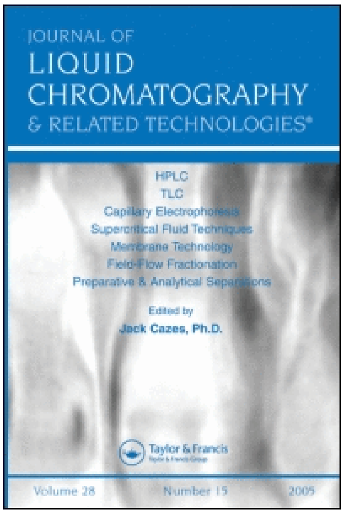

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DETERMINATION OF BENZOYL PEROXIDE IN WHEAT FLOUR BY ION CHROMATOGRAPHY WITH PRECOLUMN DERIVATIZATION

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ABSTRACT

A novel method for the determination of benzoyl peroxide bleach in wheat flour by using high performance anion exchange chromatography (HPIC) after decomposition to benzoic acid was proposed. Benzoyl peroxide was effectively extracted from wheat flour with absolute ethanol by ultrasonication under neutral condition and converted to benzoic acid in the presence of potassium hydroxide solution. The chromatographic separation of benzoic acid was performed on a Dionex OmniPac PAX-100 separation column and the determination by ultraviolet absorbance detection at 222 nm. Good linearity between the concentration of benzoyl peroxide and peak area response was achieved in the range of 0.12-20 μg/mL. The detection limit (signal-to-noise ratio 3:1) was 19 ng/mL for benzoyl peroxide. The method has been successfully applied for the analysis of real samples, and the average recoveries for various samples ranged from 81 to 92%.

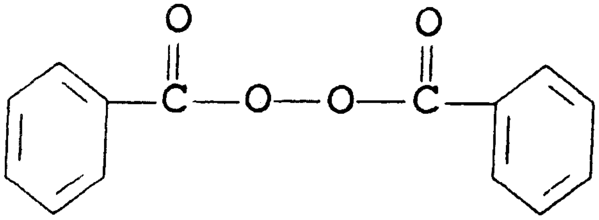

Figure 1. The structure of benzoyl peroxide.

INTRODUCTION

Benzoyl peroxide has been widely used as a flour bleacher in the food industry in many developed countries including the United States and the United Kingdom (the molecular structure of benzoyl peroxide is illustrated in Figure 1).[1] It can give an effective bleach when added to flour in an appropriate proportion.

The Chinese regulations permit the use of benzoyl peroxide in the manufacture of flour below the ratio of 0.3 g/Kg,[2] and in practical application the usage is generally not more than 0.06 g/Kg.[3]

The methods for the determination of benzoyl peroxide bleach in flour were rarely reported in the literature. The official final action AOAC method was based on the reduction of the bleach to benzoic acid in an acidified ether solution and the quantification of benzoic acid was carried out by colorimetry.[4]

Gas chromatography was also used for the determination of benzoyl peroxide after decomposition to benzoic acid in cheese[5] and wheat flour.[6] Besides, benzoyl peroxide in wheat flour could be determined by reversed-phase thin-layer chromatography in combination with UV detection.[7] All these methods were tedious and time-consuming that contained multi-step extraction and clean-up procedures, thus not suitable for the routine analysis.

To our knowledge, no ion chromatographic method for the determination of benzoyl peroxide has been reported. In this paper, a relatively simple method was proposed for the determination of benzoyl peroxide bleach in wheat flour by using high performance anion exchange chromatography (HPIC) in combination with precolumn derivatization, and applied to the analysis of various real samples with satisfactory results.

MATERIALS AND METHODS

Apparatus

A Dionex Model DX-500 ion chromatograph (Sunnyvale, CA, USA) equipped with a 50 μL sample loop was employed along with a Dionex PeakNet chromatography workstation for instrument control as well as data acquisition and processing.

The chromatographic separation was performed by a Dionex OmniPac PAX-100 separation column, and the detection by a Dionex AD20 absorbance detector with detection wavelength set at 222 nm. The eluent was 3 mmol/L sodium carbonate + 2% methanol with a flow rate of 1.0 mL/min.

The spectrophotometric experiments were carried out by using a Shimadzu UV-120-02 spectrophotometer (Kyoto, Japan) with 1 cm quartz cells.

Reagents

The benzoyl peroxide stock solution (0.5 mg/mL) was prepared by dissolving accurately 0.1000 g of benzoyl peroxide (purity: >98%, obtained from Xuchang Pharmaceutical Factory, Henan Province, China) in absolute ethanol and diluted to 200 mL with the same solvent. The working solution (15 μg/mL) was prepared by dilution of the stock solution with absolute ethanol weekly. The other reagents were of analytical reagent grade or higher purity. Distilled deionized water was used throughout.

Preparation of Calibration Graphs

Into 25 mL volumetric flasks 0.2, 0.4, 1, 5 mL of benzoyl peroxide working solution (15 μg/mL) as well as 0.6, 1 mL of benzoyl peroxide stock solution (0.5 mg/mL) were transferred and 2 mL of 0.1 mol/L KOH was added. All the solutions were mixed by ultrasonication for 2 min at room temperature, diluted to volume with water and set aside overnight.

The solutions were injected into the HPIC system for calibrations. All these solutions were prepared freshly. In this study, the peak area measurements for all calculation were carried out.

Procedure

All the samples including two ordinary wheat flours (Sample A and B), two dumpling flours (Sample C and D) and one special flour for stuffed buns (Sample E) were purchased from the local market. Approximately 1 g of flour sample was weighed accurately into a 100 mL dried stopper-attached conical flask, and 8.0 mL of absolute ethanol was added. After the stopper was capped, the solution was shaken vigorously by wrist action for 1 min and then extracted by ultrasonication for 15 min at room temperature. The suspension was transferred completely into a screw cap polyethylene plastic conical centrifuge tube with a disposable pipette, and centrifuged at 4000 rpm for 20 min. Four milliliters of supernatant was transferred accurately into a 25 mL volumetric flask, and 2 mL of 0.1 mol/L KOH was added. The solution was mixed in an ultrasonic water bath for 2 min at room temperature, diluted to volume with water and set aside overnight. The final sample solution was injected into the HPIC system after filtering through a 0.45 μm filter.

RESULTS AND DISCUSSION

Selection of Separation System

From the theoretical point of view, it is very difficult to determine benzoyl peroxide directly by ion chromatography because of its strong hydrophobicity that was also confirmed by our experiments. The most commonly used method for the determination of benzoyl peroxide bleach in flour was based on its decomposition reaction to benzoic acid, and the latter could be detected by various methods.[4-6] As an organic weak acid which dissociation constant is 6.46×10^{-5},[8] benzoic acid can be determined by ion chromatography.[9-15] Hence benzoyl peroxide can be determined indirectly through the quantification of benzoic acid after precolumn derivation reaction. Because benzoyl peroxide is a strong organic oxidant and liable to explode, in practical application it is always used as a mixture in which it has been diluted with some inorganic fillers,[1] such as carbonate or phosphate along with sulfate.[3] In order to avoid these potential interferences, firstly, a Dionex IonPac ICE-AS6 separation column on which the retention mechanism is ion exclusion was employed. Because under this condition, these inorganic strong acid anions were all eluted out within void volume. Unfortunately, benzoic acid was retained so strongly on this column that it could not be eluted out by using commonly used eluents such as diluted sulfuric acid + methanol, which may be caused by its weak acidity together with its strong hydrophobicity.

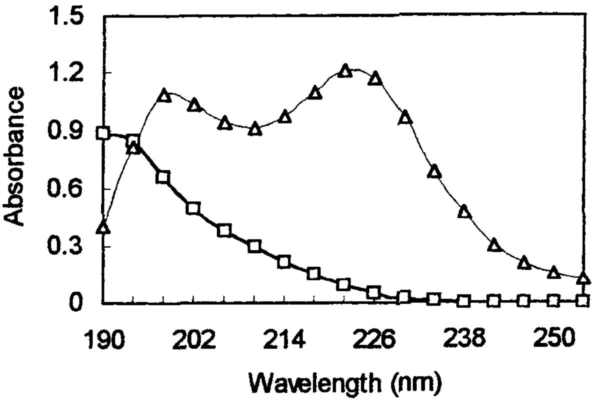

Figure 2. The absorption curves of 20 μg/mL benzoic acid (Δ) against eluent blank and of eluent (□) against water. Eluent: 3 mmol/L sodium carbonate + 2% methanol. Spectrophotometric measurements were performed by using a Shimadzu UV-120-02 spectrophotometer with 1 cm quartz cells.

To date, all the ion chromatographic methods for the determination of benzoic acid were HPIC,[9-15] and the Dionex OmniPac PAX-100 anion exchange separation column is designed to separate a variety of aromatic acids including benzoic acid.[16] In order to wet the hydrophobic substrate on this column and maintain the maximum column performance, it is essential that the eluent always has, at a minimum, 1% organic solvent such as methanol.[16]

So, in this study, a Dionex OmniPac PAX-100 separation column was employed and 3 mmol/L Na_2CO_3 + 2% methanol was used as eluent for separation of benzoic acid.

Under the specified experimental conditions, benzoic acid can be eluted out within a very short time, and the carbonate anion in real samples within the void volume. In addition, phosphate as well as sulfate anions did not possess ultraviolet characteristic. So these anions did not interfere with the determination of benzoic acid.

Selection of Detection Wavelength

Ultraviolet absorbance detection is the first choice for the detection of benzoic acid in HPIC because its conductivity signal is very low. Figure 2 shows the absorption curve of benzoic acid against eluent blank obtained by using a spectrophotometer. The absorbance of benzoic acid reaches a maximum at 222 nm at which the background absorbance of eluent is very low. Hence 222 nm was chosen for the further study.

Study on Precolumn Derivation Reaction

So far, the most conventional decomposition reaction of benzoyl peroxide to benzoic acid that can be used for practical application was the reaction with potassium iodide in an acidic matrix[6,7] that was rapid, simple, and complete. But the complete elution of the excess iodide anion in the decomposition reaction required at least 35 min under the experimental conditions, though iodide anion did not interfere with the detection of benzoic acid. If potassium iodide + methanol solution was chosen as eluent, this problem could be avoided, and the excess iodide anion, as reductant, could be eluted within void volume resulting in an apparent system peak in chromatogram.

On the other hand, because the ultraviolet absorption of iodide in eluent was very strong in the range of 190-250 nm, the detection sensitivity of analyte of interest (benzoic acid) became very poor. Hence the decomposition reaction was not studied further. It was commonly considered that anionoid reagents such as trialkylammonium could react with benzoyl peroxide similar to iodide.[17] In addition, several basic (NaOH, KOH and Na_2CO_3) and acidic solutions (H_2SO_4 and acetic acid), along with reductant solution ($FeSO_4$), were employed to decompose benzoyl peroxide to benzoic acid.[6] Preliminary experiments showed that a precipitation occurred in the benzoyl peroxide solution when ascorbic acid or oxammonium hydrochloride reductant solution was added, and the mechanism was not yet clear.

In this study, the effects of various precolumn derivation reagents and reaction time on the decomposition ratio of benzoyl peroxide to benzoic acid were studied as follows: 1 mL of 0.5 mg/mL benzoyl peroxide stock solution was transferred into a 25 mL volumetric flask and 1 mL of 0.1 mol/L derivation reagent was added; the solution was mixed in an ultrasonic water bath for 2 min at room temperature prior to dilution to volume with water. The decomposition ratios were calculated by comparing the peak area responses of benzoic acid, converted from benzoyl peroxide, with the peak area responses of equivalent benzoic acid working solution (19.8 μg/mL). The detailed results are summarized in Table 1.

We can find that the decomposition ratios increase by increasing the reaction time for all reagents and after 0.5 d, the decomposition ratio remains approximately constant except for sodium carbonate. The incomplete

Table 1

Effect of Precolumn Derivatization Reagent and Reaction Time on the Decomposition Ratio of Benzoyl Peroxide to Benzoic Acid

Time	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min	2 H	3 Hr	4 Hr	0.5 D	1 D	2D	3 D
Ammonium hydroxide	33.89	36.90	39.29	42.43	43.88	46.77	51.58	58.15	58.00	58.56	59.00	58.46	60.53
Trimethylammonium	38.53	42.76	44.95	46.91	49.00	52.05	62.49	64.21	64.97	68.21	69.46	71.24	70.32
TMAOH[a]	57.35	59.40	59.67	60.96	61.76	61.01	62.69	63.63	63.73	88.90	89.69	89.71	87.28
TPAOH[b]	54.76	56.15	56.09	56.91	60.15	59.69	63.15	65.40	76.76	89.79	90.33	89.38	87.46
TBAOH[c]	55.24	56.07	57.09	55.30	57.62	57.67	60.88	64.73	75.20	89.16	89.14	87.85	86.62
Na_2SO_3	58.48	64.02	65.81	67.26	68.94	70.85	76.09	82.02	85.60	85.37	83.96	82.84	82.79
Na_2CO_3	52.76	52.90	53.14	54.39	54.13	54.99	57.36	58.53	60.66	69.05	75.44	83.96	82.63
KOH	60.04	60.96	62.24	62.13	62.98	62.70	67.93	70.44	73.98	89.81	88.81	88.68	88.58

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[a] TMAOH = Tetramethylammonium hydroxide

[b] TPAOH = Tetrapropylammonium hydroxide

[c] TBAOH = Tetrabutylammonium hydroxide

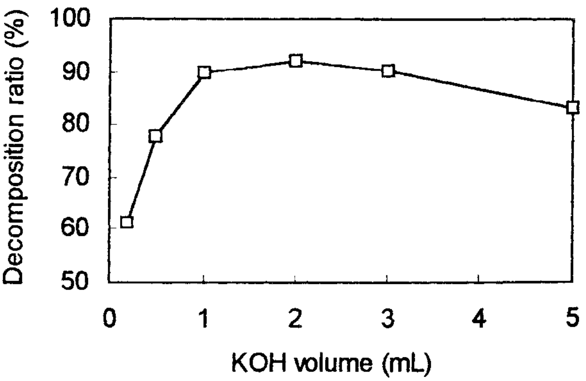

Figure 3. The effect of addition volume of 0.1 mol/L KOH on decomposition ratio of benzoyl peroxide. The experimental conditions as the text.

conversion of benzoyl peroxide to benzoic acid can be explained by the small amounts of carbon dioxide, benzene, phenyl benzoate, and biphenyl which may also be formed in reaction.[5] These compounds were retained strongly on the separation column, except carbon dioxide. Therefore, the separation column should be washed by 50 mmol/L potassium hydroxide + 50% methanol for 30 min when the analysis is completed per day to avoid the increase of column pressure and the loss of column efficiency.

From the viewpoint of decomposition reaction, sodium sulfite seemed to be the most suitable derivation reagent because it could reach its constant within the shortest time. However, the excess of sulfite anion, which possesses ultraviolet characteristic, should be eluted out from the separation column though it did not interfere with the detection of benzoic acid, and it needed at least 10 min. Finally, 0.1 mol/L of potassium hydroxide was preferred and the reaction time was chosen as 0.5 d. Additionally, the effect of the addition volume of 0.1 mol/L potassium hydroxide on the decomposition ratio was also studied and the results are shown in Figure 3. Hence 2.0 mL of potassium hydroxide was used in all experiments.

Selection of Extraction Solvent

The extraction solvents which were used in the above-mentioned method[4-7] were ether solvents. The procedures were tedious and time-consuming because multi-step clean-up operations were necessary after solvent

Table 2

Analysis of Real Samples

Sample	Content[a] (μg/g)	Added (μg)	Recovery (%)[b]
A	16.28 ± 0.62	60	81.81 ± 2.53
		300	83.63 ± 0.90
		500	85.65 ± 1.28
B	20.60 ± 0.89		
C	29.69± 1.27	60	89.84 ± 2.22
		300	86.24 ± 1.46
		500	91.32 ± 1.21
D	6.13 ± 0.38		
E	24.94 ± 0.73		

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[a] Average of four determinations ± standard deviation.
[b] Average of six determinations ± standard deviation.

extraction to remove fat-soluble interfering materials in foods which could also dissolve in ethers. Because benzoyl peroxide can dissolve in absolute ethanol,[18] in which fat solubility was poor, in this study, the absolute ethanol was used as extraction solvent for a trial. The operation of clean-up was not needed because the fat-soluble interfering materials were reduced and the recoveries for spiked real samples were satisfactory. Therefore, absolute ethanol was adopted as extraction solvent.

Linearity, Precision and Detection Limits

Under the optimized experimental conditions, linear relationship between the concentrations (0.12-20 μg/mL) of benzoyl peroxide and peak area responses gave a correlation coefficient of 0.9998. The detection limit for benzoyl peroxide was 19.0 ng/mL at a signal-to-noise ratio of 3. That is to say, 0.00095 g of benzoyl peroxide in per kg of flour can be detected, which indicates that the proposed method is suitable for the routine analysis. The precision was evaluated by performing seven replicate analyses of benzoyl peroxide working solutions which had been done as the solutions for calibration graphs. The relative standard deviations were 0.62% and 0.49% when the concentrations were 12 μg/mL and 20 μg/mL, respectively.

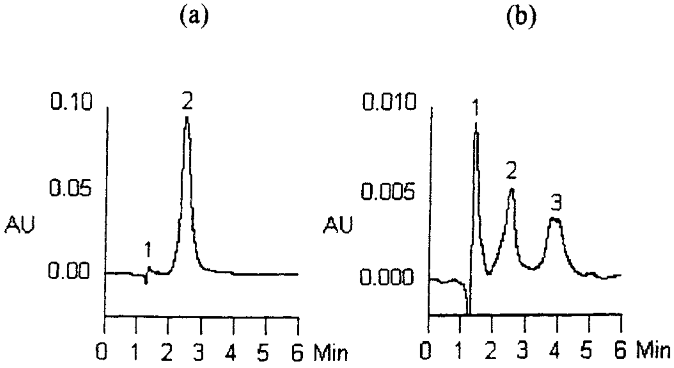

Figure 4. The chromatograms of 12 μg/mL benzoyl peroxide standard solution which was used for calibrations (a) and of Sample C final solution (b). Peaks: 1 = System peak; 2 = Benzoic acid; 3 = Unknown peak.

Analysis of Real Samples

All the samples were analyzed in accordance with the optimum experimental conditions. The results obtained are summarized in Table 2 and the chromatogram of Sample C final solution shown in Figure 4.

We can find that the amounts of benzoyl peroxide added in all flours do not exceed the Chinese regulation. Besides, spike recovery studies were carried out by using 1 g of Sample A and C, and the results also shown in Table 2 are satisfactory.

CONCLUSIONS

A novel ion chromatographic method for the determination of benzoyl peroxide bleach in flour, based on the precolumn derivatization reaction, with ultraviolet absorbance detection has been developed. It has been applied to the analysis of real samples with satisfactory results.

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REFERENCES

1. D. J. Wallington, "Cereals and Cereal Products," in **Food Industries Manual**, M. D. Ranken, R. C. Kill, eds., Blackie Academic & Professional, London, 1993, pp. 169-170.

2. National Standard of the People's Republic of China, GB 2760-86, Hygienic Standards for Food Additives in Use, 1986.

3. Z. G. Liu, Y. X. Gao, **Fundamental Food Additives**, the Chinese Light Industry Publishing House, Beijing, 1994.

4. K. Helrich, **Official Methods of Analysis of the Association of Official Analytical Chemists**, Association of Official Analytical Chemists, Inc., Arlington, 1990.

5. A. B. Karasz, F. DeCocco, J. J. Maxstadt, J. Assoc. Off. Anal. Chem., **57**, 706-709 (1974).

6. S. Ogawa, H. Suzuki, M. Toyoda, Y. Ito, M. Iwaida, Nippon Shokuhin Kogyo Gakkaishi, **31**, 356-359 (1984).

7. K. X. Wang, R. C. Zang, Z. Y. He, Fenxi Huaxue, **20**, 19-22 (1992).

8. D. R. Lide, **CRC Handbook of Chemistry and Physics**, CRC Press, Inc., Boca Raton, 1992.

9. Y. Hoshino, H. Saitoh, K. Oikawa, Bunseki Kagaku, **32**, 273-276 (1983).

10. S. Matsushita, Y. Tada, N. Baba, K. Hosako, J. Chromatogr., **259**, 459-464 (1983).

11. K. Fung, D. Grosjean, Anal. Lett., **17**, 475-482 (1984).

12. K. Fujimura, M. Tsuchiya, Bunseki Kagaku, **37**, 56-59 (1988).

13. G. Havner, D. K. Pannell, J. G. Tarter, J. Liq. Chromatogr., **12**, 299-305 (1989).

14. Y. Zhu, Fenxi Huaxue, **19**, 313-316 (1991).

15. Z. F. Gao, C. G. Fu, Fenxi Ceshi Xuebao, **13**, 32-36 (1994).

16. Installation Instructions and Troubleshooting Guide for the OmniPac PAX-100 Analytical Column, Dionex Corporation, Sunnyvale, CA, 1991.

17. K. Tokumaru, Chem. Lett., (**4**), 523-526 (1992).

18. D. R. Lide, G. W. A. Milne, **Handbook of Data on Organic Compounds**, CRC Press, Inc., Boca Raton, 1994.

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