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Parameter Selection for Detection Benzoyl Peroxide

Effective Detection of Benzoyl Peroxide in Flour - Based on Parameter Selection of Raman Hyperspectral System

Xiaobin Wang\(^1\), Chunjiang Zhao\(^1\), Wenqian Huang\(^2\), Qingyan Wang\(^2\), Chen Liu\(^2\), Guiyan Yang\(^2\)

\(^1\)College of Information and Electrical Engineering, Shenyang Agricultural University, Shenyang, China
\(^2\)Beijing Research Center of Intelligent Equipment for Agriculture, Beijing, China
\(^3\)National Research Center of Intelligent Equipment for Agriculture, Beijing, China
\(^4\)Key Laboratory of Agri-informatics, Ministry of Agriculture, Beijing, China
\(^5\)Beijing Key Laboratory of Intelligent Equipment Technology for Agriculture, Beijing, China

Corresponding author Chunjiang Zhao E-mail: zhaocj6404@126.com

Abstract

Penetration depth and spatial resolution of Raman hyperspectral imaging system were studied for effective detection of benzoyl peroxide in flour. The determinations of parameters were achieved by using the single band background-correct image of a benzoyl peroxide Raman characteristic band and a simple threshold method. The selected parameters were used to detect mixture samples with different concentrations. Percentage of detected benzoyl peroxide pixels was positively correlated to its concentration. The result shows that parameters selected in this study are effective for the detection of benzoyl peroxide additive in flour and can be used for quantitative analysis in the future.
KEYWORDS: Raman hyperspectral system; Penetration depth; Spatial resolution; Benzoyl peroxide; Flour

INTRODUCTION

Flour is one of the main food ingredients in the world, and its products are known for their color, taste, and aroma. There is a high requirement on the degree of whiteness in flour, and at the same time, most consumers judge the quality of flour based on how they see the color. The whiter and finer the flour and its products, the more they are favored by the consumer. Therefore, flour whiteness a key area of attention for wheat processing enterprises.\textsuperscript{[1,2]} Because wheat contains lutein, most freshly milled flour is yellowish in color. The conjugated double bond in the lutein molecule is a chromophore and absorbs light. As it slowly oxidizes in air, the color fades gradually. This process requires a long storage period, often lasting up to several months, which is not commercially feasible.\textsuperscript{[3–5]}

In order to meet the needs of consumers, flour processors widely use brightening agents to increase the degree of whiteness of flour, and benzoyl peroxide (BPO) is a commonly used flour brightening agent. BPO is a strong oxidant that quickly oxidizes the lutein in flour to fade the yellow color of flour and make it appear whiter.\textsuperscript{[6]} However, excessive BPO addition can destroy some existing nutrients such as carotene, vitamin A, vitamin B, vitamin E, among others. Long-term consumption of whitened flour will cause vitamin
deficiency, resulting in certain diseases such as angular cheilitis, neuritis, and in severe cases, it may cause cumulative damage to the central nervous system, liver failure, and others.\textsuperscript{[7,8]}

Currently, methods used to detection BPO additives in flour include gas chromatography (GC),\textsuperscript{[9]} high-performance liquid chromatography (HPLC)\textsuperscript{[10]} and liquid chromatography - mass spectrometry (LC-MS).\textsuperscript{[11]} These are traditional methods that are relatively high in detection precision. However, these methods are generally limited to laboratories due to complicated pre-processing, long detection time, and requirement of the skilled operators. With the development of spectroscopy, some spectroscopy methods, such as infrared spectroscopy,\textsuperscript{[12,13]} fluorescence spectroscopy,\textsuperscript{[14,15]} Terahertz spectroscopy\textsuperscript{[16,17]} and Raman spectroscopy,\textsuperscript{[18,19]} have been adequately applied for the detection of BPO additive in flour. These spectroscopy methods are single-point detection, and the spatial coverage does not meet the demand associated with whole samples.

Raman hyperspectral imaging technology is a highly fusion method that combines Raman spectral technology and hyperspectral imaging technology, encompassing the advantages of both methods.\textsuperscript{[20–23]} In addition to obtaining the Raman spectra, a visualizable result is also obtained. This technology is widely applied to the detection of food additives and dopants.\textsuperscript{[24]} Dhakal et al.\textsuperscript{[25]} used a point-scan Raman system to detect
different concentrations of melamine in milk powder. The lower limit of detection was 0.005% and a linear relationship with a correlation coefficient of 0.99 was established between the melamine detection concentration and the actual concentration in the mixture.

Qin et al. [26] used the Raman hyperspectral imaging system to detect four types of adulterants (ammonium sulfate, dicyandiamide, melamine, and urea) in skimmed milk powder. Spatial distribution of adulterant particles was shown based on self-modeling mixed analysis and Raman chemical images. The above works have carried out research on the detection of adulterants in milk powder. But for the effective detection of mixture particles, its physical properties need to be considered. If the mixture sample is too thick, the laser cannot penetrate the sample and the Raman signals at the bottom cannot be effectively detected. In addition, the physical property of the mixture also affects the spatial distribution of the mixture grains. Therefore, the spatial resolution is also critically important in selecting an effective detection method for mixtures.

In this work, in order to realize the effective detection of BPO additive in flour, we studied the depth of penetration and spatial distribution of a Raman hyperspectral imaging system. To ensure that every chemical particle was detected at the bottom of the mixture sample, penetration depth evaluation was performed using 785nm line laser. To determine the particle distribution in the mixture, the spatial resolution was assessed. As
an example, the selected depth of penetration and spatial resolution was used for
detection in mixture samples with three different concentrations of BPO in flour.

MATERIALS AND METHODS

Instruments And Reagents

The Raman hyperspectral imaging system for detection of BPO in flour is shown in Fig.
1. It consists of a 785nm laser (I0785MM8000MF-1X20B-SCAN, Innovative Photonic
Solutions, Monmouth Junction, NJ, USA), 785nm beam splitter (Semrock, Rochester,
NY, USA), spectrometer (ImSpector R10E, Specim, Oulu, Finland), and 16-bit camera
(iKon-MDU934P-BEX2-DD, Andor Technology, Belfast, NI, UK). The working
principle is as follows: After the line laser is projected onto a beam splitter placed at a
45-degree angle, the line laser reflected by the beam splitter vertically strikes the surface
of the sample, and the sample emits scattering light. The line laser is approximately 200
mm long and 1 mm wide on the sample surface. The Raman scattering signals through
the beam splitter are collected by the spectrometer and camera. The data is transferred to
the computer via a USB cable. The sample is placed on a single-axis moving platform
with a movement range of 0~30 cm controlled using a stepping motor connected to the
computer. In order to prevent the influence of light during the collection process, the
entire system, with the exception of the computer, was placed in a black box. System
control and data collection are accomplished using the Spectral Image-VNIR-R software.
BPO (≥99%) was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Flour was obtained through flour processing companies (Jingyang Flour Co., Ltd) using laboratory-grown wheat to ensure that without any additives, and the particle size is about 90 ~ 150um. Culturing dish, diameter 38 mm and depth 5 mm, was purchased from Crystalgen Biotech Ltd. Aluminum alloy ring, inside diameter 35mm, outside diameter 45mm and thickness 1mm, was purchased from Shanghai Haijia Standard Parts Factory. The vortex mixer, Vortex-Genie 2, was obtained from Scientific Industries, Inc., USA.

**Sample Preparation**

(1) Bi-layer samples

BPO powder was transferred to the culturing dish and the dish was filled to the brim. The aluminum alloy ring was placed on top of the culturing dish so that the lower edge of the aluminum alloy ring and the top edge of the culturing dish are fitted with their circle centers on the same straight line. The ring was then filled with flour to the brim. The number of aluminum alloy rings placed on top of the culturing dish was varied to prepare flour layers of different thickness. The number of aluminum alloy rings used was 1, 2, 3, 4, and 5, and flour thicknesses of 1, 2, 3, 4, and 5mm were obtained. Five bi-layer samples were prepared.
(2) Mixture samples

A specific amount of BPO and flour samples were loaded into a 50 ml centrifuge tube and mixed with the vortex mixer to obtain 4 mixture samples with different BPO additive concentrations (0.4, 0.6, 0.8, and 1.0%, w/w). Each mixture sample weighed 10 g; e.g., the highest concentrated mixture was prepared using 0.1 g BPO and 9.9 g flour and the least concentrated mixture was prepared using 0.04 g BPO and 9.96 g flour. The mixture sample was transferred to a custom 45 mm × 45 mm square aluminum alloy container and the container was filled to the brim for test. The sample volume capacity of the square container was about 2.2 g.

**Data Collection**

The prepared sample was placed on the single-axis moving platform in the center of the line laser. The focal distance between the lens and the sample surface was 20 cm. Raman spectra collection range was 785~1000 nm (corresponding to Raman shift of 0~2728 cm⁻¹). The laser output power was 10 W, the spectral resolution was 0.278 nm, and the integration time was 3000ms. Wherein, information from the bi-layer samples was collected under identical spatial resolution to verify the effective penetration depth. Furthermore, information about the 1% mixture samples was collected under three different spatial resolutions to verify the spatial resolution. Then, the confirmed
penetration depth and spatial resolution were used to detect mixture samples of different concentrations of BPO in flour.

Data Processing

In order to reduce the calculation complexity and time, the region of interest (ROI) and spectral range were first selected. Then, the three-dimensional hyperspectral image was transformed to two-dimensional Raman spectroscopy signals for pre-processing. Due to a change in the laser light source intensity, CCD thermal stability noise, and external stray light during the Raman signal collection process, a significant level of high-frequency noise was present.\textsuperscript{[27–29]} This study was used a Savitzky-Golay filter with 2nd-order polynomial and 5 data point window width to reduce the high-frequency noise and improve the Raman signal quality.\textsuperscript{[30]} Laser light interacted with the sample to generate Raman signals accompanied by fluorescent signals. The relatively high fluorescent background signal significantly interfered with the identification of Raman bands. An adaptive iteratively reweighted penalized least squares (aiPLS) method was reported in the literature and used to correct the fluorescent background signal in this study.\textsuperscript{[31]}

The two-dimensional Raman signals after denoising and fluorescence correction were transformed to three-dimensional hyperspectral image for image processing. A non-overlapping and stronger Raman band was selected as the BPO Raman characteristic
band through a comparison of the Raman signals of pure BPO and pure flour. Single band image of the BPO Raman characteristic band was selected from the pre-processed hyperspectral image. A simple threshold method was used to distinguish between BPO pixels and flour pixels. In order to prevent false positive (where flour pixels are mistaken for BPO pixels) and false negative (where BPO pixels are mistaken for flour pixels), the BPO characteristic band intensity and flour characteristic band intensity of each pixel was compared to give a final threshold. The data processing flow chart for detection of BPO in flour is shown in Fig. 2. The entire process was performed based on a program written using MATLAB 7.11.

For the bi-layer sample, 90 × 90 pixels of ROI were selected from the sample surface for data processing. The binary image was created using the threshold of 50 and then calculates the transmittance of the laser to the different thickness of the flour layer. For the mixture sample, 25mm × 25mm region of ROI was selected from the sample surface for data processing. The binary image was created using the threshold of 158 to achieve spatial resolution determination and BPO pixel detection.

RESULTS AND DISCUSSION

Raman Spectra Of Flour And Benzoyl Peroxide
The mean Raman spectra of BPO and flour are shown in Fig. 3. As shown in this figure, the greater fluorescence background present in the flour Raman signal caused the baseline to drift. An obvious Raman signal at 477 cm\(^{-1}\), which was caused by the vibration of the C-C-C in the starch. \(^{32}\) Different from the Raman spectrum of flour, the Raman spectrum of BPO has a flat baseline as well as greater number and higher intensity of Raman bands. This provided a basis for the detection and identification of BPO in flour. In the Raman spectrum of BPO, highest intensity of the Raman band was at 999 cm\(^{-1}\) and can be attributed to the ring breathing vibration of the two mono-substituted benzenes. \(^{33}\) In addition, the band at 999 cm\(^{-1}\) did not overlap with the Raman band of flour and was selected as the BPO Raman characteristic band.

**Depth Of Penetration**

The mean background-corrected Raman spectra of bi-layer samples with different thickness flour layers are shown in Fig. 4. After fluorescence background correction, the mean Raman spectra have relatively flat baselines. In the mean Raman spectra, the selected 999 cm\(^{-1}\) Raman characteristic band can be observed when the thickness of the flour layer was increased from 1mm to 4mm. The intensity of the Raman characteristic band was the greatest when the flour thickness was 1mm and decreased with flour thickness. When the flour thickness was 5mm, the intensity of the characteristic band was extremely low and difficult to identify. Therefore, in bi-layer samples, the laser was able
to penetrate a maximum flour layer thickness of 4mm to detect the BPO Raman signals at the bottom.

The binary image of bi-layer sample with different thickness flour layers are shown in Fig. 5. Wherein white pixels above this value were identified as BPO and black pixels below this value were taken as flour. As shown in the figure, when the flour thickness was 1mm, all pixels in the image were white, indicating that laser fully penetrated the flour layer to detect the BPO Raman signal, the transmittance was 100%. When the flour thickness was 2mm, eight pixels in the image were black and the rest were white, indicating that only eight scanning points were not penetrated by the laser, the transmittance was 99.9%. When the flour thickness was 3mm, the number of black pixels drastically increased. A search revealed a total of 3309 white pixels, showing that 3309 BPO pixels were scanned, the transmittance was 40.85%. When the flour thickness was 4mm, most pixels in the image were black, only 38 scanning points were penetrated by the laser, the transmittance was 0.47%. The above study revealed that when the flour thickness was 1mm, the laser fully penetrated the flour layer; and when the flour thickness was 2mm, it was similar to the laser penetrating through the whole flour layer. Therefore, 2mm sample depth was selected for analysis of mixture samples to ensure that the laser could penetrate the sample to detect the bottom Raman signals.
Spatial Resolution

The selection of an effective spatial resolution is an important factor regarding the detection of single BPO particles in flour. Fig. 6 shows the binary images for detection of 1% BPO in flour at different spatial resolutions. Wherein black pixels represented BPO particles and white pixels represented flour particles. As shown in the figure, with the reduction in spatial resolution, the detected BPO pixels decreased. In the 0.5 mm/pixel spatial resolution images, the number of detected BPO pixels was the least, with 34. Due to the lack of BPO pixel detection at this resolution, this resolution was inappropriate as an effective detection method. In the 0.125 mm/pixel spatial resolution image, the number of detected BPO pixels was the greatest, and a small number of BPO pixels are clustered together, which is not conducive to the identification of BPO pixels. Compared to 0.125 mm/pixel, BPO pixels in 0.25 mm/pixel spatial resolution image were fewer. However, the spatial distribution trend was consistent and also can demonstrate the spatial distribution of BPO pixels. In the same detection region, the greater the spatial resolution, the longer the time it took to collect the spectral images. Considering the testing time and data processing efficiency, spatial resolution of 0.25 mm/pixel was selected to detect BPO in mixture images.

Detection Of Mixture Samples
With reference to the above study, the determined depth of penetration and spatial resolution parameters were used to test three different concentrations of BPO (0.4%, 0.6% and 0.8%, w/w) in flour. The binary images for detection of different concentrations of BPO in flour are shown in Fig. 7. BPO pixels were distributed on the entire surface and the number of pixels increased with BPO concentration. Calculation showed that the number of BPO pixels accounted for 0.65% of the total pixels in a 0.4% concentration sample. Similarly, the number of BPO pixels in the binary images of samples at 0.6% and 0.8% were 0.95% and 1.4% respectively. Result shows that the number of detected BPO pixels in the flour was positively correlated to BPO concentration and could be used for the quantitative analysis of BPO in flour in the future. Compared with the traditional Raman spectroscopy in the detection of BPO in flour, the Raman hyperspectral system with the parameters selected in this paper can effectively realize the identification and spatial distribution of BPO in flour, and successfully answered the question of “what” and “where”.

CONCLUSION
This study realized the effective detection of BPO in flour by using the selected penetration depth and spatial resolution of line-scan Raman hyperspectral system. The mean Raman spectra of BPO and flour showed that the 999 cm\(^{-1}\) Raman band can be used as the Raman characteristic band for the detection of BPO in flour. The effective
penetration depth of the 785 nm laser into the sample was 2 mm and the transmittance was 99.9%. The spatial resolution of 0.25mm/pixel was selected to show the spatial distribution of BPO particles, and can shorten the time of detection and data processing. Based on the selected penetration depth and spatial resolution, the concentration of BPO in the mixture samples was positively correlated with the percentage of BPO pixels in the binary image. The above results indicated that the parameters selected in this study can be used for the effective detection of BPO in flour. Positive correlation between the detection rate of BPO and the actual concentration of BPO in flour indicated that the method developed in this study can be used for quantitative analysis in the future. The research methods and ideas can provide a reference for the identification and spatial distribution of additives in food.

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REFERENCES


Figure 1. Raman hyperspectral imaging system for detection of benzoyl peroxide in flour.
Figure 2. Data processing flow chart for detection of benzoyl peroxide in flour.
Figure 3. Mean Raman spectra of (a) benzoyl peroxide and (b) flour.
Figure 4. Mean background-corrected Raman spectra of bi-layer samples with different thickness flour layers, (a) ~ (e): 1, 2, 3, 4, 5 mm.
Figure 5. Binary images of bi-layer samples with different thickness flour layers, (a) ~ (d): 1, 2, 3, 4 mm.
Figure 6. Binary images for detection of 1% benzoyl peroxide in flour at different spatial resolutions, (a) ~ (c): 0.5, 0.25, 0.125 mm/pixel.
Figure 7. Binary images for detection of different concentrations of benzoyl peroxide in flour, (a) ~ (c): 0.4%, 0.6%, 0.8%.
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