

Research Article

The Sensor Construct of Colorimetric Sensor Array for Rapid Evaluation of Fish Freshness

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Abstract: A Colorimetric Sensor Array (CSA) system was developed for the evaluation of fish freshness using metalloporphyrin as sensor. SPME-GC/MS experiments were carried out to provide the VOCs component information for the theoretical investigation. Density Functional Theory (DFT) method at B3LYP/LANL2DZ level was performed to investigate the binding property between metalloporphyrin and VOCs to construct a proper CSA sensor for the evaluation of fish freshness. Finally, pattern recognition method was used to evaluate the effect of metalloporphyrin on the detection of fish freshness. Results show that the classification accuracies are 78.3, 78.3 and 81.7%, respectively for the data without MnP data, ZnP data and CoP data, respectively, 85.0 and 83.3% for the data without CuP data and FeP data. The evaluation result is in good agreement with the theoretical study. This research suggests that theoretical study is useful for the design of CSA sensor and the construction of CSA sensor will be helpful for quality evaluation of fish freshness.

Keywords: Colorimetric sensor array, density functional theory, fish freshness, SPME-GC/MS

INTRODUCTION

Recent trends of fish freshness in production, processing, marketing and consumption are vital from the food nutrition and safety view. The Food and Agriculture Organization of the United Nations (FAO) in the World Health Organization (WHO) declares food safety along the total production chain from the storage to consumption (Badii and Howell, 2002). VOCs produced by microbiological spoilage and autolytic enzyme decomposition are used as an important indicator of fish freshness to evaluate the product quality. In the last decade, the techniques (Epstein and Walt, 2003; Winquist *et al.*, 2000; Vlasov *et al.*, 2002; Buryak and Severin, 2005) based on sensors have been developed in the various applications for the freshness detection in food products (Winquist *et al.*, 2005; Esbensen *et al.*, 2004; Berna *et al.*, 2004; Ampuero and Bosset, 2003). These technologies are discriminating one analyte from each other by the sensor array reacting with sample. Comparison of the traditional component-by-component detection (e.g., GC-MS, chromatography) (Wang and Sporns, 1999; Gokbulut and Karabulut, 2012), the sensor technology is cheaper and more convenient and applications in the freshness detection have been found. However, these sensors are mostly based on electrochemical sensors or conductive

polymers, which are dependent on the weak interaction between sensors and analyte.

In recent years, CSA sensors based on color changes are developed to detect a wide range of VOCs, with the advantages being low-cost, sensitive, simple and convenient. This novel technique is based on the stronger chemical interactions and has been provided to be the potentially powerful technology in the detection of food quality, such as fish freshness (Huang *et al.*, 2011), soft drink analysis (Zhang and Suslick, 2007; Zhang *et al.*, 2006) and natural amino acids (Huo *et al.*, 2010). A proper CSA sensor is essentially important for the CSA technology. The traditional design of colorimetric sensor array relies on two fundamental requirements:

- Sensors and VOCs possess a strong molecular interaction
- Strong molecular interaction must be strongly related to the color changes. Three classes of dyes meet these requirements:
 - Lewis acid/base dyes (i.e., metal ion-containing dyes)
 - Brønsted acidic or basic dyes (i.e., pH indicators)
 - Dyes with large permanent dipoles (i.e., zwitterionic solvatochromic dyes) (Suslick *et al.*,

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2007; Janzen *et al.*, 2006). It will take a long time and money to select a proper CSA sensor for a special VOCs based on the experiment

In this study, three steps were carried out to construct a proper CSA sensor and evaluate the detection results. SPME/GC-MS experiments were carried out to obtain the VOCs-components to select proper molecular for the theoretical calculation below. DFT-based methods were performed to investigate the binding ability of CSA sensor with VOCs at B3LYP/LANL2DZ level. CSA experiments were carried out to detect the VOCs volatilized by the *Parabramis pekinensis* samples and the CSA sensor were evaluated by the pattern recognition method. Furthermore, this study was intended to provide a proper CSA sensor for rapid detection of fish freshness.

RESEARCH METHODOLOGY

CSA sensor array and molecular model: A special CSA (Fig. 1a and b) was designed from our previous research for the detection of fish freshness by the comparison of the test results. It was composed by 8 kinds of metalloporphyrins and 4 kinds of pH indicators. Dyes were printed on a hydrophobic C₂ reverse phase silica gel and a 4×3 colorimetric sensor array was made. Because of the differences of solubility and polymerization capacity, the diffusion degrees of dyes are different. Figure 1c is the difference images, which was obtained from the after-exposure image to subtract the before-exposure image, almost being identified by the eyes. When the data is processed by pattern recognition tool, it can be up to ppb levels of VOCs. However, it needs a lot of time and money to decide the proper detection system by the previous design strategies.

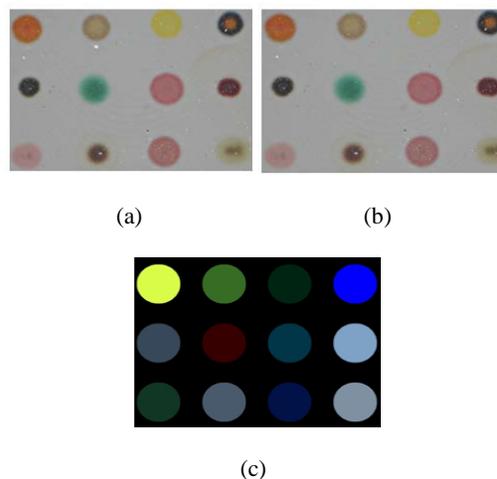


Fig. 1: Color variations of the sensor array before (a) and after (b) exposure to a fish sample, difference images (c) of (a) and (b)

Figure 2 shows the optimized structures for metalloporphyrin (Fig. 2a) and its complex binding with oxygen (Fig. 2b). Chlorine atom was selected as the axial ligand for each metalloporphyrin. This atom was chosen because of its most commonly used in the CSA sensor and the price is lower and easy to purchase it. When VOCs coordinate to an metalloporphyrin, the angle between the VOCs and the Fe-N_{porphyrin} is 45°, which is in agreement with the previous reports (Jensen and Ryde, 2004; Jensen and Ryde, 2005). The binding property of the other metalloporphyrins and VOCs are similar to it.

Sample pretreatment: Fish of bream ("*Parabramis pekinensis*") in the experiments were provided from a local market. This fish was chosen for its high nutrition in protein and the price is lower for the Chinese people

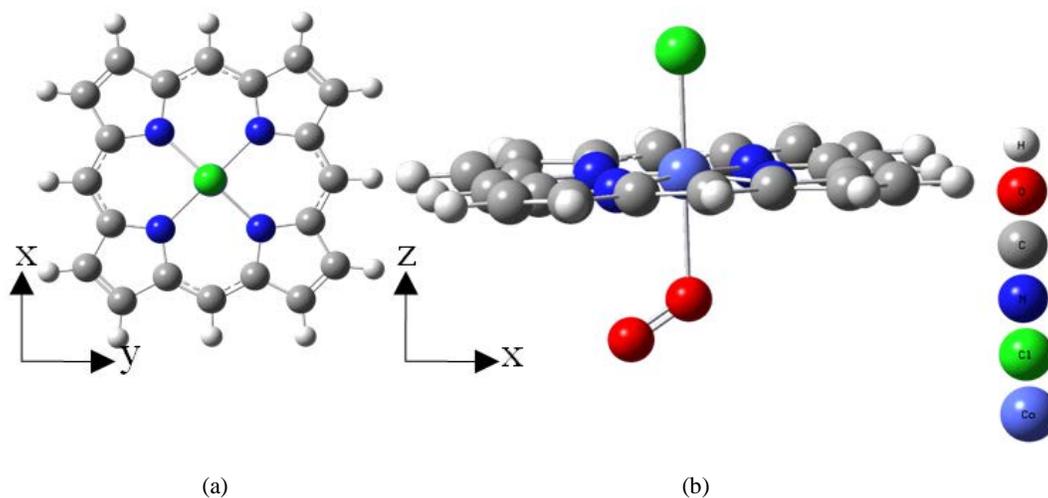


Fig. 2: Optimized structure of CoP and its complex CoP-O₂; all the other complexes are similar, (a) is on the top view to porphyrin plane structure, (b) is at the side view to show the binding property of CoP and CoP-O₂

throughout the year. The weight for each fish is about 400 ± 10 g. Then they were cleaned, gutted and stored in cold storage at 4°C . The experiments were carried out at each 24 h from the first test and lasting for 12 days.

VOCs detection: A sample was provided from the back for each fish about 4 g and 4 fish were selected for each day. Then the sample was taken into hermetically vial (15 mL) to be heated on the plate of PC-420D (Corning, America). A SPME fibre was employed to extract VOCs from the hermetically vial using an 40 min adsorption period at 60°C with shaking the sample. Then sample was desorbed for 5 min at 250°C in the GC-MS instrument (6890N, Agilent Technologies). VOCs were adsorbed into a DB WAX capillary column ($60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$). The VOCs was carried by helium gas at a constant flow rate (0.8 mL/min). Then the temperature program started at 40°C for 4 min, increased to 90°C at 5°C/min , reached to 230°C at 10°C/min , then held at 230°C for 10 min. The splitless mode was used for injection and the temperature of interface is 250°C . The detection instrument is MS with electron impact ionization (5973 Network Mass Selective detector, Agilent Technologies). Mass spectra was collected over m/z range of 33-450 amu and ionization energy was 70 eV.

Theoretical calculation: All the calculations were performed at the Gaussian 09 program package. The initial geometry structures were obtained from Cambridge Crystallographic Data Centre (CCDC). The metalporphyrins molecule is placed on x, y plane (Fig. 1b). The influence of the VOCs on metalporphyrins was simulated by adding the gas atom 2-4 Å above the center of CoP molecule. DFT and B3LYP/ LANL2DZ method (Goh and Nam, 1999; de Visser, 2006) without orbital symmetry constraints was used to optimize molecular structures at different three possible spin multiplicities: low-spin (singlet), intermediate-spin (triplet) and high-spin (quintet) states to obtain the ground state structure of CoP and COP-VOCs.

RESULTS AND DISCUSSION

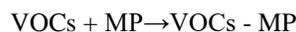
VOCs analysis: Although this study was not carried out the research to determine quantitative data, the compound is absent or present below the detection limits in concentration indicating the absence of the compound. After storage at 4°C , the VOCs detected in the storage period of *Parabramis pekinensis* gives an indication of the freshness and the chemical component that will be used in the design of CSA sensor. Characterizing VOCs for *Parabramis pekinensis* during for different storage days are mainly derived from 69 components: alcohols from C_2 to C_9 ; hydrocarbons from C_5 to C_{17} ; esters from C_4 to C_9 ; ketones from C_3 to C_8 through microbiological spoilage and autolytic enzyme decomposition (Gram and Huss, 1996). Among

these detected compounds, alcohols, hydrocarbons ketones and esters were the main components that can be found in Fig. 3. The trimethylamine was found at each test in Fig. 3 and it was usually used as the indicator of fish freshness for the concentration increasing during the storage period. In our previous study, the freshness of *Parabramis pekinensis* was divided into 5 levels. This result was used to evaluate the effect of metalloporphyrin below. Trimethylamine, propanol, propane, butanone were selected to represent the detected VOCs in the class level including trimethylamine. The molecular model were obtained from Cambridge Crystallographic Data Centre (CCDC).

Relative energy: Relative energy were used to confirm most stable structure for the further theoretical calculation. Geometry structures of Metalloporphyrin-VOCs with different cations were optimized at three possible spin multiplicities: low-spin (singlet or doublet), intermediate-spin (triplet or quartet) and high-spin (quintet or sextet) states of the VOCs-Metalloporphyrins complexes. The spin multiplicities and relative Energies are shown in Table 1. The relative energies of Metalloporphyrins-VOCs at different spin states shows that the low-spin state is the lowest energy state for most of CoP and ZnP and their complexes with VOCs. For most models of FeP, CuP and their complexes, the intermediate-spin state is found to be the lowest energy states. And the lowest energy states at high-spin state is mainly observed in the MnP and its complexes with VOCs.

Furthermore, the energy gaps among the lowest energy states and the other states of FeP, CuP and CoP with VOCs are much smaller than others. According to the previous researches, the metalloporphyrins binding with VOCs require the higher energy states of spin multiplicity changing to the ground states (Sun *et al.*, 2009). Therefore, the smaller energy gap will makes the binding processes through more than one pathway, which leads to a faster and more efficient reaction.

Binding energy: The process of VOCs binding to metalloporphyrin can be described as below:



The Binding Energy (BE) corresponding to the process can be written as:

$$\text{BE} = E_{\text{VOCs}} + E_{\text{MP}} - E_{\text{MP-VOCs}}$$

which is shown in Fig. 4.

All of the energies are obtained from the optimized structures of product and reactants. Figure 5 displays BE (kcal/mol) of all complexes. As shown in Fig. 5, the binding energies for CoP-L4, CoP-O₂, FeP-O₂ are 10.388, 3.845, -5.815 kcal/mol, respectively which is in good agreement with the previous study (10.2 kcal/mol for CoP-L4, 5.765 kcal/mol for CoP-O₂

Table 1: Spin Multiplicity (Ms), relative energies relating to the most stable model (the energies are given in kcal/mol)

	Empty	O ₂	N ₂	H ₂ S	L1	L2	L3	L4	L5	Ms
CoP	0	23.69	0	0	0	0	0	0	0	1
	11.52	0	42.64	12.96	21.72	19.48	7.38	28.95	20.93	3
	32.22	34.36	26.56	16.97	35.45	32.71	27.19	72.40	35.40	5
MnP	60.73	71.95	312	88.01	28.35	59.68	78.07	52.57	52.68	1
	0	16.60	18.41	17.03	100.99	8.02	20.04	11.92	12.14	3
	73.58	0	0	0	0	0	0	0	0	5
FeP	10.20	1.65	30.36	10.15	1.50	0	10.69	0	26.31	2
	0	6.70	0	0	0	7.23	0	9.74	0	4
	1.62	0	68.18	25.13	0.06	48.49	50.88	46.52	50.13	6
CuP	19.20	66.67	39.58	19.18	18.82	19.59	44.36	19.33	29.00	1
	0	20.06	0	0	0	0	0	0	0	3
	56.13	0	63.62	55.38	62.69	56.94	56.30	59.21	59.16	5
ZnP	0	35.47	0	0	0	0	0	864.50	0	2
	34.76	0	32.81	75.16	32.40	75.67	35.39	0	73.01	4
	89.93	71.91	86.97	127.22	83.56	129.76	91.79	51.92	125.17	6

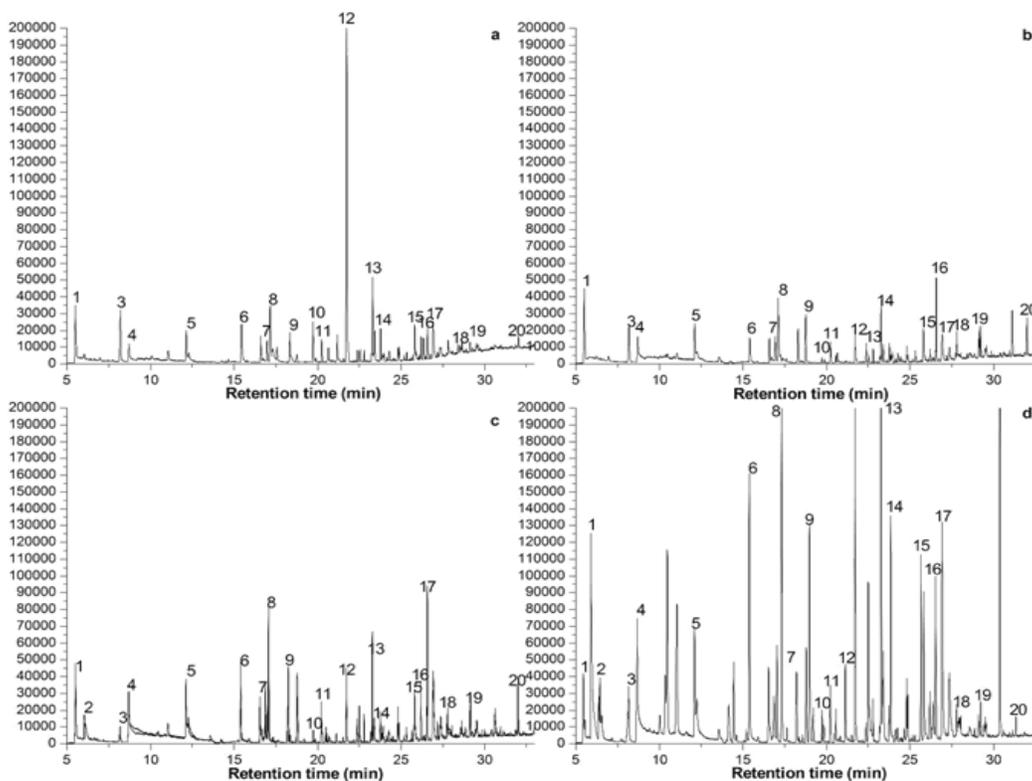


Fig. 3: TIC chromatogram of *Parabramis pekinensis* samples obtained by SPME/GC/MS, (a) fresh fish, (b) 4 day of storage, (c) 7 day of storage, (d) 7 day of storage

1: Trimethylamine; 2: Methanethiol; 3: 2-methyl-propanal; 4: Acetone; 5: Ethyl alcohol; 6: Hexanal; 7: p-xylene; 8: 1-penten-3-ol; 9: 3-methyl-1-butanol; 10: 1-pentanol; 11: 3-hydroxy-2-butanone; 12: 1-hexanol; 13: 1-octen-3-ol; 14: 1, 2, 4, 5-tetramethyl-benzene; 15: 1-nonanol; 16: Heptadecane; 17: Methoxy-phenyl-oxime; 18: 15-crown-5; 19: 1-methyl-naphthalene; 20: Tributyl phosphate

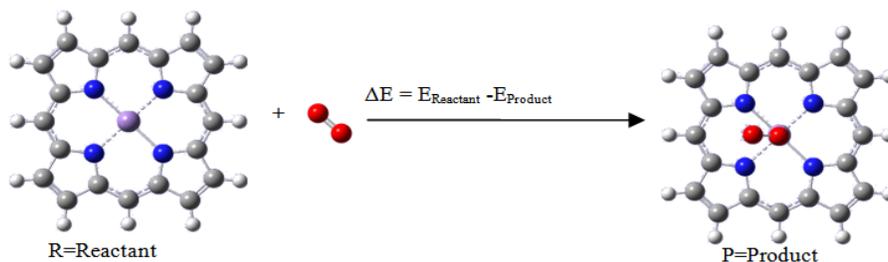


Fig. 4: Binding Energy (BE) associated with the binding process

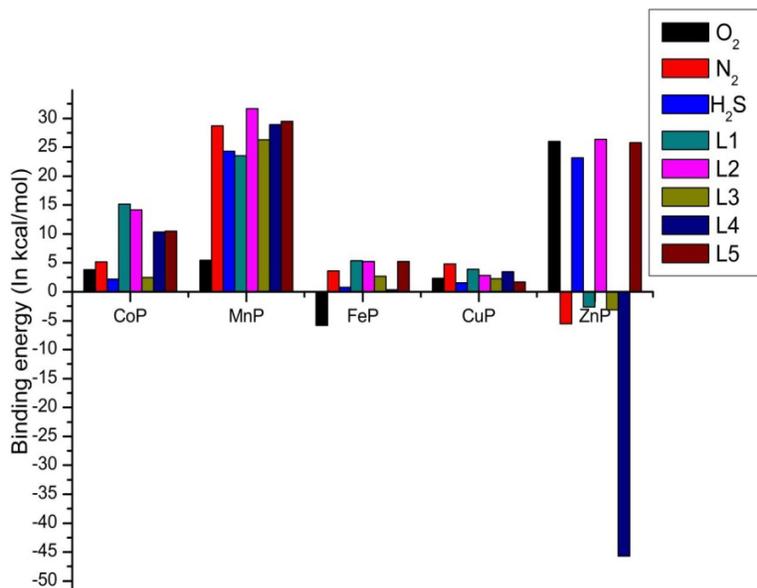


Fig. 5: The Binding Energy (BE) for the most stable models of metalloporphyrins-VOCs in kcal/mol (the energies are given in kcal/mol)

Table 2: SVM classification of fish freshness

	Group 1	Group 2	Group 3	Group 4	Group 5	Total	Classification accuracy (%)
Total	2	1	2	1	1	7	88.3
Data1	5	1	2	2	3	13	78.3
Data2	2	2	3	1	5	13	78.3
Data3	4	1	1	1	2	9	85.0
Data4	2	-	2	1	5	10	83.3
Data5	2	1	4	2	2	11	81.7

Total: The CSA measurement with all metalloporphyrins; Data1: The CSA measurement without MnP; Data2: The CSA measurement without ZnP; Data3: The CSA measurement without CuP; Data4: The CSA measurement without FeP; Data5: The CSA measurement without CoP

and -7.840 kcal/mol for FeP-O₂) (Li *et al.*, 2008; Orellana, 2013; Sun *et al.*, 2011). Furthermore, Figure 5 displays the binding energy of 5.466 kcal/mol that is a little smaller than the previous result (8.763 kcal/mol) (Orellana, 2013). It is interesting that the BE of metalloporphyrins can be divided into two groups. ZnP, MnP and CoP possess the largest BE and FeP, CuP possess a significant smaller BE. Different BEs caused by the different cation in the center of metalloporphyrin and the liganded VOCs can indicate that we can use different cation to modulate the binding of the metalloporphyrins with VOCs. This finding could be utilized in the design of new metalloporphyrin-based CSA sensor for the detection of fish freshness.

Classification with SVM method: The digital data represent the difference between the images before-exposure and after-exposure were evaluated by SVM. The measurements can be classified into four groups, corresponding to day 1, 2 (group 1), day 3, 4, 5 (group 2), day 6, 7, 8 (group 3), day 9 (group 4), day 10, 11, 12 (group 4), respectively. This classification scheme was depended on the trimethylamine experiment, which was carried out in our previous study. The classification results of fish sample with SVM are shown in Table 2.

The overall classification accuracy was 97.5% in train data and 88.3% in test data. The result had lower classification accuracy for samples in the data without MnP data, ZnP data and CoP data; it misclassified 2 in train data and 13 in test data for the data without MnP, 3 in train data and 13 in test data for the data without ZnP, 5 in train data and 11 in test data for the data without CoP. However, data 3 (without CuP data) and data 4 (without FeP data) had a relative better classification, which mean that CuP data and FeP data are not the important as MnP data, ZnP data and CoP data. This difference between the classification results produced by the binding ability, which can be found in Fig. 3.

CONCLUSION

A CSA sensor capable of evaluating the fish freshness during storage was constructed by us. GC-MS result showed that alcohols, hydrocarbons ketones and esters were the main components, that will be used in the theoretical calculation. The ability profiles of metalloporphyrin binding VOCs was divided into two groups corresponding to binding energy. ZnP, MnP and CoP possess the strong binding ability and FeP, CuP

possess a significantly smaller capability. The effect evaluation of metalloporphyrin on the CSA sensor was analyzed using SVM analysis. An overall classification accuracy of 88.3% was achieved. The classification accuracy of the data without corresponding metalloporphyrin data is good in agreement with the theoretical study. Results indicate that theoretical study is useful for the design of CSA sensor and the constructing CSA sensor will be helpful for quality evaluation of fish freshness.

ACKNOWLEDGMENT

This study was sponsored by the Scientific Research Foundation of Chuzhou University (Project No. 2014qd042); Major Program of National Natural Science Foundation of Anhui Province (Project No. KJ2014ZD20); Key University Science Research Project of Jiangsu Province (Project No. 14KJA550001); National Natural Science Foundation of China (Project No. 31071549); Priority Academic Program Development of Jiangsu Higher Education Institutions.

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