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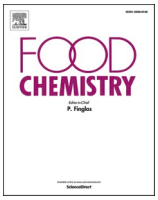

Headspace measurement of dimethyl sulfide in beer through paper-based smartphone-colorimetry

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ABSTRACT

Beer ranks among the most popular and most widely consumed beverages globally. During its production through brewing and fermentation, its natural characteristic style and inherent flavors are produced. Dimethyl sulfide (DMS) contributes significantly to the flavor profile of beer. Its presence is desirable at low concentration levels. However, it becomes an off-flavor at high concentrations. The concentration of DMS in beer was measured through smartphone-based colorimetry. A paper-based analytical device (PAD) was fabricated by immobilizing a chromogenic reagent phase consisting of alkaline nitroprusside in a gelatin hydrogel. Headspace sampling and subsequent reaction with the immobilized reagent enabled the detection of low levels of DMS. By capturing the digital image of the PAD, the concentration was determined by utilizing the RGB color value. A linear behavior was observed in the Green color value (G value) in the concentration range with 5 to 120 μg DMS L^{-1}, with a Pearson coefficient of 0.995 and limit of detection was 2.7 μg L^{-1}. High precision and accuracy were demonstrated, with RSD ranging from 0.79 to 1.36 % ($n = 6$) and trueness values between 92.5 and 104.4%, respectively which also satisfied AOAC requirements for low levels of analyte. The results of smartphone-based colorimetry agreed well with the results of chromatographic analysis of DMS in lager and all-malt beer samples. This method is an inexpensive alternative to the gas chromatography method for DMS measurement and will allow small-scale brewers and craft beer producers to monitor off-flavor in their products.

1. Introduction

Beer consists of hundreds of volatile and nonvolatile constituents of varied chemical functionalities produced from brewing of malt, hops, and yeast. The quality of a beer highly depends on its sensory attributes. The holistic perception of flavor and aroma character is derived from the contribution of the flavor compounds present in the beer making it a desirable beverage (Hughes, 2009; Humia et al., 2019).

Among the salient flavor components of beer are the sulfur-containing compounds, the most abundant of which is DMS (Kucharczyk et al., 2020). Though present at μg L^{-1} level concentrations, DMS significantly contributes to the aroma and flavor profile of the beer (Bamforth, 2014). Its level in most beer products is usually maintained below its sensory threshold of 30 μg L^{-1}; however, lager beers contain DMS levels of 50 to 100 μg L^{-1} (Marconi et al., 2011). Beyond this concentration level, the powerful cabbage-like odor of DMS becomes noticeable, causing off-flavor and lowering the sensory quality of the beer (Scarlata & Ebeler, 1999). Breweries, therefore, analyze the DMS content of their product to ensure consistent flavor quality and consumer satisfaction.

DMS measurement in beer has been carried out using several analytical methods. The typical analysis of DMS in beer involves gas chromatography employing several detectors including a flame photometric detector, (Svoboda et al., 2017) chemiluminescence detector (Baldus et al., 2013) photoionization detector (Liu et al., 2011) and mass spectrometry detector (Marconi et al., 2011). Mass spectroscopy has also been explored to detect abnormal DMS levels in the beer headspace using a fingerprint mass spectrometry-type electronic nose (Kojima et al., 2005). A simple method that utilizes UV/Vis relies on the colored complex formed when DMS reacts with sodium nitroprusside to measure DMS in beer (Grigsby & Palamand, 1977).

Gas chromatography coupled with headspace sampling (HS) has also been used for the measurement of low concentration of DMS. (Liu et al., 2011).This technique works by extracting of volatile compounds, such as DMS, into the gas phase from the liquid sample system (Rodinkov et al., 2020). The extraction procedure preconcentrates the volatile

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constituents of the test system, separating them from the nonvolatile substances left in the solid or liquid system. It reduces the number of peaks in the gas chromatogram, which now features only peaks of the volatile components, simplifying identification and quantification. Headspace sampling is also being coupled with solid-phase extraction (SPE) to improve the analytical performance of headspace gas chromatography for DMS measurement in beer (Scarlata & Ebeler, 1999) In this technique, a solid-phase gas sorbent or extractant selectively isolates the compound of interest from the other volatiles in the headspace. The utilization of solid-phase extraction leads to enrichment of the volatile analyte, resulting in an enhanced sensitivity and an improved limit of detection.

This paper adopts the HS sampling technique in smartphone-based colorimetric method for DMS measurement in beer. A PAD containing the immobilized chromogenic reagent was used which selectively binds DMS molecules present in the headspace sample. The extracted DMS in the immobilized reagent generated a pink color upon acidification and quantification was carried out directly through smartphone-based digital image colorimetry (DIC).

The smartphone has become a popular platform for colorimetric measurements for a variety of analytes (Purim et al., 2018; Salcedo & Sevilla, 2018). It presents a cost-effective and compact alternative to the diffuse-reflectance spectrophotometers (Santiago & Sevilla, 2022). Smartphone-based colorimetry has also been used for the assessment of beer freshness with the use of disposable polymer films for the detection of furfural (Rico-Yuste et al., 2016). In utilizing smartphone-based colorimetry, the digital image of the colored solid-state reagent is captured by the smartphone camera, and through digital imaging analysis, values are generated in the red (R), green (G), and blue (B) color space. The generated RGB results are associated with the radiation intensity across the range of wavelengths.

2. Materials and methods

2.1. Reagents

All reagents were analytical grade chemicals and were used as purchased without performing any other purification. Dimethyl sulfide (≥99.0 % purity), gelatin (CAS: 9000-70-8), polyethylene glycol (average molecular weight 3,350), and carboxymethylcellulose salt (CAS: 9004-32-4) were purchased from Sigma Aldrich (Missouri, U.S. A.). Sodium hydroxide (Cas—No: 1310-73-2), sodium nitroprusside (Cas—No: 13755–38-9), ethanol (98 % purity) and hydrochloric acid (37 % assay) were all purchased from Merck (Darmstadt, Germany). Lager beer and all-malt beer were purchased from the local market.

A stock solution of DMS standard was prepared by introducing 100 μL of DMS to 90 mL water maintained at 4 °C in a 100-mL volumetric flask placed in an ice bath. The mixture was subsequently diluted to volume. Serial dilution of the stock solution was done to prepare the calibration standards. To avoid volatilization of DMS, these solutions were maintained in an ice bath. Stock solution and calibration standards are freshly prepared prior to use.

2.2. Preparation of the solid-state reagent

The solid-state reagent consisted of filter paper (Whatman Grade 1) impregnated with the colorimetric reagent. The colorimetric reagent was formulated by dissolving sodium nitroprusside (0.33 % *w/v*) in an aqueous solution consisting of 0.05 g gelatin per mL of 10 % NaOH solution. The reagents for the preparation of PAD are freshly prepared before use. Sodium nitroprusside is a health hazard, additional precautions must be observed when handling the chemical. The required amount of reagent was prepared in small amounts. The filter paper was soaked in the sensing reagent for at least 30s. It was then laid on a flat surface for support and oven-dried for 1 h at 40 °C. It was cut into small squares (5 mm × 5 mm), which were then supported on a thin flexible plastic strip with 50 mm × 5 mm dimension (Supplementary Material, Fig. 1).

The same procedure was utilized for the preparation of solid-state reagent using polyethylene glycol (PEG) and carboxymethyl cellulose (CMC). Each of the 2 reagents were used in place of gelatin during the preparation.

2.3. Colorimetric measurement using a smartphone

Headspace vapor was generated by injecting 1.00 mL of the DMS standard solution (or beer sample) into a 1 L polyvinyl fluoride gas sampling bag that has been previously fully purged with nitrogen gas. The sampling bag was submerged in a water bath at 40 °C for 10 min. to allow the vaporization of DMS.

One milliliter of the headspace vapor was withdrawn from the bag using a gas-tight syringe. It was transferred into the 20 mL glass vial containing the sensor paper fixed in the plastic strip. The said glass vial was previously sealed with a silicon stopper and an aluminum crimp prior to injection to avoid any losses. The time for the gas-solid reaction to complete is 10 min. After the reaction, the reagent paper was taken out of the glass vial, and 10 μL of 6 M HCl solution was dropped on the sensing paper. The color was allowed to develop for 5 min.

The digital image of the colored reagent paper was captured inside a fabricated light box using the camera of a smartphone (Apple iPhone 6S Plus). A PVC board (Sintra, low-gloss matte finish) was used to construct the light box (10 cm × 17 cm × 7 cm) which was equipped with two 3-chip LED strip modules (Samsung, 0.75 watts). During image capture, the smartphone camera is stationed on the exterior, at the top of the box aligning with the light source. A hole was cut into the box to accommodate the camera lens. The paper was positioned at 5 cm directly below the camera lens (Supplementary Material, Fig. 2). The JPEG images was subjected to analysis using Image J software to obtain the RGB color space values (Santiago & Sevilla, 2022).

2.4. Gas chromatography measurement

The identical headspace vapor produced using the method as in the smartphone measurements was subjected to analysis using gas chromatography (GC) to establish the correlation of GC with digital imaging colorimetry (DIC). The headspace vapor peak area response was obtained using the Shimadzu Gas Chromatograph equipped with Pulsed Flame Photometric Detector (PFPD) set in square root of peak area mode. The conditions for the gas chromatographic analysis of were based on the study of Li et al.

For the comparison of the results of analysis of GC and DIC, beer samples were analyzed via GC following the reagent preparation, calibration and sample preparation procedure from Methods of Analysis of American Society of Brewing Chemist (ASBC) Methods of Analysis for the analysis of DMS (Beer 44). A Shimadzu GC-PFPD was utilized with the conditions for the GC obtained in the study of Li et al. (Li et al., 2008).

3. Results and discussions

3.1. Paper-based analytical device

The solid-state reagent contained the alkaline nitroprusside colorimetric reagent immobilized in a gelatin hydrogel on a paper substrate. A solid-gas reaction took place between the solid-state reagent and the DMS vapor in the gas phase. A complexation reaction occurs in the paper-based reagent phase, similar to that in solution (Filipovic et al., 2013), resulting in the development of a pink color.

$$\left[(CN)_5FeNO\right]^{2-} + R_2S \rightarrow \left[(CN)_5FeN(O)SR_2\right]^{3-}.$$

A hydrophilic polymer, such as gelatin, CMC and PEG provided a

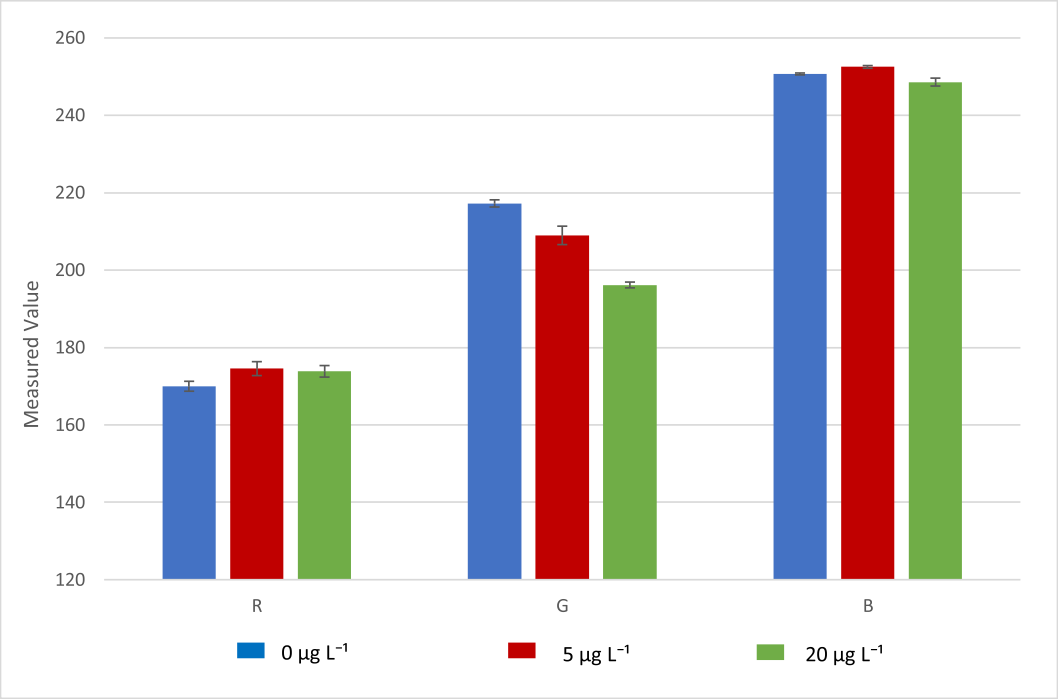

Fig. 1. RGB profile of the sensing reagent phase exposed to different DMS concentrations. Error bars indicate the standard deviation of triplicate analysis.

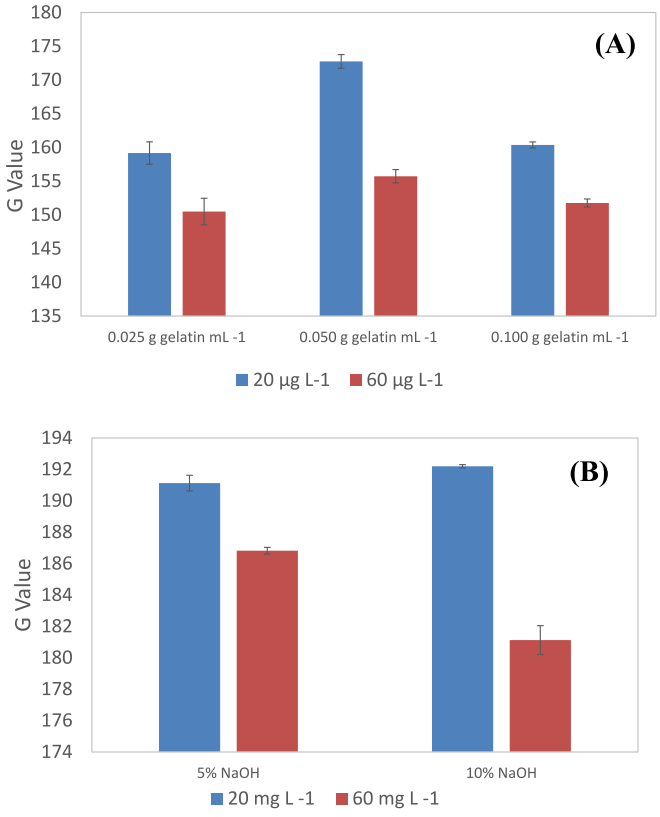

Fig. 2. Effect of (A) the concentration of the hydrogel and (B) the NaOH concentration on the G value. Error bars indicate the standard deviation of triplicate analysis.

suitable immobilizing agent for the colorimetric reagent. It entraps the reagent system in the hydrophilic cavities in the polymeric network comprising its matrix. The inherent porosity of the polymers allows the diffusion of the gaseous analyte into the entrapped reagent phase while maintaining the optimal medium necessary for the reaction. In contrast, the evaluation of PAD without hydrogel revealed an absence of color formation. This is likely attributable to the reduced retention of the colorimetric reagent on the filter paper, as the absence of hydrogel compromises its immobilization.

The gelatin reagent paper produced a more intense coloration, compared to reagent papers with PEG and CMC, and was therefore used for the measurement of DMS vapor. The amphiphilic nature of the polypeptide structure of gelatin enabled it to retain nitroprusside molecules and maintain an alkaline environment needed in the chromogenic reaction. Gelatin exhibits antioxidant properties (Sinthusamran & Benjakul, 2018) which also promoted the stability of the entrapped nitroprusside molecule.

A qualitative filter paper (Whatman Grade 1) was used as the substrate of the hydrogel-based reagent. It provided porosity and particle retention capacity sufficient for effective impregnation with a thin layer of the colorimetric reagent (Singh et al., 2018).

3.2. *Reagent colorimetric response*

The PAD which has been exposed to DMS developed an intense pink color upon acidification. The smartphone captured the digital image of the PAD. Analysis of the digital images revealed changes in the color values in the RGB color channels in response to exposure with DMS vapor. A slight increase was observed in the R-value, an appreciable decrease occurred in the G-value, and no change was seen in the B-value (Fig. 1).

The notable change in the G value can be attributed to the shift in the reflected radiation intensity in G (green) color space which peaks at the wavelength of 530 to 545 nm (Fan et al., 2021) The decrease in the radiation intensity can be attributed to the absorption of radiation by the reagent phase. This phenomenon aligns with the absorption spectrum of solutions of the DMS-nitroprusside complex in the visible region, characterized by the peak at 520 nm (Grigsby & Palamand, 1977).

The G value changed significantly when the concentration of DMS was increased from 5 to 20 µg L^{-1}, but not the R and B values, as shown

in Fig. 1. This observation demonstrates that the G value has the greatest sensitivity to DMS concentration, providing a basis for its choice as the color channel to be measured for the determination of DMS vapor.

3.3. Optimization of the reagent composition

The response of the solid-state reagent phase in the presence of DMS vapor was affected by the composition of the reagent. The **concentration of hydrogel** used in the preparation of the reagent affected the sensitivity of the solid-state reagent to DMS concentration. Fig. 2-A shows that the greatest sensitivity was exhibited by the reagent phase prepared using 0.05 g gelatin mL^{-1}. Increasing the concentration of the hydrogel caused an increase in the amount of the entrapped reagent. However, a too-high concentration produced a viscous reagent mixture which did not impregnate the paper substrate well, leading to a lower amount of immobilized reagent.

The **concentration of NaOH** also influenced the sensitivity of the solid-state reagent. Good sensitivity was observed in the reagent phase containing 10 % NaOH (Fig. 2-B). The highly basic concentration is believed to promote the unprotonated DMS structure formation which exhibits a strong affinity to adduct formation with nitroprusside anion (Filipovic et al., 2013). A higher NaOH concentration (15 %) caused deterioration of the sensing paper. This can be attributed to the solubility of cellulose in NaOH solutions (10 % and higher % NaOH) (Keshk, 2014). No deterioration was observed in the reagent with 10 % NaOH since the effective OH^- concentration must have been reduced by its deprotonating action on sodium nitroprusside and by its reaction with gelatin, which exhibits acidic properties (Fan et al., 2021; Sinthusamran & Benjakul, 2018). Hence, 10 % NaOH was used for subsequent measurements.

3.4. Method development

Minimizing the potential interference from other sulfides present in beer such as hydrogen sulfide, dimethyl disulfide and dimethyl trisulfide which reacts with the chromogenic reagent, is achieved by carefully controlling the heating temperature of the sample. The sampling bag was heated at the **temperature** of 40 °C, which is sufficient to cause the

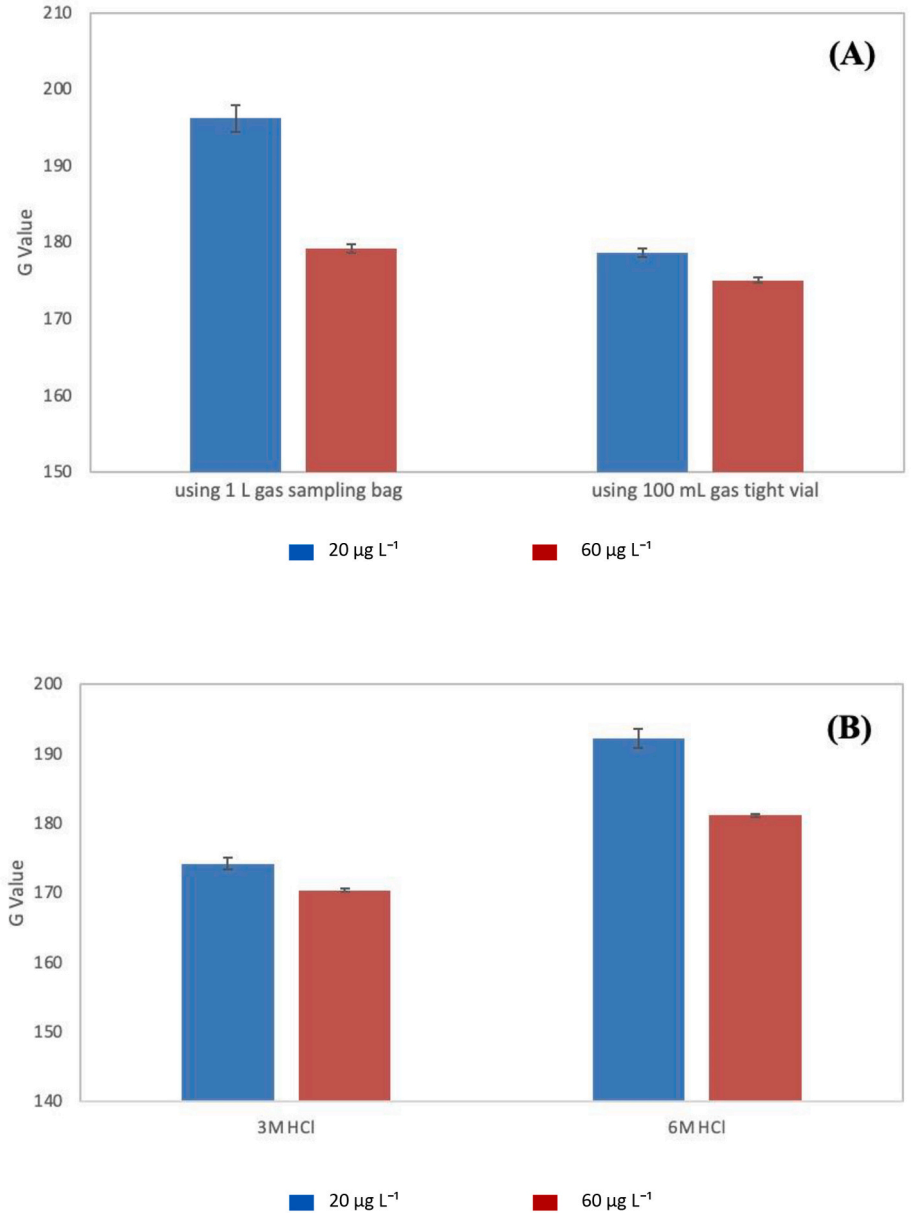

Fig. 3. Effect of (A) the volume of the vapor generation vessel and (B) the HCl concentration. Error bars indicate the standard deviation of triplicate analysis.

volatilization of DMS and hydrogen sulfide, as both of these compounds have a boiling temperature of less than 40 °C (Li et al., 2008). Although hydrogen sulfide may be present in beer, its concentration is typically less than 1 μg kg^{-1} which is below its sensory threshold (Oka et al., 2008). This temperature also restricts the volatilization of sulfur compounds with higher boiling temperatures including dimethyl disulfide and dimethyl trisulfide, which were not extracted when the same headspace sample of the commercial beer sample analyzed by DIC was injected into the GC. The selectivity of the DIC method was examined and demonstrated good selectivity for DMS compared to other sulfur-containing organic and inorganic compounds including sulfites, sulfates, and sulfoxide. (Santiago & Sevilla, 2022).

A static headspace sampling was adopted in the measurement of DMS. This technique involved the generation of headspace vapor from a liquid sample in a suitable vessel. The volume of the **vapor generation** vessel affected the G-value obtained in the analysis. Headspace sampling from 1000 mL gas sampling bag led to a higher sensitivity to DMS concentration than that from a 100-mL gas-tight vial sealed with a silicon septum (Fig. 3-A). A larger headspace volume leads to the generation of less concentrated vapor than in a smaller headspace volume, allowing greater discrimination of the concentration of the components in the headspace.

The **acid concentration** affected the intensity of the G value obtained. Generally, higher sensitivity was obtained when using 6 M compared to 3 M HCl (Fig. 3-B). It was also observed that lower G values were attained when 3 M HCl was used compared to 6 M. Use of 9 M HCl was excluded since its high concentration was observed to have caused the sensing paper to be torn off. Hence 6 M HCl was used throughout the study.

The **drying time** of sensing paper before image capture affected the G value of the colored reagent phase. The pink color of the paper became more pronounced and intense upon air drying of the sensing paper. Hence, a decline in G value was observed as the drying time was lengthened from 0 to 15 min. The greatest sensitivity was achieved by allowing the paper to air dry for 5 min before image capture. Such amount of drying time allowed full-color development.

3.5. Analytical performance characteristics

The resulting G values of the varying concentrations of DMS solutions demonstrated high precision. Specifically, 50, 70, and 110 μg L^{-1} of DMS were analyzed and obtained a range of 0.79 to 1.36 % relative standard deviation ($n = 6$). The satisfactory precision is attributable to the controlled image acquisition conditions created by the lightbox.

A linear decrease of the G-value with the increase in DMS concentration was manifested in the range of 5 to 120 μg L^{1} (Fig. 4). Minimal response variation was observed at concentrations exceeding 120 μg L^{1}. The linear equation: $G = -0.456\ C_{DMS} + 207.9$ also demonstrated a Pearson linear coefficient of 0.995. The LOD calculated based on a $3s_{blank}$ / m criterion was determined to be 2.70 μg L^{-1}.

Synthetic unknown solutions demonstrate accurate and repeatable results across three concentration levels of 50, 70, and 110 μg L^{-1}. For six replicate measurements, the percent trueness fell within 92.5–104.4 %, while the percent relative standard deviation (RSD) was 2.78 to 5.23 %. These values adhere to the AOAC criteria which is 15 % and 80 % to 110 % for RSD and trueness correspondingly (Horwitz & Latimer, 2023).

The G values derived from a series of DMS solutions correlated well with the peak area in the chromatogram obtained in the gas chromatographic analysis of these solutions (Fig. 5). This correlation indicates an agreement in the analytical results between the two methods of DMS measurement.

3.6. Analysis of real samples

The results of the analysis of lager beer and all-malt beer samples using smartphone-based digital colorimetry agreed well with those obtained from GC measurements (Table 1). The Paired *t*-test results indicated that there is no statistically significant difference between the two data sets at 95 % confidence. Taking the gas chromatographic method as the reference, the percent trueness of the values obtained through digital imaging colorimetry fell within the acceptable range specified by the AOAC (Horwitz & Latimer, 2023). These results indicate that the analytical performance of the smartphone-based headspace digital colorimetry method is comparable to that of GC method in the DMS concentration occurring in beer.

4. Conclusion

Headspace sampling and PAD enhanced the analytical performance of a smartphone-based colorimetric method for DMS. The DMS levels in beer which is as low as 5 to 120 μg L^{-1} could be measured employing a paper-based immobilized alkaline nitroprusside reagent as a solid-state reagent. This method could be useful in ensuring that beer has DMS levels below the flavor threshold of 30 μg L^{-1}, and that lager does not contain higher than 100 μg DMS L^{-1} levels, which causes off-flavor. It presents an alternative method to the more expensive GC method of DMS measurement. Small-scale producers of craft beer can utilize their smartphones to monitor off-flavor caused by DMS in their products,

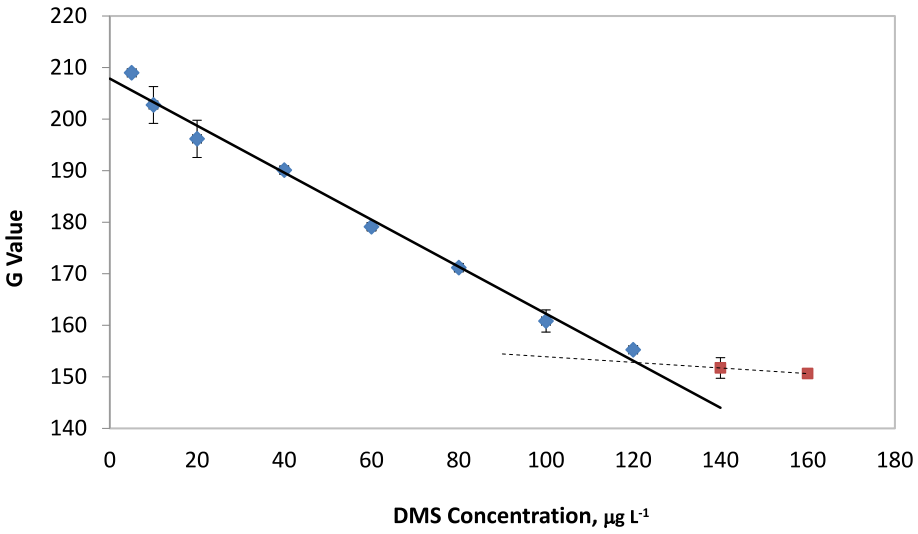

Fig. 4. Graph of DMS concentration vs. G value at the range of 5 to 120 μg L-^{1}. Error bars indicate the standard deviation of triplicate analysis.

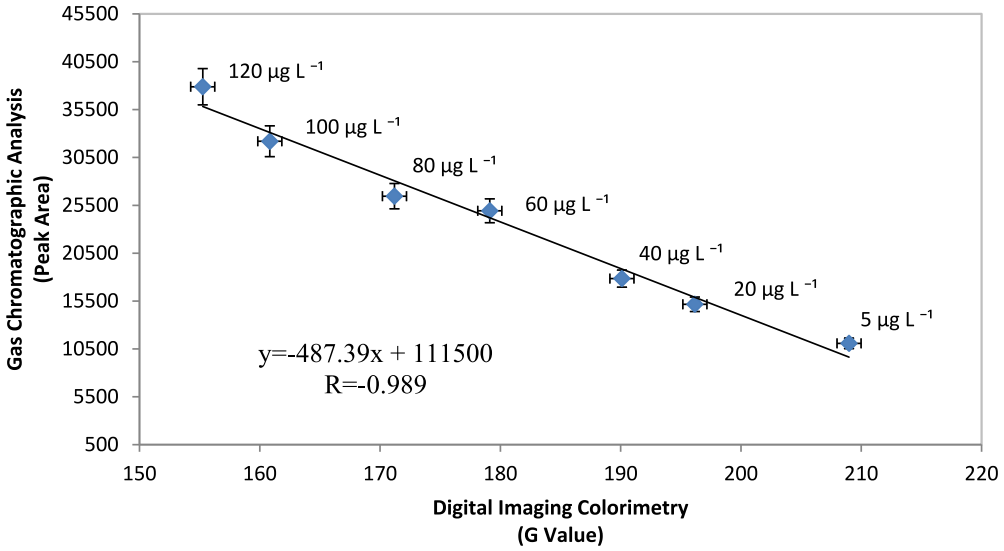

Fig. 5. Correlation plot of the G values obtained in digital imaging colorimetry and the peak area obtained in gas chromatographic analysis at the range of 5 to 120 µg L^{-1}. Error bars indicate the standard deviation of triplicate analysis.

Table 1
Comparison of the Digital Imaging Analysis and Gas Chromatography.

SAMPLE	DMS CONCENTRATION, µg L^{-1}		Percent trueness
	Digital Imaging Analysis	**Gas Chromatographic Analysis**	
BRAND A	57.34	55.57	103.2
BRAND B	73.38	74.32	98.7
BRAND C	67.05	69.47	97.9
BRAND D	117.62	118.2	99.5
BRAND E	70.81	71.61	98.9
BRAND F	46.89	46.74	100.3
BRAND G	84.86	83.55	101.6
Paired t-test: calculated t-value = 0.70; critical t-value =2.45			**AOAC Acceptable percent trueness = 80–110 %**

allowing them to conduct the analysis onsite and reduce the amount of time for analytical testing.

CRediT authorship contribution statement

Jamila B. Santiago: Writing – original draft, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Fortunato B. Sevilla:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Compliance with ethical standards

The presented research did not involve human participants and/or animals.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144391.

Data availability

No data was used for the research described in the article.

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