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Determination of Formaldehyde in Dried Shiitake Mushrooms by a Modified Pararosaniline Spectrophotometric Method

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This study established a simple and efficient method for the determination of formaldehyde in dried shiitake mushrooms. Formaldehyde in mushrooms was extracted by a wet pretreatment procedure, followed by Ultraviolet-visible (UV-Vis) spectrophotometric analysis using an acidified pararosaniline reagent. The proposed method had a high sensitivity (a detection limit of 0.02 mg L^{-1}) and a high recovery (90.3–96.5 %) with a relative standard deviation (%RSD) of 1.8–5.1 %. Formaldehyde levels in dried shiitake mushrooms collected in local markets (Ho Chi Minh City, Vietnam) ranged from 169.1 to 402.5 mg kg^{-1}.

1. Introduction

Formaldehyde (FA), the simplest aldehyde, has been illegally used in various foods (Rahman et al., 2023), including fish (Bhowmik et al., 2017), milk (Rezende et al., 2017), meat, egg (Jung et al., 2021), beverages, vegetables, and fruits (Nowshad et al., 2018) for antiseptic and preservation purposes (Rahman et al., 2023). With its concerning health risks, the International Agency for Research on Cancer (IARC) has classified FA as a group 1 human carcinogen (Protano et al., 2022). The World Health Organization (WHO) and the US Environmental Protection Agency established the acceptable daily consumption rates of FA for humans as 0.15 mg kg^{-1} body weight per day and 0.2 mg kg^{-1} body weight per day (Rahman et al., 2023). The FA contents in some food items were analyzed using various techniques and briefed in Table 1.

Table 1: The contents of FA in some food items

Geographical location	Analytical method	Limit of detection (LOD)	Food type	FA content (mg kg^{-1})	Reference
Bangladesh	HPLC–PDA	0.32–1.75[a]	Rice, fish, vegetables, fruits	2.5–26.2	Wahed et al., 2016
	HPLC	0.0069[b]	Fish	5.1–39.68	Bhowmik et al., 2017
Brazil	Spectrophotometry	0.02[a]	Mushrooms	27.4–1052	Pinto et al., 2018
	Electrochemical oxidation of DDL on GCE	0.13[a]	Mushrooms	30–493	Pinto et al., 2019
China	HPLC–DAD	0.005[a]	Mushrooms	119–494	Liu et al., 2005
	Spectrophotometry	0.0029[a]	Seafood, vegetables, mushrooms	12.3–86.0	Cui et al., 2007
	Spectrophotometry	0.1[b]	Mushrooms	0–270	Shao et al., 2024
Portugal	Sensor	0.2[a]	Milk	< 0.6–2.8	Veríssimo et al., 2020
Taiwan	GC-MS	2.0[b]	Squid	4.1–48.5	Yeh et al., 2013
USA	PDMS	2.0[a]	Fish, meat, mushrooms	5.4–187.9	Weng et al., 2009
		5.0[b]			

[a] presented in mg L^{-1}; [b] presented in mg kg^{-1}.

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Shiitake (*Lentinus edodes*) is the second most consumed mushroom globally due to its health and nutraceutical benefits (Salwan et al., 2021). It is a rich source of nutrients, typically carbohydrates, protein (Li et al., 2018), and a range of vital minerals, vitamins, bioactive compounds, and antitumor polysaccharides (Ahmad et al., 2023). On the other hand, shiitake mushrooms were reported to contain high FA levels (up to 478 ± 12 mg kg^{-1} for dried samples (Pinto et al., 2018)). There are several potential explanations for the presence of FA in mushrooms: (i) illegally added to mushrooms to extend their shelf life (Wahed et al., 2016), (ii) a residual trace from air disinfection during seed inoculation, and (iii) a natural byproduct of the shiitake mushroom's metabolism (Liu et al., 2005).

FA in food or mushrooms can be extracted by wet sample pretreatment or steam-distilled methods, then derivatized using different reagents, and finally analysed by a wide range of analytical methods (Rahman et al., 2023). Although HPLC and GC-MS offer significant advantages such as high sensitivity, excellent selectivity, and accurate results (Su and He, 2017), their implementation can be challenging due to expensive instruments and heavy equipment operator skills (Pinto et al., 2019). Colorimetric sensors offer fast and convenient ways to quantify FA (Wongniramaikul et al., 2018); their accuracy can be compromised due to a lack of specificity and selectivity (Wahed et al., 2016). Spectrophotometry provides a compelling combination of simplicity, cost-effectiveness, sensitivity, and selectivity, making it a popular choice for various analytical applications (Yuan et al., 2022). A major drawback of current spectroscopic techniques is their reliance on harsh conditions (using strong acids and high temperatures) (Hladová et al., 2019). Many spectroscopic techniques utilizing reagents with milder analytical conditions or simple procedures have been continuously developed. The modified pararosaniline method stands out for its ease of use, high sensitivity, and selectivity, being effective for the determination of FA in air (Huynh et al., 2024).

Being popular in Asian cuisine, dried shiitake mushrooms are a staple in meals across China, Japan, Thailand, and Vietnam (Liu et al., 2005). The FA contents in dried shiitake mushrooms were currently analysed by acetylacetone spectrophotometric methods, for instance the two recent reports from Thailand (Wongniramaikul et al., 2018), and China (Shao et al., 2024). There is still a lack of FA data for dried shiitake mushrooms from Vietnam, this study established optimal conditions for an acidified pararosaniline (PRA) UV-vis spectroscopic method to determine FA levels in some dried shiitake mushrooms in Vietnam.

2. Experimental

2.1 Optimization of FA derivatization using PRA reagent

The PRA processes were performed on a sample containing an initial FA concentration of 1.00 mg L^{-1} and a blank containing only distilled water. Reagents at various initial concentrations (1.5–15 mM for pararosaniline hydrochloride; 1.2–9.6 M for hydrochloric acid; 7.9–79 mM for sodium sulfite) were added to the samples, and then processed at various temperatures (30 °C, 50 °C, 70 °C, and 90 °C) for 5–120 min.

2.2 Sample preparation and FA determination

Dried shiitake mushrooms were collected from local markets in Ho Chi Minh City (Vietnam). FA in dried shiitake mushrooms was extracted by a wet sample preparation method using distilled water. After being cut and blended into small pieces (2 mm × 5 mm × 5 mm), dried shiitake mushroom sample (0.500 g) was transferred to a 20 mL test tube with a cap, and soaked in 10.0 mL of distilled water at various temperatures (30–100 °C) for 40 min. After being cooled down quickly, the sample solution was filtered through a syringe filter and then diluted (10-fold) with distilled water to form the diluted extract solution.

For the quantification of FA in the diluted extract solution: 0.50 mL of a PRA reagent (6.2 mM pararosaniline in 2.4 M hydrochloric acid) was added into a test tube containing 5.00 mL of the diluted extract solution. After mixing the solution well, 0.50 mL of 16 mM sodium sulfite was added. The tube was capped, and the mixture was again thoroughly mixed. It was left to stand at room temperature (approximately 30 °C) for 30 min to reach colour stability. The absorbance of the resulting solution was recorded at a wavelength (λ_{max}) of 576 nm on a Biochrom Libra S22 UV-Vis Spectrophotometer (USA). The results were reported as average values in triplicate.

3. Results and discussion

3.1 Processing a PRA method for FA determination

The derivatization reaction between FA and PRA can be influenced by operating factors, including concentrations of pararosaniline hydrochloride, hydrochloric acid (HCl), sodium sulfite (Na_2SO_3), as well as temperature, and reaction time. This section aimed to optimize these conditions to achieve high sensitivity for samples while low signal (absorbance) for blank solutions.

Regarding the effect of pararosaniline hydrochloride concentration, its initial concentrations were varied in a range of 1.5–15 mM (in 2.4 M HCl). As shown in Figure 1a, the sensitivities of both sample and blank solutions increased with an increase in pararosaniline concentration. According to Monro et al. (2012), when pararosaniline hydrochloride is dissolved in an aqueous solution, it produces a magenta solution. This solution turns a pale biscuit colour when acidified with HCl due to diminished conjugation. The incomplete bleaching can happen when adding more pararosaniline hydrochloride at the same HCl concentration. As an acceptable value for the blank absorbance (< 0.2 absorption unit), the pararosaniline hydrochloride concentration of 6.2 mM was rationally selected.

The effect of HCl on the colour-forming reaction was examined by using pararosaniline solution (6.2 mM) and adding HCl at different concentrations (1.2–9.6 M). Figure 1b indicated that the absorbances of both sample and blank solutions were high at HCl low concentrations and dropped gradually at HCl high concentrations. The studies by Miksch et al. (1981) and Georghiou et al. (1983) showed that the use of excess [H^+] decreased the sensitivity of the mixture solution because it could lead to an equilibrium shift that generated a large number of cation radicals on an acidified pararosaniline molecule. The 6.2 mM pararosaniline in 2.4 M HCl solution (prepared by dissolving 20 mg pararosaniline hydrochloride in 2.00 mL of concentrated HCl and diluting to 10.0 mL with distilled water) was used for further studies.

Sodium sulfite was also crucial to the formation of complex solutions. Initially, a pale purple solution was produced when FA and PRA reacted to form iminium ions. The addition of sodium sulfite caused a highly conjugated alkyl amino-sulfonic acid, resulting in a rich purple solution (Monro et al., 2012). Figure 1c showed the effect of sodium sulfite concentrations (7.9–79 mM) on the complexation reactions. The absorption tended to gradually decline at high sulfite concentrations. As a result, the sulfite concentration of 16 mM was selected for the best sensitivity in sample processing.

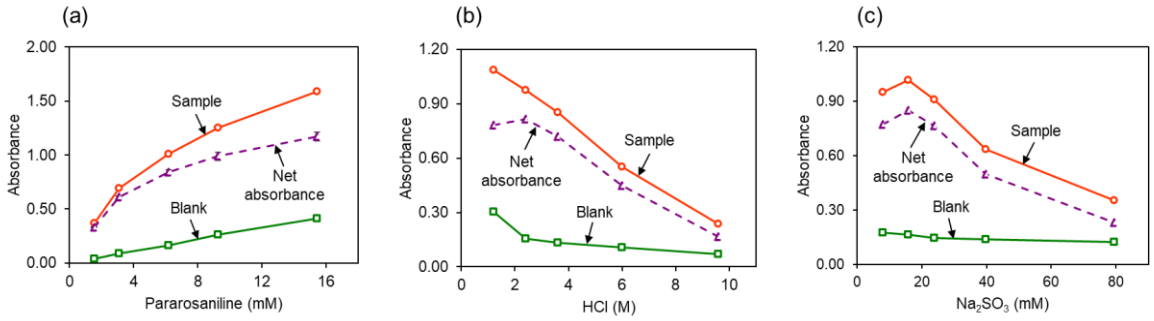

Figure 1: Effect of factors: a) pararosaniline (1.5–15 mM); b) HCl (1.2–9.6 M); c) Na_2SO_3 (7.9–79 mM) on absorbance

Using the optimized concentrations of PRA and sulfite, the influences of temperature and reaction time on the colour-forming process were investigated at low (0.10 mg L^{-1}) and high (1.00 mg L^{-1}) FA standard solutions. Figure 2a showed that the net sensitivities (the difference in absorbance between the sample (A) and blank (A_0), $\Delta A = A - A_0$) were high at low temperatures (30 °C and 50 °C) and decreased when the temperature of the reaction increased (70 °C and 90 °C). The temperature of 30 °C was selected for the complexation reaction. Figure 2b showed the effect of time on the complexation reaction at 30 °C. The absorbances increased from approximately 0.7 to 0.9 for the first 30 min and lightly decreased to approximately 0.8 when the reaction time reached 120 min. These results permitted selecting the reaction time of 30 min.

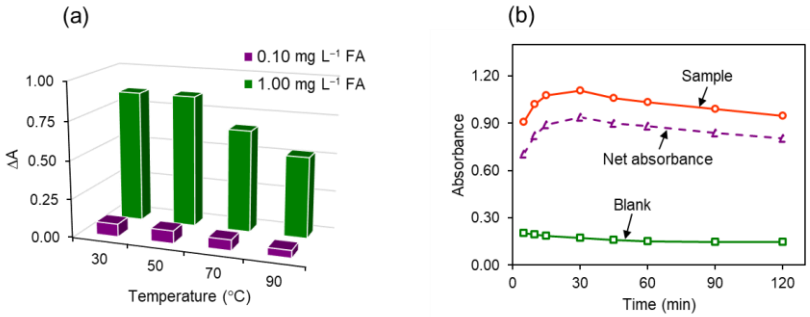

Figure 2: Effect of factors: a) temperatures (30–90 ℃); b) time (5–120 min) on the complexation reaction

Using optimized conditions of the complexation reaction, a standard curve with the FA concentration ranging from 0 to 2.00 mg L^{-1} was established (Figure 3), with good coefficient of determination (R^2 > 0.995). The limit of detection (LOD) of 0.02 mg L^{-1} (Eq(1)), and the limit of quantification (LOQ) of 0.06 mg L^{-1} (Eq(2)) were estimated via replicate analysis of blank solutions containing distilled water with the reagents (Nguyen et al., 2023).

$$\mathrm{LOD} = \frac{3\times \mathrm{SD}}{\mathrm{b}} \quad (1)$$

$$\mathrm{LOQ} = \frac{10\times \mathrm{SD}}{\mathrm{b}} \quad (2)$$

where SD = the standard deviation, and b = slope of the calibration curve.

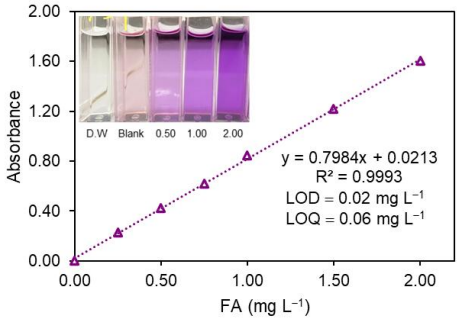

Figure 3: The calibration curve and colours of standard FA solutions (D.W = distilled water; Blank = distilled water with the reagents; 0.50, 1.00, and 2.00 = FA concentrations of 0.50, 1.00, and 2.00 mg·L^{-1} with the reagents)

3.2 Determination of FA in shiitake mushrooms

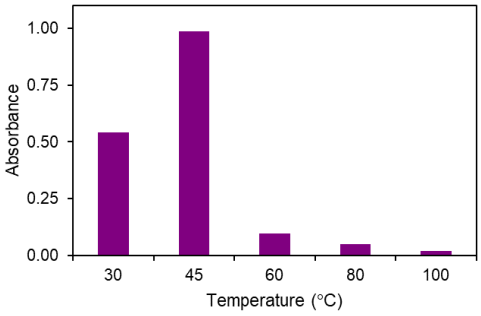

Figure 4: Effect of temperature on FA extraction from dried shiitake mushrooms

According to Liu et al. (2005), shiitake mushroom samples were pretreated with a high ratio of solvent-to-sample for a prolonged time. Since the FA levels in the mushroom samples from the previous study (Table 1) were high, only a small sample size (0.500 g of sample in 10.0 mL of distilled water) was examined. At high temperatures (over 60 °C), FA could be evaporated from the extraction solution or converted into other intermediates, considerably reducing absorbance. This was similar to the study done by Mason et al. (2007), in which the FA content significantly decreased (from 32 to 85 %) after frying 6 min. The FA extraction from such dried shiitake mushrooms should be carried out at 45 °C.

The recovery study on dried shiitake mushroom samples spiked FA at three different levels (50, 250, and 500 mg kg^{-1}) demonstrated good values with the recoveries ranging from 90.3 to 96.5 % (%RSD from 1.8 to 5.1 %) (Table 2) within the acceptable ranges established by the Association of Official Agricultural Chemists. Liu et al. (2005) also reported high recoveries (80–102 %) for the FA extraction from fresh shiitake mushrooms by water using ultrasonication and its quantification by liquid chromatography.

The results of FA concentrations in seven dried shiitake mushroom samples purchased from different local markets in Ho Chi Minh City (Vietnam) were reported in Table 3, ranged from 169.1 mg kg^{-1} to 402.5 mg kg^{-1}.

The results were similar to the previous studies (mushroom samples from Brazil, China, and USA), as shown in Table 1. In conclusion, the FA content in mushrooms is mostly of natural origin.

Compared to HPLC and GC-MS methods (Table 1), the present method has some advantages such as low-cost, and simple experimental procedure. This method has a high sensitivity with a low limit of detection (LOD = 0.02 mg L^{-1}), a good accuracy (via the recovery of 90.3–96.5 % at three different FA levels), and a good repeatability (via %RSD of 1.8–5.1 %) in compliance with the Association of Official Agricultural Chemists. Based on an acetylacetone spectrophotometric method for the FA determination in dried shiitake mushrooms, Pinto et al. (2018) reported a longer extraction procedure (60 min) with the LOD of 0.02 mg L^{-1} and the recovery of 92–114 %, evidencing that the present pararosaniline spectrophotometric method is more effective and efficient.

Table 2: Recovery study

FA spiked (mg kg^{-1})	FA detected [a] (mg kg^{-1})	Recovery (%)	RSD (%)
0	334.0 ± 2.7	-	-
50	379.1 ± 2.3	90.3	5.1
250	575.3 ± 8.8	96.5	3.7
500	815.1 ± 8.5	96.2	1.8

[a] an average value ± standard deviation (for three replicates)

Table 3: FA levels in dried shiitake mushrooms

Sample No.	FA [a] (mg kg^{-1})
1	272.5 ± 5.4
2	336.4 ± 4.9
3	319.3 ± 5.1
4	402.5 ± 4.4
5	169.1 ± 5.6
6	200.2 ± 4.5
7	285.0 ± 5.7

[a] an average value ± standard deviation (for three replicates)

4. Conclusions

This study developed a reliable method to measure FA levels in dried shiitake mushrooms. The procedure involved soaking 0.500 g of blended mushrooms in 10.0 mL of distilled water at 45 °C for 40 min, achieving the high recoveries of FA from 90.3 to 96.5 % at different FA levels. The complexation reaction using 6.2 mM pararosaniline (in 2.4 M hydrochloric acid) and 16 mM sodium sulfite could be performed at room temperature (approximately 30 °C) for 30 min. The studied method is simple, it can be widely applied for the FA determination in dried shiitake mushrooms. In practice, with seven shiitake mushroom samples collected from local markets in Ho Chi Minh City (Vietnam), the FA levels ranged from 169.1 to 402.5 mg kg^{-1}, evidencing that the FA contents in such mushroom samples are mostly of natural origin. The present method can be extended to a variety of foods with a minor modification due to the differences in sample matrix and FA content.

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