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Rapid analysis and quantification of fluorescent brighteners in wheat flour by Tri-step infrared spectroscopy and computer vision technology

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ABSTRACT

Fluorescent brightener, industrial whitening agent, has been illegally used to whitening wheat flour. In this article, computer vision technology (E-eyes) and colorimetry were employed to investigate color difference among different concentrations of fluorescent brightener in wheat flour using DMS as an example. Tri-step infrared spectroscopy (Fourier transform-infrared spectroscopy coupled with second derivative infrared spectroscopy (SD-IR) and two dimensional correlation infrared spectroscopy (2DCOS-IR)) was used to identify and quantitate DMS in wheat flour. According to color analysis, the whitening effect was significant when added with less than 30 mg/g DMS but when more than 100 mg/g, the flour began greenish. Thus it was speculated that the concentration of DMS should be below 100 mg/g in real flour adulterant with DMS. With the increase of the concentration, the spectral similarity of wheat flour with DMS to DMS standard was increasing. SD-IR peaks at 1153 cm^{-1}, 1141 cm^{-1}, 1112 cm^{-1}, 1085 cm^{-1} and 1025 cm^{-1} attributed to DMS were regularly enhanced. Furthermore, it could be differentiated by 2DOS-IR between DMS standard and wheat flour added with DMS low to 0.05 mg/g and the bands in the range of 1000–1500 cm^{-1} could be an exclusive range to identify whether wheat flour contained DMS. Finally, a quantitative prediction model based on IR spectra was established successfully by Partial least squares (PLS) with a concentration range from 1 mg/g to 100 mg/g. The calibration set gave a determination coefficient of 0.9884 with a standard error (RMSEC) of 5.56 and the validation set presented a determination coefficient of 0.9881 with a standard error of 5.73. It was demonstrated that computer vision technology and colorimetry were effective to estimate the content of DMS in wheat flour and the Tri-step infrared macro-fingerprinting combined with PLS was applicable for rapid and nondestructive fluorescent brightener identification and quantitation.

1. Introduction

Fluorescent brightener is frequently used in textile, paper making, and plastic industry. It not only can reflect visible light, but also can absorb, transform ultraviolet light and then release purple blue or cyan visible light to offset yellow color to whiten materials. Therefore, related manufactures use the fluorescent brightener to enhance the whiteness and bright degree of the processing products [1]. Although there is still no conclusion that the fluorescent brightener could cause human cancer, but as industrial agents, it is absolutely forbidden to be added to foods.

As China is a big country of wheat production and consumption, wheat is one of the most important foods for Chinese. Color and luster is an important indicator for the quality of wheat flour foods especially Chinese traditional wheat flour noodles, steamed bread and dumplings [2]. For a long time, Chinese people have the habit of eating noodles with high whiteness [3–6]. As a result, some flour manufacturers mindless added fluorescent brightener in the wheat flour for grabbing market and increasing sales [7]. In 2011, China National Health and Family Planning Commission put fluorescent brightener in the list of "Non-food substances illegally added or

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food additives easily abused in food", and pointed out that generally food adulterated with fluorescent brightener were wheat flour, mushrooms, etc. Given that no established test standard of fluorescent brightener in wheat flour is currently available, it would be essential to develop an adequate technique approach for regulating flour industry through rapid identifying whether wheat flour contains fluorescent brightener.

Since fluorescent brightener is a physical whitening product, so computer vision technology and colorimetry can be used for color analysis. Electronic eye, a computer vision technology converting the image into digital image, uses the image sensor instead of the human eye to collect images of objects, and use computer simulation criterion to identify the image avoiding the subjective deviation of human eye [8]. The colorimetry method use numerical to represent color though the value of *Lab* which combine *L* (Luminosity, the percentage of black), *a* (red–green, +127 is red whereas −128 is green), *b* (yellow–blue, +127 is yellow whereas −128 is blue). All colors are described by the three values [9]. In this work, we employed computer vision technology and colorimetry speculate on the content of fluorescent brightener DMS in graham flour, and build the prediction model of DMS content based on the infrared spectra.

Fourier transform infrared spectroscopy (FT-IR), a quick, easy to handle and nondestructive technique, has an advantage of macroscopic identification of complex system as a whole [10–12]. To delineate the overlapped spectra in FT-IR, second derivative infrared spectroscopy (SD-IR) can be used to improve the apparent resolution. Two dimensional correlation infrared spectroscopy (2DCOS-IR) expands the signal to a second dimension which can not only further enhance the resolution of infrared spectra, but also provide dynamic chemical structural information potentially for identification and quantification [13]. At present, some literature have applied high-performance liquid chromatography (HPLC), ultraviolet spectrophotometry, fluorescence spectrophotometry to determine fluorescent brightener in the flour, but these approach could not achieve rapid qualitative and quantitative analysis simultaneously. In this work, we adopted a Tri-step infrared spectroscopy (FT-IR, SD-IR and 2DCOS-IR) coupled with chemometrics to identify and quantitate DMS in wheat flour in a holistic manner.

2. Experimental

2.1. Apparatus and materials

Thermo Scientific Nicolet iS5 FT-IR, equipped with single-point ATR, in 400–4000 cm^{-1} rang with a resolution of 4 cm^{-1}. Spectra were recorded at 16 scans. Tiny vortex mixing apparatus. Konica Minolta CR-400 color difference meter; Alphamos IRIS VA300 computer vision technology (E-eyes); brown bottle.

This experiment used fluorescent brightener DMS which belongs to triazinylaminostilbene stilbenes with high yield in the domestic purchased from Shenzhen Lijing Bio-Chem Technology Co. Limited.

Wheat flour samples were purchased from supermarkets in Shanghai.

2.2. Pretreatment

Fluorescent brighter DMS taking quality range between 0 and 0.8 g (0.00025 g, 0.005 g, 0.025 g, 0.05 g, 0.15 g, 0.25 g, 0.35 g, 0.45 g, 0.50 g, 0.80 g) with 5 g wheat flour respectively put in brown bottle then sealed and blended 30 min with micro vortex mixing apparatus, respectively. The final concentration of samples were 0.05 mg/g (D-1), 1 mg/g (D-2), 5 mg/g (D-3), 10 mg/g (D-4), 30 mg/g (D-5), 50 mg/g (D-6), 70 mg/g (D-7), 90 mg/g (D-8), 100 mg/g (D-9), 160 mg/g (D-10), respectively.

2.3. Procedure

2.3.1. Content analysis

Powder accessories were filled with samples and then hang the back cover tightly. The pretreatment of color analysis were all used powder accessories.

2.3.1.1. Whiteness analysis. Firstly, input *L*, *a*, *b* value of standards board to the color difference meter. Secondly, adjusted lens against standards board, pressed key TARGET to complete the calibration. Thirdly, put the lens against the powder accessories with under test sample, pressed key TARGET, recorded the values of *L*, *a*, *b*. Each sample were measured three times.

2.3.1.2. Color analysis. After focal length adjusting and color calibration, images of wheat flour samples in the powder accessories were collected in a large measuring chamber (42 cm × 56 cm), which illuminated and closed to ensure controlled light conditions without influence of external light. Each sample was measured four times. The background of each picture was automatically removed using a specific threshold to keep only the wheat flour colors. For each picture, the machine vision software created a color spectrum by using a 4096 color scale.

2.3.2. Infrared spectrum measurement

Samples were pulverized to around 200 meshes. The FT-IR spectra of the samples were collected at room temperature by single-point ATR. Second derivative IR spectra were obtain 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.

3. Results and discussion

3.1. Whiteness and color analysis of different contents of DMS in wheat flour

3.1.1. Whiteness analysis

As the accurate content of DMS frequently added in wheat flour has not been reported yet, the whiteness of samples was determined for speculating the content range of DMS through whiteness analysis. With increasing the content of DMS, *Lab* (whiteness), *L* (brightness) and *a* (greenness) values increased but *b* (yellowness) values decreased (Fig. 1), suggesting that the whiteness, brightness and greenness of samples were strengthened while the yellowness were weakened. When DMS content was 160 mg/g, the *Lab*, *L* and *b* values of interrogated samples did not reach the level of white flour. However, *a* value of the samples exceeded that of the white flour. Subsequently, it can be speculated that the DMS content added in the flour illegally should be less than 100 mg/g. Thus, 100 mg/g was selected as the upper limit content of DMS in wheat flour for building quantitative models based on infrared spectra.

3.1.2. Color analysis

The proportion of various colors of each sample was analyzed using principal component analysis (PCA) in order to extract the main differences in color between samples (Fig. 2). The sensors of PCA were Colors-4077, 3820, 3804, 4078 and 4094 and