

Rapid Semi-Quantitative Analysis of Formaldehyde in Food by Digital Image Colorimetry

Jinda Rungroadsri, Wadcharawadee Limsakul, Worawit Wongniramaikul, and Aree Choodum

Abstract—Digital image colorimetry was successfully applied for the rapid semi-quantitative analysis of formalin in fresh food. A built-in digital camera in mobile phone was used to image a colorimetric product of formaldehyde, while a custom-built color analysis program was used to analyze the color intensity of the digital image. A colorimetric product (yellow) was obtained from the colorimetric reaction between the colorimetric reagents entrapped within a custom-built sensor and formaldehyde. A wide linear range (1 to 50 mg/L) with good linearity (>0.99) was obtained for formaldehyde quantification. Good inter-day precision (1.30-4.37%RSD) with low detection limit (0.016 mg/L) were obtained. The method was applied for semi-quantitative analysis of formaldehyde in fresh foods from fresh markets in Phuket. The concentrations of formaldehyde in the foods were lower than their natural occurrence.

Index Terms—Digital image colorimetry, formaldehyde, mobile phone, colorimetric sensor.

I. INTRODUCTION

Formaldehyde is a widely used industrial chemical due to its high reactivity and relatively low cost [1]. It is mainly used as a precursor in many industries such as textile, plastic, and wood industries [2]-[4]. It is also commonly used as a preservative in medical laboratories, mortuaries, and consumer products. Formaldehyde can cause severe health issues. It irritates to eyes and nose, damages central nervous system, and disorders immune system [1]. The International Agency for Research in Cancer (IARC) has classified it as carcinogenic to human.

Several inappropriate use of formaldehyde for extending food shelf-life has been reported in many countries including Thailand. For example, there was a contamination of formaldehyde in squid and prawn sold at a fresh market in Chiangmai [5]. The concentration of 18.87 ± 1.38 ppm and 19.32 ± 1.70 ppm were found in the samples which are higher than their natural occurrence (*i.e.* 1.8 ppm in squid and 1.0-2.4 ppm in prawn), [6]. The determination of formaldehyde in food is then required.

A wide range of instrumental methods has been reported for the determination of formaldehyde, *e.g.* gas chromatography [7], high performance liquid

chromatography [8], capillary electrophoresis [9], and sensors [10]. These methods need to be done in laboratory using expensive instruments.

Recently, a low-cost colorimetric method has been used in conjunction with digital image analysis as a real-time and rapid quantitative method for various analytes [11]-[13]. It is based on the analysis of RGB values (Red Green Blue basic color) from digital images of the colorimetric product. Due to the reflected light from the colorimetric product passes through RGB filters during imaging. This makes the light separated into three spectral ranges. The separated lights are detected and recorded as individual RGB values by the image sensor located behind each filter. The combination of recorded RGB data generates a final color digital image of the colorimetric product. Analysis of the digital images using color analysis program, *e.g.* Matlab [14], Kylix [15], Visual basic [16], or Adobe Photoshop [17] provided a recorded RGB value. These values can be used as the analytical data to produce a calibration graph for quantitative analysis of interested analyte.

In this work, a digital image colorimetry (DIC) was developed for a rapid semi-quantitative analysis of formaldehyde in foods from fresh markets in Phuket.

II. EXPERIMENTAL

A. Colorimetric Test of Formaldehyde

Formaldehyde solution (37% w/v, Supelco Bellefonte, PA) was used to prepare a stock solution (1000 mg/L). Standard working solutions were prepared daily by diluting of the stock solution in ultrapure water (Barnstead EasyPure II, Thermo fisher scientific, OH) to appropriate concentrations.

A custom-built formaldehyde test kit was used for colorimetric test of formaldehyde. It was developed based on the entrapment of colorimetric reagents within polymer matrix at the bottom of small sample vial (2.5 mL). Acetylacetone (Sigma-Aldrich, USA) in ammonium acetate (Sigma-Aldrich, USA) was used as a colorimetric reagent. Sample solution can be directly added into the test kit for colorimetric detection of formaldehyde.

B. Photographic System and Procedure

A custom-built photographic box [18] was used throughout the experiment. Three sample vials (sensors) were hung at the top of the box. The colorimetric product from the kits were imaged under reproducible illumination using a built-in camera of mobile phone (Samsung Galaxy Core II) which was put in the slot at the front wall of the box.

The RGB values of the digital images were analyzed using a custom-built RGB analysis program installed on the

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computer.

C. Quantification of Formaldehyde

Calibration curve for quantification of formaldehyde was investigated. A series of known concentration formaldehyde standard solutions was added into three sensors to produce color products. The products were photographed at 1 minute post reaction. RGB values from these images were then analyzed using a custom-built color analysis program. Ratios between intensity of blue and red (I_B/I_R), blue and green (I_B/I_G), and blue and total (I_B/I_{TOTAL}) were plotted against the concentrations of formaldehyde solutions to establish a standard curve.

D. Analytical Performance and Method Validation

Sensitivity, linearity, linear range, limit of detection (LOD) and limit of quantification (LOQ) [19] were investigated. Precision was studied in terms of percentage relative standard deviation (%RSD) for RGB values. The accuracy was evaluated by analyzing the known concentration of formaldehyde standard (7.5 mg/L) against the established standard curve.

E. Real Sample Analysis

Fresh foods from fresh markets in Phuket were tested using the formaldehyde sensor coupled with the DIC. The foods were immersed in water for 15 minutes. The water samples were then tested with formaldehyde sensor and DIC. The foods were washed further until clean and ready to cook. They were then immersed in water for further 15 minutes. The water samples were then tested as described.

III. RESULTS AND DISCUSSION

A. Colorimetric Test of Formaldehyde.

Due to custom-built formaldehyde sensor used in this work based on the Hantzsh reaction, yellow product (Fig. 1) was obtained [1], [20]-[22]. Colorimetric reagents consisted of acetylacetone, ammonium acetate, and acetic acid were entrapped within polymer matrix. Acetylacetone was used as β -diketone to be reacted with formaldehyde because it could produce more intense color product and react faster than methyl acetoacetate [1]. When formaldehyde sample solution was added into the sensor, the entrapped chemical reagents were dissolved allowing the colorimetric reaction occurred. Darker yellow products were obtained with increasing formaldehyde concentrations.

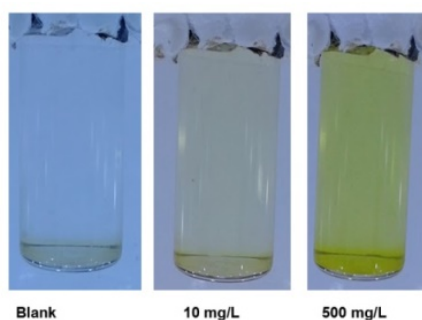


Fig. 1. Color products from custom-built formaldehyde sensors.

B. Digital Image Colorimetry for Formaldehyde

Individual RGB values were obtained from systematic

analysis of digital images of colorimetric products. Intensity of blue channel (I_B) was higher than green (I_G) and red (I_R) channels at low concentrations of formaldehyde (<25 mg/L). Darker yellow products obtained at high concentrations (>25 mg/L) provided higher I_G and I_R than I_B . Intensities of red and green lights seemed to be constant with increasing the concentrations of formaldehyde, but I_B was decreased (Table I). These indicated that the yellow products absorbed the light at the blue range (400-500 nm) which was in agreement with spectrophotometric results (λ_{max} at 462 nm). When more products produced at higher concentrations, the darker color products absorbed more blue light, thus provided less intensities.

TABLE I: INDIVIDUAL RGB INTENSITIES FROM YELLOW PRODUCTS AT VARIOUS CONCENTRATION OF FORMALDEHYDE

Concentration (mg/L)	R (a.u.)	G (a.u.)	B (a.u.)
0	107.1	146.5	195.8
1	123.8	138.3	158.5
5	126.6	139.7	153.8
10	130.7	140.7	146.9
25	132.8	141.0	134.3
50	130.4	140.6	110.5
100	128.7	140.7	97.3
250	126.6	139.9	82.5
500	125.6	141.4	67.6

Total intensity (I_{TOTAL} : $I_R+I_G+I_B$) was also investigated. It was found that I_{TOTAL} decreased when the concentration of formaldehyde was increased as shown in Fig. 2. This was due to the higher amount of color product produced at higher concentration of formaldehyde could absorb more light.

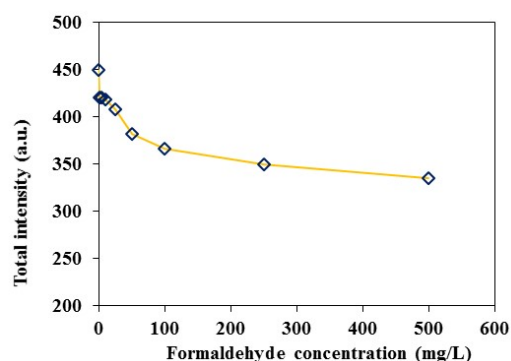


Fig. 2. The relationships between I_{TOTAL} ($I_R+I_G+I_B$) with formaldehyde concentrations.

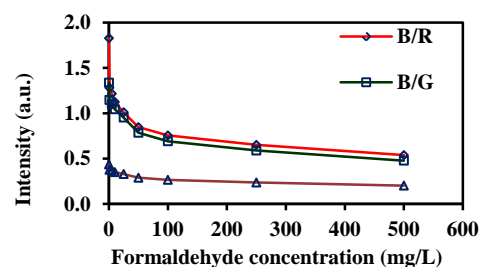


Fig. 3. The relationships between I_B/I_R , I_B/I_G , and I_B/I_{TOTAL} with formaldehyde concentrations.

The relationships of I_B/I_R , I_B/I_G , and I_B/I_{TOTAL} with formaldehyde concentrations are shown in Fig. 3. I_B/I_R was higher than I_B/I_G and I_B/I_{TOTAL} . When concentration of formaldehyde was increased, the I_B/I_R , I_B/I_G , and I_B/I_{TOTAL}

were decreased because of less reflected light from darker products.

The relationships of formaldehyde concentration and I_B/I_G , and I_B/I_{TOTAL} in the range of 1 to 50 mg/L was founded to be linear with linearity of 0.99, and 0.9874, respectively (Fig. 4).

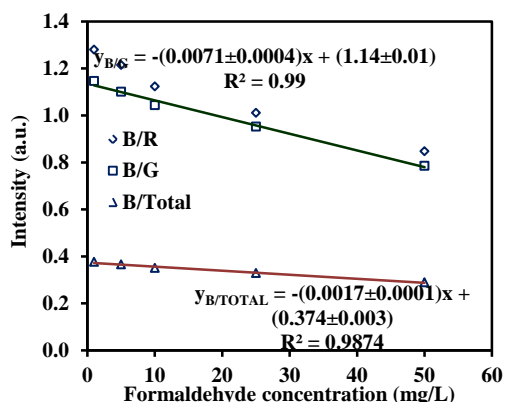


Fig. 4. The relationships between I_B/I_R , I_B/I_G , and I_B/I_{TOTAL} with formaldehyde concentrations in the range of 1 to 50 mg/L.

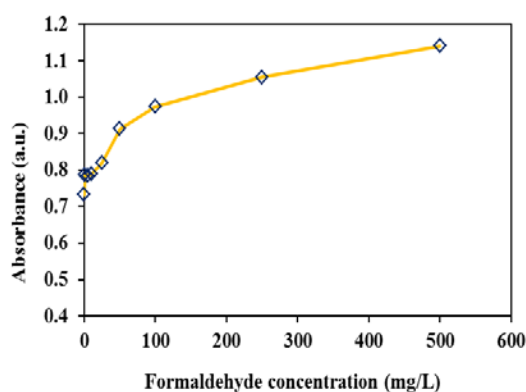


Fig. 5. The relationships between A_{TOTAL} ($A_R+A_G+A_B$) with formaldehyde concentrations.

TABLE II: INDIVIDUAL AND TOTAL RGB ABSORBANCE FROM YELLOW PRODUCTS AT VARIOUS CONCENTRATION OF FORMALDEHYDE

Concentration (mg/L)	R (a.u.)	G (a.u.)	B (a.u.)	Total (a.u.)
0	0.38	0.24	0.11	0.73
1	0.31	0.27	0.21	0.79
5	0.30	0.26	0.22	0.78
10	0.29	0.26	0.24	0.79
25	0.28	0.26	0.28	0.82
50	0.29	0.26	0.36	0.91
100	0.30	0.26	0.42	0.97
250	0.30	0.26	0.49	1.05
500	0.31	0.26	0.58	1.14

When the molecular absorption of the yellow products was calculated [15], [16], the blue channel (A_B) which was increased with concentration of formaldehyde revealed the highest level of absorbance (Table II). This was a good agreement with spectrophotometric result which revealed the maximum absorption of the product in the blue range of 462 nm.

Total absorbance (A_{TOTAL} : $A_R+A_G+A_B$) was also investigated. A_{TOTAL} increased when the concentration of formaldehyde was increased (Fig. 5). The relationships of A_B/A_R , A_B/A_G , and A_B/A_{TOTAL} with formaldehyde concentrations (Fig. 6) revealed that A_B/A_G was higher than

A_B/A_R and A_B/A_{TOTAL} . When concentration of formaldehyde was increased, the A_B/A_G , A_B/A_{TOTAL} , and A_B/A_R were increased because the darker products absorbed more light. The relationships of formaldehyde concentration and A_B/A_G , and A_B/A_R in the range of 1 to 50 mg/L was founded to be linear with linearity of 0.9965, and 0.9888, respectively (Fig. 7).

C. Calibration Curve for Quantification of Formaldehyde

The linear relationships of formaldehyde concentration and I_B/I_G , I_B/I_{TOTAL} , A_B/A_G , A_B/A_R in the range of 1 to 50 mg/L could be used as standard curves for quantification of formaldehyde. However, the relationships of A_B/A_G revealed better sensitivity and linearity, it was selected for quantitative analysis of formaldehyde in real sample. The calibration equation as in (1) was used.

$$y = -(0.0125 \pm 0.0004)x + (0.78 \pm 0.01) \quad (1)$$

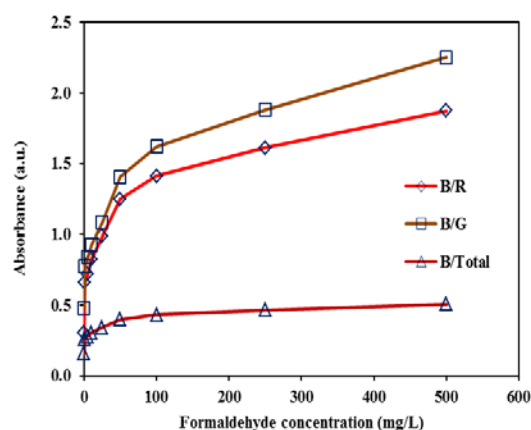


Fig. 6. The relationships between A_B/A_R , A_B/A_G , and A_B/A_{TOTAL} with formaldehyde concentrations.

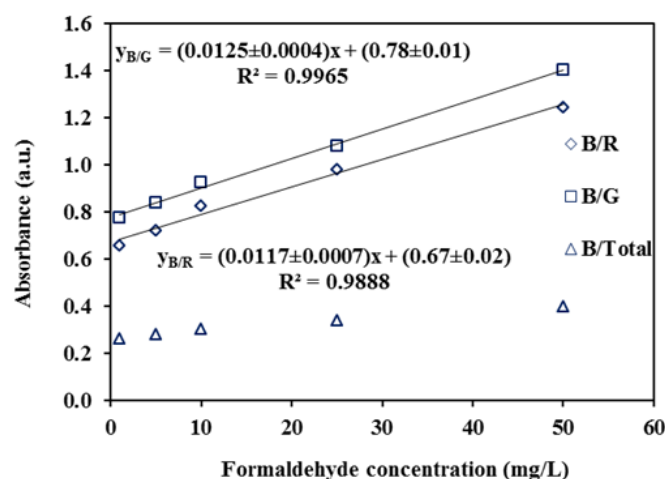


Fig. 7. The relationships between A_B/A_R , A_B/A_G , and A_B/A_{TOTAL} with formaldehyde concentrations.

D. Analytical Performance and Method Validation

Analysis of formaldehyde using a custom-built formaldehyde sensor and DIC provided a linear quantification equation in the range of 1 to 50 mg/L with good linearity (>0.99). The developed method provided sensitivity of 0.0125 ± 0.0004 a.u./mg/L. LOD and LOQ of 0.016 and 0.053 mg/L were obtained indicating a good performance of the method for quantification of formaldehyde in food. Good intra-day (0.28-2.87%RSD)

and inter-day precisions (1.30-4.37%RSD; $n=3$) were obtained. When control samples of 7.5 mg/L were tested, the formaldehyde concentration of 6.5 mg/L was quantified indicating a good accuracy. These results indicated that the use of DIC together with custom-built formaldehyde sensor could be used for quantification of formaldehyde in food. The method made a quantification of formaldehyde easier and cheaper because it required only a daily used mobile phone compared to other expensive laboratory instruments.

TABLE III: CONCENTRATION OF FORMALDEHYDE IN REAL SAMPLES

Sample	Concentration of formaldehyde (mg/kg)
Tomato	0.1-0.7
Shred ginger	0.04-0.1
Jew's ear mushroom	0.5-0.7
Shrimp	0.5-0.6
Fish	0.3-0.4
Squid	Non detectable

E. Real Sample Analysis

Fresh food samples including tomato, shred ginger, jew's ears mushroom, shrimp, fish, and squid from fresh markets in Phuket were tested using the developed method. Formaldehyde concentrations in a range of 0.04 to 0.7 mg/kg were obtained (Table III). The highest formaldehyde concentrations were found in tomato and mushroom samples in the ranges of 0.1 to 0.7, and 0.5 to 0.7 mg/kg, respectively. These quantified concentrations were lower than their natural occurrence.

IV. CONCLUSION

A digital image colorimetry (DIC) was successfully developed for a rapid quantification of formaldehyde in fresh foods from fresh markets in Phuket. The analytical performance and method validation were found to be effective for determination of formaldehyde in foods. Analysis of fresh foods collected from fresh markets in Phuket showed lower concentration of formaldehyde than their natural occurrence.

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