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Original Article

A rapid and sensitive spectrophotometric method for the determination of benzoyl peroxide in wheat flour samples

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ABSTRACT

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. Under the selected conditions, the linear range for quantification of BPO was observed between 0.2–1.0 mg L^{-1} with $r^2 = 0.998$. The limit of detection (LOD) was 0.025 mg L^{-1}. The developed method obtained superior precision (relative standard deviation < 2%) using 11 repeatability at 0.2 mg L^{-1}, 0.6 mg L^{-1}, and 0.8 mg L^{-1}. The proposed methodology was successfully applied to determine BPO in wheat flour samples.

1. Introduction

Benzoyl peroxide (BPO) is widely used as an initiator and catalyst for polymerization processes [1–4]. Moreover, it is commonly used as a food additive in flour, resulting in a bright white flour color, because of its bleaching property [5]. BPO has been used as an acne treatment because it works as a peeling agent. It increases skin turnover, clears pores, and reduces bacterial count (specifically *Propionibacterium acnes*) as well as acts directly as an antimicrobial [6,7].

An excessive BPO can not only annihilate nutrients in flour, but it has effects on human health especially when BPO is in flour. It can decompose into benzoic acid and other

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deleterious substances, such as biphenyl and phenylbenzoate, that cause tissue damage and diseases [8,9].

The specific maximum concentration of BPO in food is 0.05 g kg^{-1} in the USA and UK [10]. In 2009, the Codex Alimentarius Commission determined that the amount of BPO in wheat flour was < 75 mg kg^{-1} [9]. Recently, BPO has been strictly forbidden as an additive to flour in the European Union and China (since May 1, 2011) [8]. Hence, a rapid, sensitive, and selective methodology for quantification of BPO in food samples needs to be developed.

There are many analytical methods for determining BPO. The Association of Official Analytical Chemists method [11] is the standard method for quantification of BPO in wheat flour. This method is based on dissociation of BPO to benzoic acid in the presence of iron and hydrochloric acid as the catalyst. The benzoic acid product is detected by colorimetric reaction. Even though this method is simple, it has low sensitivity for measuring the analyte. Chromatographic techniques such as liquid chromatography [12,13], high-performance liquid chromatography (HPLC) [14–19], gas chromatography [20], capillary electrophoresis [21], and ion chromatography [22] have been developed and used for the detection of BPO in flour samples. Therefore, chromatographic method can determine many analytes simultaneously [23]. Moreover, these methods are provided high accuracy and high precision. Flow injection analysis (FIA) is an alternative method for the determination of BPO, and it is rapid, automatic, and has a high sample throughput [24–26]. Generally in FIA, the reagents continuously flow in narrow tubing aided by the operation of a peristaltic pump in order to obtain a continuously generated baseline, which results in a lot of wasted generations. Chemiluminescence [10,27–29], fluorescence [8,30], differential pulse voltammetry [31], and amperometry [6,32] have been reported as useable methods and highly sensitive for determining BPO. Colorimetric reactions based on chromogenic reagents such as *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPDA) [24,33], *N*-ethyl-2-naphthylamine (NENA) [34], *N,N*-dimethyl-*p*-phenylenediamine [35], iodine in acidic medium [26], ferric thiocyanate [36], β-cyclodextrin [37], and 3,3′,5,5′-tetramethylbenzidine (TMB) [9] have been developed for the determination of BPO. These methods provide high sensitivity and selectivity, low detection limit, and high precision and accuracy. However, to the best of our knowledge, the use of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a chromogenic reagent has not been presented for BPO detection.

ABTS is a chromogenic substrate for enzyme peroxidase activity measurement similar to TMB which can be oxidized by hydrogen peroxide in the presence of peroxidase enzyme as a catalyst. The color of the solution changes from light yellow-green to a blue-green color. The maximum absorption wavelength (λ_{max}) was observed at 415 nm. Therefore, a novel method of utilizing ABTS as a reagent detectable by spectrophotometry for BPO in food samples was investigated.

2. Materials and methods

2.1. Reagents and chemicals

The chemical reagents used throughout this study were analytical grade and utilized without any further purification. The deionized water was purified by Milli-Q, Millipore apparatus. ABTS was obtained from Sigma-Aldrich (Sigma-Aldrich,

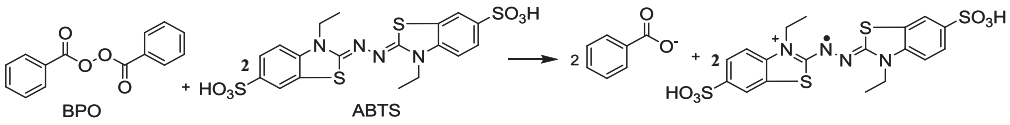

Fig. 1 – The possibility reaction of the proposed system.

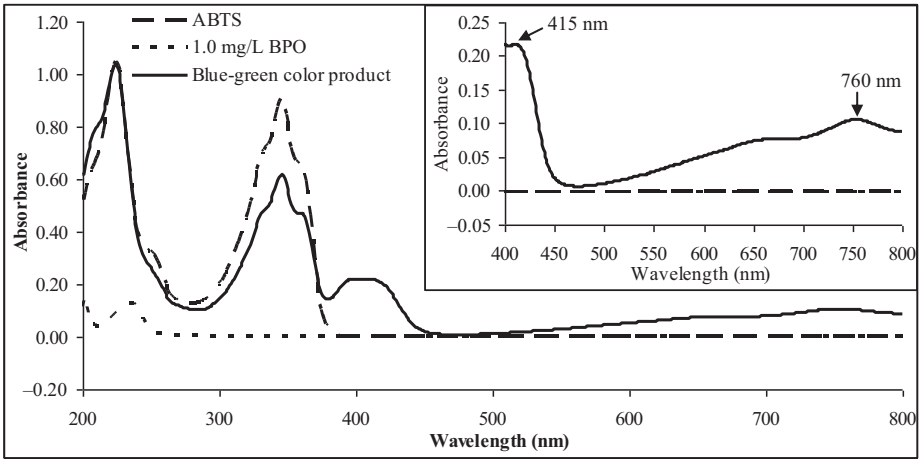

Fig. 2 – Absorption spectra of 1.0 mg L^{-1} BPO (dotted line), ABTS 10 mg L^{-1} (broken line) and the blue-green color solution after mixed 1.0 mg L^{-1} BPO with 10 mg L^{-1} ABTS (unbroken line). ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BPO = benzoyl peroxide.