



## Research article

## Smartphone-based portable device for multi-sample colorimetric determination of nitrite in sausage

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### Abstract

**Importance of the work:** A custom-designed light box with a hat-shaped cover ensured consistent illumination for image analysis using a smartphone-based method.

**Objectives:** To develop a simple, rapid and accurate smartphone-based method for detecting nitrite in sausages.

**Materials and Methods:** Samples were analyzed on a microplate and images were captured using a smartphone camera. Green color intensity was measured and correlated with the nitrite concentration. The method was validated against the Association of Official Analytical Chemists (AOAC) standard, demonstrating high sensitivity and accuracy.

**Results:** Using the Griess reaction, the method formed an azo dye compound and provided a portable, user-friendly and cost-effective alternative to conventional techniques. A custom-designed photo box with LED lighting and a hat-shaped light fixture enabled simultaneous analysis of multiple samples. The method provided excellent sensitivity and accuracy, with a linear correlation between green color intensity and nitrite concentration. The addition of 0.003% Brilliant Blue solution substantially expanded the calibration curve's linear range. Validation against the AOAC method confirmed the reliability of the method, highlighting its potential for rapid, on-site food analysis.

**Main finding:** The custom-designed, hat-shaped light fixture effectively minimized specular reflections from Light emitting-diode lighting, enabling the use of cost-effective alternatives to iPads or tablets as illumination sources for simultaneous multi-sample colorimetric measurements.

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## Introduction

Portable devices for chemical analysis are available in various formats, such as microfluidic paper-based analytical devices ( $\mu$ PADs), indicator papers and well-plate reaction vessels. These analytical tools have been widely used in medical diagnostics, environmental monitoring, food analysis and security applications. Traditional detection methods, including polymerase chain reaction (PCR), high-performance liquid chromatography-mass spectrometry (HPLC/MS) and enzyme-linked immunosorbent assay, offer high accuracy and sensitivity; however, these methods are costly, bulky, time-consuming and require trained personnel, limiting their applicability for point-of-care use. Consequently, the development of simpler, more accessible detection devices is of great interest. Among these, smartphones have emerged as promising alternatives, particularly for optical detection when integrated with analytical tools (Nonno and Ulber, 2021).

A low-cost, *in situ* digital imaging approach using smartphone-based spot testing was developed to determine ascorbic acid in fruits from the Brazilian Amazon, involving the reduction of Fe(III) by ascorbic acid, followed by complexation with 1,10-phenanthroline (Vagner et al., 2019). Additionally, a smartphone-based spectrophotometer was designed to detect trace levels of chlorine and nitrite in water samples. This system, constructed from Plexiglas, utilized two light-emitting diodes (LEDs) as a light source, a Digital versatile disc piece as a dispersion element and a smartphone battery as the power source, with spectral analysis performed using freely available software (Sargazi and Kaykhail, 2020). Kuhlmann et al. (2021) reported substantial inaccuracy and variability in spatial orientation sensor data (pitch and roll) across different smartphone models, resulting from differences in both hardware and software. Although their study focused specifically on orientation sensors, it highlighted an important point—sensor data collected from different smartphone models can vary widely. This observation suggests that similar challenges may arise when using photographs taken with different smartphones for color-based analysis in substance quantification. Variations in camera sensors, lens systems and built-in image processing across models could lead to inconsistencies in color representation. Consequently, substance quantification based on color data from images captured by different smartphone models may produce inconsistent analytical results.

Nitrite is an inorganic compound commonly used as a food additive in cured meat products to enhance flavor, stabilize curing color and inhibit bacterial growth. It is a white-to-slightly-yellowish, odorless, hygroscopic, crystalline powder with high water solubility and at concentrations as low as 3–5 mg/kg can cause pink discoloration in non-cured meat products. Improper use has been reported at levels of 30–50 mg/kg in cured meats such as low-quality sausages, bacon and processed poultry products (Feiner, 2016). Nitrite is also present in the natural environment, with dietary intake being the primary exposure route for humans (Agency for Toxic Substances and Disease Registry, 2017). Sodium nitrite is a potent oxidizing agent that can induce hypotension and impair oxygen transport by forming methemoglobin, leading to methemoglobinemia, commonly known as “brown blood disease” (Aguilar et al., 2017). Furthermore, the International Agency for Research on Cancer classifies nitrites as “probably carcinogenic to humans” (Group 2A) under conditions that promote endogenous nitrosation, potentially leading to the formation of carcinogenic N-nitroso compounds (Agudo et al., 2010). The acceptable daily intake for nitrite is set at 0.06 mg/kg body weight/d by the European Commission’s former Scientific Committee for Food and 0.07 mg/kg body weight/day by the Joint FAO/WHO Expert Committee on Food Additives (Aguilar et al., 2017;). Yenuthok et al. (2023) proposed a novel diazotizing agent, *m*-anisidine, for spectrophotometric nitrite determination in sausages.

Recent advancements in nitrite detection methods aim to improve analytical efficiency. Various techniques, including chemiluminescence, capillary electrophoresis, chromatography, colorimetry and electrochemical methods, have been used to measure nitrite levels (Moorcroft et al., 2001). Among these, colorimetry is widely used due to its simplicity and sensitivity. Nitrite determination often relies on the diazotization-coupling reaction, commonly known as the Griess reaction, in which nitrite ions react with sulfanilamide under acidic conditions to form a diazonium salt. Then, this intermediate couples with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED), producing a red-violet azo dye in an alkaline environment. This reaction remains one of the most widely used methods for nitrite analysis (Qiu-Hua et al., 2017). The Association of Official Analytical Chemists (AOAC) Method 973.31, uses spectrophotometry and also utilizes the Griess reaction, serving as a widely recognized standard for nitrite determination in cured meats (Association of Official Analytical Chemists, 1996). However, this established

protocol has inherent limitations, primarily due to its single-sample processing nature (which restricts throughput) and its unsuitability for rapid, on-site field measurements. These constraints necessitate the exploration of alternative analytical strategies that are more conducive to high-throughput and field-deployable applications. Puangpila et al. (2018), developed a smartphone-based colorimetric system for nitrite determination in water, incorporating an LED-illuminated box to ensure consistent lighting and utilizing mobile phone software to enhance measurement accuracy.

The current study aimed to develop a smartphone-based portable system for nitrite determination in sausages. The proposed system features a custom-designed photo light box, with the smartphone serving as the detector. The detection process is based on the reaction between nitrite and the diazotizing agent *m*-anisidine, followed by coupling with NED. The red, green and blue (RGB) values of captured images are analyzed to determine nitrite concentration. Compared to previous methods, such as those by Sandeep et al. (2015) and Qiang et al. (2024), which used iPads or tablets as light sources for simultaneous sample analysis, the proposed method offers considerable advantages, being more cost-effective, easier to assemble and utilizing commercially available lighting components.

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## Materials and Methods

### *Reagents and standards*

All chemicals used were of analytical grade and were utilized without further purification. Deionized (DI) water with a specific conductance of 18 M $\Omega$ ·cm (Vivagen; Republic of Korea) was used to prepare all solutions. A standard stock solution of 500  $\mu$ g/mL nitrite was prepared by dissolving 0.1631 g sodium nitrite (Kemaus; Australia) in 200 mL of DI water. Working solutions of nitrite were freshly prepared by appropriately diluting the stock solution with DI water.

The diazotizing agent was prepared by dissolving 184.8 mg of *m*-anisidine (Merck; Germany) in 50 mL of ethanol (Merck; Germany). The coupling NED reagent was prepared by dissolving 20.0 mg of N-(1-naphthyl) ethylenediamine dihydrochloride (ITW Reagents; Italy) in 20 mL of glacial acetic acid (Carlo Erba; Italy) and diluting to 100 mL with DI water.

A 1.0 M hydrochloric acid solution was prepared by diluting the appropriate volume of 37% HCl (RCI Labscan; Ireland) in DI water. A 1.5 M phosphate buffer solution (PBS) was prepared by dissolving 20.41 g of potassium phosphate monobasic (Kemaus; Australia) in approximately 90 mL of DI water. The pH was adjusted using NaOH (Kemaus; Australia) and the solution was diluted to 100 mL with DI water.

A 2% ethylenediaminetetraacetic acid (EDTA) solution was prepared by dissolving 1.00 g of EDTA in 50 mL of DI water. A 0.003% Brilliant Blue (BrB) solution was prepared by diluting 0.20 mL of a 0.75% BrB FCF solution (Sky Blue Food Color; Winner; Thailand) in 50 mL of DI water.

The AOAC diazotizing agent, sulfanilamide, was prepared by dissolving 600 mg of sulfanilamide (DC Fine Chemicals; UK) in 50 mL of hot water. After cooling, 20 mL of glacial acetic acid was added and the volume was adjusted to 100 mL with DI water.

### *Colorimetric determination of nitrite using smartphone-based detection*

#### *Portable device fabrication and image processing*

Sample solutions were placed in a 96-well opaque microplate, which was positioned inside a photo studio light box and elevated using a laboratory lifting platform. Images were captured using a Samsung Galaxy Note 10 Plus™ smartphone camera (model SM-N975F, 12 MP, backside-illuminated CMOS sensor; Samsung, Seoul, Republic of Korea). The camera settings were fixed to f/1.5 aperture, 1/4 s exposure time, ISO-400, with flash and HDR turned off and automatic white balance enabled, to achieve reproducible results. Images were saved in JPEG format.

The RGB color intensities of the images were measured using the “RGB Color Detector” mobile application on the Samsung Galaxy Note 10 Plus™. Measurements were taken at the center of each sample well within a circular region of interest with a radius of 20 pixels, as illustrated in Fig. S1. The linear relationship was evaluated by plotting the RGB values of each nitrite standard calibration solution on the y-axis against nitrite concentrations in the range 0.0–5.0  $\mu$ g/mL on the x-axis. Mathematical transformations of the color intensities were applied to determine the most accurate correlation, based on the coefficient of determination ( $R^2 > 0.995$ ) derived from linear regression analysis.

### *Optimization of colorimetric method*

Nitrite detection in the sausage samples was based on a method developed by Yenuthok et al. (2023), with modifications to enhance visibility and expand the linear range of the azo dye by incorporating BrB alongside NED. BrB acts as a background dye and the intensity of the resulting green hue (formed with the yellow azo dye) is directly proportional to the nitrite concentration, enhancing visibility and expanding the linear range.

A sausage sample ( $5.00 \pm 0.005$  g) was homogenized and placed in a glass vial. Then, 25 mL of DI water was added and the mixture was gently swirled for 2 min. Then, the mixture was boiled for 20 min, allowed to cool to room temperature, filtered into a 50 mL Falcon tube and centrifuged at 6,000 revolutions per minute for 5 min. The clear supernatant was carefully transferred to a 50.0 mL volumetric flask and diluted with DI water to the desired volume.

A 2.0 mL aliquot of the extracted solution was transferred to a 10.0 mL volumetric flask. Then, 0.40 mL of 2% EDTA, 1.0 mL of *m*-anisidine (for the sample blank, 1.0 mL of ethanol was used instead) and 0.50 mL of 0.1 M HCl were added. The solution was mixed thoroughly and allowed to stand at room temperature for 10 min. Subsequently, 2.0 mL of 1.0 mM NED reagent, 0.50 mL of pH 6 PBS and the appropriate volume of 0.003% BrB were added. The volume was adjusted to the mark with DI water, shaken and allowed to stand for another 10 min. Then, 200  $\mu$ L of the sample solution was transferred into a microplate for image capture and the RGB values were measured against a sample blank.

The impact of different volumes of 0.003% BrB on calibration solutions was evaluated to determine its effect on the linearity of the calibration curve. A calibration curve was plotted between nitrite concentrations on the x-axis and the appropriate mathematical transformation of RGB values on the y-axis. Only calibration curves with an  $R^2$  value greater than 0.995 were considered acceptable.

### *Method validation of smartphone-based colorimetric assay*

The proposed method was validated to assess its performance in nitrite determination in sausage samples. The validation parameters (linear range, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision) were evaluated following the Eurachem guidelines (Bertil and Ulf, 2014). Subsequently, the results were compared with those obtained using the AOAC standard method (Mohamed et al., 2008) in 10 sausage samples from a local market in Bangkok,

Thailand. Statistical analyses, including the F test and t test, were performed to evaluate differences between the methods.

### *AOAC colorimetric method for nitrite in cured meat*

This method is based on AOAC (1996). A 5.0 mL aliquot of the extracted solution (from Section 2.2.2) was transferred to a 25 mL volumetric flask, followed by the addition of 2.5 mL of sulfanilamide in 20% acetic acid (87  $\mu$ mol). The mixture was homogenized and allowed to stand at room temperature for 15 min. Then, 2.5 mL of NED reagent (27  $\mu$ mol) was added and the volume was adjusted to 25 mL with DI water. After 15 min to allow for complete color development, absorbance was measured at 540 nm using a 1 cm glass cuvette against a blank solution with a Genesys 40 visible spectrophotometer, Thermo Scientific, USA. The nitrite concentration was determined from a calibration curve.

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## **Results and Discussion**

### *Colorimetric determination of nitrite using smartphone-based method*

#### *Portable device fabrication and image processing*

Smartphones can be considered to offer considerable advantages for analytical applications due to their portability and ease of use. However, a dark box was added to the method used in the current study to minimize external light interference and ensure consistent image capture, with the microplate directly illuminated by an LED light. Initial experiments with a commercial photo studio light box encountered challenges, including light leakage and reflections from the LED light on the sample surface, which led to fluctuations in RGB values.

To address these issues, a custom-designed cubic photo light box was fabricated and used in the current study (Supplementary Fig. S2A). The enclosure was constructed from black-painted wood, with external dimensions of 42 cm  $\times$  42 cm  $\times$  42 cm (length, width, height) and internal dimensions of 40 cm  $\times$  40 cm  $\times$  40 cm (length, width, height). Mounted on the inner surface of the top lid were a circular LED light source and a bespoke hat-shaped lighting fixture (Supplementary Fig. S2B). The fixture comprised a circular disc made from black corrugated plastic ("future board") with a diameter of 16 cm and a central aperture. A hollow black cardboard cylinder, measuring 5 cm in height and 10 cm in diameter, was inserted through the aperture to further control light direction and minimize reflections.



In the top-view configuration with the lid removed, a standard 96-well microplate (127.8 cm × 85.5 cm) was positioned 13 cm from the inner left wall and 15.7 cm from the bottom wall of the box. The microplate was elevated 20 cm above the base using a laboratory lifting platform, which was specifically designed to accommodate smartphones with varying focal lengths. This design improved the system's flexibility and compatibility across different mobile devices, while ensuring optimal image capture conditions. Images were captured using the Samsung Galaxy Note 10 Plus™. The schematic representation of the smartphone-based colorimetric determination process using a custom-designed photo light box is shown in Fig. 1

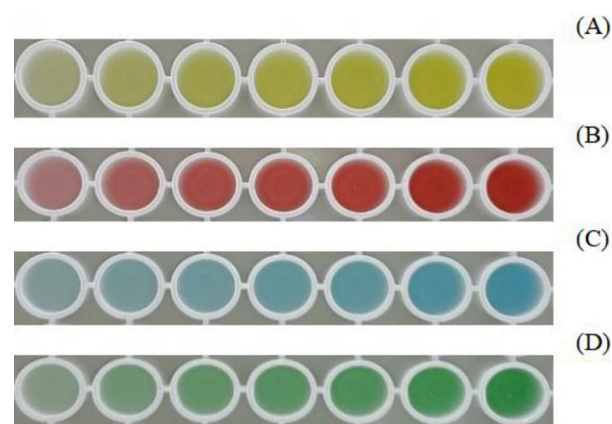
These adjustments considerably improved image quality, as confirmed by the RGB values of the four primary colors (Supplementary Table S1). The percentage relative standard deviation (%RSD) of these primary RGB values was maintained below 5% to ensure measurement reproducibility. This criterion determined an optimal simultaneous sample size of 56, leading to increased efficiency and throughput compared to traditional spectrophotometry, which processes one sample at a time. Despite initial challenges with lighting conditions, the optimized smartphone-based approach had notable advantages in terms of convenience and throughput compared to traditional spectrophotometry.

Next, the optimized conditions were applied to evaluate the linearity of each primary color (yellow, red, blue and green). Based on the results, there was a strong linear relationship ( $R^2 > 0.995$ ) for all colors. Thus, the smartphone-based

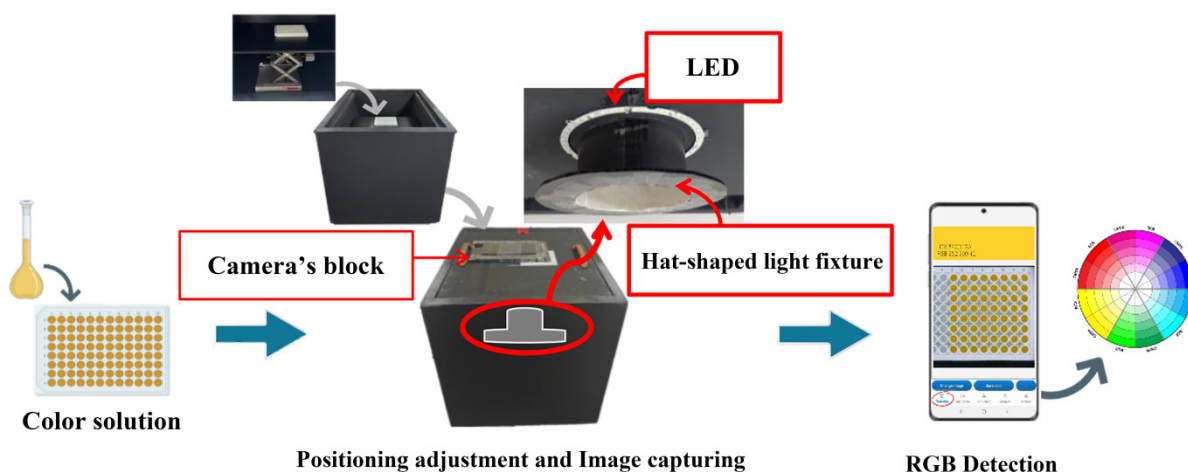
colorimetric method effectively analyzed colorimetric reactions with high accuracy. Representative images of the primary color solutions are shown in Fig. 2.

#### *Selection of optimal RGB channel and data transformation*

The nitrite standard solutions were transferred to 10 wells of a microplate (200  $\mu$ L each) and an image was captured. Nitrite concentrations (0.0  $\mu$ g/mL, 0.05  $\mu$ g/mL, 0.1  $\mu$ g/mL, 0.2  $\mu$ g/mL, 0.5  $\mu$ g/mL, 1.0  $\mu$ g/mL, 2.0  $\mu$ g/mL, 3.0  $\mu$ g/mL, 4.0  $\mu$ g/mL and 5.0  $\mu$ g/mL) were plotted on the x-axis, while the RGB values were plotted on the y-axis to investigate their relationship with nitrite concentration. The experiment was performed in triplicate to ensure reproducibility.

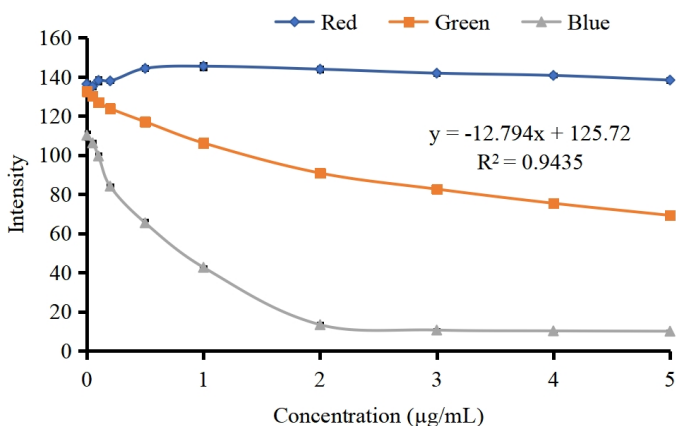


**Fig. 2** Representative images of primary color solutions: (A) yellow; (B) red; (C) blue; (D) green



**Fig. 1** Schematic representation of smartphone-based colorimetric determination process using a custom-designed photo light box, where RGB = red, green and blue

The graphical representation of the correlation between the RGB values and the nitrite concentration is illustrated in Fig. 3.



**Fig. 3** Plot of nitrite concentration versus RGB values, with corresponding solution colors, where  $R^2$  = coefficient of determination

The G value had the strongest near-linear relationship. Therefore, G values were selected for data transformation to establish the best-fitting model for nitrite quantification. The transformation is presented in Equation 1:

$$(G_0 - G_x) / G_x \quad (1)$$

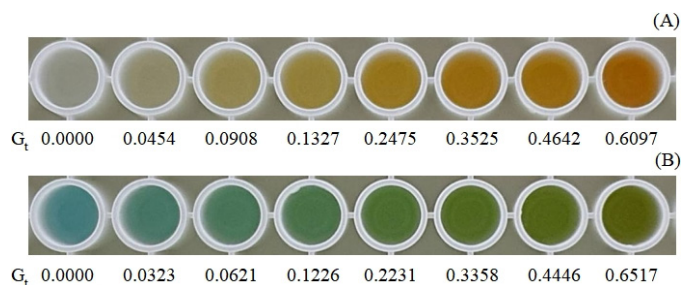
where  $G_0$  is the green value of the blank and  $G_x$  is the green value of the sample.

This transformation greatly enhanced the correlation between analytical response and nitrite concentration, producing a linear calibration curve with a higher  $R^2$  than the untreated data. However, two limitations were noted. First, the blank solution's G value was influenced by

the background color of the microplate. Second, the narrow concentration intervals in the first three steps and signal saturation at nitrite concentrations above 2.0  $\mu\text{g/mL}$  led to a lower linear correlation. As a result, the linear range of the calibration curve was set up to 2.0  $\mu\text{g/mL}$ , achieving an  $R^2$  of 0.995. Table 1 presents a comparative analysis of the raw and transformed RGB channel values plotted against nitrite concentration. Based on these data, the transformed G channel had the strongest correlation with nitrite concentration.

#### Optimization of the colorimetric method

The linearity of the calibration curve was affected by background color variations, leading to changes in the RGB values. A blue-colored 0.003% BrB solution was introduced as the background in the blank solution to improve the  $R^2$  value. According to the RYB color model, mixing yellow and blue produces green. This transformation changed the initial yellow hue (Fig. 4A) into a green hue (Fig. 4B) in solutions containing nitrite.



**Fig. 4** Effect on calibration solution colors of adding 0.003% Brilliant Blue solution: (A) before addition; (B) after addition, where  $G_t$  = transformed G values

**Table 1** Comparative analysis of raw and transformed RGB channel values for different nitrite concentrations

Concentration ( $\mu\text{g/mL}$ )	Red	Green	Blue	$(R_0 - R_x)/R_x$	$(G_0 - G_x)/G_x$	$(B_0 - B_x)/B_x$
0.00	136.05	133.06	111.93	0.0000	0.0000	0.0000
0.05	135.85	130.41	107.31	0.0015	0.0203	0.0431
0.10	136.57	127.57	100.52	-0.0038	0.0430	0.1136
0.20	137.27	124.46	83.82	-0.0089	0.0691	0.3355
0.50	144.33	117.05	66.18	-0.0573	0.1368	0.6915
1.00	145.14	106.13	43.02	-0.0626	0.2537	1.6020
2.00	143.80	90.85	13.52	-0.0539	0.4645	7.2767
Slope	4.508	-20.631	-48.391	-0.0312	0.2283	3.4686
intercept	137.38	129.85	101.80	-0.0093	0.0155	-0.4702
$R^2$	0.5806	0.9746	0.9304	0.5835	0.9965	0.9199

$R_0$ ,  $G_0$  and  $B_0$  = the red, green and blue value of the blank, respectively

$R_x$ ,  $G_x$ , and  $B_x$  = the red, green and blue value of the sample, respectively

$R^2$  = coefficient of determination.

However, at higher nitrite concentrations, the green color darkened, requiring dilution for samples above 3.0 µg/mL. Various volumes (0–1,000 µL) were tested in triplicate to optimize the BrB volume. The  $R^2$  value of the calibration curve was evaluated for each condition. The best linear correlation was achieved with 500 µL of 0.003% BrB (Supplementary Table S2). This adjustment extended the linear range of the calibration curve from 2.0 µg/mL to 3.0 µg/mL, improving the method's applicability.

### *Ion interference study*

Potential interference from various ions was assessed using a nitrite concentration of 1.5 µg/mL. A 2% EDTA solution was used as a masking agent to minimize interference. Based on the results, EDTA effectively reduced interference, maintaining the method's specificity. Tolerance limits for each interfering ion are summarized in Table 2.

The method maintained a relative error below 5% for all tested ions, confirming its robustness. Based on these findings, an optimized reagent composition was established (Table 3).

**Table 2** Tolerance limits of interfering ions in smartphone-based nitrite detection method

Ion interference species	Form added	Tolerance limit (mg/kg)
Na <sup>+</sup>	NaCl	>105
K <sup>+</sup>	KNO <sub>3</sub>	7,730
Ca <sup>2+</sup>	CaCl <sub>2</sub>	2,500
Mg <sup>2+</sup>	MgCl <sub>2</sub>	2,000
Mn <sup>2+</sup>	MnCl <sub>2</sub>	1,000
Zn <sup>2+</sup>	ZnSO <sub>4</sub> 7H <sub>2</sub> O	1,130
Fe <sup>2+</sup>	FeCl <sub>2</sub>	750
Cl <sup>-</sup>	NaCl	>1.5 × 10 <sup>5</sup>
F <sup>-</sup>	NaF	1,000
I <sup>-</sup>	NaI	1,000
CO <sub>3</sub> <sup>2-</sup>	Na <sub>2</sub> CO <sub>3</sub>	1,250
SO <sub>4</sub> <sup>2-</sup>	ZnSO <sub>4</sub> 7H <sub>2</sub> O	1,670
NO <sub>3</sub> <sup>-</sup>	KNO <sub>3</sub>	1.39 × 10 <sup>4</sup>

**Table 3** Reagent composition for a 10 mL sample solution in smartphone-based nitrite detection method

Reagent component	Volume used (mL)
Supernatant	2.0
2% EDTA	0.40
0.03 M <i>m</i> -Anisidine	1.0
0.1 M HCl	0.50
1.0 mM NED	2.0
1.5 M PBS pH6	0.50
0.003% Brilliant blue	0.50

NED = N-(1-naphthyl) ethylenediamine dihydrochloride; PBS = phosphate buffer solution.

### *Validation of nitrite determination in sausage samples*

The optimized smartphone-based method established a very good linear relationship between nitrite concentrations (0.1–3.0 µg/mL) and the transformed G values ( $R^2 = 0.9978$ ). The method's LOD and LOQ values were 0.04 µg/mL and 0.1 µg/mL, respectively. Accuracy assessments showed recovery percentages within the acceptable AOAC range at both the LOQ (0.1 µg/mL) and maximum limit (1.6 µg/mL). Precision analysis, including repeatability and intermediate precision, demonstrated excellent reproducibility, with the Horwitz ratio values consistently below 2. Overall, the validated results confirmed the high accuracy and precision of the smartphone-based colorimetric method for nitrite determination in sausage samples (Table 4).

**Table 4** Analytical performance of smartphone-based detection method for nitrite determination in sausage samples.

Parameter	Value
Linear range / (µg/mL)	0.1 – 3.0
Coefficient of determination	0.9978
Regression equation	$(G_0 - G_x) / G_x = 0.2081$ $[\text{NO}_2^-] + 0.0043$
Limit of detection (LOD) (µg/mL)	0.04
Limit of quantification (LOQ) (µg/mL)	0.1
Accuracy (%Recovery)	
- at LOQ / (%)	86.28–105.87
- at maximum limit (ML)(%)	98.78–102.72
Precision (Horwitz ratio)	
- at LOQ (%RSD)	0.850
- ¼ ML (%RSD)	0.535
- ½ ML (%RSD)	0.337
- at ML (%)	0.377

### *Application to real samples and comparison with reference methods*

The smartphone-based method was compared to the AOAC standard method using the standard F and t tests. The nitrite concentrations in 11 sausage samples were determined and expressed as mean ± SD values. Based on these results (Table 5), 8 of the 11 samples had nitrite concentrations exceeding the LOQ. Statistical comparisons were conducted using four replicates per sample. At the 95% confidence level, there were no significant differences between the two methods ( $p > 0.05$ ). Thus, the smartphone-based detection method was a reliable alternative to the AOAC method for determining the nitrite concentrations in sausage samples.

**Table 5** Comparison of nitrite concentrations in sausage samples determined using smartphone-based detection method and AOAC spectrophotometric method.

Sample number	Nitrite concentration (mg/kg)		<i>p</i> value	
	Smartphone-based	AOAC	F test	t test
1	24.99 ± 1.47	25.67 ± 0.67	0.11	0.43
2	58.30 ± 1.56	57.36 ± 1.78	0.41	0.46
3	<LOD	<LOD	-	-
4	<LOQ	<LOQ	-	-
5	12.22 ± 0.78	11.46 ± 0.42	0.17	0.13
6	34.09 ± 1.67	34.49 ± 0.86	0.15	0.69
7	32.65 ± 1.02	31.49 ± 0.77	0.32	0.12
8	14.57 ± 0.24	14.74 ± 0.31	0.33	0.42
9	55.05 ± 1.51	55.82 ± 0.68	0.11	0.38
10	<LOD	<LOD	-	-
11	14.71 ± 0.78	14.18 ± 0.35	0.11	0.26

## Conclusion

This study introduced a novel smartphone-based colorimetric method for nitrite determination in sausage samples, utilizing the Griess reaction with *m*-anisidine and NED to form an azo dye compound. Unlike traditional methods that require sophisticated equipment and trained personnel, this approach offers portability, user-friendliness and cost-effectiveness. A custom-designed photo box with LED lighting and a hat-shaped light fixture ensured consistent and accurate measurements, enabling the simultaneous analysis of multiple samples in a microplate.

The intensity of the green (G) channel, detected via the smartphone application, was transformed using  $(G_0 - G_x) / G_x$  and produced a strong linear correlation with nitrite concentration for precise quantification. The addition of 0.003% BrB extended the linear range of the calibration curve compared to the original yellow solution. Comparison with the AOAC standard spectrophotometric method revealed no significant differences, confirming the reliability and accuracy of the proposed approach. Additionally, this method could be used as an effective preliminary screening tool when visual assessment is required.

In conclusion, the smartphone-based colorimetric method provided a reliable, simple and rapid alternative for nitrite analysis, with potential applications for improving efficiency and accessibility in on-site food safety monitoring. While this approach offers advantages in terms of portability and user-friendliness, it has certain limitations, notably a relatively higher limit of detection and a restricted linear dynamic range compared to conventional spectrophotometric techniques. Nonetheless, the method's performance should be adequate for

detecting nitrite concentrations commonly encountered in food products. Future research will aim to not only to overcome these current limitations but also to expand the method's applicability to the analysis of other relevant analytes through the development of optimized colorimetric reactions.

## Conflict of Interest

The authors declare no conflicts of interest.

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