RESEARCH ARTICLE

Novel Method for the Determination of Preservative (Formaldehyde) in Bovine Milk through Smart Phone-Based Colorimetric Technology

Vipin K Gupta[1], RS Aulakh[2], SS Tomar[3]

Abstract

The use of formaldehyde as a preservative is a very common practice to decrease the microbial load and to increase the shelf life of the milk. Its addition in food has immense adverse public health significance. The present study explored a simple, low-cost, rapid, reproducible, and field applicable Smartphone-based colorimetric technology, which was standardized and in-house validated for the quantitative determination of formaldehyde in milk samples. The method has simple steps of spot-test reaction and digital image analysis with the Red Green Blue approach. The linearity of the method was shown by analytical curves ranging from 0.25–4 ppm that were characterized by $R^2 > 0.99$. The limit of detection of 0.31 ppm demonstrated the sensitivity of the method to estimate formaldehyde residues in milk. Thus, we developed an innovative technology that uses an easily available device with potential of on-site quantification of formaldehyde in the supply chain. This technique is not only beneficial for end-users but also helps in achieving extension goals, which emphasize on the transfer of technology, *i.e.,* moving out of the lab to the land.

Keywords: Formaldehyde, Milk, Preservative, Quantification, Smartphone-based colorimetry.

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[1]Dept. of Veterinary Public Health, College of Veterinary Science and AH, Mhow, Indore, India

[2]Director, School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

[3]Dept. of Animal Genetics & Breeding, College of Veterinary Science and AH, Mhow, Indore (India)

Corresponding Author: Vipin K. Gupta, Dept. of Veterinary Public Health, College of Veterinary Science and AH, Mhow, Indore, India, e-mail: drvipin80@gmail.com

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Introduction

The challenges of maintaining milk safety are now recognized globally. Increasing consumer demand for fresh produce, complexity of supply networks, lack of rapid transport, bad cold storage and refrigeration facility, and the tropical climatic condition has led to being an increase in adulterating and counterfeiting practices, particularly preservation. The use of formaldehyde as a preservative in milk is a very common practice. Usually, it is used as a sterilizing agent, an antibacterial agent, a disinfectant, a hardening, and a reducing agent, a corrosion inhibitor, and also use in the preservation of biological specimens (Herschkovitz *et al.*, 2000). Its use in milk to decrease the microorganism and to increase the keeping quality has been reported (Gupta *et al.*, 2013). Formaldehyde is among most commonly occurring frauds in milk as it not only preserves but also improves the appearance and keeps it odorless (Nascimento *et al.*, 2015). The addition of formalin in milk may cause vomiting, diarrhea, and abdominal pain in humans. Larger doses may cause decreased body temperature, shallow respiration, weak irregular pulse, and unconsciousness. It also affects the optic nerve and causes blindness. It is one of the potent carcinogens (Gwin *et al.*, 2009) and hence, detrimental to human health (International Agency for Research on Cancer (IARC), 2004).

With the advancement of information technology and electronics sector, the place of personal computers has been occupied by smartphone devices (Sumriddetchkajorn *et al.*, 2014). It has now become an integral part of every home. It is having facility of digital color camera and computation; its applicability has been widened to different areas like telemedicine (Martinez *et al.*, 2008; Preechaburana *et al.*, 2010), microscopy (Breslauer *et al.*, 2009; Tseng *et al.*, 2010; Lee *et al.*, 2011), and fluorescent imaging (Zhu *et al.*, 2011) etc. The colorful display of a mobile phone is also useful for the analysis of foods and beverages (Iqbal and Bjorklund, 2011). Colorimetry is not a new phenomenon, Smartphone-based colorimetric quantification has been proposed and demonstrated by many workers (Shen *et al.*, 2012; Sumriddetchkajorn *et al.*, 2014)

In India, regulatory agencies routinely perform the chromogenic qualitative test during inspections of milk. Merely qualitative detection is no confirmatory evidence of adulteration. It should be quantified with a validated analytical method. The availability of such type of technique and portable handheld devices, which can collect and analyze data within food supply chains at targeted points, is lacking. Keeping in view above points, the present study was undertaken for the development and validation of colorimetric based method with a smartphone for preservative (formaldehyde) determination in milk.

Materials and methods

A novel, interesting, and inexpensive technology for the quantitative determination of milk preservative (formaldehyde) was explored. In recent years, digital image analysis (DIA) or digital image-based (DIB) methods have been developed and applied in analytical chemistry for quantitation of analyte of interest (Benedetti *et al.,* 2015). The same principle of smartphone-based chemistry instrumentation; digitization of colorimetric measurements (Chang 2012) was extended here for assessing the concentration of formaldehyde in milk. A brief description of the materials used and methodologies adopted was as under.

Apparatus and Instrumentation

A smartphone embedded with Android 6.0.1 operating system, equipped with 13 MP CCD camera, 1.5 GB RAM and 1.2 GHz processor. A black plastic mobile platform is containing a white light source, batteries, a white plastic light diffuser, a flat mirror, a sample bottle holder, and an observing window. Image J software program, Electronic weighing balance, test tube stands, and pipettes were used. Various glasswares like a beaker, test tubes, etc. (Borosil) were used in the study for collection, dilution, storage, and spot tests.

Chemicals and Reagents

All the chemicals were of analytical reagent (AR) grade and were obtained from Moly-Chem India and Loba-Chemie India. Triple distilled water was used for making various preparations. The chemicals and reagents used in the study were: 2-4 dinitro-phenyl hydrazine solution, Standards solutions of formaldehyde; from reference material, a stock solution of formaldehyde in water was prepared using de-ionized water. For a matrix-matched standard solution, milk samples were spiked with different concentrations ranging from 0.25–4 ppm. All the working standard solutions were derivatized with acetonitrile and 2-4 DNPH solution.

Estimation of Formaldehyde in Milk

Analysis of digital image data

The spot test reactions were first performed. The digital image of the colorimetric reaction was then taken by a smartphone using a black plastic plate-form. Captured pixs were converted into numerical values by using Image J. software; the user can select the most homogeneous region in the image (usually middle portion) defining the coordinates for the selected region, and to be used for all the images. The software scans all the pixels (column by column) to extract the Red, Green, and Blue (RGB) components for each pixel and calculates a mean value for each RGB component. These values change relatively with developed color in colorimetric spot reaction. Color generated was proportional to the concentration of the substance of interest/analyte.

Preparation of analytical curve

Quantitative estimation of formaldehyde using a smartphone-based colorimetric method requires preparation of a calibration curve. For it, different concentrations of formaldehyde (0.25 to 4.0 ppm) were used. The pixs of developed yellow color was taken by smartphone using a black plastic plate-form. The blank was prepared by taking authentic milk and treating it with the same procedure. To validate the method for the tested analyte, parameters like sensitivity, linearity, range, limit of detection (LOD) and limit of quantification (LOQ) were evaluated by using prepared calibration curve for formaldehyde estimation. The accuracy, precision was also determined.

Results and discussion

In the present study, a simple, low-cost, rapid, reproducible, and field applicable smartphone-based colorimetric technology was standardized and in house validated for the quantitative estimation of formaldehyde in milk samples. The method had simple steps of spot-test reaction and digital image analysis with a RGB approach. Our study brings together the talents of the spot test (fast, low volume and screening evaluation) and digital pix (color quantification, low-cost gadgets, portability, and area applicability). The method revealed great analytical performance in terms of sensitivity, linearity, range, the limit of detection (LOD), and limit of quantification (LOQ).

Analysis of Digital Image Data

Digital images for formaldehyde

In the presence of 2–4 dinitro-phenyl hydrazine solution aliquot changed its color to yellowish, which was captured by a smartphone. Digital images for five standard solutions with different concentrations were depicted in Figure 1. All images represent a selected area equal to 25×28 pixels. On visual examination, the images present a difference in intensity for the yellow color, relative to the formaldehyde concentration in each calibration solution.

The intensity of the green "G" component decreases with increasing concentrations of the standard solution, the intensities associated with the red "R" and the blue "B" components remain practically almost constant (Figure 2).

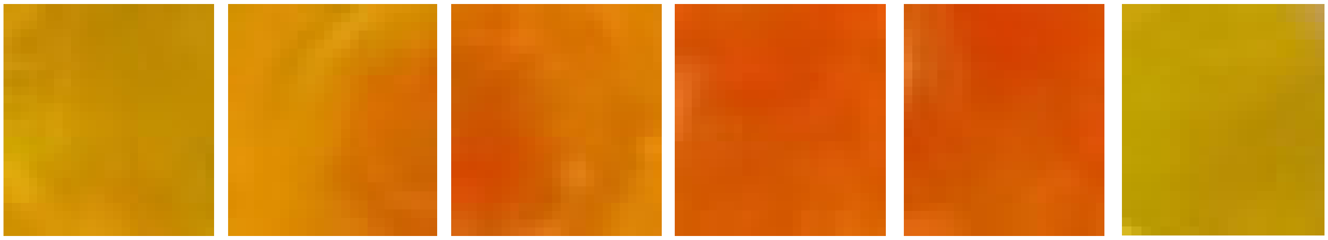

Figure 1: Digital images captured for different standard concentrations (25x28 pixel selected area)

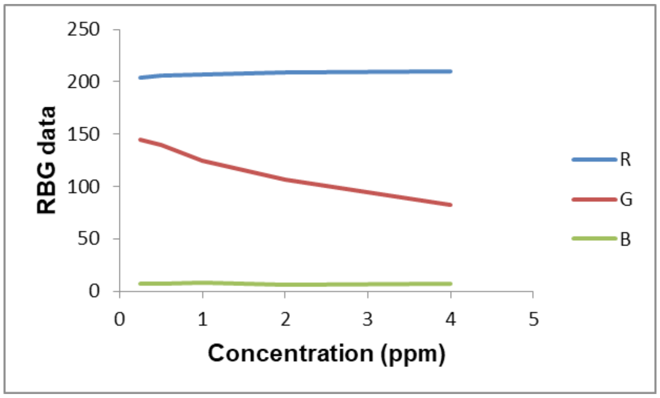

Figure 2: RGB data of the standard concentrations (0.25 to 4.0 ppm)

Analytical Curve

The analytical curve for formaldehyde was constructed using the analytical response calculation (R) as described in the literature (Kohl *et al.,* 2006 and Gomes *et al.,* 2008).

$R = \log (I_0^{RGB}/I^{RGB})$

Where I_0^{RGB} and I^{RGB} are the mean values of the intensities for the R, G, or B components in the selected area associated with both the blank and standard solutions, respectively.

Linear regression was found between the concentration of the formaldehyde (ppm) and analytical response (R) obtained from mean values of the intensities for the G (Green) component (Figure 3). Other relationships between the concentration of formaldehyde and the response of those concerned with mean values of the intensities for the R or B

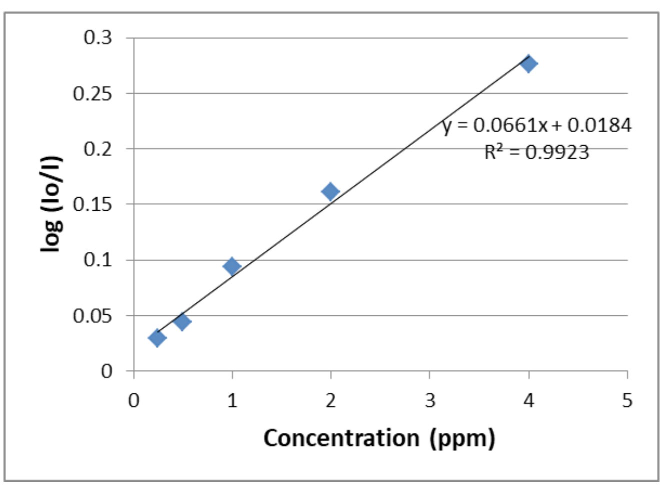

Figure 3: Standard curve for formaldehyde estimation

Table 1: Method validation parameters for formaldehyde determination

Parameter	*Formaldehyde*
Linear regression equation	y = 0.0661x + 0.0184
R^2	0.9923
Range (ppm)	0.25 to 4.0
LOD (%)	0.31
LOQ (%)	1.04
Accuracy (% recovery)	92.02
Precision (% RSD)	3.51

components were also studied to maximize precision. But, in every case, the results were poor compared to those using "G" (Green) for formaldehyde determination.

Application

The present method obtained a satisfactory analytical curve for the determination of formaldehyde in milk samples with the regression equation y = 0.0661x + 0.0184; where y = R is the analytical response and x is the analyte concentration in ppm of formaldehyde. The linear correlation coefficient, R^2 was 0.9923 in the range between 0.25 ppm (mg/L) and 4.0 ppm (mg/L). The method showed a linear regression at studied concentration i.e., up to 4.0 ppm. For estimation of the concentration of formaldehyde up to that level, the above described linear equation can be used; however, if the unknown sample contains a higher concentration of formaldehyde in milk, such samples were required to be diluted.

The LOD of method for formaldehyde determination was found as 0.31 ppm. The mean % recovery of analyte was found to be 92.02 and mean % RSD to be 3.51. No other investigation was observed in the literature review for comparison of formaldehyde determination in milk through smartphone-based colorimetry. All the method validation parameters are presented in Table 1.

Conclusion

A simple, low-price analytical technology for quantification of formaldehyde in milk based on mixture of a colorimetric spot-check and a digital image-based (DIB) method is in house validated. The digital snapshots from spot-test reactions were captured with the use of a smartphone camera on a transportable plastic plate-form designed with inner lighting control. Pix were then converted into numerical values by using software, which provides a concentration of the analyte in milk through linear regression equation, *i.e.*, quantitative detection strategies for on-site milk fraud evaluation – shifting out of the lab, onto the land (milk supply chain).

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