of the standard anisole and the electron-impact (70 eV) mass spectrum with the parent peak at m/z 108 (M^+) obtained from this peak the same as that of anisole, as shown in Figure 2A. On the other hand, each mass spectrum obtained from the peaks with retention times of 3.3 and 5.6 min is shown in parts B and C, respectively, of Figure 2. As can be seen in either mass spectrum, the parent peak (M^+) is exhibited at m/z 124, and what is more surprising, significant difference between two fragmentation patterns, except the intensity of ions, was scarcely observed. Therefore, it could be presumed that such parent peak difference of these reaction products and anisole resulted from the replacement of anisole by a hydroxy group, and both products would perhaps be isomeric with each other. The confirmations of mass spectra obtained from these peaks were performed by comparison with those of the standards of 2-, 3-, and 4-hydroxyanisole using GC–MS. In con-

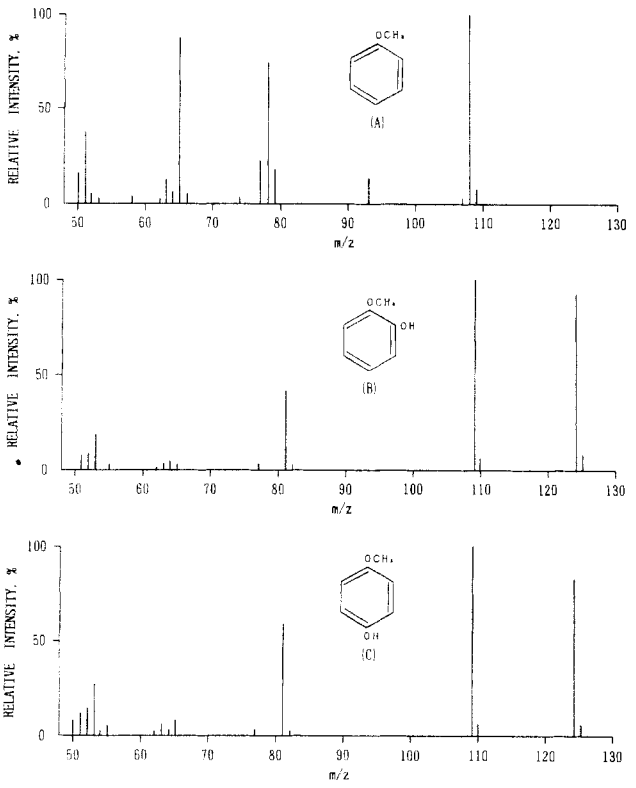

Figure 2. Mass spectra of anisole (A), 2-hydroxyanisole (B), and 4-hydroxyanisole (C). Ionization voltage 70 eV.

sequence, the peaks with retention times of 3.3 and 5.6 min on the gas chromatogram were in fair agreement with those of 2-hydroxyanisole for the former and 4-hydroxyanisole for the latter, respectively, but 3-hydroxyanisole was not. Furthermore, the fragmentation patterns of parts B and C in Figure 1 were the same as that with parent peak corresponding to the molecular weight of 2- and 4-hydroxyanisole, respectively. Therefore, the shift of peaks from m/z 124 to 53 for both hydroxyanisoles could be attributed to a characteristic phenol degradation compound [m/z 109 ($M^+ - CH_3$; a) 81 (a – CO; b), 53 (b – CO)]. From these results, it was concluded that the reaction products under the conditions previously described were 2- and 4-hydroxyanisole.

Further derivatization of hydroxyanisole with sufficient volatility and electron-capture response to permit its subsequent determination by GLC-ECD was considered a necessary step. Since phenol benzoates are briefly formed by Shotten–Baumann reaction from the phenol and benzoyl chloride in the presence of an alkaline, this most common approach was investigated with PFB-Cl. Salts such as NaOH, KOH, Na_2CO_3, and $NaHCO_3$ and these concentrations on the pentafluorobenzoylation were studied. The constant formation and the highest production for both PFB derivatives of the hydroxyanisoles were found only when $NaHCO_3$ was used in the concentration range 6–8%, although above 9% it gradually decreased, and these average yields (n = 4) for the standards of 2- and 4-hydroxyanisole at the level of 5 μg were almost quantitative, 97.3% for the former and 99.1% for the latter, respectively; an adequate volume of 8% $NaHCO_3$ solution was found to be 5 mL. In addition, the amount of PFB-Cl and the time of pentafluorobenzoylation for both hydroxy derivatives were investigated after proceeding to the hydroxylation stage as described under Analytical Procedure using 4.0 μg of H_2O_2 corresponding to 1 μg/mL of sample extract. The PFB-Cl amount was examined for 5, 10, 20, 40, 60, 80, and 100 μL with a reaction time of 2 min in a total volume of 5 mL of 8% $NaHCO_3$ solution. A minimum amount of 10 μL of PFB-Cl was required for complete pentafluorobenzoylation, so to ensure an excess of reagents, 40 μL of PFB-Cl was used. The amount of PFB-Cl was satisfactory if more H_2O_2 were present (for example, 10 μg of H_2O_2). Both PFB derivatives could be easily prepared in a short time, and the reaction time was found to be complete after 2 min at room temperature.

Various trials on the selection of the GLC column for the determination of both PFB derivatives were carried out as the following steps. Column packings of 5% (w/w) DC-200, DC-550, OV-17, OV-210, OV-101, OV-225, QF-1, SE-30, and XE-60 on Chromosorb W (HP) were tested. Although the columns, except OV-225, showed the peaks for both PFB derivatives, the use of a mixed-phased OV-17 and OV-210 (13:10) column (1.5 m) at 150 °C gave good peak characteristics and sensitivity. In addition, both PFB derivatives separated well with symmetrical peaks on the chromatogram, which allowed peak height to be used for calculations, and the retention times of the PFB derivatives of 2- and 4-hydroxyanisole relative to that of β-BHC as an internal standard were 0.57 and 0.72, respectively, as shown in Figure 1A. Under the conditions proposed for chromatographing, it was determined that the PFB derivatives of 2- and 4-hydroxyanisole would yield 45.8% and 28.0% of the full-scale deflection at $10^2 \times 16$ sensitivity, respectively, when 1 ng of each product was injected, and these sensitivities suggested that H_2O_2 may be measured reliably at the trace level. The PFB derivative of 2-hydroxyanisole was about 1.6 times more sensitive than that of 4-hydroxyanisole. After pentafluorobenzoylation, the n-hexane extract should be injected into the gas chromatograph as soon as possible; the PFB derivatives were stable for at least 1 week, provided they were kept at 5 °C in a refrigerator, but at room temperature after 4 days the contents of the PFB derivatives of 2- and 4-hydroxyanisole decreased to 98.3% and 64.6%, respectively. The PFB derivative of 4-hydroxyanisole gave a slightly yellow color, suggesting that it had gradually decomposed.

To determine whether these PFB derivatives had decomposed during the GLC process, the identities of both products prepared by the procedure described above were confirmed by GC–MS. A molecular ion peak with the expected m/z value (318; M^+) was observed for either PFB derivative, and other significant fragment ion peaks which were useful from structure elucidation were m/z 195 (base peak), 167, 123, 109, and 95 in a like manner. Significant difference between fragmentation patterns of either PFB derivative, except the intensity of each ion, was scarcely observed.

Varying conditions for quantitative reaction of H_2O_2 and anisole were studied next. A series of experiments was conducted with 4.0-μg quantities of H_2O_2 to establish the optimum reaction pH, time, temperature, and reagent amount required for maximum production of the hydroxy derivative. After hydroxylation and pentafluorobenzoylation according to the described procedure, two PFB derivatives were subjected to GLC-ECD. Since anisole in acidic solution was found to react with H_2O_2 to produce 2- and 4-hydroxyanisole in the presence of $K_3Fe(CN)_6$, derivatization reactions at various pH values were examined by adding all of the reagents, followed by heating at 60 °C for 40 min. As can be seen from Figure 3, a constant formation over the approximate pH range 1.5–4.0 was obtained with the yields (n = 5) of 41.3 ± 2.5% for 2-hydroxyanisole and 59.1 ± 7.1% for 4-hydroxyanisole. Above pH 4.5 both of them decreased in the same manner,

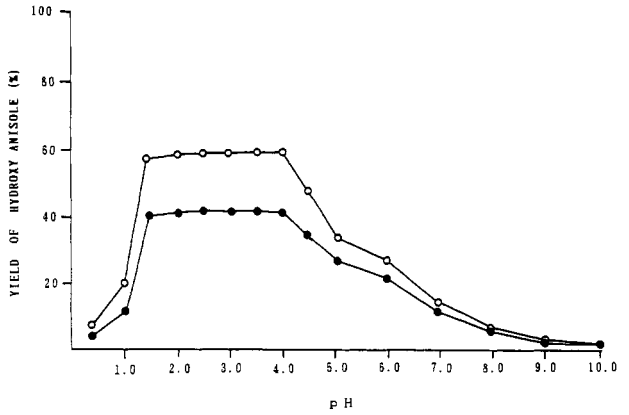

Figure 3. Effect of pH for the formation of 2-hydroxyanisole (●) and 4-hydroxyanisole (○) on the reaction with H_2O_2 and anisole. To 4.0 μg of H_2O_2 was added to 40 μL of anisole and 0.3 mL of 2% $K_3Fe(CN)_6$ solution at 60 °C for 40 min. Both hydroxyanisoles were determined by GLC-ECD after pentafluorobenzoylation, and yields of them were determined by comparison with the calibration curve, which was constructed from each PFB derivative after pentafluorobenzoylation of known amounts of authentic 2-hydroxyanisole or 4-hydroxyanisole. (Correlation coefficient was 0.999 for PFB derivative of 2-hydroxyanisole and 0.998 for that of 4-hydroxyanisole.) Reactions and GLC-ECD conditions are as for Figure 1.

suggesting that this hydroxylation would take place in positions 2 and 4 of the anisole at the same time. In our procedure, if 0.5 mL of acetic acid is added to the buffer solution (pH 4.0) as the extraction solvent, the pH of the solution is about 3.0. The addition of acetic acid was also preferable for an increase of the solubility of anisole in the sample extract. The efficiency of the derivatization reaction was also examined in relation to time, temperature, and reagent amounts. H_2O_2 and all of the reagents mixed in total of 4 mL of buffer solution were permitted to react for 10, 20, 40, 60, 90, and 120 min at 60 °C. Each yield similar to that described above for both hydroxy derivatives was obtained after 30 min of reaction and could not be increased by longer reaction time or increase in reagent concentration. Therefore, the reaction was found to be complete after 30 min; however, in practice, 40 min was used. Furthermore, although the yields of both hydroxy derivatives increased with an increase in reaction temperature, the reaction mixture subsequently darkened, and therefore the use of a long reaction time at high temperatures was not desirable; in practice, 60 °C was adopted as optimal. Subsequent experiments were examined for the influence of reagent quantities [$K_3Fe(CN)_6$ and anisole] on the hydroxylation of anisole. From these results, a minimum of 1.5 mg of $K_3Fe(CN)_6$ was required for complete reaction, and the additional amount of anisole in the range 10–50 μL was adequate as derivatizing agent in this method; to ensure an excess of reagents, 6 mg of $K_3Fe(CN)_6$ and 40 μL of anisole were used.

Various solvents such as ethyl acetate, benzene, chloroform, dichloromethane, diethyl ether, *n*-hexane, and toluene were tried for the extraction of both the hydroxy and PFB derivatives. When ethyl acetate, diethyl ether, and dichloromethane were used for the hydroxy derivative, the yields were high, but with *n*-hexane, the yield was low. However, dichloromethane was selected because it did not cause coextraction for various foods, and *n*-hexane was used for the extraction of the PFB derivatives for the same reason. Furthermore, as a shaking time of 1 min was the minimum for complete extraction of both hydroxy and PFB derivatives, mechanical shaking for 3 min was used.

Most workers have used extraction techniques with distilled water or methyl alcohol for separation of H_2O_2 from foods. Tanada et al. (1971) have reported that the use of distilled water led to a significant decomposition of H_2O_2 after extraction from foods, although methyl alcohol did not. So we tried methyl alcohol as extraction solvent but it gave poor yields for the hydroxylation of anisole. On the other hand, Ogata (1964) reported that H_2O_2 was most stable at about pH 4.0. Therefore, we chose 0.2 M sodium acetate–acetic acid buffer solution (pH 4.0) as reasonable extraction solvent for removing H_2O_2 from foods.

Table I. Effect for the Formation of 2-Hydroxyanisole and 4-Hydroxyanisole in the Presence of Various Substances or Ions

interfering ion or substance	interfering ion or substance concn, ppm	rel yield,[a] % 2-hydroxyanisole	rel yield,[a] % 4-hydroxyanisole
none	0	100.0	100.0
Na^+	100	90.3	104.9
K^+	100	108.9	98.3
Ca^{2+}	100	92.4	26.7
Mg^{2+}	100	97.8	96.7
Zn^{2+}	100	92.1	46.7
Cu^{2+}	20	105.6	104.0
Fe^{3+}	50	90.6	69.1
NH_4^+	250	94.4	89.8
Cl^-	250	109.7	104.9
PO_4^{3-}	100	89.3	103.3
SO_4^{2-}	100	108.1	98.5
S^{2-}	10	101.1	103.1
NO_2^-	10	89.9	88.8
NO_3^-	100	100.0	99.2
BrO_3^-	10	91.1	64.4
L-ascorbic acid	15	90.1	82.1
formaldehyde	15	105.3	103.1
succinic acid	100	103.5	107.1
L-(+)-tartaric acid	100	94.5	94.6
oxalic acid	100	109.9	153.3
citric acid	100	97.6	100.0
butylhydroxytoluene	30	105.6	154.9
butylhydroxyanisole	30	108.9	102.3
sorbic acid	50	98.9	102.4

[a] Each value represents an average of duplicate analyses.

Next our concern was the extent to which any of various ions or substances might inhibit the reaction. So tests were done with various possible interfering ions or substances that actually occur in the sample extract. Four micrograms of H_2O_2 was added to 40–1000 μg of various ions or substances, 40 μL of anisole, and 0.3 mL of 2% $K_3Fe(CN)_6$ solution in a total of 4 mL of buffer solution, and each mixture was analyzed by GLC-ECD after the hydroxylation and pentafluorobenzoylation. From the data in Table I, it is evident that the tested substances affected the yields of 2- and 4-hydroxyanisole somewhat differently. For 2-hydroxyanisole, 10 of the substances reduced the yield to less than 95%, but only 2 reduced it below 90%. For 4-hydroxyanisole, eight of the substances reduced the yield below 95%, but more importantly, seven of these eight reduced it below 90%, sometimes far below. However, it should be kept in mind that these ions or substances are present at relatively high levels. The method described was tested on the analysis of fish products and noodles, and the results of analysis of the sample digest by GLC-ECD before and after the formation of the PFB derivatives are shown in Figure 1B–E. The derivatized extract obtained from various samples gave gas chromatograms with good peak characteristics.

For the GLC-ECD assay using the described procedure, a linear relationship between peak height and amount of H_2O_2 could be found in the concentration range 0.1–1.0 μg/mL of working solution by using β-BHC as an internal

Table II. Recovery Rate for H_2O_2 Added to Various Foods

added level,[a] ppm	av recovery, % ± SD					
	noodle (udon)	boiled fish paste (kamaboko)	boiled fish paste (hanpen)	fish jelly product (narutomaki)	rolled fish cake (chikuwa)	overall
0.2	87.2 ± 3.3	90.9 ± 2.2	87.1 ± 4.2	88.8 ± 3.8	89.6 ± 3.0	88.7 ± 3.4
0.5	94.7 ± 2.8	91.4 ± 2.9	93.2 ± 1.8	97.1 ± 5.6	95.1 ± 5.6	94.3 ± 3.6
1.0	97.3 ± 3.0	95.5 ± 2.5	93.0 ± 3.1	93.3 ± 0.9	96.9 ± 5.4	95.2 ± 3.5
overall mean ± SD	93.1 ± 5.2	92.6 ± 3.2	91.1 ± 4.2	93.1 ± 5.1	93.9 ± 4.8	

[a] H_2O_2 was added to 4 mL of each sample solution prepared with buffer solution.

standard. This suggested that β-BHC is suitable as an internal standard. A calibration curve constructed by the peak height of the PFB derivative of 2-hydroxyanisole had a correlation coefficient of 0.999 ith the reproducibility to be satisfactory for quantitative determination, whereas a calibration curve constructed by the peak height of the PFB derivative of 4-hydroxyanisole had a correlation coefficient of 0.703. Although elucidations of these experimental results were not attempted in detail here, they will be reported in the future. Therefore, in this method the measurement of the PFB derivative of 2-hydroxyanisole was preferable for the determination of H_2O_2 in the real-world samples.

On the basis of these results, to evaluate the reproducibility of the method and the efficiency of the recovery of H_2O_2 from various foods, known amounts of H_2O_2 added to 4-mL extracts of food prepared with buffer solution were determined by performing five replicate assays on each of the foods listed in Table II. H_2O_2 amounts calculated from the PFB derivative of 2-hydroxyanisole gave a reasonable coefficient of variation below 4.9%. Furthermore, the recoveries that were determined on the basis of the PFB derivative of 2-hydroxyanisole ranged from 87.1% to 97.3% with an average of 92.7%, as shown in Table II. Somewhat lower recoveries were obtained from each food level of 0.2 ppm. Although rigorous recovery studies were not carried out at levels below 0.1 ppm, a distinguishable small interfering peak, depending on reagent, was noted at the retention time of the PFB derivative of 2-hydroxyanisole on the gas chromatogram (see Figure 1A), and this peak was equivalent to about 0.03 ppm of H_2O_2 sample extract. Therefore, 0.06 ppm, on the basis of twice the background interference, was noted as the detection limit for H_2O_2 of this method. Reduction of the blank values as reported by Kobayashi and Kawai (1982) will be the subject of a future study.

On the other hand, standards were injected before each sample until a constant response was obtained and after each sample to ensure reproducibility; syringes were cleaned between samples with alcoholic KOH and subsequently rinsed with methyl alcohol, ethyl acetate, and *n*-hexane to eliminate previously reported (LeBel and Williams, 1979) contamination problems. This was especially important when switching from high- to low-concentration solutions.

H_2O_2 contents of different foods determined by the present method are summarized in Table III. A preliminary survey of various fish products and noodles purchased commercially was carried out to obtain an indication of presence of H_2O_2 residue in foods. From the results, it can be seen that all of the foods investigated contain relatively small amounts of H_2O_2 (ranging from 0.10 to 3.10 ppm). H_2O_2 contents of these foods obtained were somewhat higher than the range reported by Toyoda (1982). The results demonstrate that our method is effective in detecting the trace amount of H_2O_2 residue in various foods. The most significant advantage of the method presented is that the GLC-ECD analysis is simple and has good sensitivity and would probably be applicable to microassay in other food, environmental, and biological samples along with H_2O_2, although this was not tested in this work. Furthermore, derivative preparation is simple, and the derivatives formed are stable for a relatively long time. Therefore, it is concluded that the method can be proposed as a new alternative for the analysis of fish products and noodles containing H_2O_2 residue.

Table III. H_2O_2 Contents of Various Japanese Noodles and Fish Products Obtained from Commercial Sources

sample no.	kind of food	brand	H_2O_2 content,[a] ppm
1	noodle (udon)	A	0.10
2		B	0.25
3		C	0.10
4		D	0.50
5		E	0.20
6	boiled fish paste (kamaboko)	A	0.75
7		A	1.01
8		B	0.30
9		C	2.30
10		D	0.10
11	boiled fish paste (hanpen)	A	0.20
12		B	0.10
13		C	0.10
14		D	0.10
15		E	0.30
16	fish jelly product (narutomaki)	A	0.59
17		B	2.50
18		C	3.10
19		C	0.30
20		D	0.72
21	rolled fish cake (chikuwa)	A	0.20
22		B	0.55
23		C	0.10
24		D	0.50
25		E	0.10

[a] Each value represents an average of duplicate analyses.

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