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Rapid determination and chemical change tracking of benzoyl peroxide in wheat flour by multi-step IR macro-fingerprinting

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ABSTRACT

BPO is often added to wheat flour as flour improver, but its excessive use and edibility are receiving increasing concern. A multi-step IR macro-fingerprinting was employed to identify BPO in wheat flour and unveil its changes during storage. BPO contained in wheat flour (<3.0 mg/kg) was difficult to be identified by infrared spectra with correlation coefficients between wheat flour and wheat flour samples contained BPO all close to 0.98. By applying second derivative spectroscopy, obvious differences among wheat flour and wheat flour contained BPO before and after storage in the range of 1500–1400 cm^{-1} were disclosed. The peak of 1450 cm^{-1} which belonged to BPO was blue shifted to 1453 cm^{-1} (1455) which belonged to benzoic acid after one week of storage, indicating that BPO changed into benzoic acid after storage. Moreover, when using two-dimensional correlation infrared spectroscopy (2DCOS-IR) to track changes of BPO in wheat flour (0.05 mg/g) within one week, intensities of auto-peaks at 1781 cm^{-1} and 669 cm^{-1} which belonged to BPO and benzoic acid, respectively, were changing inversely, indicating that BPO was decomposed into benzoic acid. Moreover, another autopeak at 1767 cm^{-1} which does not belong to benzoic acid was also rising simultaneously. By heating perturbation treatment of BPO in wheat flour based on 2DCOS-IR and spectral subtraction analysis, it was found that BPO in wheat flour not only decomposed into benzoic acid and benzoate, but also produced other deleterious substances, e.g., benzene. This study offers a promising method with minimum pretreatment and time-saving to identify BPO in wheat flour and its chemical products during storage in a holistic manner.

1. Introduction

Benzoyl peroxide (BPO) used as flour improver for the first time posted on the commission for approval and formulated the ADI value of 0–40 ppm, at the 16th FDA/WHO in 1983. Part of BPO added to wheat flour reacted with water under the action of air and enzyme in flour. The reaction product oxygen destroyed carotenoid pigment though oxidation reaction to whiten the flour [1]. Simultaneously, BPO converted into benzoic acid for preservation. FDA declared that bleaching ingredients (including BPO) may be added in a quantity not more than sufficient for bleaching. Health Canada stipulated that the content of BPO in wheat flour added less than 150 mg/kg. The Ministry of Health, Labour and Welfare of Japan stipulated that BPO should be mixed with one or more of the following: potassium alum, calcium sulfate, magnesium carbonate, sodium aluminum sulfate, calcium phosphate, starch, and calcium carbonate. The content should be less than 300 mg/kg. The Ministry of Health in China allowed the use of BPO as wheat flour improver and the content should be less than 300 mg/kg in 1986 and be reduced to 60 mg/kg in 1996 for deterring abuse. But since May 2011, it is prohibited to add BPO as food additives in wheat flour. With the progress of variety improvement and flour processing technology, the technology has been able to across the consumers' needs of white degree [2–4]. With the improvement of living standards, consumers required wheat flour to keep its original color, aroma, taste and nutrition for pursuing of natural health and as much as possible to reduce the intake of chemical substances [5].

At present, some previous studies have applied high-performance liquid chromatography (HPLC) [6–8], mass spectrometry, ion chromatography [9], chemiluminescence method [10,11], electrochemical method [12] and fluorescence method to determine BPO in wheat flour. These approaches could reach low limit of detection for quantitation but could not achieve rapid and non-destructive qualitative analysis simultaneously.

Fourier transform infrared spectroscopy (FT-IR) has an advantage of macroscopic identification of complex system as a whole and combined with second derivative spectroscopy (SD-IR) and two-dimensional infrared correlation spectroscopy (2DCOS-IR) can not only reveal the

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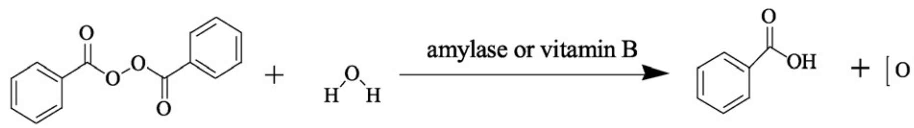

Fig. 1. Formation of benzoic acid and active oxygen by the reaction between BPO and H_2O with amylase and vitamin B [16]. Vitamin B as a composition of coenzyme A.

main constituents in sample but also distinguish the varieties and contents of chemical constituents in highly similar matrices [13–15]. In this work, we adopted a multi-step IR macro-fingerprint approach to directly identify BPO in wheat flour and track the changes of BPO in wheat flour during storage and monitor the chemical change of BPO under heating conditions for comprehensive BPO determination in wheat flour.

2. Experimental

2.1. Apparatus and materials

Thermo Scientific Nicolet iS5 FT-IR, in 400–4000 cm^{-1} rang with a resolution of 4 cm^{-1}. Spectra were recorded at 16 scans. Micro vortex mixer. Brown bottle. Drying oven.

BPO was purchased from Sinopharm Chemical Reagent Co. Limited. Wheat flour samples were purchased from wheat flour producer to avoid additives.

2.2. Pretreatment

0.5 mg and 3.0 mg BPO were mixed with 10 g wheat flour, respectively, in brown bottles, then sealed and blended for 30 min with micro-vortex mixing apparatus. The final concentration of samples was 0.05 mg/g and 0.30 mg/g, respectively, and then samples were stored in the bottles in drying oven for further use.

2.3. Procedure

Samples were pulverized to around 200 meshes. The FT-IR spectra of the samples were collected at room temperature by single-point ATR. The pressure of the ATR-FTIR acquisition was 70 ± 2 psi. Second derivative IR spectra were obtained after 7-point Savitsky–Golay smoothing of original IR spectra.

To obtain 2DCOS-IR spectra, 1–2 mg of each sample was blended with KBr powder, grounded, and pressed into a tablet. The prepared tablet was put into the sample pool of a temperature controller. The temperature range was from 30 °C to 60 °C with a heating rate of 2 °C/min. The dynamic original spectra at different temperatures were collected at an interval of 5 °C and then processed by SpectraCorr software to obtain 2DCOS-IR spectra. The temperature of moving-window 2DCOS-IR spectra of BPO was from 30 °C to 100 °C.

3. Results and discussion

3.1. IR spectra of wheat flour with BPO

Generally, part of BPO turned into benzoic acid and reactive oxygen atoms under the action of amylase and vitamin B with water molecules (Fig. 1). But the peaks in the spectra of wheat flour containing BPO before and after storage in the concentration of 0.30 mg/g were similar to wheat flour spectrum (Fig. 2a). There was no evident peak showed corresponding to BPO or benzoic acid (Fig. 2 & Table 1).

3.2. Second derivative IR spectra of flour with BPO

Second derivative spectroscopy can enhance the apparent resolution of IR spectrum or even separate previously overlapped peaks [15,18]. Second derivative IR spectra in Fig. 3 showed the differences concerned with the range of 1440 cm^{-1}–1460 cm^{-1} among wheat flour, wheat flour containing BPO before and after storage, BPO and benzoic acid. There were two peaks which belonged to the spectra of wheat flour which had similar intensity in the range of 1440–1460 cm^{-1}, which belonged to frame vibration of benzene ring. When wheat

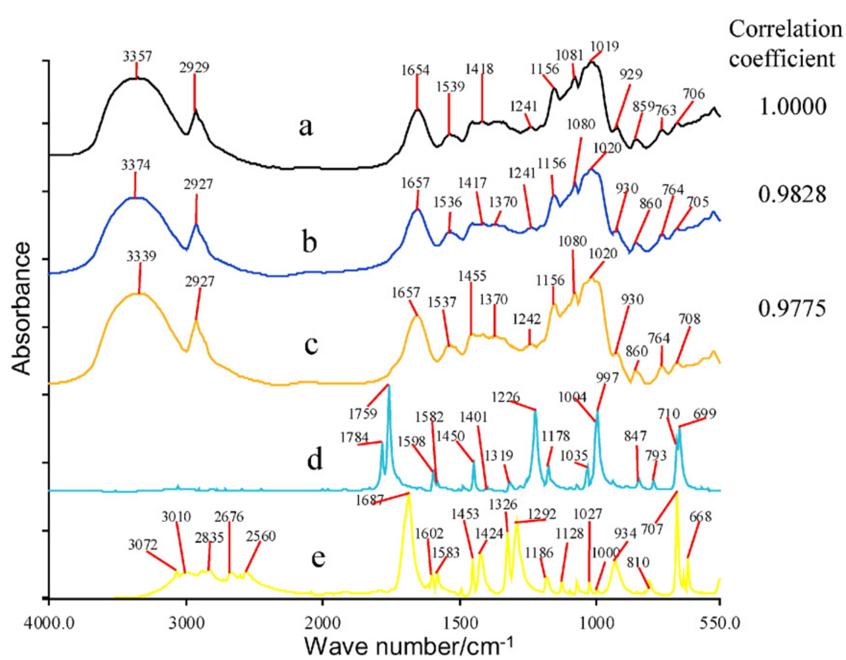

Fig. 2. IR spectra of wheat flour (a), wheat flour containing BPO before storage (b), and wheat flour containing BPO after storage (c) in the concentration of 0.30 mg/g, BPO (d) and benzoic acid (e).