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Rapid Spectrophotometric Methods for the Determine Benzoyl Peroxide from the Wheat Flour

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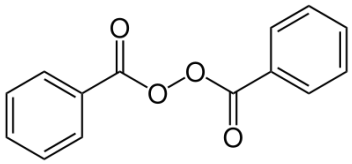
ABSTRACT

New spectrophotometric method for the determine Benzoyl peroxide from the wheat flour was developed recently. Here in this reaction potassium iodide oxidized by Benzoyl peroxide, it generates coloured iodine. There was maximum absorption peak in 580 nm wavelenghth. Potassium iodide system determine Benzoyl peroxide(Result). Other methods for the detection is based on Benzoyl peroxide reacted with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to obtain a blue-green colored product that was detected at 415 nm by spectrophotometry. In phosphoric acid medium potassium iodide oxidized by benzoyl peroxide oxidation then generated colored iodine and starch there was maximum absorption peak in 585 nm wavelength there by the starch potassium iodide system spectrophotometry was established to determine benzoyl peroxide. Hu et al. have developed method for naked eye detection of benzoyl Peroxide from wheat flour using 3,3',5,5'-tetramethylbenzidine as a chromogenic agent. they have invented naked eye detection of benzoyl peroxide in wheat flour using 3,3′,5,5′-tetramethylbenzidine as a chromogenic agent. Dugan et al.[12] have inventd spectrophotometric determination method for microgram amounts of lauroyl and benzoyl peroxide, And after that we can know absorbance from spectrophotometer. Ju.H,2013 Have obtained superior precision (relative standard deviation<2%) using 11 repeatability at 0.2 mg/L 0.6 mg/L, and 0.8 mg L. The proposed methodology was successfully applied to determine BPO in wheat flour samples. This method has been used for quantification of BPO in flour samples.

Keywords: Spectrophotometer, Benzoyl peroxide, Wheat flour, ABTS, TMB, KI.

I. INTRODUCTION

Benzoyl peroxide (BPO) is an organic compound in the peroxide family. It consists of two benzoyl groups bridged by a peroxide link. Its structural formula is $(C_6H_5CO)_2O_2$. It is one of the most important organic peroxides in terms of applications and the scale of its production. Benzoyl peroxide is used as an acne treatment, for bleaching flour, hair and teeth, for cross-linking polyester resins, and for many other purposes. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.



uses

Other common uses for benzoyl peroxide include

- Bleaching hair
- Tooth whitening systems
- The preparation of bleached flour
- As a convient oxidant in organic chemistry

- An initiator and catalyst for polyester thermoset resins, as an alternative to the much more hazardous methyl ethyl ketone peroxide.
- A hardener in order to start the polymerization process in resins. For instance, PMMA resins can be polymerized with benzoyl peroxide.
- Removing ink and dye stains on vinyl dolls.
  In the U.S., the typical concentration for benzoyl peroxide is 2.5% to 10% for both prescription and over-the-counter drug preparations that are used in treatment for acne. Higher concentrations are used for hair bleach and teeth whitening. Benzoyl peroxide, like most peroxides, is a powerful bleaching agent. Contact with fabrics or hair can cause permanent color dampening almost immediately. Even secondary contact can cause bleaching; for example, contact with a towel that has been used to wash off benzoyl peroxide-containing hygiene products.[11]

Side effect

Concentrated benzoyl peroxide is potentially explosive like other organic peroxides, and can cause fires without external ignition. The hazard is acute for the pure material, so the compound is generally used as a solution or a paste. For example, cosmetics contain only a small percent of benzoyl peroxide and pose no explosion risk.

The carcinogenic potential of benzoyl peroxide has been investigated. A 1981 study published in the journal *Science* found that although benzoyl peroxide is not a carcinogen, it does promote cell growth when applied to an initiated tumor. The study concluded, "caution should be recommended in the use of this and other free radical-generating compounds".

In a 1977 study using a human maximization test, 76% of subjects acquired a contact sensitization to benzoyl peroxide. Formulations of 5% and 10% were used.
The U.S. National Institute for Occupational Safety and Health has developed criteria for a recommended standard for occupational exposure to benzoyl peroxide, benzoyl peroxide (BPO) has been used for over 50 years as a bleaching agent inflour, whey processing and milk for Italian cheese making. It was used for bleaching flour and cheese at concentrations of up to 40 mg/kg, while bleaching of Cheddar cheese whey has been done successfully using 20 mg/kg BPO and holding for an hour at 60-63 °C. As benzoyl peroxide is almost totally converted (> 91%) to benzoic acid during cheese making and any remaining traces would further be reduced by processing of whey. Therefore the intake assessment should be made on the additional benzoic acid incorporated in the diet from the use of benzoyl peroxide to bleach whey. JECFA has evaluated the use of BPO as a bleaching agent in flour and concluded that treatment at concentrations up to 40 mg/kg was acceptable (WHO, 1964) & as per FSSAI guideline we can use maximum 40 p.p.m. benzoyl peroxide in wheat flour (or in Maida). Moreover, at the 59th meeting JECFA concluded that benzoyl peroxide was of "no safety concern" when used as a flavouring agent (based on current levels of intake) (WHO, 2002).

Concentration of benzoyl peroxide commercially used is much lower than 100 mg/kg. Only 15% of the world's cheese production is coloured and hence is subject to use BPO. Besides, not all of the coloured whey undergoes bleaching process before drying.

II. METHODS OF STUDYING

1. First Method

Here, from the study of different determination methods, have check samples of wheat flour which was borrowed from the market & have checked Benzoyl peroxide's level by different spectrophotometric methods. In the different methods researchers have used different chromogenic agents like 3-ethylbenzothiazoline-6-sulfonic(ABTS) , TMB(tetra methyl benzidine) , Adapalene etc. Here we have used common solvent-KI for the determine Benzoyl peroxide , these

method will be used wide because KI is common solvent & it is available easily. It is common iodometry reaction. Here spectrophotometer is used for the determine new method.Proposed methodology The proposed methodology was successfully applied to determine Benzoyl peroxide in wheat flour samples. This method has been used for quantification of Benzoyl peroxide in flour samples. The sample preparation procedures are described in the Materials and methods section. In order to eliminate the matric interference, ethanol was utilized as extraction solvent for the real sample. Because of inorganic salts, starch and fat are poorly dissolved in ethanol that is used as the extraction solvent and detection media. The samples were spiked with Benzoyl peroxide standard at different concentrations. The quantification of Benzoyl peroxide content depends on the formation of cation radical. However, there are non oxidative agents or other bleaching agents such as ammonium per sulfate, calcium phosphate, nitrogen dioxide, chlorine dioxide, nitrogen dichloride, and calcium peroxide that can be affected by the determination of Benzoyl peroxide by the approach method. Because the solubility of inorganic salts were poor in ethanol extract solutions other peroxides cannot be reacted with KI without adding other enzyme peroxidases. The detectable reaction of the proposed method for examination of Benzoyl peroxide in flour samples is based on redox reaction between KI and Benzoyl peroxide in an alcoholic medium.

Non additive wheat flour (flour blank) and flour samples were purchased from kalupur local markets in Ahmedabad, Gujarat, India . All samples were stored at 4^0C until prepared for quantitative assay. Flour sample (0.5 g) and spiked samples were transferred to a centrifuge tube and 5 mL of ethanol was added. Then, sonication of the sample for 5 minutes was completed followed by shaking of the solution for 5 minutes with a vortex mixer. The supernatant was collected after being centrifuged at 2000 rpm for 15 minutes.

The analytical procedures for the quantification of Benzoyl peroxide were started by pipette 10 mL of extracted solution to 100 ml volumetric flask. Then, 10 ml of 3 M KI solution was added. Finally, the solution was made up to 10 ml with 94% ethanol and the solution was reacted for 1 minute. The solution developed from blue to dark blue or violete color immediately without any catalysts, which provided maximum absorbance at 580 nm. The content of Benzoyl peroxide in the real

sample was calculated using the linear regression equation of standard curve.

Afterward, the 94% ethanol was utilized to adjust volume to 10 mL.

2.Second method

A simple, rapid, and sensitive spectrophotometric method for the determination of benzoyl peroxide (BPO) in wheat flour samples was developed. The detection principle is based on BPO reacted with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to obtain a blue-green colored product that was detected at 415 nm by spectrophotometry. The effect of factors influencing the color reaction was investigated.

3. Third method

Hu et al.[10] have developed method for naked eye detection of benzoyl Peroxide from wheat flour using 3,3',5,5'-tetramethylbenzidine as a chromogenic agent. they have invented naked eye detection of benzoyl peroxide in wheat flour using 3,3′,5,5′-tetramethylbenzidine as a chromogenic agent In this work, here first discovered that 3,3′,5,5′-tetramethylbenzidine (TMB) can be used as a novel chromogenic agent for sensitive, convenient and rapid determination of benzoyl peroxide (BPO) in wheat flour & take the reading in spectrophotometer at 450-580 nm.

4. Fourth method

Dugan et al.[12] have inventd spectrophotometric determination method for microgram amounts of lauroyl and benzoyl peroxide. A rapid, simple colorimetric method is presented for the quantitative estimation of microgram quantities of lauroyl peroxide and benzoyl peroxide. The reaction appears to be N,N-dimethyl-p-phenylenediamine sulfate + methanol → colored complex, which reaction is catalyzed in the presence of the named peroxides. Since it is a rate reaction, time and temperature must be held constant. Here after that we have taken results in spectrophotometer at 450nm to 580nm & note the absorbance value.

5. Fifth method

Ju.H,2013 Have obtained superior precision (relative standard deviation<2%) using 11 repeatability at 0.2 mg/L 0.6 mg/L, and 0.8 mg L. The proposed methodology was successfully applied to determine BPO in wheat flour samples. This method has been used for quantification of BPO in flour samples. In order to eliminate the matric interference, ethanol was utilized as extraction solvent for the real sample. Because of inorganic salts, starch and fat are poorly dissolved in ethanol that is used as the extraction solvent and detection media. The samples were spiked with BPO standard at different concentrations. The quantification of BPO content depends on the formation of ABTS cation radical. However, there are non oxidative agents or other bleaching agents such as ammonium per sulfate, calcium phosphate, nitrogen dioxide, chlorine dioxide, nitrogen dichloride, and calcium peroxide that can be affected by the determination of BPO by the approach method. Because the solubility of inorganic salts were poor in ethanol extract solutions other peroxides cannot be reacted with ABTS without adding other enzyme peroxidases. Therefore, the high percentage recoveries between 87-104% and 88-109% were obtained for the proposed method and HPLC method, respectively.

III. RESULTS & DISCUSSION

1. METHOD

The detectable reaction of the proposed method for examination of Benzoyl peroxide in flour samples is based on redox reaction between liberated KI and starch in an alcoholic medium. After Benzoyl peroxide was reduced by a chromogenic agent such as KI. It became benzoate anion. Therefore, the possibility of this color reaction was shown. The complex of violet colour formed because of starch & iodine. Here maximum absorption peak we have observed at 580 nm & maximum absorbance was 0.24 at 30 ppm. The calibration graph was created by plotting absorbance (y-axis) with concentration of Benzoyl peroxide in mg/L (x-axis). Here 10ppm to 30 ppm sample sets were prepared & got the linear regression with r^2=0.8.

Repeatable results have also got.

Table 1

Number	concentration	Absorbance
1	10 ppm	0.7
2	20 ppm	1.2
3	30 ppm	2.4
4	40 ppm	2.3
5	50 ppm	1.8

Table 2

Concentration	Absorbance
10 PPM	0.64
20 PPM	1.32
30 PPM	2.41
40 PPM	2.37
50 PPM	1.75

2. METHOD

Under the selected conditions, the linear range for quantification of BPO was observed between 0.2 - 1.0 mg / L with R^2= 0.998. The limit of detection (LOD)

was 0.025 mg/ L. The developed method obtained superior precision (relative standard deviation<2%) using 11 repeatability at 0.2 mg / L, 0.6 mg/ L and 0.8 mg/ L. The proposed methodology was successfully applied to determine BPO in wheat flour samples.

Non additive wheat flour (flour blank) and flour samples werepurchased from local markets in Maha Sarakham Province, Thailand. All samples were stored at 4 ^{0} c until prepared for quantitative assay. Flour sample (0.5 g) and spiked sample were transferred to a centrifuge tube and 5 mL of ethanol was added. Then, sonication of the sample for 5 minutes was completed followed by shaking of the solution for 5 minutes with a vortex mixer. The supernatant was collected after being centrifuged at 4070gfor 10 minutes. 2.4

The analytical procedures for the quantification of BPO were started by pipette 1000 mL of extracted solution to 10 mL volumetric flask. Then, 1000mL of 100 mg /l ABTS was added. Finally, the solution was made up to 10 mL with 96% ethanol, and the solution was reacted for 1 minute. The solution developed from light-green to a green color immediately without any catalysts, which provided maximum absorbance at 415 nm. The content of BPO in the real sample was calculated using the linear regression equation of standard curve.

Calibration graph

The calibration graph for the determination of BPO was investigated in the range 0.2e1.0 mg / L by dilution with appropriate volume of 100 mg/ L standard BPO between 20mL and 100mL followed by adding 1000mL of 100 mg /L ABTS.

Afterward, the 96% ethanol was utilized to adjust volume to 10 mL. The calibration graph was created by plotting absorbance (y-axis) with concentration of BPO in mg / L(x-axis). The detectable reaction of the proposed method for examination of BPO in flour samples is based on redox reaction between ABTS and BPO in an ethanol medium. After BPO wasreduced by a chromogenic agent such as TMB or ABTS, it became benzoate anion. Therefore, the possibility of this color reaction was shown in Fig. 1. Fortunately, this colorimetric reaction can occur without any catalysts. This reaction is rapid and simple. ABTS was oxidized by BPO as a strong oxidizing agent to provide ABTS radical cation as a blue-green color product. From the previous reports, the ABTS chromophore consists of conjugate dp-double bonds system in molecule. Therefore, it absorbs certain wavelengths at visible light. The characteristic strong absorption peak at 380 - 435 nm (yellow-green color) and board absorption peak from 650 nm to 780 nm (blue-green color) was observed as presented. In order to achieve high sensitivity, the concentration of BPO in the sample can be quantified by measuring the absorbance at 415 nm. Because the highest molar extinction coefficient for ABTS radical cation at 415 nm and less background absorption at this wavelength.

3. Method

A simple colorimetric method based on the color reaction between TMB and BPO was developed. Factors influencing the color reaction were examined. Under the optimum conditions, the calibration linear range for detecting BPO was 0.67–16.00 μg mL^{-1} with a detection limit of 0.45 μg mL^{-1} in aqueous solution. The method was successfully applied to detect BPO in wheat flour, with recoveries between 97% and 118%. More significantly, the existence of BPO can be evaluated by the naked eye easily without the aid of any instrumentation and requiring no sample preparation operation. TMB was proved to be an efficient and robust chromogenic reagent for the detection of BPO in wheat flour.

4. Method

Under controlled conditions the color intensity developed obeys Beer's law as the peroxide concentration is varied from 5 to 30 μg. per ml. in the case of benzoyl peroxide, and from 10 to 100 μg. per ml. in the case of lauroyl peroxide. Here we have got

the linear range of absorbance from 0.20 to 0.41 so we can evaluate the R^2=0.9.

5. Method

The chromatograms of standard BPO 50 µg/g, one sample spiked with standard BPO 90 µg/g and without spiked BPO are illustrated. It was found that the retention time of benzoyl peroxide was 9.750±0.016. The other compounds and/or other flour-bleaching agent were not co eluted with BPO. Wheat flour samples were analyzed by the HPLC method for comparison, The obtained results from both methods were in good agreement, which evaluated by t-test at the 95% confidence limit indicating that there is no significant difference between the two methods. Possibility of colour reaction. The detectable reaction of the proposed method for examination of BPO in flour samples is based on redox reaction between ABTS and BPO in an ethanol medium. After BPO was reduced by a chromogenic agent such as TMB or ABTS, it became benzoate anion. Therefore, the possibility of this color reaction was shown in Fig. 1. Fortunately, this colorimetric reaction can occur without any catalysts. This reaction is rapid and simple. ABTS was oxidized by BPO as a strong oxidizing agent to provide ABTS radical cation as a blue-green color product. From the previous reports the ABTS chromophore consists of conjugate dp-double bonds system in molecule. Therefore, it absorbs certain wavelengths at visible light. The characteristic strong absorption peak at 380-435 nm (yellow-green color) and board absorption peak from 650 nm to 780 nm (blue-green color) was observed. In order to achieve high sensitivity, the concentration of BPO in the sample can be quantified by measuring the absorbance at 415 nm. Because the highest molar extinction coefficient for ABTS radical cation at 415 nm and less background absorption at this wavelength. The colorimetric reaction for the determination of BPO using ABTS as the chromogenic reagent was successfully developed. This procedure provided a simple, rapid, and sensitive method for the determination of BPO in wheat flour samples. The results were satisfactory when compared with the HPLC method. Therefore, the proposed method is an alternative procedure for application to determine BPO in real flour samples.

IV. CONCLUSION

The colorimetric reaction for the determination of BPO using ABTS as the chromogenic reagent was successfully developed. This procedure provided a simple, rapid, and sensitive method for the determination of BPO in wheat flour samples. The results were satisfactory when compared with the HPLC method. Therefore, the proposed method is an alternative procedure for application to determine BPO in real flour samples. Many determination methods used in different modes. Different methods have different agents used in it & it have its own cheractoristic property. Freshly milled wheat flour has a pale yellow color due to its carotenoids content. Benzoyl peroxide is a bleaching agent typically used to give such flour a better appearance. Benzoyl peroxide has been used for over 50 years as a bleaching agent in flour. Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the use of Benzoyl peroxide as a bleaching agent in flour and concluded that treatment at concentrations up to 40 mg/kg was acceptable (WHO, 1964). The colorimetric reaction for the determination of Benzoyl peroxide using KI as the chromogenic reagent was successfully developed. This procedure provided a simple, rapid, and sensitive method for the determination of Benzoyl peroxide in wheat flour samples. The results were satisfactory when compared with the other methods. Therefore, the proposed method is an alternative procedure for application to determine Benzoyl peroxide in flour samples.

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