Using 2D-COS Based on ATR-MIR to Identify Mixed Surimi

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***Abstract*—In this study, 2D-COS (two-dimensional correlation spectroscopy) combined with ATR-MIR (attenuated total reflection mid-infrared) spectroscopy was used to quantitatively classify sliver carp surimi adulterated with different levels of hairtail surimi. ED (Euclidian distance) and CC (correlation coefficient) between 2D correlation spectrums of different surimi samples were used for this purpose. Results showed that the sliver carp surimi adulterated with different levels of hairtail surimi samples (5%, 10%, 20%, 40%, 60%, 80%) all could be classified correctly. From above we could conclude that the 2D-COS combined with ATR-MIR spectroscopy had the potential in classifying adulterated surimi samples.**

***Index Terms*—2D-COS, ATR-MIR, adulterated surimi, quantitative analysis**

I. Introduction

The purpose of economic adulteration is mostly to obtain higher profits, replacing high-cost ingredients with cheaper, inferior and lower quality ingredients. Although economic adulteration rarely results in health hazard, it still should not be accepted and forgiven. Because it disturbed the market order and defrauded the consumers, economic adulteration detection is very necessary in the food industry. Surimi and meat paste are such kind of "value-added" products, which were very easy to be adulterated and difficult to be inspected.

Currently, most of meat adulteration detections were focused on detailed and expensive methodologies such as liquid/gas chromatography [1], mass spectrometry [2] and highly-specific PCR assay [3], [4] to identify unique chemicals which could distinguish one ingredient from another. But most of these chemical methods couldn't be used to carry out quantitative analysis. Spectral analysis, including visible and near-infrared spectroscopy [5], mid-infrared spectroscopy [6], Raman spectroscopy [7] and hyperspectral imaging [8], was a kind of powerful tool that could be used for qualitative and quantitative analysis of samples and requires minimal sample preparation and destruction. But quantitative analysis of samples based on spectral usually involves building mathematical analysis model. However, the issue of calibration model transfer has not been effectively resolved [9], [10]. Considering of the high cost and limited resources, develop a calibration model for each kind of instrument was not practical. So, in practice, the application of spectral modeling analysis was considerably limited [11].

2D-COS was firstly developed by Noda [11] and has been employed in many research fields including the research of proteins, natural and synthetic polymers, pharmaceuticals, colloids, and solutions, under various perturbation methods [11]. Compared with traditional one-dimension spectra, 2D-COS has many advantages such as enhancing spectral resolution, separating severely overlapping bands, probing the order of spectral intensity changes and so on [11]. Because 2D-COS could be used to detect and analyses perturbation-induced dynamic spectra, the sample`s characteristic spectral responses under perturbations would present in their two-dimensional correlation spectrum. So 2D-COS was suitable to distinguish highly similar samples.

Theoretically, when the samples and the spectrum acquisition conditions are exactly the same, their 2D-COS spectrograms are identical also. The greater dissimilarity samples exist, the more significant difference their 2D-COS spectrogram present. So, through quantifying the differences of sample`s 2D-COS spectrogram, we can make qualitative and quantitative analysis of samples. In some researches [12]-[14], ED (Euclidian distance) and CC (correlation coefficient) were introduced to quantify the difference between 2D-COS spectrogram. The result showed that these two parameters could be used to quantify the similarity among the 2D correlation spectra of sweet and dry red wines [12], astragalus from different habitats [13], and adulterated milk [14].

In this research, we firstly explored the potential of 2D-COS in the field of minced meat adulteration detection. The adulterated surimi was discriminated and quantified through quantifying the differences between 2D-COS spectrogram.

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II. Experiment

A. Apparatus

All spectra were collected using a Fourier transform infrared (FTIR) spectrometer system (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a liquid nitrogen-cooled MCT (mercury-cadmium-telluride) detector. This spectrometer system also provides a removable inverted ATR detection device equipped with a single bounce germanium crystal. When collecting the samples were placed on the ATR plate and clamped between the ATR plate and germanium crystal. Before collecting the spectrum, we need to create a suitable pressure through pressure numerical display control operation, in order to make sure that no air is trapped between the samples and the ATR crystal.

The spectrometer was controlled by Omnic picta Software and all spectra were collected under the same ATR pressure. The spectra were collected over the range from 650 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The number of scans for each measurement was 64 resulting in a total integration time of 12s. Each spectrum of the sample was converted to absorbance units using a single-beam background spectrum collected of a clean, dry ATR crystal. Before spectral acquisition of each sample, the ATR plate and the germanium crystal were cleaned with analytical grade absolute alcohol and then dried. A RT600/HW temperature controller (Huozi Instrument Technology Shanghai Co., Ltd.) was arranged to perform the thermal perturbation at a steady temperature (45 ℃) for 60min. And the series of thermo-perturbation dynamic spectra were collected at each interval of 5 min [15].

B. Materials

TABLE I. The Categories, Number and Doping Level of Surimi Samples

Nu-mber	Cate-gory	Number	Doping level	Number	Doping level
1-1	ZJ	A1-1	5% ZJ	A1-4	40% ZJ
1-2	ZJ	A2-1	5% SD	A2-4	40% SD
1-3	ZJ	A3-1	5% AH	A3-4	40% AH
2-1	SD	A1-2	10% ZJ	A1-5	60% ZJ
2-2	SD	A2-2	10% SD	A2-5	60% SD
2-3	SD	A3-2	10% AH	A3-5	60% AH
3-1	AH	A1-3	20% ZJ	A1-6	80% ZJ
3-2	AH	A2-3	20% SD	A2-6	80% SD
3-3	AH	A3-3	20% AH	A3-6	80% AH

Note: "A1-1", "A" represents "adulterated"; for example, "5% ZJ" means ZJ adulterated with 5% hairtail surimi.

One kind of A grade pure hairtail surimi sample and three kinds of AAA grade pure sliver carp surimi samples, all containing approximately 4% sorbitol and 4% sucrose as cryoprotectant, were bestowed by Zhejiang Haichuan Aquatic Products Co. Ltd. Three kinds of AAA grade sliver carp surimi were respectively made of sliver carp from Shandong (SD), Anhui (AH) and Zhejiang (ZJ). A total of 37 surimi samples were prepared, 9 pure sliver carp surimi samples and 28 adulterated surimi samples (Table I). Before the mixed surimi was made, frozen surimi was thawed out completely and tempered to room temperature individually. Then, the hairtail surimi and sliver carp surimi were weighed separately and then mixed in a blender, during homogenization the surimi sticking on the wall of the blender was scraped and mixed again. For convenience, the hairtail surimi was chosen as adulterant. The mixed surimi was made by adding hairtail surimi to sliver carp surimi in concentration range of 5%, 10%, 20%, 40%, 60% and 80%. The samples were placed in labeled polyethylene bags and stored at 4℃ until spectral analysis.

C. Methods

1) Difference quantization of 2D-COS spectrogram based on ED

(1) The 2D-COS spectrogram of all surimi samples was obtained according to the procedure and method presented in reference [16].

(2) Calculating the ED between pure meat samples according to the equation 1 and denoted as one group of inner-EDs.

$$D_{xy}=\left\{\frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n}\left|d_{ij}\right|^2\right\}^{\frac{1}{2}}=\left\{\frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n}\left|S_{ij}^{x}-S_{ij}^{y}\right|^2\right\}^{\frac{1}{2}} \quad (1)$$

where S_{ij}^{x} represents the two-dimensional correlation spectroscopy matrix of sample x; and S_{ij}^{y} represents the two-dimensional correlation spectroscopy matrix of sample y; n represents the dimension of matrix *S*.

(3) Calculating the EDs of each adulterated surimi sample to all pure surimi samples according to the equation 1 and denoted as one group of outer-EDs. There was a total of 6 groups of outer-EDs were achieved.

(4) Significance test of difference among 7 groups of EDs was carried out using multiple comparison method and ensured significant differences ($p<0.05$) existed among all groups.

(5) Unknown sample detection: calculating the EDs of unknown samples to all pure surimi samples and denoted as one group of unknown-EDs. And then, significance test of difference of all groups of EDs including inner-ED, outer-ED and unknown-ED was carried out using multiple comparison method. We can conclude whether the surimi samples were adulterated and the adulterate level according to the significant difference level.

2) Difference quantization of 2D-COS spectrogram based on CC

$$r_{xy}=\frac{\sum_{i=1}^{n}\sum_{j=1}^{n}\left(S_{ij}^{x}-\overline{S_x}\right)\bullet\left(S_{ij}^{y}-\overline{S_y}\right)}{\sqrt{\sum_{i=1}^{n}\sum_{j=1}^{n}\left(S_{ij}^{x}-\overline{S_x}\right)^2}\bullet\sqrt{\sum_{i=1}^{n}\sum_{j=1}^{n}\left(S_{ij}^{x}-\overline{S_y}\right)^2}} \quad (2)$$

$$\overline{S_x}=\frac{1}{n^2}\sum_{i=1}^{n}\sum_{j=1}^{n}S_{ij}^{x}\quad \overline{S_y}=\frac{1}{n^2}\sum_{i=1}^{n}\sum_{j=1}^{n}S_{ij}^{y}$$

Except the calculation formula (equation 2) of CC, the procedure of difference quantization of 2D-COS spectrogram based on CC was quite similar to that of ED.

III. Results and Discussions

A. 2D-COS Spectrogram of Surimi Samples

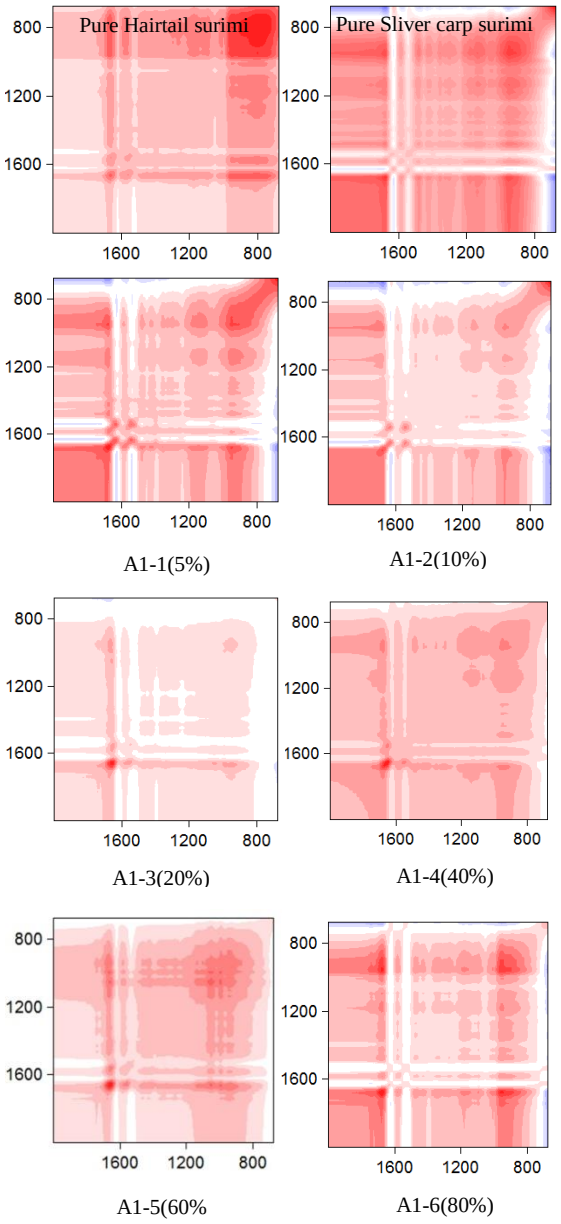

Figure 1. Synchronous 2D correlation spectrums of some surimi samples

Fig. 1 showed the synchronous 2D-COS spectrogram of some surimi samples (2000-650 cm^{-1}). In Fig. 1 the 2D-COS spectrogram of pure hairtail surimi and silver carp surimi samples had very significant differences. With the increasing adulterate ratio of hairtail surimi, the 2D-COS spectrogram of adulterated surimi samples looked more and more similar to that of pure hairtail surimi samples, but it was quite the opposite for pure silver carp surimi samples. That provided theoretical basis for differential quantization of the 2D-COS spectrogram.

B. Difference Quantization Based on ED

The inner-EDs and the outer-EDs of surimi samples were calculated and Fig. 2 showed the distribution of them. From Fig. 2 we could see that some inner-EDs overlapped with outer-EDs, and the value of outer-EDs increased with the adulterate level.

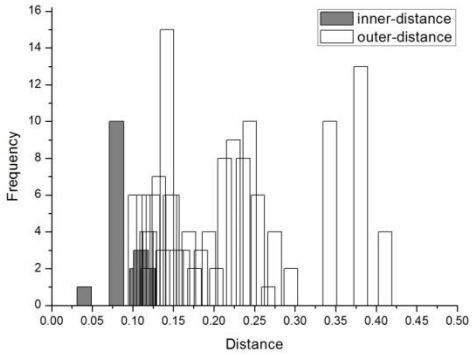

Figure 2. Distribution of all groups of ED

In order to verify the difference of all groups of ED values, the LSD (Least Significant Difference) variance analysis of all sets of EDs were performed. Firstly, the homogeneity of variance was performed using Levene's test and the results were showed that the probability is 0.114 which was greater than the significant level of 0.05, which indicated that the total variance of each group of ED was equal.

Then the single factor variance analysis of adulterated level on each set of EDs was performed. And the results showed that the value of F was 65.981, and the corresponding value of probability was 0.000, which was much smaller than significant level of 0.05. So, the adulterate level gave significant changes to each group of ED values.

TABLE II. LSD Test Results Of All Groups Of Ed Values

	0	5%	10%	20%	40%	60%	80%
0		.001	.000	.000	.000	.000	.000
5%	.001		.016	.000	.000	.000	.000
10%	.000	.016		.042	.000	.000	.000
20%	.000	.000	.042		.000	.000	.000
40%	.000	.000	.000	.000		.009	.000
60%	.000	.000	.00	.000	.009		.000
80%	.000	.000	.000	.000	.000	.000	

Although significant differences existed among 7 groups of the EDs, it was difficult to know the adulterated level affected all groups of EDs or only a few of them. Therefore, detailed influence of adulterated level on each group of EDs was explored through multiple comparison method LSD test and Table II showed the results. The results showed that there existed significant differences ($P<0.05$) among each group of ED values. The adulterated hairtail surimi affected the ED values significantly.

Sample identification based on ED

Three samples U1 (Selecting one kind pure sliver carp surimi randomly among three kinds of pure sliver carp

surimi and then adulterated with 6.5% hairtail surimi), U2 (adulterated with 20% hairtail surimi) and U3 (adulterated with 60% hairtail surimi) were prepared and tested through 2D-COS spectrogram difference quantization. Firstly, calculating unknown-EDs of three samples respectively; then significance test of difference of 10 groups of EDs was carried out using multiple comparison method. And the results were showed in Table III. The ED group of U1 had no significant differences with that of silver carp surimi sample adulterated with 5% (P=0.529>0.05) and 10% (P=0.267>0.05) hairtail surimi, while significant differences (P<0.05) were observed among ED group of U1 and the rest ED groups. The ED group of U2 had no significant differences with that of silver carp surimi adulterated with 20% (P=0.349>0.05) hairtail surimi, while significant differences (P<0.05) were observed among ED group of U2 and the rest ED groups. And the same with that of U3 which had no significant differences with silver carp surimi adulterated with 60% hairtail surimi. Three surimi samples U1 (6.5%), U2 (20%) and U3 (60%) were all identified correctly. From above we could conclude that 2D-COS spectrogram difference quantization based on ED could be used in quantitative and qualitative identification of adulterated surimi. And the limit of determination was 5%; it means that the silver carp surimi samples with an interval of adulterate level less than 5% could not be identified correctly.

TABLE III. LSD TEST RESULTS OF ED BETWEEN UNKNOWN AND KNOWN SILVER CARP SURIMI SYNCHRONOUS 2D CORRELATION SPECTRUM

	0	**5%**	**10%**	**20%**	**40%**	**60%**	**80%**
U1	0.001	**0.529**	**0.267**	0.010	0.000	0.000	0.000
U2	0.000	0.000	0.022	**0.349**	0.000	0.000	0.000
U3	0.000	0.000	0.000	0.000	0.021	**0.650**	0.000

C. Difference Quantization Based on CC

The inner-CCs and the outer-CCs of surimi samples were also calculated (the method of calculation is same as the calculation of inner-EDs and the outer-EDs) and Fig. 3 showed the distribution of them. From Fig. 3 we could see that the value of outer-CC decreased with the increasing adulterate level, but not declined in proportion with the increase of adulterate level of hairtail surimi.

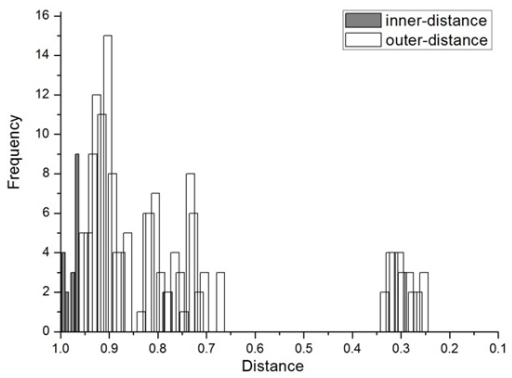

Figure 3. Distribution of All Groups of CC

Similarly, in order to verify the difference of all groups of CCs values, the LSD variance analysis of all sets of SS values were performed. Firstly, the homogeneity of variance was performed using Levene's test and the results were showed that the probability 0.746 was greater than the significant level of 0.05, which indicated that the total variance of each group of CC values was equal.

Single factor variance analysis was also carried out. The value of F was 866.053, and the corresponding value of probability was 0.000, which was much smaller than significant level of 0.05. So, the adulterate level gave significant changes to each group of CC values.

Multiple comparison method LSD test was also used to study detailed influence of adulterated level on each group of CCs values. And table 4 showed that significant differences (P<0.05) among each group of CC values, that was to say the adulterated hairtail surimi affected the CC values significantly.

TABLE IV. LSD TEST RESULTS OF ALL GROUPS OF CC VALUES

	0	**5%**	**10%**	**20%**	**40%**	**60%**	**80%**
0		.004	.000	.000	.000	.000	.000
5%	.004		.000	.000	.000	.000	.000
10%	.000	.000		.001	.000	.000	.000
20%	.000	.000	.001		.000	.000	.000
40%	.000	.000	.000	.000		.000	.000
60%	.000	.000	.000	.000	.000		.000
80%	.000	.000	.000	.000	.000	.000	

Sample identification based on CC

TABLE V. LSD TEST RESULTS OF CC BETWEEN UNKNOWN AND KNOWN SILVER CARP SURIMI SYNCHRONOUS 2D CORRELATION SPECTRUM

	0	**5%**	**10%**	**20%**	**40%**	**60%**	**80%**
U1	0.001	**0.156**	**0.190**	0.000	0.000	0.000	0.000
U2	0.000	0.000	0.001	**0.387**	0.000	0.000	0.000
U3	0.000	0.000	0.000	0.000	0.000	**0.149**	0.000

The unknown-CCs of three samples U1, U2, U3 were also calculated. And then significance test of difference of 10 groups of CCs was carried out using multiple comparison method. And the results were showed in Table V. Also, the CC group of U1 had no significant differences with that of silver carp surimi sample adulterated with 5% (P=0.156>0.05) and 10% (P=0.190>0.05) hairtail surimi, while significant differences (P<0.05) were observed among CC group of U1 and the rest CC groups. The CC group of U2 had no significant differences with that of silver carp surimi adulterated with 20% (P=0.387>0.05) hairtail surimi, while significant differences (P<0.05) were observed among CC group of U2 and the rest CC groups. And the same with that of U3 which had no significant differences with silver carp surimi adulterated with 60% hairtail

surimi. Three surimi samples were all identified correctly. From above we could conclude that 2D-COS spectrogram difference quantization based on CC could be used in quantitative and qualitative identification of adulterated surimi. And the limit of determination was 5%; it means that the silver carp surimi samples with an interval of adulterate level less than 5% could not be identified correctly.

IV. Conclusions

This study explored the feasibility of 2D-COS combined with ATR-MIR in classification of sliver carp surimi samples which adulterated with different levels of hairtail surimi. This method was based on the characteristics of 2D-COS spectrogram and the samples were identified by calculating the difference between the 2D-COS spectrogram of different samples. As for the quantitative analysis of sliver carp surimi adulterated with different levels of hairtail surimi, when adulterated level intervals was more than 5%, the samples could be classified correctly. Compared with the traditional chemical methods, this method was environmentally, friendly and the detection cost was low; compared with the traditional chemometric methods based on spectroscopy, the detection accuracy and sensitivity were all improved [14], and when the method was established, the sample size was small, and there was no worry about model invalidation. So 2D-COS combined with ATR-MIR has potential applications in the field of minced meat quality inspection.

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