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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

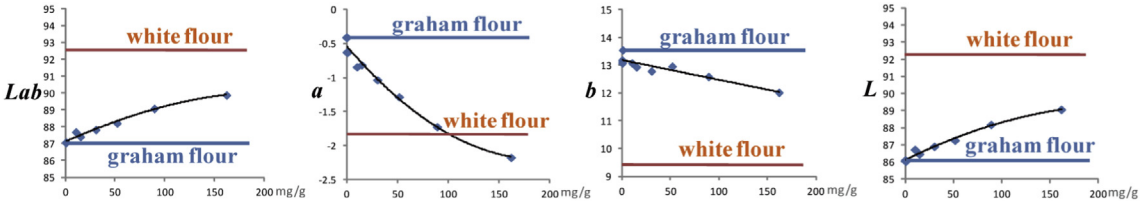

Fig. 1. Trends of *Lab*, *L*, *a*, *b* values of wheat flour added with DMS less than 160 mg/g.

discrimination index of samples was 96, indicating that different concentrations of samples were successfully differentiated. Because of the first principal component (PC1) accounted for 66.38%, the main differences of samples focused on the horizontal axis, namely the bigger the distance on the horizontal axis, the greater the difference between the samples. Specifically, graham flour and D-4 (10 mg/g) distributed in an area separately and far from the other samples, suggesting that the two had obvious differences in colors from the others. D-5 (30 mg/g) was also far away from others with a minimal PC2 score. With the concentration increase of DMS, differences between the samples of D6, D7, D8 and D9 decreased with a decreasing PC1 score and an increasing PC2 score. The results confirmed that the magnitude of the color differences was increasing followed by decreasing.

According to the contribution of sensors for graham flour and D-4, colors-3804, 3820, 4077 were the main factors. With the increase of DMS content, the contribution percentages of the three sensors were dropping. Specifically, the proportions of colors-3804 and 3820 were close to zero in D-9 indicating that DMS covered up the yellowness of graham flour. However, the proportions of colors-4078 and 4094 were rising. Furthermore, the rising rate of colors-4094, one type of greenness, was much higher than colors-4078, which was in consistent with the results of whiteness analysis.

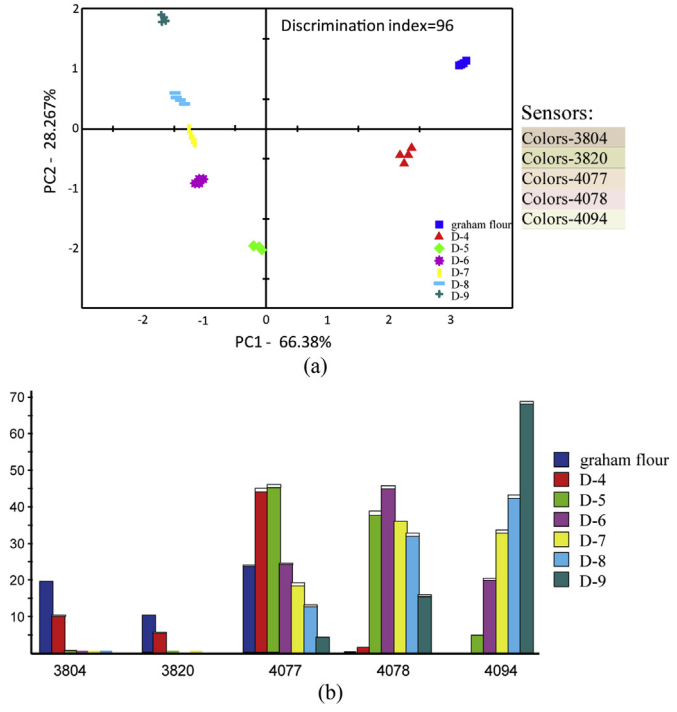

Fig. 2. Color map of various wheat flour samples (a) and color histogram (b) based on principal component analysis (PCA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. IR analysis for DMS identification and quantitation

3.2.1. IR spectra of wheat flour with DMS

With the increase of the DMS concentration, the similarity of spectra of wheat flour with DMS to DMS standard spectrum was increasing especially in 1600–1300 cm^{-1} in which the characteristic peaks of DMS gradually emerged (Figs. 3 and 4, Table 1). FT-IR spectra could be thus used to preliminarily determine whether the flour contained DMS. In order to further reveal the change rule of DMS in wheat flour with different concentrations, corresponding second derivative spectra inherent with enhanced spectral resolution were calculated and analyzed.

3.2.2. Second derivative IR spectra of flour with DMS

Generally, second derivative infrared spectroscopy can enhance the spectral resolution and amplify tiny differences in IR spectrum [14]. Overlapped absorption peaks and shoulder peaks can be separated by using second derivative spectral analysis [15]. As the concentration of DMS increased, peaks particularly at 1153 cm^{-1} (ν(C–C), δ(=CH)) ,1141 cm^{-1},1112 cm^{-1},1085 cm^{-1}, 1025 cm^{-1} (δ(C–O–C)) were intensified gradually (Fig. 5). Therefore, the second derivative infrared spectra (SD-IR) were used to build the DMS quantitative prediction model in this work.

3.2.3. Analysis of 2DCOS-IR spectra of DMS in wheat flour

In order to identify whether the flour samples contained

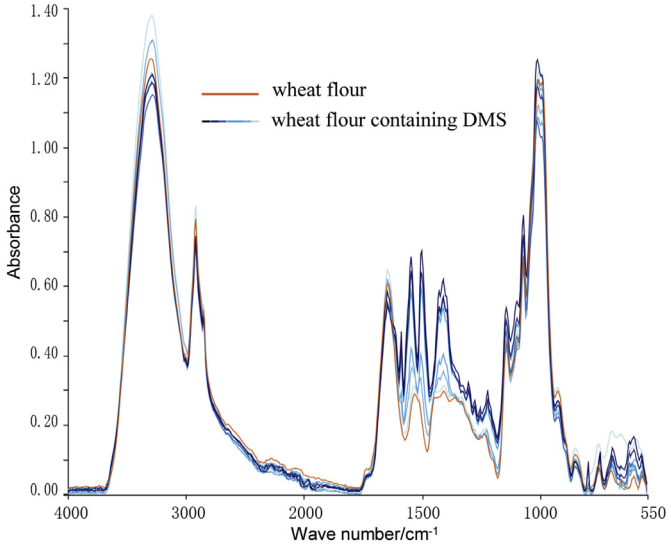

Fig. 3. FTIR spectra of wheat flour added with various contents of DMS and wheat flour blank in the range of 4000–400 cm^{-1}.

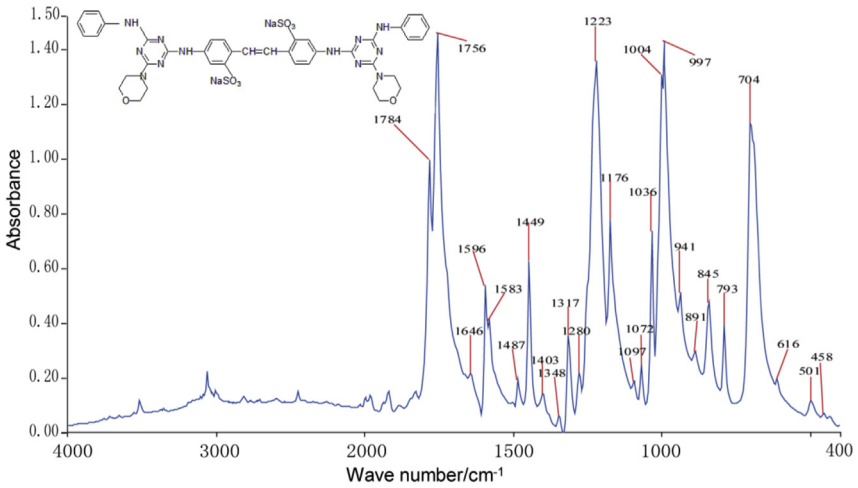

Fig. 4. FTIR spectra of DMS in the range of 4000–400 cm^{-1} and its chemical structure.

fluorescent brighteners more remarkably and convincingly, 2DCOS-IR was employed. 2DCOS-IR can improve the resolution of spectrum and provide more information by showing the influences of the perturbation on each of molecules in the sample [16–18]. 2DCOS-IR correlation spectrum illustrates the sensitivity for each IR band or functional group and correlation between functional groups, as well as the sequence of responses, when the investigated system is subjected to a given perturbation.

For 2DCOS-IR spectrum (Fig. 6 and Table 2), the main peaks were located at 1032 cm^{-1} (C–O–C symmetric stretching vibration of ether structure), 1445 cm^{-1}(–CH–H bending vibration) and 1476 cm^{-1}. Two weak autopeaks generated in this region at 1424 cm^{-1} due to –CH–H bending vibration of benzene structure and 1392 cm^{-1}. While the spectrum of wheat flour has one strong autopeak at 1071 cm^{-1} (C–O symmetric formation vibration of ether structure) and two weak autopeak at 1141 cm^{-1} and 1226 cm^{-1} not found in DMS. The spectrum of wheat flour with 0.05 mg/g DMS not only has a strong autopeak at 1071 cm^{-1} corresponding to that in the flour spectrum, but also has 1442 cm^{-1} and 1477 cm^{-1} corresponding to those in DMS spectrum. Therefore, this range can be used for identifying whether the flour samples containing DMS.

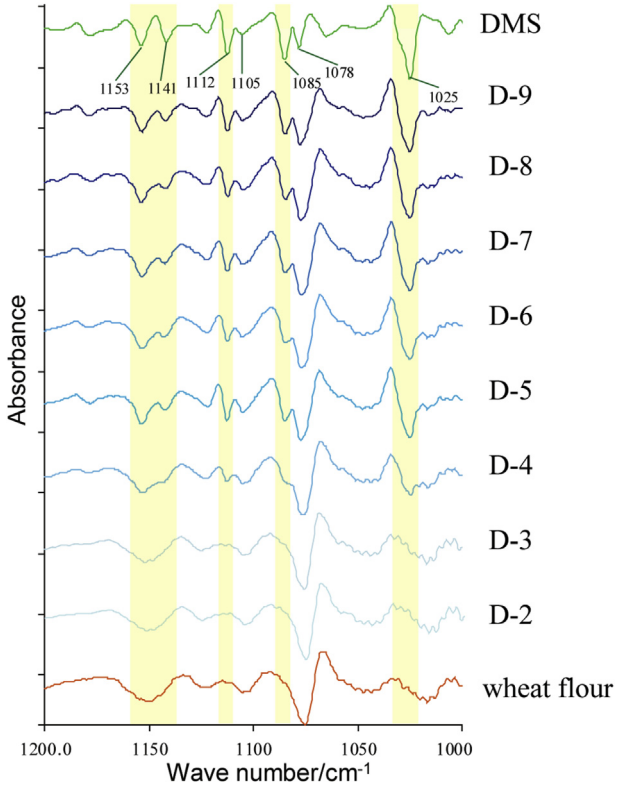

Fig. 5. SD-IR of wheat flour with various contents of DMS and wheat flour blank in the range of 1200–1000 cm^{-1}, D-2: 1 mg/g; D-3: 5 mg/g; D-4: 10 mg/g; D-5: 30 mg/g; D-6: 50 mg/g; D-7: 70 mg/g; D-8: 90 mg/g; D-9: 100 mg/g.

3.2.4. Quantitative analysis

To establish a quantitative prediction model for DMS in wheat flour, sixty-one samples (forty-five for calibration and sixteen for validation; concentration range was from 1 mg/g to 100 mg/g) were investigated and the ranges of 826–807 cm^{-1},1031–1009 cm^{-1}, 1105–1082 cm^{-1} were selected for Partial Least Squares (PLS) analysis. The parameters for establishing DMS quantitative model were summarized in Table 3. As shown in Fig. 7, the prediction values by the constructed model were very close to the actual values of DMS content in the flour samples with $R^2 > 0.98$ and RMSEP 5.73.

To verify the applicability of the model, the flour samples with four different DMS concentrations (2.99 mg/g, 41.27 mg/g, 64.81 mg/g and 71.56 mg/g) were prepared for testing and each sample was measured six times. The results were shown in Table 4. Slight deviations between predicting values and real values can be observed for the reason that different particle sizes between wheat flour and DMS led to uneven mixtures. The measured values of the samples were further tested by *t*-Text method to investigate if any significant difference existed between the calculated results of the model and the real values.

$$s^2 = \left[(n_1-1)^2 s_1^2 + (n_2-1)^2 s_2^2\right] \Big/ (n_1+n_2-2); \quad t = |d_1-d_2|[n_1 n_2/(n_1+n_2)]^{1/2} \Big/ s$$

For A, B, C samples, the values of *s* were 2.1377, 3.8977, 2.2882, 3.8977 and the values of *t* were 0.1308, 1.4190, 0.4882, 1.4190. When $f = n_1 + n_2 - 2 = 10$, if$\alpha = 0.05$, $t_{0.05}^{10} = 1.812$. As can be seen from the

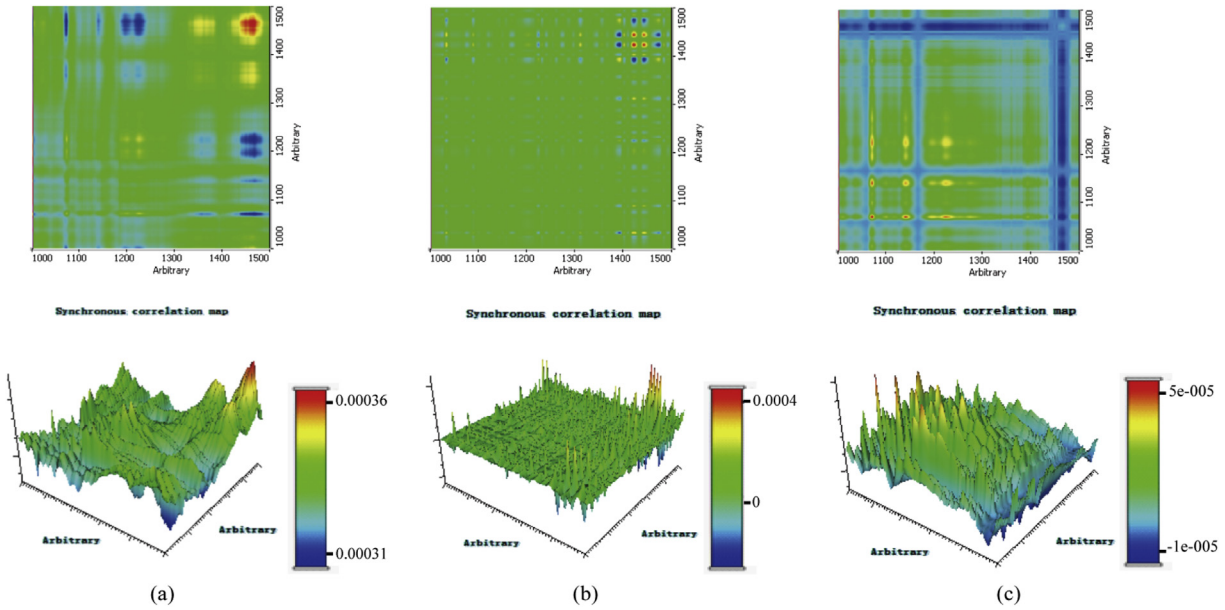

Fig. 6. 2DCOS-IR synchronous correlation spectra of wheat flour with DMS (0.05 mg/g) (a), DMS (b) and wheat flour (c) in the range of 1500–1000 cm^{-1}.

Table 1
The preliminary assignment of main characteristic absorption peaks of DMS FT-IR spectra.

Peak position (cm^{-1})	Base group and vibration mode
1784, 1756	ν(C=N)
1596, 1449	ν(C=C), ν(C–N)
1223, 1036	V_f(C–C), ν_l(C–O–C), δ(C–O)
1176, 616	$\nu_{as}(-SO_3)$

Table 2
Auto-peaks of 2DCOS-IR synchronous correlation spectra of wheat flour with DMS (0.05 mg/g), DMS and wheat flour blank in the range of 1500–1000 cm^{-1}.

Samples	Auto-peak/cm^{-1}							
DMS in flour		**1071**		1226			**1422–1485 (1442, 1477)**	
DMS	**1032**				1392	1424	**1445**	**1476**
Flour		**1071**	1141	1226				

Note: Peaks in bold are the strong auto-peaks.

results, the values of t_A, t_B, t_C, t_D were less than $t_{0.05}^{14}$, indicating no significant difference between the IR measuring values and the true values in the A, B, C, D samples. Therefore, the model was verified to predict the results accurately.

4. Conclusion

Color information of wheat flour samples were obtained quickly and accurately by computer vision technology and colorimetry, so as to provide basic information for quantitation wheat flour color, and assist determining the content of DMS in wheat flour quickly. According to the result of color trend, the range DMS content adulterated in wheat flour should be less than 100 mg/g. With the increasing of DMS content, the similarity of spectra of wheat flour with DMS to DMS standard spectrum was increasing and the –C–O vibration attributed to DMS in SD-IR spectra of 1200–1000 cm^{-1} region was enhanced gradually. The dissimilarities between wheat flour and wheat flour containing 0.05 mg/g DMS were clearly observed via 2DCOS-IR spectra. PLS quantitative model based on IR spectra could accurately predict the content of DMS in the flour quickly.

Tri-step infrared spectroscopy combined with chemometrics can qualitative and quantitative determine fluorescent brighteners in wheat flour quickly and effectively, having high potential for the rapid on-site detection of fluorescent brighteners in a holistic manner.

Table 3
Parameters for DMS quantitative model based on PLS.

Parameters	Values
Analysis range/cm^{-1}	826–807, 1031–1009, 1105–1082
Scaling (spectra)	Mean
Smooth	–
Baseline correction	Second derivative, 7 point
Normalization	MSC (multiplicative signal correction)

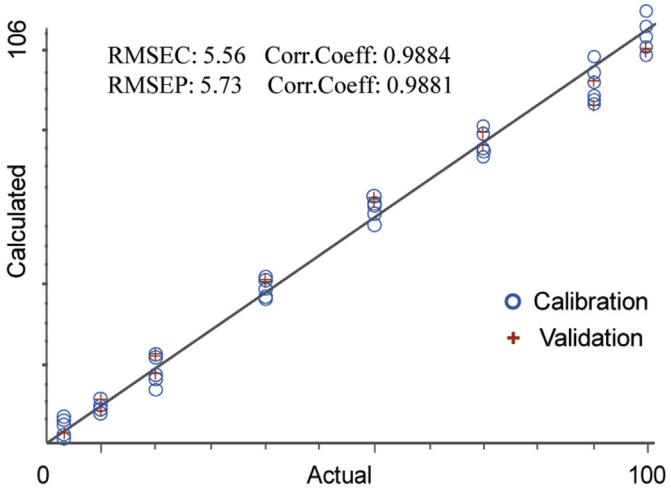

Fig. 7. PLS quantitative plot based on FT-IR spectra for determining DMS content in wheat flour.

Table 4
The accuracy test of DMS quantitative model.

Sample	Content (mg/g)	Measured value/(mg/g)						Mean (mg/g)	RSD/%
A	2.99	0.23	3.68	2.38	2.02	5.40	5.20	3.15	0.63
B	41.27	32.28	36.80	35.70	48.88	36.43	38.37	38.08	0.15
C	64.81	67.45	68.16	65.01	62.17	64.24	65.70	65.46	0.03
D	71.56	79.17	66.72	68.66	62.57	67.09	65.99	68.37	0.08

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