Challenge
Simple and rapid determination of the potassium bromate concentration in bread

Solution
The SPECORD 50 PLUS, equipped with the 6-cell changer, enables fast and simple spectrophotometric determination of the potassium bromate concentration in bread

Intended audience
This Appnote is aimed to users from the food industry

Spectrophotometric determination of potassium bromate concentration in bread based on the redox reaction between potassium bromate and promethazine in an acidic environment

Introduction

Bread has been part of the human nutrition for over ten thousand years. It is a basic food worldwide and an important source of energy thanks to its carbohydrate content. It also contains valuable vitamins such as thiamine, riboflavin and niacin, as well as minerals (e.g., magnesium, potassium) and trace elements (e.g., selenium, iodine). The elasticity of bread dough is due to the presence of gluten and its ability to trap gases. The resulting rising of the dough is considered a desirable characteristic of high-quality bread. To strengthen the gluten and obtain the desired dough properties, the oxidation process is essential. In the past, the dough was stored in the air for several days or weeks for this purpose. Nowadays, chemical oxidizing agents are added for oxidation, as they shorten the ripening time. Potassium bromate is such an oxidizing agent and a food additive frequently used in the bakery industry. It has a positive effect on the structure and rheological properties of the dough and therefore also on the end product, bread. However, potassium bromate is considered a carcinogenic and nephrotoxic substance and was classified as a category 2B carcinogen as possibly carcinogenic to humans by the International Agency for Research on Cancer in 1999. Starting in the 1990s, numerous countries banned the use of potassium bromate in food, including the European Union, Canada, Brazil and China. In the USA, however, potassium bromate is still a permitted and used food additive. The US Food and Drug Administration (FDA) restricts the use of potassium bromate and allows up to 50 mg of potassium bromate per 1 kg of flour with the justification that potassium bromate is converted into non-carcinogenic bromide during baking. If the bread is not baked long enough or at too low temperature, a residual amount of potassium bromate remains.

It is therefore important to monitor the low potassium bromate levels in flour products and baked goods. There are various methods for the photometric determination of the potassium bromate concentration.

This application note describes the spectrophotometric determination of the concentration of potassium bromate in bread based on the redox reaction between potassium bromate and promethazine in an acidic environment.

Materials and Methods

All measurements were carried out using a SPECORD 50 PLUS spectrophotometer equipped with a 6-cell changer and 10 mm glass cells. The absorbance of the standards and samples was measured using the photometry module of the ASpectUV software. The software settings are listed in Table 1.

Table 1: Software settings ASpect UV

Parameter	Settings for the determination of $KBrO_3$
Measurement mode	Absorption
Wavelength [nm]	515
Integration time [s]	0.1
Regression	Linear

Reagents

All reagents used were of analytical quality. The following reagents were required for the preparation of the standards and for sample measurement:

- Promethazine hydrochloride (PTZ) ($C_{17}H_{20}N_2S{\cdot}HCl$)
- Potassium bromate ($KBrO_3$)
- 12 mol/L hydrochloric acid (HCl)
- Distilled water

Preparation stock solution

For the stock solutions, PTZ and potassium bromate were weighed out (Table 2), transferred to a 1000 mL volumetric flask and filled up to the mark of the volumetric flask with distilled water and shaken.

Table 2: Weigh-in stock solution

Stock solution	Weigh-in [g]
0.01 mol/L PTZ	3.21
50 mg/L Potassium bromate	0.05

Preparation standards

For the standards, the potassium bromate stock solution was transferred to 10 mL volumetric flasks, mixed with 1 mL PTZ stock solution and made up to 10 mL with distilled water. 200 µl of 12 mol/L hydrochloric acid was then added and shaken. The volume used and the concentration of the standards are listed in Table 3.

Table 3: Volume and concentration of the standards

Standard	Volume $KBrO_3$-Stock solution [µL]	Concentration [µg/mL]
Standard 1	100	0.5
Standard 2	200	1.0
Standard 3	400	2.0
Standard 4	600	3.0
Standard 5	800	4.0

Samples and sample preparation

A total of four different bread samples were analyzed. The four bread samples were purchased in the United Arab Emirates. Three of the samples came from different supermarkets and one sample from a restaurant.
The bread samples, which were dried at room temperature, were first ground into powder using a mortar. For further sample preparation, approx. 5 g of each bread sample was weighed. The weights can be found in Table 4.

Table 4: Weigh-in samples

Sample	Sample mass [g]
Bread 1	5.00
Bread 2	5.00
Bread 3	5.01
Bread 4	5.02

The weighed samples were transferred to a 100 mL volumetric flask and made up to 100 mL with distilled water, shaken and then filtered through a ash-free filter paper, grade 41. From the filtrate, 8.8 mL were taken three times, each mixed with 1 mL promethazine hydrochloride stock solution and 200 µl hydrochloric acid and shaken for 1 minute.
The color reaction takes 15 min and the color complex is stable for approx. 60 min at room temperature. Standards and samples must be measured within this period.

Measurements

Preliminary tests
To eliminate a possible effect of the cuvettes, the glass cuvettes were tested before the standard and sample measurements. For this purpose, 10 glass cuvettes were rinsed with ethanol and gently dried under a gentle stream of air. The cuvettes were filled with distilled water and the absorption spectrum in the 450 - 550 nm range was measured against air as a reference. After detailed evaluation of the results, only the cuvettes with the lowest absorption deviation from each other (ΔA) were used for further measurements.

Creation of the calibration curve
The reference measurement was carried out against distilled water. To create the calibration curve, the cuvettes were rinsed three times with the standard and then filled with this standard. All standards were measured three times. The calibration curve was created in the photometry module of the ASpect UV with the software settings listed in Table 1.

Measurement of the samples
Each sample was analyzed in triplicate using the software settings in Table 1. The reference measurement was performed against distilled water. Before each measurement, the cuvette was rinsed three times with the prepared sample, then filled and measured. The ASpect UV software automatically determines the average value and the standard deviation of the samples.

Results and Discussion

Preliminary tests
As described in the „Materials and Methods" section, the cuvettes used were tested in advance. Checking the purity of the cuvettes is an important step in order to exclude any possible influences of the cuvettes and to achieve an optimal correlation value of the calibration curve. When testing the cuvettes, it is important to use the same solvent that was used for the standard and sample measurements, as impurities or deviations in the cuvette are amplified or attenuated depending on the polarity of the solvent.
The measured spectra were examined in detail for significant absorption differences. As the measurements are taken at 515 nm, the differences in absorbance values at this wavelength are of particular interest. The wavelength can be easily defined using the ASpect UV software, thus facilitating the evaluation. The cuvettes with the smallest deviation from each other were used for further measurements. In the following measurements, it was ensured that the orientation of the cuvettes was always identical.

Creation of the calibration curve
To determine the concentration, the first step was to create a calibration curve for a concentration range of 0.5 - 4.0 µg/mL. For this purpose, five defined potassium bromate standards (see Table 3) were prepared as described in the literature, which were measured according to the software settings of the ASpect UV. The determined absorbance is plotted on the y-axis against the concentration on the x-axis. The measured calibration line with a correlation coefficient (R^2) of 0.999 is shown in Figure 1.

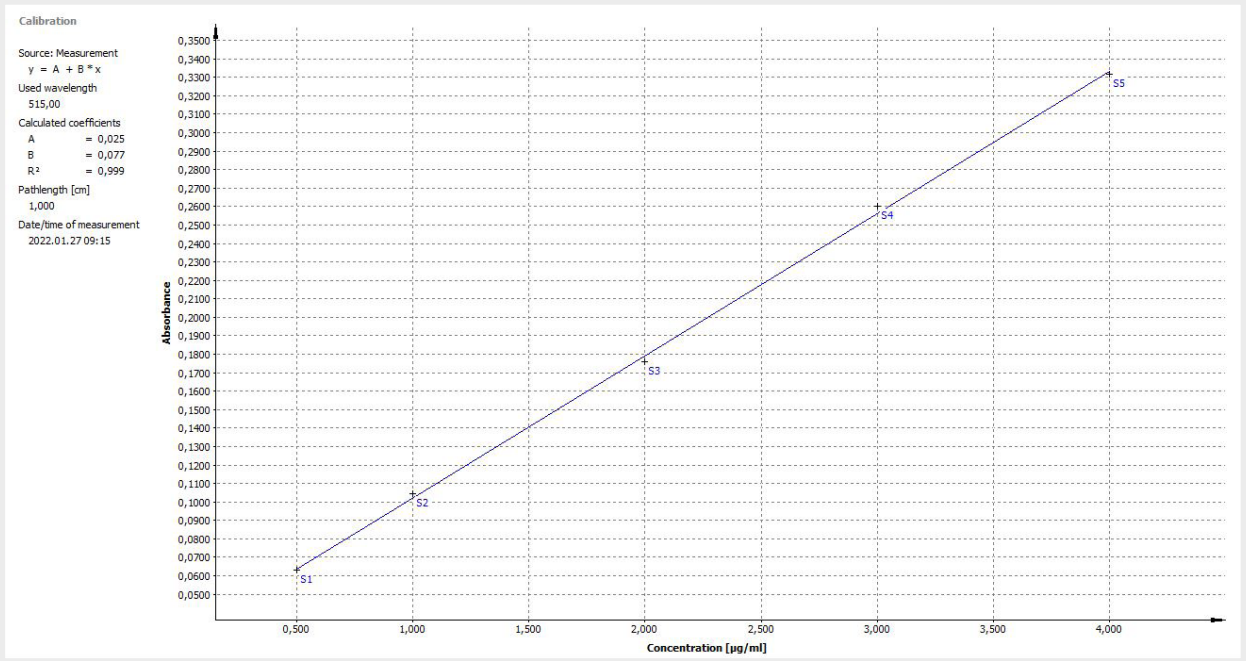

Figure 1: Calibration curve

To minimize laboratory working time, the calibration curve created was then linked to the measurement method for the samples. This means that the standards do not have to be newly prepared and a new calibration curve created for each sample measurement.

Results of the sample measurement

Table 5 shows the results measured with the ASpect UV. The low standard deviation shows that the SPECORD 50 PLUS is suitable for determining the concentration of potassium bromate.

Table 5: Measurement results of the samples

Standard	Concentration [µg/mL]	Absorption 515 nm	Standard	Concentration [µg/mL]	Absorption 515 nm
Sample 1	1.829	0.166	Sample 3	0.269	0.046
Sample 1	1.845	0.167	Sample 3	0.238	0.044
Sample 1	1.757	0.161	Sample 3	0.254	0.045
Average value	1.810	0.165	Average value	0.254	0.045
Standard deviation	0.038	0.003	Standard deviation	0.013	0.001
Sample 2	0.103	0.033	Sample 4	0.172	0.039
Sample 2	0.177	0.039	Sample 4	0.151	0.037
Sample 2	0.073	0.031	Sample 4	0.197	0.040
Average value	0.118	0.034	Average value	0.173	0.039
Standard deviation	0.044	0.003	Standard deviation	0.019	0.001

Results of the sample measurement

Formula 1 was used to calculate the potassium bromate concentration in µg per g of bread. The average value of the measured concentration [µg/mL] was always used for the calculation.

$$C_{KBrO_3} = \frac{cAUV_{KBr0_3}}{(\frac{W_{bread}}{V})} * DF \quad [1]$$

C_{KBrO_3} = Concentration potassium bromate [µg/g]

$cAUV_{KBrO_3}$ = Concentration of potassium bromate measured in the ASpect UV [µg/mL]

W_{bread} = Weigh-in bread [g]

V = Volume [mL]

DF = Dilution factor

The results calculated using Formula 1 are shown in Table 6. Potassium bromate was detected in all bread samples, with sample 1 showing the highest concentration and sample 2 the lowest.

Table 6: Potassium bromate concentration of the samples

Sample	Potassium bromate concentration in bread [µg/g]
Sample 1	41.14
Sample 2	2.68
Sample 3	5.76
Sample 4	3.92

Summary

This paper describes a simple, fast and validated method for determining the concentration of potassium bromate, so that it can be recommended for routine monitoring of bread. This application note shows that measurements can be carried out rapidly, reproducibly and cost-effectively using the SPECORD PLUS.

Figure 2: SPECORD 50 PLUS spectrophotometer

Recommended device configuration

Table 7: Overview of devices and accessories

Article	Article number	Description
SPECORD 50 PLUS Spectrophotometer with split-beam technology	822-0050P-2-R	Powerful double beam spectrophotometer with split-beam-technology (SBT), providing excellent measurement results Analysis of liquid, gaseous, powderous and solid samples possible
6-fold cuvette changer, non-temperature controlled, without stirrer	820-60335-P	Cuvette changer for the accommodation of 6 cuvettes with 10 mm path length, non-temperature controlled, without stirrer

References

[1] El. Harti et al.; A simple and rapid method for spectrophotometric determination of bromate in bread; Journal of Materials and Environmental Science; 2011; Vol. 2/ 71-76

[2] Chauhan D., Jain P.A.; Scientific study of genotoxic-carcinogenic impacts of Potassium Bromate as food additive on human health; Int. Res. J. Eng. Tech. 2016; 3:1136–1139

[3] Shanmugavel, V et al.; Potassium bromate: effects on bread components, health, environment and method of analysis, a review, Food Chemistry (2019)

Headquarters
Analytik Jena GmbH+Co. KG
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

Version 1.0 · Author: SaWu
en · 02/2024