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RESEARCH ARTICLE

Determination of the Presence, Concentration, and effects of Formalin on Raw milk around Nyeri Municipality

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ABSTRACT:
Milk adulteration is done to increase the milk quantity or its shelf life. This research focused on the latter, accomplished by adding chemicals such as formalin. This is a great global concern and it causes detrimental effects such as cancer, myeloid leukemia. The chemical effects of formalin on milk include reducing its pH, protein, and fat percentage. Five samples were collected from several milk vendors around Nyeri Municipality and a sample directly from the cow. The pure milk had the fastest reduction time (18.3±1.53minutes) in Methylene Blue Reduction Test. The milk samples had an average of 3.3929±2.0579ppm formalin. In UV-Vis, the LOD and LOQ, R^2 were 0.001M and 0.004M, 0.9868 respectively. Both the butter fat, and pH values decreased with formalin concentration increase. Their mean was 3.1±0.4% and 5.47 ± 0.806 respectively. Out of the six samples, 83.33% of them were adultered with formalin.

KEYWORDS: Formalin; Raw milk; Butter fat; Bacteria.

1. INTRODUCTION:
According to research, formalin can be used as a preservative[1]. Formalin helps in milk preservation by reducing the amount or killing the microorganisms (preferably bacteria)[2,3] and curbing bacterial growth as well as other pathogens in the milk hence there is reduced action of bacteria on the sugars contained in milk[3]. This reduces faster milk spoilage increasing its shelf life. It has however been observed to have serious negative effects on the milk properties and human health[4]. This is according to the National Toxicology Program in conjunction with other agencies, including Food and Drug Administration and The National Cancer Institute, where they depict that formalin is a human carcinogen and leads to myeloid leukemia[5]. The aspect of food preservation especially in milk has narrowed down to milk adulteration[6]. Milk adulteration is a technique used or conducted to degrade the quality and at times increase the quantity of milk, all aimed at making illegal profits[2,6].

Milk and milk products are under a great assault by formalin addition, which it affects milk properties and causes great side effects on the consumers of such products[7]. Studies show that the mortality experience evaluation of 26,561 workers working in ten formaldehyde-producing facilities, with an average of 600,000 workers per year, showed health issues as they were followed up to January 1,1980[8,9]. Infants are dependent on milk as the main source of nutrients required by their bodies[10]. Some parents buy milk for their children as supplementary food[11].

Des Raj and Singhal used the Gerber method and obtained results that showed low-fat value for milk preserved using formalin[12]. As demonstrated by research and experiments, the formalin presence could be detected by methods including Methylene Blue Reduction Test, quantified by UV-Vis Spectroscopy. The two methods were observed to be effective and accurate[13,14]. According to[15], 10% of the 41 samples taken for milk adulteration detection were adultered. This data was found significant and reliable, so the authors opted to use these methods in their research.

2. MATERIALS AND METHODS:
2.1. Milk Samples:
The milk samples were collected from several dairy shops and milk vendors around Nyeri Municipality, Kenya. The samples were collected from the following areas; Catholic Hostel shops near Dedan Kimathi University of Technology-Nyeri, Embassy, Bomas, Kieni Dairy Shop in Nyeri Town, Nyaribo and a pure sample from a farmer. The latter sample was collected immediately after milking and the milking was done in the presence of the authors.

2.2. Formalin Presence Test:
This test was conducted using the Methylene Blue Reduction Test, where 10mL of each raw milk sample was measured and 1mL of methylene blue dye was added into test tubes. Cotton wool was used to seal the test tubes tightly to avoid oxygen entry. Swirling was done to mix the contents, and the test tubes were placed in a water bath maintained at 37°C for 30 minutes and 1 hour. The incubation time was noted as the time taken for the colour change to be white. The procedure was carried out in triplicate. The results were positive when the bacteria present in the milk led to a colour change within a certain time and a negative one when there was no colour change[16,17].

2.3. Formalin Quantification Test:
UV-Vis Spectrophotometer was used to quantify formalin, and it was first calibrated before using it. Standards of 10, 15, 20, 25, and 30ppm of formalin were run at a wavelength of 276nm to obtain a calibration curve which was used in obtaining the formalin concentration of the five collected samples. The samples were then placed in the cuvette, and each procedure was carried out in triplicate. An absorbance was observed, and the data was recorded for analysis, representation and interpretation[18–20].

2.4. Effects of formalin on Butter fat and pH:
2.4.1. pH Test:
A pH meter was first calibrated using a buffer of pH 4, 7 and 10. The pH of a 10mL pure fresh milk sample was measured in triplicate, and the results were recorded. This was followed by measuring four 40mL pure milk samples to obtain four samples from the pure raw milk sample. Formalin (2.5%) was added to the four samples in amounts of 10, 20, 30 and 40mL, respectively. A volume of 10mL was measured from the 40mL samples to obtain 3 samples, and the readings of pH readings for each sample and each addition were recorded in triplicate[21,22].

2.4.2. Butter Fat Test:
The procedure involved preparing four 40mL of the pure raw milk sample. The butter fat was determined by adding 10.94mL of 90% H_2SO_4 in a butyrometer, followed by adding 10.94mL of milk in a slanting position of the pipette, and then 1mL 80% Amyl Alcohol was added to facilitate separation of fats from other solids. Tight corking was done, and then shaking to mix the components properly. This was followed by centrifugation for 5 minutes, and then the readings were done. The procedure was repeated for the three other samples upon the addition of 10, 20 and 30mL of 2.5% formalin, respectively, and each procedure was done in triplicate.[23–26]

3. RESULTS AND DISCUSSION:
3.3. Formalin Presence Test:
The observations for colour change were noted, and the colour change for sample F was observed at 18, 20, and 17 minutes for trials 1, 2, and 3, respectively. There was a colour change for sample D at 57, 59 and 54 minutes for trials 1, 2 and 3, respectively. The shorter reduction time indicated the presence of a large number of bacteria hence showing the absence or lower amounts of formalin where the concentration of the formalin was determined using a UV-Vis Spectrophotometer[17,27]. The other samples did not show a significant colour change after the 60 minutes, indicating a lower number of bacteria, hence the slower reductions—this implied presence of some concentration of formalin.

Table 1: Methylene Blue Reduction Time in the collected samples

Sample	Average time for colour change during 60 minutes run
Sample A (Catholic)	-ve
Sample B (Embassy)	-ve
Sample C (Bomas)	-ve
Sample D (Kieni Dairy Shop)	56.7 ± 2.52 minutes
Sample E (Nyaribo)	-ve
Sample F (Pure sample)	18.3 ± 1.53 minutes

Note: **-ve** means there was no significant colour change at a specific time, indicating formalin presence.

3.4. Formalin Quantification Test:
The mean formalin concentration of the samples collected was obtained, and its value was 0.000113± 0.0000685M. The curve was linear, as indicated by the coefficient of determination, R^2. *Uncertainty* indicates the range of possible or probable values where the true measurement value lies[29]. Uncertainty of the y-intercept was 0.02975 while that of the slope was 46.137. The LOD and LOQ using the standard deviation of intercept was 0.001 M and 0.004, respectively. The Regression sum of squares and Residual sum of squares were 0.4127 and 0.0055, respectively. According to research for the quantitative analysis of formaldehyde using UV-Vis spectrophotometer measured at 207nm, the analysis was done using Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR), and the correlation factor were 0.98 and 0.94 with a predicted accuracy of 84% and 83.45% respectively. Formalin was observed in street vendors' milk at a concentration of

10% in four samples; hence, the mean was 2.5% and absent in farmers' house milk and farm milk[30]. These tests were conducted using Wynther Blyth test and qualitative disc assay method . This affirms this study's finding that street vendors of milk are the largest and main group that performs milk adulteration using formalin[30].

Table 2: Formalin quantification test results

Sample	Concentration (M)	Concentration (ppm)
Sample A (Catholic)	0.000201 ± 0.00000252	6.0362 ± 0.075598
Sample B (Embassy)	0.000136 ± 0.00000153	4.0842 ± 0.045826
Sample C (Bomas)	0.000108 ± 0.000000577	3.2433 ± 0.12124
Sample D (Kieni Dairy Shop)	0.0000819 ± 0.00000250	2.4595 ± 0.075050
Sample E (Nyaribo)	0.000151 ± 0.000000577	4.5347 ± 0.017321
Sample F (Pure sample)	0.00 ± 0.00	0.00 ± 0.00
Mean	0.000113 ± 0.0000685	3.3929 ± 2.0579
Median	0.000122	3.6638
Standard Deviation	0.0000685	2.0579
Variance	4.7×10^{-9}	4.2348
RSD	60.650	60.650

3.5. Effects of formalin on Butter fat and pH:
### 3.5.1. pH Test:
Formalin affects milk acidity. The pH of the raw milk decreased with an increase in formalin concentration. This depicted an increase in acidity with increase in the concentration of formalin. With a lower acidity, milk is able to take long before going bad hence the ability of formalin being used as a preservative[31,32]. The mean pH of the samples obtained from the research was 5.47 ±0.806. The testing of the physical and chemical quality of milk sold in Kanpur city showed a mean pH of 5.84 and a mean butter fat of 3.20%. This milk was found to have some amounts of formalin used as a preservative[33].
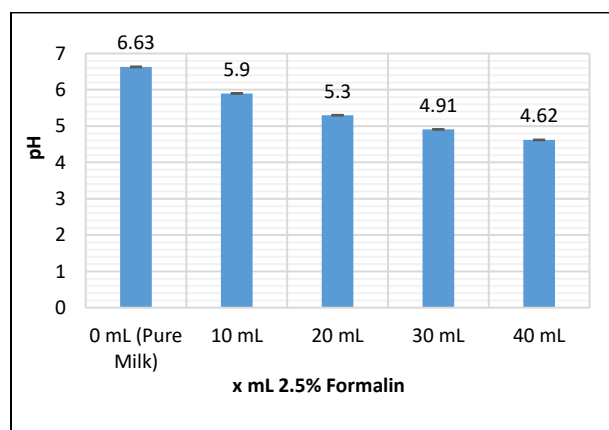
Figure 1: Graph of pH against Formalin Concentration in Milk

3.5.2. Butter Fat Test:
Formalin reduces the butterfat of milk. The higher the concentration of formalin, the lower the butter fat percentage[1]. This was clearly depicted by the experiment conducted. The butter fat percentage decreased with an increase in formalin concentration. This shows the harmful effects of formalin on the butter fat percentage in raw milk when used as a preservative[34]. The mean butter fat percentage obtained from the research was 3.1 ±0.44%.
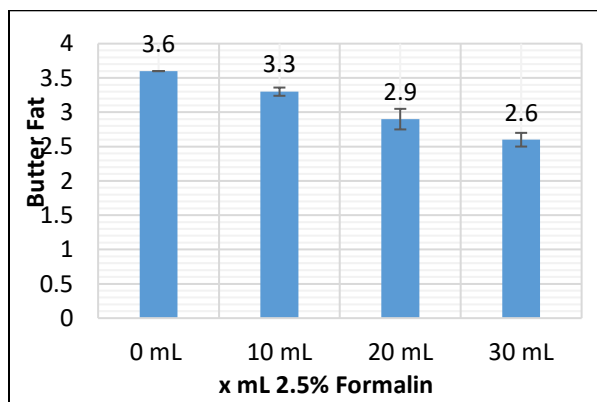
Figure 2: Graph of butter fat against Formalin Concentration in Milk

4. CONCLUSION:
The formalin detection test showed the presence of formalin in milk which was accurately determined using UV- Vis spectroscopy. Out of the six samples collected, five were adultered using formalin, accounting for 83.33% of the total samples. Pure milk had faster reductions of methylene blue, and its formalin concentration was 0.00M. The mean formalin concentration of samples collected was 0.000113± 0.0000685M (3.3929±2.0579ppm). Milk sold by milk vendors around Nyeri municipality was adultered using formalin, and milk adulteration is still practiced despite being prohibited and considered harmful. Formalin reduces the pH and butter fat of milk. It is vital to have quality milk sold and consumed by consumers to eradicate the side effects of using formalin as a preservative. This should start from the door step of dairy products producers to milk vendors to milk companies and processors.

5. FUNDING:

6.DECLARATION OF COMPETING INTERESTS:
The authors affirm that they are free of any known financial conflicts of interest or personal ties that might have appeared to affect the research presented in this study.

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