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An Alternative and Fast Method of Nitrite Determination in Meat Sausages Using the PhotoMetrix® Smartphone Applicative for Digitized Image Processing

Gleisson A. Almeida[1], Antônio J. Demuner[1], Daiane E. Blank[1], Cristiane I. Cerceau[1], Bianca L. Sousa[1], Iara F. Demuner[2], Marcela R. Coura[2], Maria J. M. Firmino[1], Marcelo H. Santos[1*]

[1]Chemistry Department, Federal University of Viçosa, Viçosa, Brazil
[2]Pulp and Paper Laboratory, Federal University of Viçosa, Viçosa, Brazil
Email: *marceloh.santos@ufv.br

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Abstract

Due to the potential threats of nitrite to public health and safety, in this work, we proposed, for the first time, the application of PhotoMetrix® software for the detection of nitrite in meat products. Nitrite monitoring in food matrices has been carried out by expensive analytical methods. Aiming mainly speed and cost reduction, colorimetric analysis software has been developed using digital imaging. Among them, the PhotoMetrix®, a free software based on templates univariate and multivariate mathematicians, has been standing out for its functionality. Herein, we evaluate the efficiency of PhotoMetrix® in the monitoring of nitrite concentration in meat sausages. The method was compatible with spectrophotometric data and showed a high correlation coefficient ($r^2 = 0.9940$) for the Red channel, high selectivity and sensitivity (limit of detection = 0.02 $mg \cdot L^{-1}$). Besides, the nitrite values determined for different brands of meat sausages are in agreement with the Brazilian legislation (IN 51/2006). Thus, our findings indicate that the PhotoMetrix® software is a viable alternative for monitoring the quality of meat sausages.

Subject Areas

Analytical Chemistry, Food Science & Technology

Keywords

PhotoMetrix®, Nitrite, Food Analysis, Meat Sausages

1. Introduction

The determination of nitrite (NO_2^-) is an imperative approach in samples food analysis due to the health problems posed by the presence of these chemical food additives [1] [2] [3] [4]. Though the nitrite is one of the most common nitrogen-containing compounds found in humans, NO_2^- that carries the label of highly harmful to food, may cause harm to humans such as cancer [5] [6] [7] [8] [9]. After consumption of products containing excessive NO_2^-, such as meat products, nitrite can interact with amines in the digestive tract, producing highly carcinogenic N-nitrosamine compounds [7]. In addition, some diseases such as infant methemoglobinemia and the central nervous system birth defects are associated with ingestion NO_2^- [8] [9].

Therefore, the search for simple and effective techniques for detecting and monitoring nitrite content is essential for public health and safety [1]-[6]. Indeed, a range of methods for NO_2^- detection have been reported for many authors, including chromatography [10] [11] [12], colorimetry [13] [14] [15], electrochemical [16] [17] [18], fluorescence detection [19] [20], capillary electrophoresis [21] and spectrophotometric procedures [22]. Nonetheless, these techniques display low mobility, spend large amounts of chemical reagents and require expensive equipment [10] [23]. Aiming mainly to get around the high costs, a novel methodology using PhotoMetrix® has been related, showing a simple operation, cheaper equipment, a lower limit of detection, and high selectivity [23] [24]. In this context, colorimetric analysis software and a range of chemical analysis have been developed based on digital imaging, aiming to obtain greater agility and cost reduction [23] [24]. Among the color models, RGB is one of the most known models for the generation of all colors in the visible spectrum by three channels: red (R), green (G) and blue (B) [25].

Many studies about technological advances in both acquisition and processing of images have aroused the interest of researchers, mainly due to the effectiveness of their results in front of traditional colorimetric equipment and methods. For instance, it is possible to capture and process images in chemical determinations involving color change by chemical reactions, resulting in faster and lower cost analysis, being an alternative to the use of robust and sophisticated equipment, such as spectrophotometers and colorimeters [26]. Indeed, [27] develops an effective method to determine NO_2^- in water and food using digital images acquired using a desktop scanner.

In this work, we proposed, for the first time, the application of PhotoMetrix® in nitrite analysis in meat sausages acquired commercially in the Viçosa-MG. In general, the application of the PhotoMetrix® as a detector in chemical analysis by colorimetric methods is an alternative to spectrophotometer usage. Methodologies involving this detection system can also be very attractive in the routine analysis due to its low cost as highlighted before.

Our findings demonstrated that NO_2^- contents in meat sausages samples determined by the PhotoMetrix® method were below the maximum limits allowed by Brazilian legislation [28] (Agência Nacional de Vigilância Sanitária (ANVISA) Resolution, Normative Instruction number 51, December, 2006, IN 51/2006) and compatible with the results found by spectrometer readings. In addition, the portable PhotoMetrix® was successfully applied to nitrite detection in solutions, exhibiting high selectivity and sensitivity. Finally, it is very important to highlight that PhotoMetrix® was very effective in the NO_2^- determination in food samples. The coupling between digital images from a common smartphone and PhotoMetrix® software to determine nitrite concentration in food samples showed to be very attractive financially, and might be useful in routine analysis.

2. Materials and Methods

2.1. Reagents, Samples and Instruments

Sodium Nitrite P.A., potassium ferrocyanide, sodium tetraborate and zinc acetate were acquired from Vetec (Rio de Janeiro, Brazil). Labcon test kit for Labcon Test, containing Reagent 1 (sulphanyl acid, acetic acid and distilled water) and Reagent 2 solutions (alpha-naphthylamine and ethyl alcohol) were purchased from Alcon (Camboriú, Brazil). All samples were commercially purchased (Viçosa, Minas Gerais, Brazil). A single-beam Ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, UV Mini 1240) equipped with a 1-cm quartz cuvette was used to perform UV-Vis analysis. A Samsung Galaxy J2 Prime cell phone with an 8.0 MP resolution camera and containing the PhotoMetrix® applicative was used to perform digital image analysis.

2.2. Preparation of Solutions

Nitrite solutions were prepared in distilled water at concentrations ranging from 0 to 10 ppm and used as standard samples to constructed the analytical curve. Three solutions were produced and used for the preparation of food extracts: 1) sodium tetraborate decahydrate at 5% (w/v); 2) potassium ferrocyanide trihydrate at 15% (w/v); 3) zinc acetate dihydrate at 30% (w/v) containing 3% (v/v) of glacial acetic acid.

2.3. Preparation of Food Samples Extracts

Before you begin to format your paper, first write and save the content as a separate text file. Keep your text and graphic files separate until after the text has been formatted and styled. Do not use hard tabs, and limit use of hard returns to only one return at the end of a paragraph. Do not add any kind of pagination anywhere in the paper. Do not number text heads—the template will do that for you. Finally, complete content and organizational editing before formatting. Please take note of the following items when proofreading spelling and grammar: Extracts of six samples from different brands of meat products were analyzed: S1: smoked sausage; S2: sausage (brand 1); S3: bologna; S4: sausage (brand 2); S5: salamito; S6: sausage (brand 3). Approximately 1 g of each sample was grounded with the aid of gral and pistil and dissolved in 5 mL of 5% (w/v) sodium tetraborate and 50 mL of distilled water. After solubilization, the solution was heated in a water bath at 80˚C for 20 minutes under stirring, with the aid of a glass rod. A treatment in absence of samples was prepared in the same conditions and was used as a blank. Elapsed warm-up time, a volume of 5 mL of trihydrated 15% (w/v) potassium ferrocyanide and 5 mL of zinc acetate 30% (w/v) were added, the volume was supplemented with distilled water to 200 mL, and the system was kept under stirring. After 15 minutes, the extract solutions were obtained by filtration on filter paper. The procedure was performed in triplicate.

2.4. Determination of Nitrite

The NO_2^- concentration was determined using volumetric balloon containing 10 mL of the extract or standard solutions followed by addition of 2 drops of Reagent 1, and the mixture was kept for 5 minutes without stirring. After, 2 drops of Reagent 2 were added, a color change was observed, the volume was supplemented with distilled water to 50 mL and solution was homogenized. After 15 minutes, both spectrometric analysis (λ = 540 nm) and readings smartphone using the PhotoMetrix® coupled to a dark box containing LED daylight were performed as previously described [23]. The analytical curves, one obtained by each method, were used to calculate the NO_2^- concentrations ($mg \cdot kg^{-1}$ of sample).

2.5. Application of Portable PhotoMetrix

The PhotoMetrix® smartphone applicative was purchased for free from the Google Play Store. The sequence of options "univariate analysis", "collecting multiple channels" and "calibration" was executed to construct the analytical curve. In the last menu, the number of standard solutions (n = 7) was added and the concentrations of these samples (0 to 10 ppm) were informed by running the "capture images" option. The colorimetric measure of each solution was performed putting them in the data collection cabin, the "save" option was executed and the program generated the analytical curve. The measurements of the analyte of interest in the matrices food were performed using the sequence: "univariate analysis", "collecting multiple channels" and "sampling", under the same conditions as standard solutions. Finally, the analytical curve was selected, allowing the conversion of the signal obtained into the NO_2^- concentration.

2.6. Comparison between the Methods Used to Determine Nitrite of Foods

The comparison between the traditional UV-Vis spectrophotometry method and the PhotoMetrix® application used for determination of NO_2^- in meat products was performed by analysis of variance (ANOVA) and Tukey test with a 95% confidence interval.

2.7. PhotoMetrix® Validation Method

Validation was performed for the nitrite determination of foods. The parameters evaluated were: linearity; repeatability; accuracy/recovery; and limits of quantification (LOQ) and detection (LOD) [23]. The validation of the PhotoMetrix® method in the red channel was accomplished according to the methodology described by Bazani *et al.*, 2021 [23], with modifications. To evaluate the linearity and repeatability sodium nitrite solution was used at concentrations of 0.0, 1.16, 2.09, 4.28, 6.20, 8.06, and 10.40 mg·L^{-1}. Accuracy/precision was determined from recovery tests using the sodium nitrite solution at concentrations of 1.16, 4.28, and 10.40 mg·L^{-1}.

3. Results and Discussion

In this work, a simple and low-cost method using the PhotoMetrix® program together to digital imaging was developed to determine nitrite in meat sausages acquired in Viçosa-MG, Brazil. First, standard solutions were subjeted to readings in the RGB channels of the PhotoMetrix® software, and the color intensities were used to construct the analytical curves obtained in these channels (**Table 1**).

Evaluating the analytical curves obtained in the R, G and B channels (data not shown), the analytical curve acquired using Red channel ($y = 7.3481x + 52.744$; $r^2 = 0.9940$) was chosen to determine nitrite in the samples. R channel showed the best linearity ($r^2 = 0.9940$) and high sensitivity to sodium nitrite concentration variations (angular coefficient = 7.3481). The obtained values were compared to NO_2^- concentrations found using the linear regression ($y = 0.0675x + 0.0036$; $r^2 = 0.9983$) by UV-Vis spectrophotometry ($\lambda = 540$ nm). The parameters linearity, repeatability, accuracy/recovery, LOQ, and LOD were evaluated for the nitrite determination of foods by PhotoMetrix® in the red channel. Seven sodium nitrite concentrations were used to determine the NO_2^- solution concentrations with PhotoMetrix® applicative. For data of linearity and repeatability, the coefficient of variation values (CV) obtained were lower than the maximum permitted by RE 899 (CV ≤ 5.0) [29], indicating that the method was linear and repeatable. The mean recover to accuracy/precision validation was between 97.64% and 100.20%, being considered adequate according to the Brazilian

Table 1. Nitrite concentration (ppm) and color intensities obtained in RGB channels.

Nitrite (ppm)	RED	GREEN	BLUE
0	201	202	203
1	196	192	190
2	188	185	181
4	171	166	165
6	159	157	153
8	147	142	138
10	126	121	116

Association of Technical Standards recommendations NBR14029 [30]. The LOD and LOQ determined were 0.02 and 0.06 mg·L^{-1}, indicating that the PhotoMetrix® method is able to quantify nitrite in food in small quantities. The presence of nitrite was identified in all samples of meat products, whose mean concentrations ranged from 56.00 - 124.00 ppm and 56.74 - 125.61 ppm, respectively, in UV-Vis and PhotoMetrix® analysis (**Figure 1**). In both methods, NO_2^- concentration values differed in all samples, including between different brands of the same meat sausages. The highest variations were found to brands of sausages (samples 2, 4 and 6). Satisfactorily, all processed foods displayed nitrite amounts inferior to allowable levels established by Brazilian legislation IN 51/2006 (150.00 mg·Kg^{-1}) [28], and the highest concentrations were found in salamite (sample 5). On the other hand, smoked sausage (sample 1) displayed the lowest NO_2^- content (56.00 mg·Kg^{-1}). It is interesting to highlight that NO_2^- content in salamito was about 1.4-fold higher than in bologna.

Although the products have been satisfactory according to the Brazilian legislation (IN 51/2006) [28], the NO_2^- levels are still worrying since other countries legislations establish maximum limits lower than 150 mg kg^{-1} of nitrite in embedded foods [28]. Several industrialized meat sausages exhibit compositions variation that may be compromising the quality and standardization of products, suggesting the absence of an efficient system to quantify nitrite in products by food industries [31]. Thus, considering the human diseases caused by nitrite salts, we reinforce the need for the development of preventive measures directed to reduce the toxic effects caused by these additives. In this work, close values were found between analysis methods used, indicating that PhotoMetrix® can be a viable alternative to determine nitrite in food matrices, useful for instance in laboratories with limitations of financial resources analytical. Other studies also used digital images with smartphones, the state-of-the-art cell phones, for colorimetric determinations. The advent of technologies for the acquisition and processing of images in smartphones allowed to perform colorimetric/spectrophotometric and fluorimetric determinations without analytical instruments, with the additional possibility of performing in situ measurements [24] [32]. Indeed, Neto and collaborators [33] reported a fast, simple and low-cost method

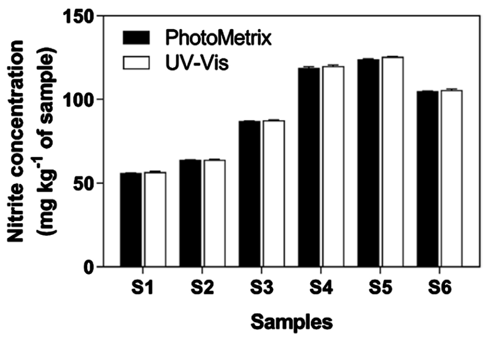

Figure 1. Comparison between nitrite concentration (mean ± standard deviation) in meat sausages samples measured by PhotoMetrix® software using Red channel and UV-Vis spectrophotometry.

for the classification of mineral waters based on the reaction between metal ions and colorimetric reagent. The authors used digital images from a smartphone and the measurements were performed by RGB system directly on the sample complex [33]. Similarly, our research group showed the usage of digital images obtained with a simple smartphone and the PhotoMetrix® program to determine total phenolics and antioxidants in tomato, strawberry and coffee at different maturation stages [23].

4. Conclusion

In the present study, we demonstrated that PhotoMetrix® applicative might be used as a detector in chemical analysis by colorimetric methods as an alternative to spectrophotometer use. The coupling between digital images from a common smartphone and PhotoMetrix® software to determine nitrite concentration in food samples showed to be very attractive financially, and might be useful in routine analysis. The efficiency of the method was proven by comparison with spectrophotometric analysis, a reference methodology.

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Conflicts of Interest

The authors declare no conflict of interest.

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