DEVELOPMENT OF INDICATOR FOR ON-SITE DETECTION OF BROMATE IN SOME BREAD SAMPLES IN ABEOKUTA METROPOLIS

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Abstract

A new highly selective indicator, Chitosan-Silver Carmoisine (CTS-AgCAM) was developed for the detection of potassium bromate in bread. The detection was based on the oxidation of Carmoisine (CAM) by potassium bromate (KBrO₃), catalysed by Silver Nanoparticles (AgNPs). The CTS-AgNPs was prepared by the reaction of silver nitrate with sodium hydroxide and stabilised in chitosan (CTS) and further addition of CAM. Batch experiments were carried out on various concentration of KBrO₃ to obtain optimal conditions for the use of the indicator. This was monitored with a UV-Vis Spectrophotometer. Fifty bread samples were then collected randomly from five different bakeries within Abeokuta, Ogun state, Nigeria and qualitative analysis was carried out on the bread samples using the prepared indicator. The UV-Vis spectrum of the CTS- AgNPs revealed surface plasmon absorption maxima at 418-420 nm while the indicator showed maximum absorption at 516 nm. From the qualitative analysis, it was observed that on addition of 2 drops of CTS-AgCAM to a bread sample solution containing KBrO₃, a pink colour of the indicator disappeared. It was also observed from the result of quantitative analysis that the fifty bread samples analyzed all contained KBrO₃ with concentration ranging between 3.23 ± 0.08 and 6.29 ± 0.91 µg/g. The indicator worked best between 1 and 5 µg/g and had a detection limit of 1 µg/g. The result showed that this indicator can be used easily for efficient on-site detection of bromate in bread.

Keywords: Potassium bromate, bread, silver nanoparticles, indicator

1. Introduction

Potassium bromate is a colourless, odourless and tasteless white powder that is used as food additives in the food industry. It is a powerful oxidizing agent in acidic solution [1], which aids the raising process and helps to produce a texture in the finished product that is appealing to the public [2]. The use of KBrO₃ has been a common choice among flour millers and bakers throughout the world because it is cheap [3]. However, it has been discovered that KBrO₃ is toxic. It is classified as a carcinogenic substance [4]. Non-carcinogenic effects include abdominal pain, diarrhoea, nausea, vomiting, kidney failure, oliguria, anuria, deafness, vertigo, and hypotension, depression of the central nervous system and thrombocytopenia [5]. It also degrades the vitamins and essential fatty acid content in flour [6]. Due to its acute toxic effect, the National Agency for Food and Drug Administration and Control (NAFDAC) banned the use of potassium bromate in 1993 with Decree no 15 of 1993 [7]. However, many Nigerian bakers continue to use it in order to enhance their profits [8]. The maximum concentration of KBrO₃ allowed in bread by the US Food and Drug Agency (FDA) is 0.02µg/g [9].

Several analytical techniques had been developed for the quantitative determination of KBrO₃ in bread which involve the use of
sophisticated and expensive equipment. They include: Ion chromatography method with ICP-MS [10,11], Redox titrimetric method [12], High Performance Liquid Chromatography [13,14], and spectroscopic methods [15,16,17]. However, there are several limitations associated with many of these currently available methods, such as being time consuming and requiring complicated instrumentation. Hence the need for a fast, reliable, accurate and on-site method of sensing bromate in finished baked goods. This study therefore develops an indicator for high selectivity visual detection of potassium bromate in bread. This is based on the oxidation of carmoisine by potassium bromate, using silver nanoparticles as a catalyst.

2. Experimental

2.1. Material and Instrument

All the chemicals used for the preparation of reagents used in this research work were of analar grade. Carmoisine, chitosan and silver nitrate (AgNO₃) were used as purchased from Sigma Aldrich, Co, St Louis, USA. The sodium hydroxide (NaOH) pellets and potassium bromate salt were obtained from the Chemistry Department Laboratory, Federal University of Agriculture, Abeokuta, Ogun State. Other reagents used were sulphuric acid and acetic Acid.

The absorbance readings of the samples were obtained using a UV-Vis spectrophotometer (Shimadzu 1650PC).

2.2. Preparation and optimization of Indicator

2.2.1 Synthesis of silver nanoparticles

Silver nanoparticles were prepared by following a previously reported method [14]. In the typical procedure, an aqueous solution of AgNO₃ (0.4 mL, 0.1 M) was added to 100 mL of an aqueous solution containing 0.1% chitosan(0.1 g in 1% acetic acid), and vigorously stirred on a magnetic stirrer for one hour after which a few drops of NaOH (0.1 M) were added. The solution was further stirred for 30 min, with its temperature maintained at 50°C. The resulting mixture was centrifuged. The residue was washed with ethanol and dried in the oven at 50 °C for one hour.

2.2.2 Preparation of CTS-AgCAM

Various proportions of AgNPs (0, 0.01, 0.05g) were each added to 12.0 mL of CAM and stirred together at 30 °C for 6 h. The solution was then filtered. The resulting solutions (indicator) were labelled as In1, In2, and In3 respectively. The spectra of the AgNPs, CAM and CTS-AgCam were recorded using a UV–Vis spectrophotometer.

2.2.3 Optimisation for Qualitative and Quantitative analysis

Each of the indicators was used on various concentrations of potassium bromate, ranging from 1µg/mL to 10 µg/mL. To 2 mL of each concentration of potassium bromate, 1.0 mL of 2.0 M H₂SO₄ was added, and 2 drops of In1 was also added. This was repeated, using In2 and In3. The absorbance of the resulting solutions were recorded.

2.3 Batch experiment

2.3.1 Preparation of standard solutions of bromate

Different concentrations of bromate solutions were prepared varying from 0.01 µg/mL to 10 µg/mL. 8.0 mL of each were then placed in a 10.0 mL standard flask. CAM (1.0 mL) and 0.2 mL of sulfuric acid were then added. Mixtures were diluted with distilled water up to 10.0 mL. Mixtures were
well shaken for one minute and the absorbance was read at 516 nm against a blank reagent. Results were used to plot the calibration curve and to determine the equation of the linear regression.

The Detection Limit (DL) was calculated using the Eq 1[18]:

\[
DL = 3.3 \times (\sigma/s) \quad \text{Eq 1}
\]

(where \(\sigma\)=Standard Deviation of blank; \(s\)=Slope of curve)

2.3.2 Application of indicator (CAM and CTS-AgCAM) under the optimised condition (mentioned in 2.2.3)

Batch experiment was carried out on different concentrations of bromate, which include: 16.7, 167, 1670, 16700 and 83500 \(\mu\)g/mL. Aliquots of 2 mL were measured into a container from the different concentrations and to each was added a 1.0 mL of 2 M \(\text{H}_2\text{SO}_4\). The mixture was well shaken, before the addition of two drops of the indicator. The colours of the resulting solutions were noted.

2.3.3 Selectivity Studies

To investigate the anion recognition ability of the indicator, some commonly existing anions such as Cl\(^-\), CN\(^-\), NO\(_3\)^-, and Ascorbic acid were tested. For this purpose, 2.0 mL of 0.01M of each anion was added to 1.0 mL of 2M \(\text{H}_2\text{SO}_4\), and to the mixture was added 2 drops of the CTS-AgCAM.

2.4 Application of CTS-AgCAM to Bread samples in Abeokuta metropolis

2.4.1 Sampling

Five loaves of bread samples were collected from five different commercial bakeries (bakeries A, B, C, D, and E) at an interval of two weeks within a month, in Abeokuta metropolis (Samplings 1 and 2). This made a total of fifty loaves of bread samples. Samples were collected randomly from each bakery in small transparent polythene nylons and transported to the laboratory in big black polythene bag. Fresh samples for qualitative analysis were analysed on arrival at the laboratory while pulverised samples for quantitative analysis were stored in small, transparent polythene sample nylon in a refrigerator.

2.4.2 Qualitative Analysis

From each bread sample was measured 1.0 g and transferred into a test tube and 10.0 mL of distilled water was added. The mixture was shaken and allowed to stand for 20 minutes. A 2.0 mL volume was decanted from the test tube and 1.0 mL of 2M \(\text{H}_2\text{SO}_4\) solution was added. The mixture was shaken vigorously, and 1.0 mL of the indicator solution was added.

2.4.3 Quantitative Analysis

A quantity of 10 g of each bread sample was measured and dried in an oven for an hour at 75°C. The dried crust was pulverized and 1g of each powdered sample was weighed and poured into 20.0 mL of distilled water, and then filtered through a filter paper. The filtrate (8.0 mL) was transferred into a 15.0 mL volumetric tube to which were added 0.2 mL of 2 M \(\text{H}_2\text{SO}_4\) and 1.0 mL of the CTS-AgCAM. The resulting solution was made up to 10.0 mL and shaken for one minute. The absorbance of the samples was taken at 516 nm using a UV-Vis spectrophotometer. The unknown concentration was calculated from the linear regression curve obtained from the standard solutions of bromate.

2.5 Data Analysis

Data obtained were analysed using SPSS and results of quantitative analysis were expressed as mean ± standard deviation.
3 Results and discussion

3.1 UV-Vis analysis of AgNPs, CAM and CTS-AgCAM indicator

The electronic spectrum of the prepared silver nanoparticles revealed a typical surface plasmon absorption maximum at 418-420 nm (Fig. 1). This is similar to previous similar studies for the synthesis of silver nanoparticles [15,19,20], thus confirming the product as CTS-AgNps.

![Fig 1: Spectrum of silver nanoparticles.](image1)

It is well known that AgNPs exhibits yellowish-brown colour in aqueous solution due to excitation of surface plasmon vibrations [19,20]. As the sodium hydroxide was mixed in the aqueous solution of the silver ion complex, a change in colour from light yellow to black was observed, due to the reduction of silver ion [21]. Formation of black colour is due to the surface plasmon resonance property of silver nanoparticles [20].

The absorbance of CAM as shown in Fig. 2 was 0.4 while that of CTS-AgCAM indicator was 0.18 as shown in Fig.3.

![Fig 2: UV-Vis spectrum of Carmoisine](image2)

This result shows a reduction in absorbance of the indicator, compared to that of carmoisine, which is shown by the reduction in the intensity of colour of the CTS-AgCAM indicator. Carmoisine gave a red
solution on dissolution in water. The red solution became lighter on addition of CTS-AgNPs. It infers that in the presence of the CTS-AgNPs, the rate of oxidation of carmoisine by potassium bromate strongly increases [15,22].

3.2 Determination of optimization level for the use of the Indicator

Fig. 4 showed the reaction of 12.0 mL of CAM with 0, 0.01 and 0.05 g of CTS-AgNPs respectively, in various concentration of bromate. On the addition of various proportions of AgNPs to 12.0 mL of CAM, it was observed that though there was a colour change from pink to colourless, the time taken for change in colour using In1 was higher than that of In2 and In3.

This means that the presence of silver nanoparticles catalysed the reaction between CAM and KBrO₃ [14]. Also, the time taken for In3 to change to colourless was faster compared to In2. It can be inferred from the observation that the higher the amount of silver nanoparticles present in the CTS-AgCAM indicator, the faster the change in colour. Hence, the use of 0.12 g of silver nanoparticles since it gave the best result.

The result of batch experiment of CTS-AgCAM indicator (In3) with different concentration of bromate (Fig 5) reveals that the higher the concentration of potassium bromate present in a solution, the faster the oxidation of the CAM, and the more rapid the colour change (Table 1).

![Fig 4: Reaction of 12.0 mL of CAM with In1(0 g of CTS-AgNps), In2(0.01 g of CTS-AgNps) and In3(0.05 g of CTS-AgNps) in various concentration of potassium bromate.](image)

![Fig 5: Addition of indicator to different concentration of potassium bromate (A-16.7; B-167; C-1670; D-16700; E-83500 µg/mL) discern](image)

**Table 1: Result of Batch Experiment**

| Concentration of KBrO₃ (µg/mL) | Time Taken for Change of Colour |
|-------------------------------|---------------------------------|
| 83500                         | Instantly                       |
| 16700                         | 1 minute                        |
| 1670                          | 5 minutes                       |
| 167 µg/mL                     | 15 minutes                      |
| 16.7 µg/mL                    | 1 day                           |

From the attempt to discern whether or not the indicator could be used for quantitative
measurements, the calibration curve (Fig 6) of potassium bromate standard was constructed. The calibration curve obeyed Beer’s law. The regression equation of the calibration plot was calculated using the equation: \( Y \ (\text{Abs}) = 0.111x \ ; R^2 = 0.983. \)

Fig 6: Calibration curve of potassium bromate standard (1-5 µg/mL)

From the Eq [18], the Detection Limit (DL) was calculated to be 1 µg/g (approximately).

3.3 Selectivity studies of indicator with anions

The reaction of CTS-AgCAM indicator with 167 µg/mL of different anions (Fig 7) showed that the anions caused no interference. The anions include Cyanide ions, Chloride ions, Nitrate ions, and Ascorbic acid. This was observed by a change in colour of the CTS-AgCAM indicator from red to light pink, pink, pink and light yellow respectively. For bromate ions, a colourless solution was observed. Hence, the indicator gives a distinct reaction with potassium bromate.

Fig 7: Observed colour change of CTS-AgCAM indicator in the presence of 167 µg/mL of some anions.

3.5 Qualitative and Quantitative analysis of Some bread samples

It was observed that on addition of the CTS-AgCAM indicator, to the acidified sample solution, the presence of KBrO\textsubscript{3} in each bread sample was confirmed by the disappearance of the pink colour of the indicator. However, 10 % samples showed pink colouration, which indicated that the level of potassium bromate is below the detection limit. The reaction is due to the oxidation of CAM by KBrO\textsubscript{3} in the acidic medium [15].

Potassium bromate was found in each of the 50 bread samples analyzed, despite the ban that has been placed on its use by National Agency for Food and Drug Administration and Control (NAFDAC). The mean content of KBrO\textsubscript{3} (Table 2) found in the samples collected in bakeries A, B, C, D, and E were 6.29±0.91, 5.69±0.25, 6.51±0.77, 4.38±0.47 and 3.23±0.08 µg/g respectively. Bread sample collected from bakery A recorded the highest amount of residual bromate (6.29 ± 0.91 µg/g) while the lowest level of residual bromate (3.23 ± 0.08 µg/g) was recorded by bread sample collected from bakery E.
Table 2: Concentration of KBrO₃ in some brands of bread obtained from some bakeries in Abeokuta

| Bakery | [KBrO₃] in Sampling 1 (µg/mL) | [KBrO₃] in Sampling 2 (µg/mL) | Mean [KBrO₃] (µg/mL) |
|--------|-------------------------------|-------------------------------|----------------------|
| A      | 5.64                          | 6.93                          | 6.29(±0.91)          |
| B      | 5.51                          | 5.86                          | 5.69(±0.25)          |
| C      | 7.05                          | 5.97                          | 6.51(±0.77)          |
| D      | 4.71                          | 4.04                          | 4.38(±0.47)          |
| E      | 3.17                          | 3.29                          | 3.23(±0.08)          |

The concentration of KBrO₃ in bread samples obtained in this study is similar to the 3.60 and 9.20 µg/g for the lowest and highest level of KBrO₃ as reported in Gwagwalada, Nigeria [11]. It is also similar to 3.70 and 12.10 µg/g as the lowest and highest level of KBrO₃ found in bread samples consumed in Kaduna metropolis respectively [23]. The concentration of KBrO₃ in bread samples obtained in this study was above the USFDA permissible limit, which is an indication that the brands of bread samples are unsafe for human consumption.

4. Conclusion

An indicator for on-site detection of high-selective detection of KBrO₃ in bread has successfully been developed. This has been achieved by the reaction of synthesized chitosan-silver nanoparticles with CAM. The characteristics of the method involve its simplicity and detection of bromate with high selectivity.

The result of this work has also confirmed that potassium bromate is still being used in bread making as it is present in all the bread samples that was analysed in Abeokuta metropolis. The result attracts a conscious effort by relevant agencies to enforce the ban on the use of potassium bromate by bakeries.

Since this study reveals a method that can be used both for the qualitative and quantitative analysis of KBrO₃ in bread, it can be recommended for the routine control of potassium bromate in bread. However, there is a need for further improvement on the development of this indicator to detect very low concentration of potassium bromate in bread samples.

Owing to the adverse effects of KBrO₃, the use of other improvers such as ascorbic acid, which is nontoxic, should be encouraged instead of potassium bromate as improvers in flour during bread making. This can be done by enlightening the bakers, and by carrying out analysis on flour before being used for bread making.

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