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Endoscope-Based Digital Image Colorimetry for Nitrite Quantification in Processed Meats

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ABSTRACT. This study introduces a novel digital image colorimetry (DIC) approach for quantifying nitrites in processed meats, specifically sausages, to meet the demand for more accessible and cost-effective testing methods in food safety. By utilizing a custom-designed, 3D-printed imaging box to ensure standardized experimental conditions and incorporating an endoscope as the image sensor to reduce variations caused by smartphone camera specifications, the DIC method enhances the accuracy and reliability of analysis. The method achieved comparable accuracy to the Griess spectroscopy method (average error <5%) while providing significant advantages in portability, affordability, and accessibility for on-site nitrite concentration monitoring. Validation against the Griess spectroscopic technique demonstrated consistent results in quantifying nitrites in both commercial sausages and standard samples. This study highlights the utility of the DIC method as a viable tool for routine nitrite testing in the food industry, supporting consumer safety and regulatory compliance. Furthermore, the proposed approach paves the way for broader applications in food safety and analytical chemistry.

Keywords: 3D-printed device, digital image colorimetry, endoscope sensor, food safety monitoring, Griess analysis, nitrite quantification

INTRODUCTION

The process of curing has been used since ancient times to enhance flavor and preserve the safety and quality of food, particularly meats (Martin, 2001). Nitrites are one of the primary curing agents, playing a vital role in preventing the formation of anaerobic spores and eliminating food pathogens (Keeton, 2017). They also react with oxygen to form nitric oxide, which helps inhibit lipid oxidation (Karwowska et al., 2019) and interact with myoglobin to enhance the pink color in meat (Wójciak et al., 2019).

However, concerns about the potential health risks associated with nitrite consumption have emerged (Shakil et al., 2022). Nitrites can undergo nitrosation under acidic conditions, forming nitrosamines, suspected as carcinogens (Bryan et al., 2012). Furthermore, elevated blood nitrite levels have been linked to increased methemoglobin (MHb) concentration, which impairs the ability of hemoglobin to transport and release oxygen. This condition can lead to fatal systemic hypoxia, metabolic acidosis, and cyanosis (Padovano et al., 2022).

In response to these concerns, the Joint FAO/WHO Expert Committee on Food Additives (2002) has set an acceptable daily intake limit of 0.07 mg/kg of body weight for nitrites. Badan Pengawas Obat dan Makanan Republik Indonesia (2013) has established a limit of 30 mg/kg for nitrite content in processed meat products. Despite these regulations, nitrite remains a common additive in processed meats, primarily due to their widespread availability and low cost (Jo et al., 2020). As a result, developing reliable methods for analyzing nitrites is essential for public monitoring, ensuring consumer safety and maintaining compliance with regulatory standards.

Several analytical techniques have been developed for nitrite detection, including Griess spectroscopy (Mohamed et al., 2008), chemiluminescence (Gill et al., 2019), ion chromatography (Coviello et al., 2020), and electrochemical detection (Han et al., 2024). Among these, Griess spectroscopic method is one of the most commonly used due to its simplicity and affordability (Wang et al., 2017). This method involves the reaction of nitrite ions with sulfanilamide and N-(1-naphthyl)ethylenediamine (NED) in an acidic solution, forming a pink or red azo dye (See **Figure 1**). The intensity of the dye is proportional to the nitrite concentration, which can be quantified using UV-Vis spectroscopy.

Despite its utility, the Griess spectroscopic method requires a UV-Vis spectrophotometer, and the Griess reagents must be frequently replaced due to the rapid degradation of aqueous sulfanilamide and NED solutions (Mako et al., 2020). More importantly, this b method is unsuitable for bon-site analysis, as

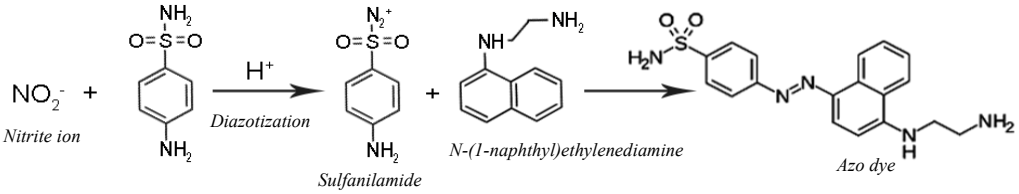

Figure 1. Schematic representation of the reaction between nitrite ions, sulfanilamide, and N-(1-naphthyl)ethylenediamine in an acidic solution, resulting in the formation of azo dyes.

it necessitates sending samples to a laboratory. With the increasing demand for portable analytical methods that can be performed quickly on-site, paper-based analysis using test strips has emerged as a more convenient alternative for nitrite quantification (Sarvestani et al., 2024). However, this method is less precise than the spectroscopic approach, as it is semi-quantitative and provides only rough estimates of nitrite levels in specific gradations (i.e. 1, 5, 10, 20, 40, and 80 mg/L) (MACHEREY-NAGEL GmbH & Co. KG, 2024). This measurement also relies on a subjective visual comparison of color changes, which can lead to inaccuracies (Fan et al., 2021).

In recent years, digital image colorimetry (DIC) using smartphones has gained significant traction as a portable and cost-effective analytical tool, offering convenience, accuracy, and the ability to collect and process data on-site without the need for specialized equipment (Thongkam & Hemavibool, 2022). To address the limitations of existing methods, we propose a new DIC method that combines the use of an endoscope sensor with a custom-designed 3D-printed imaging box. This novel approach aims to address variations in smartphone camera specifications (Nixon et al., 2020) and standardize the experimental setup to maintain consistent lighting and image capturing conditions (Permana et al., 2023). The use of endoscope sensors in digital image colorimetry (DIC) has been explored primarily in point-of-care diagnostics, such as blood flow monitoring (Kim et al., 2022), ear and oropharyngeal examinations (Cai et al., 2021), and cervical cancer screening (Kadama-Makanga et al., 2024). However, their application in chemical analysis remains limited. This study utilized endoscope technology to improve the portability and consistency of DIC analysis of nitrites in meats, especially sausages, while retaining its reliability and accuracy.

EXPERIMENTAL SECTION

Materials

Sodium nitrite (Merck, ACS grade, ≥99.0%) was used as the standard for nitrite determination in this study. The Griess reagent was prepared using sulfanilamide (Loba Chemie, AR grade, ≥99.0%), N-(1-naphthyl)ethylenediamine (Merck, ACS grade, ≥97.0%), and phosphoric acid (Xilong Scientific, AR grade, ≥85.0%). Commercial chicken sausages used in this study were purchased from local grocery stores. Sodium tetraborate (Xilong Scientific, AR grade, ≥99.0%) was used to extract nitrites from the sausages. Paper-baed nitrite quantification was performed using QUANTOFIX Nitrite 91311 semi-quantitative test strips (MACHEREY-NAGEL, Germany).

Digital Image Colorimetry (DIC) Measurements

Nitrite test strips were dipped into nitrite solutions until the reaction pads were fully submerged for 1–2 s. The strips were then shaken for 30 s to remove excess liquid and allow color development. Afterwards, the strips were placed on a test strip tray inside a 3.0 cm × 3.0 cm × 8.0 cm imaging box (custom-built using 3D-printed PLA+ filament) to ensure consistent lighting conditions during image capture. Digital images of the test strips were captured using a 720P HD endoscope (Antootan AN98A, China), operated via the Dr. Endoscope USB Camera Pro application. The endoscope's built-in LED light source was set to an illumination level of 75 ± 5 lux, which was independently measured using AS803 Lux Meter. **Figure 2** illustrates the arrangement of the imaging box, endoscope, and smartphone (OPPO F11) used for digital image colorimetry performed in this study.

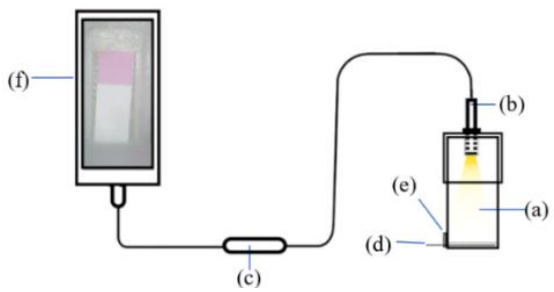

Figure 2. Experimental setup for DIC analysis, including (**a**) the imaging box, (**b**) the endoscope, (**c**) the LED illumination controller, (**d**) the test strip, (**e**) the test strip tray, and (**f**) the smartphone display.

The nitrite measurements were conducted in triplicate (i.e., three test strips were used to measure each synthetic sample and sausage extract solution), with five images captured for each strip. The RGB values of the test strip images were extracted using Adobe Photoshop CS6, focusing on a region of interest (ROI) containing at least 1,000 pixels. The average red (R), green (G), and blue (B) values were then normalized to obtain their respective *r*, *g*, and *b* color parameters using Equations (1-3):

$$r = R/(R + G + B) \quad (1)$$
$$g = G/(R + G + B) \quad (2)$$
$$b = B/(R + G + B) \quad (3)$$

Spectroscopic Measurements Using the Griess Method

Spectroscopic analysis using the Griess method was conducted to validate the DIC methods developed in this study. The Griess reagent used in this study consisted of 2.0% sulfanilamide dissolved in 5.0% phosphoric acid and 0.2% N-(1-naphthyl)ethylenediamine, following the procedure outlined by Komsta et al. (2013).

A 1,000 μL aliquot of Griess reagent was added using a micropipette (Corning, USA) to 25.0 mL of the sample nitrite solution in a volumetric flask, resulting in a reddish-pink color. The mixture was allowed to stand at room temperature for at least 10 minutes to stabilize the color. The absorbance of the colored solution was then measured using a UV-Vis spectrophotometer (Hanon i3, China) at a wavelength of 540 nm. Each sample solution was measured in triplicate, and the average values of the absorbance readings were used for analysis.

Calibration and Validation of DIC and Griess Methods

A 1,000 mg/L nitrite stock solution was prepared by weighing 0.1000 ± 0.0002 g of sodium nitrite using an analytical balance (Sartorius BCE224i-1s) and dissolving it in deionized water (Adrona B3 HPLC, Latvia) in a 100.0 mL volumetric flask. This stock solution was further diluted with deionized water to prepare both calibration standards and synthetic samples with known nitrite concentrations. The concentrations of the standard solutions used for DIC calibration were 5, 10, 20, 30, and 40 mg/L. The concentrations of the standard solutions used for Griess calibration were 0.0 (blank), 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L. Synthetic samples were prepared with concentration values selected within the respective linear calibration ranges of each method. Calibration curves for the DIC and Griess analyses were constructed by plotting the concentrations of the standard solutions against the test strip color parameter signals (*r*, *g*, or *b* values) and absorbance values, respectively.

Method validation parameters, including linearity, working range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), were evaluated following standard analytical validation guidelines (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2023). Linearity and working range were assessed from the calibration curves within the observed linear response regions. LOD and LOQ were calculated from calibration statistics based on the standard deviation of the response (S_y) and the slope of the calibration curve. Accuracy was evaluated using synthetic samples with predetermined nitrite concentrations by comparing measured values with actual concentrations, while precision was assessed using triplicate measurements as described in the corresponding experimental sections.

Nitrite Extraction and Analysis in Commercial Sausage Samples

A single, commercially available chicken sausage product was selected as a representative processed meat matrix to compare the analytical performance of the DIC and Griess methods. Nitrite extraction from sausage samples was adapted from previously reported methods (Hersa & Pratiwi, 2018; Pereira et al., 2012; Rincón et al., 2003).

15.0 g of sausage was cut, homogenized using a blender, and placed in 50 mL centrifuge tubes (Hersa & Pratiwi, 2018). Nitrite extraction from processed meats can be performed both with and without sodium tetraborate (borax) solutions (Rincón et al., 2003). In this study, different concentrations of borax solutions were used to extract nitrite from the sausage samples. For samples with borax solutions (2.5% or 5.0%), 10.0 mL of the solution was added, and the volume was adjusted with deionized water to a final volume of 50 mL. The centrifuge tubes were then heated in a water bath (Lauda Scientific GmbH, Germany), derived from the procedure used by Pereira et al. (2012). The heating process was performed at 80°C for 15 minutes.

After heating, the samples were allowed to cool to room temperature, and the nitrite extract was filtered through Grade I filter paper (Hawach, USA). The filtrate was first analyzed using test strips for DIC measurement. For Griess analysis, the same filtrate was diluted with a factor of 20 to adjust nitrite concentration to the suitable calibration range. All experimental procedures, including nitrite extraction and determination, were performed in triplicate to ensure consistency in measurements.

RESULTS AND DISCUSSION

Digital Image Colorimetry and Griess Calibration Measurements

Digital Image Colorimetry (DIC) analysis of nitrite test strips was performed using an endoscope as the image sensor. This sensor offers several advantages, such as cost-effectiveness and compatibility with various connected devices, including smartphones and computers. It should be noted that the use of an endoscope eliminates variations caused by the differing specifications or settings of smartphone cameras, ensuring more consistent image capture. Additionally, the endoscope is equipped with a built-

in, adjustable LED light source, offering better control over light intensity during analysis. Its shorter focal length and smaller size, compared to a standard smartphone camera, enables the construction of a more compact and portable imaging box (see **Figure 2**), facilitating the convenience and mobility of the DIC method.

To enhance the reliability of the DIC measurements, it is crucial to select an appropriate color space and its corresponding color parameters (Saravanan et al., 2016). Normalizing the RGB color space has been shown to effectively minimize variations caused by changes in illumination, thereby reducing the standard deviation of the color response (Chavolla et al., 2018; Halim & Tjahjono, 2023). Accordingly, the *r*, *g*, and *b* color parameters (refer to Eqs 1-3) of the test strip images were plotted against the concentrations of their respective standard solutions, as illustrated in **Figure 3**.

Although the test strips used in this study can measure nitrite concentrations up to 80 mg/L, linear regression analysis of the DIC method indicates that the color parameters show linearity only up to 40 mg/L. The calibration curves illustrating the relationship between the *r*, *g*, and *b* color parameters and nitrite standard concentrations are shown in **Figure 3**. A detailed performance comparison of these calibration curves is provided in **Table 1**. The results show that the *r* color parameter has the highest linear correlation coefficient (r^2), along with the lowest limit of detection (LOD) and the lowest limit of quantification (LOQ). This suggests that the *r* channel is the most reliable choice for DIC-based nitrite analysis, outperforming the other color parameters (*g* and *b*).

To further validate the DIC method, a Griess spectroscopic analysis was performed. A calibration curve was constructed by plotting the absorbance values of nitrite solutions at 540 nm against their respective standard concentrations. The results obtained from the Griess method are summarized in **Table 1**, with the corresponding calibration curve presented in **Figure 4**.

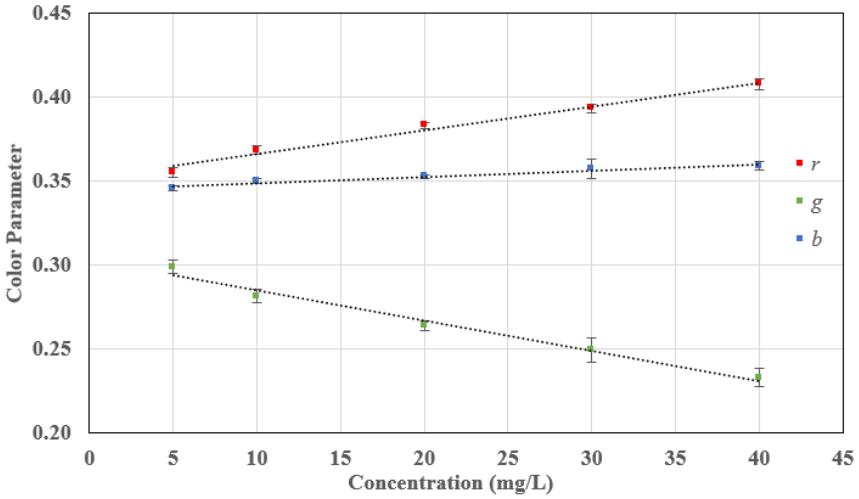

Figure 3. DIC calibration curves illustrating the relationship between the *r*, *g*, and *b* color parameters and nitrite standard concentrations (mg/L).

Table 1. Performance comparison of the linear calibration curves for the *r*, *g*, and *b* color parameters in the DIC and the Griess spectroscopic method.

Parameters	DIC			Griess Spectroscopy
	r	*g*	*b*	
Linearity range (mg/L)	5 – 40	5 – 40	5 – 40	0.0 – 2.5
Slope	0.0014	-0.0018	0.0004	0.6170
Intercept	0.3518	0.3030	0.3451	0.0160
r^2	0.9817	0.9808	0.9639	0.9968
Standard deviation (S_y)	0.0026	0.0034	0.0010	0.0365
LOD (mg/L)*	4	5	17	0.2
LOQ (mg/L)**	11	16	52	0.6

*$\mathrm{LOD} = 3.3 \times S_y/\mathrm{Slope}$
**$\mathrm{LOQ} = 10 \times S_y/\mathrm{Slope}$

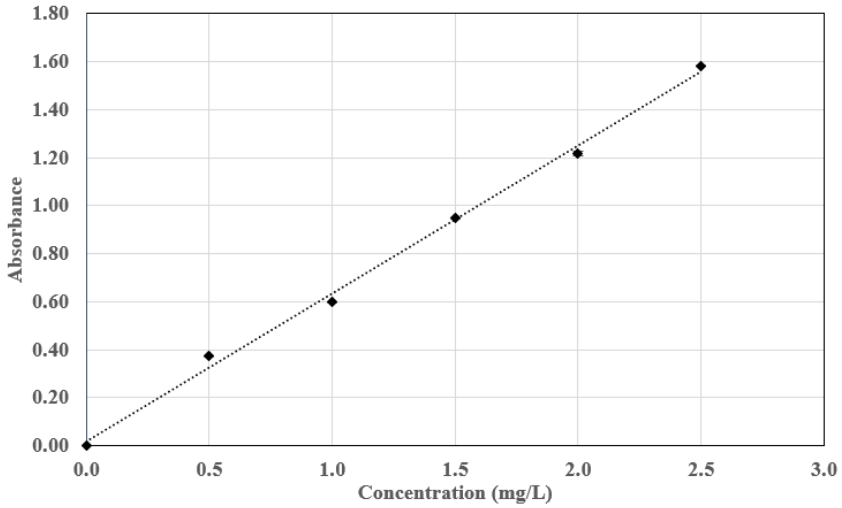

Figure 4. Standard calibration curves of Griess spectroscopic analysis.

Figure 4 shows that the Griess calibration curve exhibits good linearity in the lower concentration range (up to 2.5 mg/L), compared to the DIC method, which is linear up to 40 mg/L (see **Figure 3**). This narrower range limits the direct applicability of the Griess method for analyzing nitrite content in meat products, where nitrite levels are often much higher than the typical measurable range of the Griess method (Bahadoran et al., 2016). Consequently, sample dilution would be necessary to bring nitrite levels within the calibration range. However, it is worth noting that the Griess calibration curve still provides very high analytical precision within its narrow concentration range, thus facilitating reliable measurements. Detailed performance of the Griess calibration curves is provided in **Table 1**, including the calculated limit of detection (LOD) and limit of quantification (LOQ), which provide guidelines for the method's utility.

Accuracy Assessments of DIC and Griess Methods Using Synthetic Samples

The accuracy and reliability of both the DIC and Griess methods were evaluated using synthetic samples with predetermined nitrite concentrations. Five synthetic samples, labeled as "actual concentration" in **Table 2**, were prepared for each method. The concentrations of these samples were chosen based on the measurable linearity ranges of the DIC and Griess methods, i.e., above the limit of quantification (LOQ) but below the maximum allowable concentration limit. The test strip color parameters (*r* values) and the absorbance values of the synthetic samples were measured, and the nitrite concentrations were calculated using the corresponding calibration curves given in **Table 1**. The measured concentrations were then compared to the actual concentrations of the synthetic samples, with the calculated relative errors shown in **Table 2**.

As shown in **Table 2**, the results from the DIC analysis show a comparable accuracy to those of the Griess spectroscopy, with both methods exhibiting a relative error of less than 8% between the actual and measured nitrite concentrations. The average relative error for the DIC method was approximately 3.6% across the five synthetic samples, while the Griess analysis yielded an average relative error of about 4.6%. These results indicate that both methods provide reliable measurements of nitrite concentrations within the specified ranges. This suggests that the DIC method could serve as a favorable alternative to the Griess method, without significant compromise in accuracy.

Table 2. Comparison of the accuracy of the DIC and Griess Spectroscopy methods for quantifying nitrites.

DIC			Griess Spectroscopy		
Actual Concentration (mg/L)	Measured Concentration (mg/L)	Relative Error (%)*	Actual Concentration (mg/L)	Measured Concentration (mg/L)	Relative Error (%)*
13	12.87±0.79	1.0%	0.7	0.65±0.00	7.1%
16	15.95±1.00	0.3%	1.1	1.05±0.00	4.5%
20	20.80±1.62	4.0%	1.3	1.32±0.01	1.5%
24	22.19±1.69	7.5%	1.6	1.70±0.00	6.3%
28	26.52±2.52	5.3%	2.0	2.07±0.01	3.5%

*Relative Error (%) = (Actual Concentration − Measured Concentration/Actual Concentration) x 100%

Nitrite Quantification in Sausages

The DIC and Griess methods were subsequently applied to determine nitrite concentrations in commercial processed meat samples. Sausages were selected due to their widespread consumption and popularity as a processed meat product (Zeng et al., 2019).

Nitrites are water soluble salts that can be easily extracted from the meat matrix by dissolving them in deionized water (Cahyono et al., 2019; Pulungan, 2018). To improve the extraction process, sodium tetraborate decahydrate (borax) was added to neutralize the pH level of the sample extracts and eliminate the presence of hydrolyzed proteins, which could interfere with nitrite measurements (Rincón et al., 2008).

In this study, different concentrations of borax solutions (0%, 2.5%, and 5%) were used to extract nitrite from sausage samples, allowing a comparison measurement consistency between the DIC and Griess methods. The nitrite filtrate was initially measured using test strips for DIC analysis. The filtrates from the same aliquots were then diluted with a factor of 20 a to bring the nitrite concentration within the calibration range for the Griess method. The nitrite measurements obtained from both methods are summarized in **Table 3**. The nitrite concentration reported for the Griess method was determined by multiplying the measured concentration of the diluted sample by the associated dilution factor.

Additionally, the normalized UV-Vis spectra of the nitrite standard solution and the extracted nitrite filtrates are presented in **Figure 5**. A comparison of these spectra shows no observable interference from other substances in the filtrate, and the addition of borax did not induce major changes to UV maxima. These findings suggest that the borax treatment helps in reducing any interfering substances without significantly altering the nitrite signal.

Table 3 shows that the measured nitrite concentrations in sausage samples decrease with the addition of borax. This aligns with the previous reports by Rincón et al. (2008), which shows that hydrolyzed proteins in sausage extracts at pH≤6 could lead to false-positive results in the Griess method. The addition of borax, which raises the pH to 7-8, reduces the solubility of these hydrolyzed proteins and minimizes the occurrence of false positives.

Finally, the results in **Table 3** demonstrate that the nitrite concentrations measured by DIC analysis closely match those obtained using the Griess method, indicating that the DIC method is a reliable and accurate tool for quantifying nitrites in sausages.

Further Discussion

Both the DIC and Griess spectroscopy analysis operate on the same principle, where nitrite ions react with sulfanilamide and N-(1-naphthyl) ethylenediamine (NED) to form a red or pink azo dye under acidic conditions. In the case of nitrite test strips, the aqueous sulfanilamide and NED compounds are immobilized using cellulose and grafted onto paper-based materials to increase their stability and shelf-life (Mako et al., 2020). A comparison between the DIC and Griess spectroscopy methods is summarized in **Table 4**.

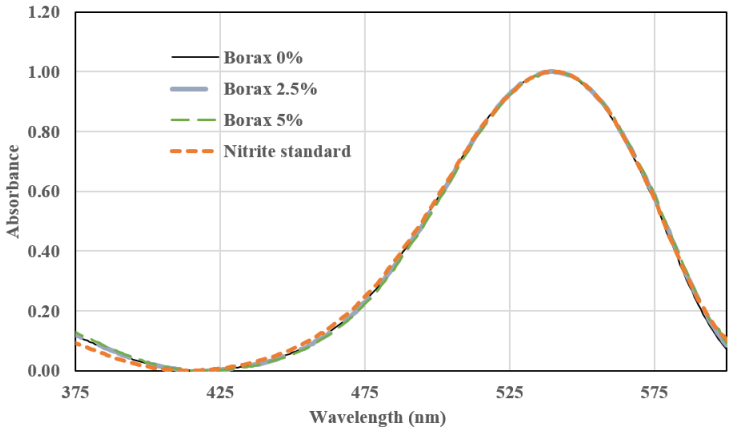

Figure 5. Normalized UV-Vis spectra of a standard nitrite solution and nitrite extracted from sausages using 0%, 2.5%, and 5.0% borax solutions.

Table 3. Nitrite concentration of sausages, as measured by the DIC and Griess spectroscopy methods.

Extraction Method	Nitrite Concentration (mg/L)	
	DIC	Griess Spectroscopy*
Borax 0%	28.46±2.11	28.79±1.73
Borax 2.5%	24.60±1.22	27.24±0.64
Borax 5%	22.77±2.09	25.83±2.84

*Actual Sample Concentration = Measured Concentration x Dilution Factor (DF = 20)

Table 4. Comparison of the DIC and Griess spectroscopy methods for nitrite analysis.

Category	DIC	Griess Spectroscopy
Main instrument(s)	Endoscope and imaging box	UV-Vis spectrophotometer
Main instrument cost	~USD 10	~USD 10,000
Supporting instrument(s) for display	Smartphone or Computer	Computer
Griess reagents	Immobilized in test strips	Freshly prepared using NED, sulfanilamide, and phosphoric acid
Consumables cost (mainly for Griess reagent)	USD 100 (per 100 pcs test strips) or USD 1 per sample measurement	USD 250 (for maximum 100 samples) or circa USD 2.5 per sample measurement
Sample measurement time	1-2 minutes	15-20 minutes
Sample volume	1-3 mL	50-100 mL
Facilitating portable analysis	Yes	No
Ease of use	Very easy	Moderate

Table 4 highlights several advantages of the DIC method over Griess spectroscopy method. First, the DIC method supports portable measurements. It utilizes an endoscope, smartphone, and compact imaging box for measurement and data collection, making it easy to transport to on-site locations for sample testing. Second, the DIC method is user-friendly. Unlike the Griess method, which requires operating a UV-Vis spectrophotometer, the DIC method does not demand specialized skills, making it more accessible to a wider range of users. Another key advantage of the DIC method is its speed. Typical DIC measurements, including color development and image acquisition, can be completed in less than two minutes, whereas the Griess method requires at least 15 minutes for reagent preparation, color stabilization, and spectroscopic analysis. The reported DIC measurement time does not include manual RGB extraction using Adobe Photoshop; however, this step can be readily automated using image-processing software.

In terms of affordability, the DIC method is significantly more economical. The instruments required for DIC, such as the endoscope, smartphone, and imaging box, are much less expensive than those needed for UV-Vis spectroscopy. Furthermore, the consumables for the DIC method primarily consist of test strips, which are more affordable than the reagents required for Griess spectroscopy. Finally, the DIC method requires a smaller sample volume and generates less chemical waste.

Overall, this study demonstrates that the DIC method provides accuracy and reliability comparable to the Griess spectroscopic method, supporting its feasibility as a proof-of-concept approach for nitrite determination in processed meats. The results highlight the potential of using an endoscope as a compact and low-cost DIC instrument for rapid, on-site analysis, particularly in resource-limited settings where access to traditional laboratory equipment may be restricted. While the present study focuses on method feasibility and comparative analytical performance against an established method (Griess), future studies will address full method validation, including expanded repeatability and measurement precision assessments, as well as evaluation across a broader range of sample matrices. Furthermore, the proposed approach may be extended to other colorimetric test-strip analyses, such as measurements of chlorine, bromine, fluoride, lead, iron, copper, and water hardness.

CONCLUSIONS

The digital image colorimetry (DIC) method presented in this study offers a promising alternative for quantifying nitrites in processed meats, particularly sausages. By utilizing a custom-designed 3D-printed imaging box, the proposed method ensures standardized experimental conditions for data collection. Moreover, the use of an endoscope as the image sensor minimizes variations caused by differences in smartphone camera specifications. The adoption of the normalized *r* color parameter further enhances the accuracy and reliability of the analysis.

Compared to the traditional Griess spectroscopy method, the DIC method demonstrated good accuracy, with an average measurement error of less than 5%, while providing significant advantages in portability, affordability, and accessibility for on-site monitoring of nitrite concentrations. Validation against the Griess spectroscopic technique demonstrated consistent results in quantifying nitrites in both commercial sausages and standard samples. These findings suggest that the proposed DIC method is a viable tool for routine nitrite testing in the food industry, supporting consumer safety and regulatory compliance. Finally, this study paves the way for extending the use of DIC beyond food safety testing.

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