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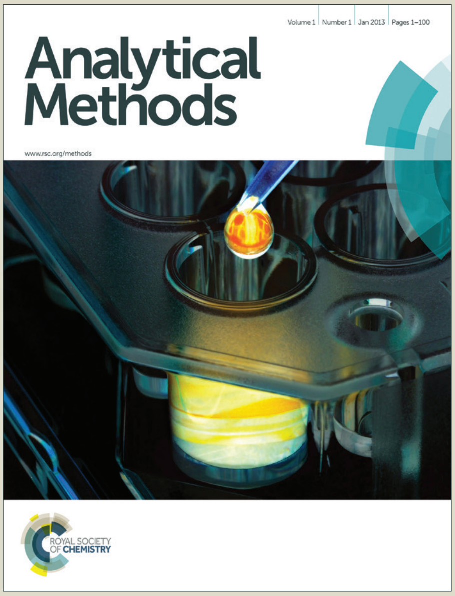

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A digital image analysis method for quantification of sulfite in beverages

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A novel, simple and low-cost analytical procedure for sulfite determination in beverage samples is presented. The approach proposed consists of image capture from a sulfite colorimetric reaction, using a system built with low-cost materials for luminosity control and digital image decomposition into the primary colors Red (R), Green (G) and Blue (B). The colorimetric reaction is based on the reduction of Fe (III) to Fe (II) in the presence of sulfite and further reaction with o-phenanthroline to form the red complex $[Fe(C_{12}H_8N_2)_3]^{2+}$. Under optimized reaction and system conditions, the analytical curve was linear in a sulfite concentration range from 8.0 to 140 mg L^{-1}, with limits of detection and quantification of 2.6 mg L^{-1} and 8.0 mg L^{-1}, respectively. The analytical method was applied to sulfite quantification in different beverage samples such as white wine, vinegar, rosé wine, cashew juice and coconut water. The results acquired were in close agreement with those obtained using iodometric titration as a comparative method, with a confidence level of 95%. Moreover, the method can be useful with regard to social and environmental impacts, due to the low generated residues (800 µL per spot-test) and employs easily available instrumentation with the potential for *in situ* determination during the application of sulfite as additives during beverage production and quality control.

Introduction

Beverage industries generally use a significant number of artificial additives in their commercial formulations, including colorants and anti-oxidants[1]. In the list of artificial compounds commonly used as additives, sulfite is a typical substance, present in juices, soft and alcoholic drinks[2]. The sulfite is applied as a preservative, inhibiting the action of bacteria, fungi and yeasts; as an anti-oxidant, inhibiting enzymatic and non-enzymatic reactions; and as an anti-browning agent; thus improving the shelf-life of these products[3-5]. Sulfites are found in food in free or bound form. The free sulphite is presented in aqueos solution as sulfur dioxide (SO_2), hydrogen sulfite or bisulfite ion (HSO_3^-) and sulfite (SO_3^{2-}) as a function of the pH of the solution [4,6]. Sulfite amounts in food samples need to be rigorously controlled, because the excessive ingestion of sulfite can generate health problems such as headaches, migraines, asthmatic attacks, and even brain damage. A high level of sulfite toxicity can contribute to the development of cancer[7, 8].

The Brazilian legislation estabilish the maximum limit for sulfite in beverages between 40 and 500 mg L^{-1}, depending of the beverage compostion[9]. Regarding to the acceptable daily intake (ADI) per unit body mass, the JECFA (Joint FAO/WHO Expert Committee on Food Additives) estabilish the limit of 0.7 mg kg^{-1} [10].

For the determination of sulfite concentration, the literature report the use of chromatography[11], spectrophotometry[12-14], capillary electrophoresis[15, 16], fluorescence[17], voltammetry[6, 18] and amperometry[19, 20]. These are adequate techniques for accurate sulfite determination. However, for portable, fast, low-cost sulfite *in situ* determination with low reagent consumption and waste generation, some of the previous techniques are unsuitable. For the development of novel analytical strategies for the sensing of different analytes, taking into account the principles of recent "Green Chemistry"[21, 22], the use of digital image analysis methods is favored[23].

Digital image analysis methods are based on the use of digital cameras, smartphones[24], webcams[25] and scanners[26] to capture photography from colorimetric reactions, *e.g.* spot-tests, and the conversion of these images into a measurable data, such as values of the primary colors red, green and blue in the RGB approach[27-30]. Advances in digital camera and cell phone technology along with the reduction of their prices have greatly stimulated the use of these devices as detectors in analytical chemistry. A survey of the literature demonstrates that digital image analysis methods have been developed and applied for the determination of different analytes in varied matrix samples, *e.g.* sodium in physiological serum, calcium in water and lithium in anti-depressive drugs[29], total acidity in red wines[31], sodium and calcium in powdered milk[32], and for estimating the age of bloodstains[33], adulterations to cow's milk[34], and the ethanol content in alcoholic drinks[35]. Flow analysis systems have also been proposed using this type of analytical approach[36].

In this work, we proposed the combination of a spot-test and a digital image analysis method for sulfite determination in varied beverage samples. This work is related with a previous work reported by our research group, which ethanol content in drink samples was determined[35]. The most important features of this analytical approach are its low-cost and the portability of the instrumentation developed for the luminosity control; its low waste generation; and the ability to perform a significant number of samples simultaneously by using only one photography. Additionally, this method can be employed *in situ* during the application of additives in beverage production for quality purposes.

Experimental

Apparatus and instrumentation

The colorimetric reactions of the spot-test for sulfite determination were conducted in a porcelain plaque of 12 reaction vessels. Eppendorf (Germany) micropipettes were used for the transfer of exact reagent volumes. The digital images of the colorimetric reactions were recorded from a Sony (Japan) 6.0 Mega Pixel (MP) digital camera model DSC-W50, and the reaction time controlled using a chronometer.

The digital image capture of the porcelain plaque, containing the spot-test reactions, was conducted in a closed system (21 × 15 × 7 cm), as illustrated in Fig. 1. The system consists of: (1) black plastic box with an opening in its upper part to enter with the objective or lens from the digital camera; (2) four ultrabright white LEDs (Bluex, 12 V, 1 W) for the light control; (3) one rechargeable battery (Unipower, 12 V with 1.3 Ah^{-1}) as power supply for the LEDs; (4) two potentiometers, variable resistors of 10 kΩ used to supply adequate electric current to the LEDs; (5) an ON/OFF switch; and (6) two connectors to the battery charger. The internal compartment of the system was spray painted matt black to eliminate reflection effects.

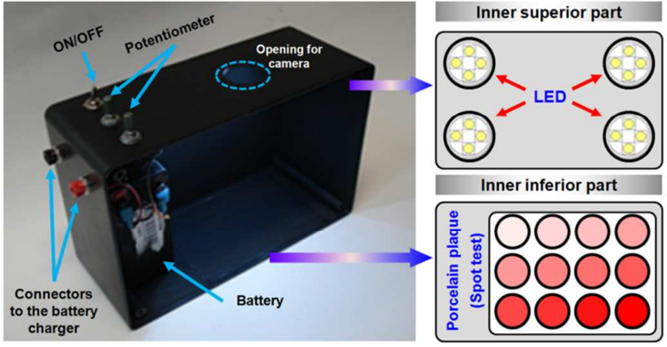

Fig. 1. Illustration of the closed system built for the image capture, indicating its components. In the system, the upper interior part is used for the fixation of the four LEDs, and the porcelain plaque (sulfite spot-test) is placed in the lower interior part.

The porcelain plaque was placed in the lower part of the system. With the system completely closed, digital images were captured from the digital camera placed at the top. The captured images were transferred to a computer *via* a USB cable or memory card, and analyzed. For image treatment the free software ImageJ was used, as well as Excel and Origin. Further details can be found in previous work[35].

Chemicals and samples

Iron (III) nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$) was purchased from Merck (Germany), 1,10-phenanthroline and anhydrous sodium sulfite (Na_2SO_3) were purchased from Synth (Brazil), and sulfuric acid from Qhemis (Brazil).

A 0.05 mol L^{-1} Fe (III) solution was prepared from dissolution of the salt $Fe(NO_3)_3.9H_2O$ in 0.5 mol L^{-1} H_2SO_4. The 0.05 mol L^{-1} 1,10-phenanthroline solution, as well the Na_2SO_3 solutions were prepared in 0.2 mol L^{-1} acetic acid/acetate buffer solution (pH 4.5). The sulfite solutions were prepared daily, to avoid oxidation. The sulfite standard solutions were standardized by back titration with sodium thiosulfate. Deionized water (resistivity > 18.0 MΩ cm) was purified using a Millipore Milli-Q system (USA). The samples of white wine, vinegar, coconut water, rosé wine, cashew juice 1 (more concentrated) and cashew juice 2 were purchased in the local market and received no treatment, except a simple dilution. Sulfite was determined in the samples as total sulfite, in the form of hydrogen sulfite (HSO_3^-), once into the acid medium (pH 4.5), all sulfite species are converted to HSO_3^-[4,6]. The iodometric titration was performed as comparative method to determine total sufite[37].

RGB data acquisition

The image treatment performed in the ImageJ software consisted of RGB decomposition of a selected area of the image (32 × 26 pixels). The RGB image decomposition provides mean and mode values for the channels red (R), green (G) and blue (B) absorbed by the solution. The final analytical signal was defined as –log (I/I_0), where I is the R, G or B value (mean or mode) from the standard or sample solution and I_0 is the R, G or B value for the blank.

Analytical procedures for sulfite quantification

The colorimetric reaction used as a spot-test for sulfite determination was the reaction of sulfite with Fe (III) of the complex $[Fe(C_{12}H_8N_2)_3]^{3+}$, previously formed by the reaction between Fe (III) and 1,10-phenanthroline. The reactions involved in the spot-test are shown in Eqs. 1 and 2. The complex $[Fe(C_{12}H_8N_2)_3]^{3+}$ absorbs radiation mainly in the ultraviolet region of the electromagnetic spectrum with negligible absorption in the visible, region, thus it is a colorless complex. After the addition of sulfite in acid medium (HSO_3^-), Fe (III) is reduced to Fe (II), and the red complex $[Fe(C_{12}H_8N_2)_3]^{2+}$ is generated.

$$2Fe^{3+}_{(aq)} + 6(C_{12}H_8N_2)_{(aq)} \leftrightarrow 2[Fe(C_{12}H_8N_2)_3]^{3+}_{(aq)} \quad Eq.1$$

$$2[Fe(C_{12}H_8N_2)_3]^{3+}_{(aq)} + HSO_3^-{}_{(aq)} + H_2O_{(l)} \leftrightarrow$$

$$2[Fe(C_{12}H_8N_2)_3]^{2+}_{(aq)} + SO_4^{2-}{}_{(aq)} + 3H^+_{(aq)} \quad Eq.2$$

The experimental conditions of the spot-test reaction were optimized as a first step in the analysis. The reaction was carried out in acid medium and the use of acetic acid/acetate buffer solutions at different pH values: 3.5, 4.5 and 5.5 was tested. The acid employed in the preparation of the 0.05 mol L^{-1} Fe (III) solution also was studied using hydrochloric acid, nitric acid and sulfuric acid, all at 0.5 mol L^{-1}. Different reaction times were evaluated, in the range from 0 to 15 min, in order to obtain a color scale to allow a fast screening analysis. Additionally, different volumes for each reaction vessel of the porcelain plaque were evaluated ranging from 500 to 1000 µL, observing the sensitivity, precision and waste generated in the test.

Under the optimum conditions, the analytical curve for sulfite was determined. The channels (R, G and B) were evaluated, and the B channel was selected which showed the best linear dependence on sulfite concentration and the higher sensitivity as demonstrated below. Interference and recovery assays were performed, in order to evaluate possible matrix effects. Moreover, the proposed procedure was applied to the sulfite quantification of varied beverage samples consisting of white wine, vinegar, rosé wine, cashew juice and coconut water. The samples also were analyzed by a reference procedure, which was the direct iodometric titration method[37]. Briefly, in this procedure, the target analyte (reductor agent) was directly titrated with a standard triiodite solution using starch as indicator.

Result and discussion

Digital image capture and treatment of data

For the digital image capture, an appropriate apparatus was designed for the control of light in the intern compartment, ensuring the reproducibility of images and consequently the measurements carried out. Thus, the effects of shadows and/or reflection on the reaction vessels were reduced. The use of LEDs also eliminates the use of flash for image capture, allowing the use of low-resolution digital cameras without flash or having poor quality flash systems. Moreover, the system was designed using low-cost materials and is portable, simple to operate, and has reduced dimensions and weight (*ca.* 1 kg).

With regard to the image treatment step, the relevant descriptions refer to the position and size of the area selected for the RGB decomposition. The area should be free of bright areas, which can be formed in some reaction vessels of the porcelain plaque by reflection from the LED. The selection of bright areas can generate higher than actual values of R, G and B, and therefore produce false results on analysis. The size of the selected area should be optimized for each type of camera. When cameras of lower resolution were used, a greater number of pixels should be used to obtain sufficient sensitivity for the sulfite determination. We optimized this parameter in this study, and an area corresponding to 32 × 26 pixels was selected.

Optimization of the reaction conditions

The effect of the composition of the aqueous media for preparation of the Fe (III), Na_2SO_3 and 1,10-phenanthroline reagent solutions was studied. In the case of Na_2SO_3 and 1,10-phenanthroline solutions, acetic acid/acetate buffer solutions at pH values of 3.5, 4.5 and 5.5 were used. The use of a buffer solution for Na_2SO_3 solution helps prevent the oxidation of the sulfite. The Fe (III) reagent solution was evaluated in 0.5 mol L^{-1} hydrochloric acid, nitric acid or sulfuric acid. An acid medium was used for the Fe (III) solution in order to prevent its hydrolysis. The reactions using the different previously prepared reagent solutions were conducted using the same reaction time and sulfite concentration, and the variation of the R, G and B values was compared. The following conditions were established for the further experiments allowing the best analytical performance: 0.2 mol L^{-1} acetic acid /acetate buffer pH 4.5 for Na_2SO_3 and 1,10-phenanthroline solutions, and 0.5 mol L^{-1} sulfuric acid for Fe (III) solution. To the best of our knowledge, this is the first time the reaction of SO_3^{2-} and Fe (III) with further complexation with 1,10-phenanthroline has been used for this analytical purpose.

The reaction time was evaluated in the range from 0 to 15 min. The goal in this study was to acquire a color scale in accordance with the sulfite concentration (screening analysis) in the lowest time possible with good sensibility. Using an equal reaction time of 5 min this effect was achieved (Fig. 2). Although this analysis time is relatively high, is necessary to emphasize that within this time, a complete calibration curve with 12 points can be obtained or four samples can be analyzed simultaneously in independent triplicates. Thus, each measurement takes approximately 25 s.

The optimum volume of the individual reaction vessels in the porcelain plaque was evaluated and 800 μL was employed because it allows high sensitivity and precision image acquisition and low waste generation. The following volumes of each reagent solution were used: 160 μL of 0.05 mol L^{-1} Fe (III), 480 μL of sulfite standard solution or beverage sample, and 160 μL of 0.05 mol L^{-1} 1,10-phenanthroline.

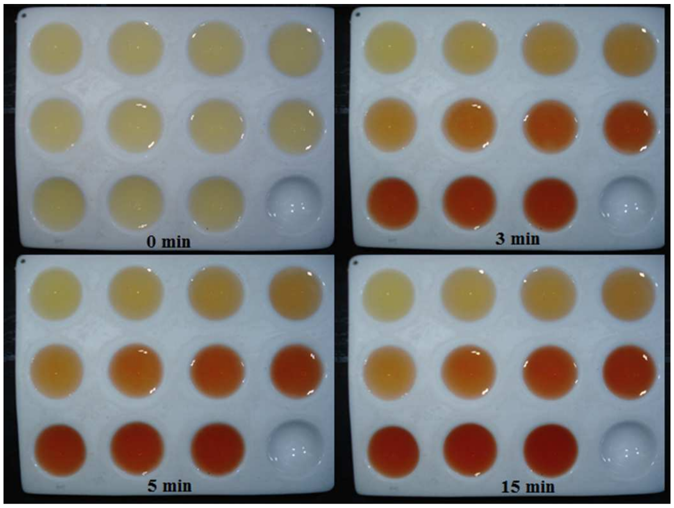

Fig.2. Images of the porcelain plaque containing spot-test reactions with sulfite concentrations from 8.0 to 240 mg L^{-1} for kinetic studies.

Analytical features

Analytical curves for sulfite quantification were first built evaluating the three channels, R, G and B. However, only the B channel produced linear analytical curves with satisfactory sensitivity. The B channel was expected to have the best response for the sulfite spot-test, because blue is the complementary color that is absorbed by the red solution.

Fig. 3 (a) shows a digital image captured for the porcelain plaque containing spot-test reactions with sulfite concentrations in the range from 8.0 to 240 mg L^{-1}. The treatment of the image presented in Fig. 3 (a) provided a linear analytical curve in the range from 8.0 to 140 mg L^{-1} (Fig. 3 (b)), following the regression equation: $-\log (I/I_0) = 0.017 + 5.7 \times [SO_3^{2-}]$, where $[SO_3^{2-}]$ is mg L^{-1}, and r = 0.995. The limits of detection (LOD) and quantification (LOQ) were calculated using the relations $3 \times \sigma/m$ and $10 \times \sigma/m$, where σ is the standard deviation of ten blank measurements and *m* the slope of the analytical curve. Thus, the LOD and LOQ were 2.6 mg L^{-1} and 8.0 mg L^{-1}.

The proposed apparatus is of low-cost: the cost of manufacturing of the closed system is approximately *ca.* 70 dollars, and cheaper digital cameras can be used, provided that the camera resolution is not a limiting factor[35]. In addition, we performed the spot-test based on a simple chemical reaction, using easily purchased reagents commonly found in analysis laboratory. Moreover, at only 5 min, a complete analytical curve was obtained with good precision and sensitivity. Additionally, the visible result allows a fast screen analysis based on the color scale produced.

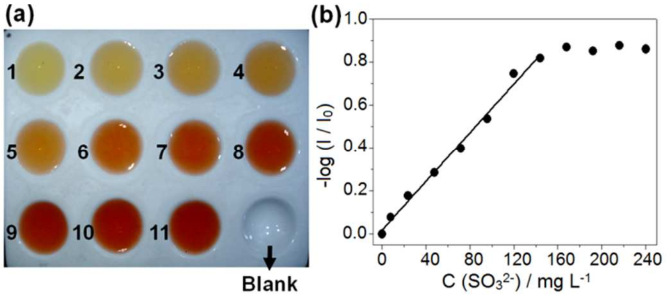

Fig. 3. Digital image (a) obtained for porcelain plaque containing spot-test reactions at different sulfite concentration levels : (1) 8.0; (2) 24; (3) 48; (4) 72; (5) 96; (6) 120 ; (7) 140 ; (8) 170; (9) 190; (10) 220; (11) 240 mg L^{-1}. (b) Analytical curve obtained for sulfite determination from treatment of the image present in (a). The analytical curve was linear in the range from 8.0 to 140 mg L^{-1}.

Interference and recovery assays

To study potential interferents substances that are commonly found in beverage samples such as ascorbic acid, fructose, sucrose and ethanol were considered. Thus, measurements for a 64 mg L^{-1} sulfite solution in the presence of the potential interferents in the proportions analyte:interferent 1:0.01, 1:0.1, 1:1, 1:10 and 1:100 were performed. Relative errors were calculated by comparison of the recovered sulfite concentration with the added sulfite concentration. The tolerable concentration of ascorbic acid that produced an error lower than 10% was 6.4 mg L^{-1}. On the other hand, for fructose, sucrose and ethanol, the tolerable level was 640 mg L^{-1}. These values were expected, since ascorbic acid has a higher reducing action than fructose, sucrose and ethanol. Moreover, the concentration of sulfite in commercial beverage samples is the major antioxidant specie in the samples.

Recovery assays were carried out, allowing evaluation of the influence of sample matrices in the sulfite quantification. Table 1 presents the recovery percentage obtained for different beverage samples containing two levels of added sulfite (32 mg L^{-1} and 64 mg L^{-1}). As can be seen, the recovery percentages ranged from 84.4 to 103%, indicating that interference of the sample matrices was not significant.

Table 1. Results of recovery obtained for sulfite determination in different beverage samples

S[a]	Added (mg L^{-1})	Measured (mg L^{-1})	Recovery (%)
A	32.0	32 ± 1	100
	64.0	55 ± 2	85.9
B	32.0	27 ± 2	84.4
	64.0	58 ± 3	90.6
C	32.0	30 ± 2	93.7
	64.0	63 ± 3	98.4
D	32.0	33 ± 2	103
	64.0	57 ± 2	89.1
E	32.0	32 ± 2	100
	64.0	60 ± 2	93.8
F	32.0	28 ± 2	87.5
	64.0	60 ± 2	93.8

[a] Sample from A to F: white wine, vinegar, coconut water, rosé wine, cashew juice 1 (more concentrated) and cashew juice 2, respectively. The samples of rosé wine, cashew juice 1 and cashew juice 2 were diluted to fit within the linear range of the digital image analysis method.

Sulfite determination in beverage samples

The digital image analysis method was applied to the sulfite determination of many beverage samples. For comparative purpose, the iodometric titration was performed. The results acquired employing both methods are presented in Table 2.

As can be seen, the results obtained using the proposed procedure were well compatible with those found using the iodometric titration method, with relative errors ranging from −4.5% to +6.2%. Moreover, the results obtained using both methods were compared using a paired *t-test*. The t_{exp} value (1.83) was lower than the $t_{critical}$ value (2.57) at a confidence level of 95%. Thus, the combined use of spot-test and digital image analysis proved a viable strategy for sulfite quantification in beverage samples with a fast, accurate, precise and low-cost analytical method.

Table 2. Results for the sulfite determination in various commercial beverage samples (n = 3)

S[a]	Comparative method (mg L^{-1})	Proposed method (mg L^{-1})	Relative error (%)
A	111 ± 1	106 ± 1	− 4.5
B	65 ± 1	69 ± 1	+ 6.2
C	86 ± 1	88 ± 1	+2.3
D	233 ± 4	242 ± 1	+3.9
E	317 ± 8	309 ± 6	− 2.6
F	160 ± 1	158 ± 5	− 1.3

[a] Sample from A to F: white wine, vinegar, coconut water, rosé wine, cashew juice 1 and cashew juice 2.The samples of rosé wine, cashew juice 1 and cashew juice 2 were diluted to fit within the linear range of the digital image analysis method.

The samples of rosé wine, cashew juice 1 and cashew juice 2 were diluted to fit within the linear range of the digital image analysis method.

Conclusions

In this work, we proposed the development of a novel approach for sulfite determination in beverage samples. The approach was performed by a combination of spot-test and digital image analysis for determination of sulfite in beverage by reduction of a Fe (III) complex. Under optimized conditions, the analytical curve for the proposed procedure was explored, and the blue channel (B) was used in the sulfite determination of samples of white wine, vinegar, rosé wine, cashew juice and coconut water. From the satisfactory results obtained, the developed procedure can be employed for sulfite determination in beverages, *e.g.* in industry and in smallholdings, due to the ease of handling, portability, fast response, and low-cost of the materials used. Moreover, the method can be employed during beverage production for quality purposes, by *in situ* analysis of sulfite, using both quantitative (RGB method) and qualitative (screen analysis in spot-test) methods. The proposed method generates lower volumes of residues (800 µL per spot-test) compared with iodometric titration. Furthermore, for the first time the reaction of SO_3^{2-} and Fe (III) with further complexation with 1,10-phenanthroline was used for digital image method.

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Notes and references

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A digital image analysis method for quantification of sulfite in beverages

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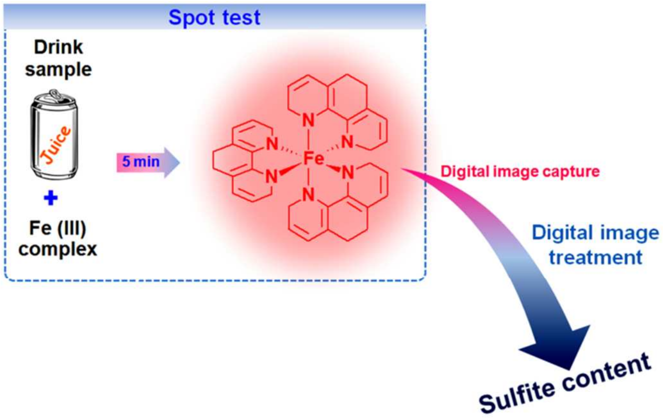

G.A. A combination of a spot-test and digital image analysis for determination of sulfite in beverages by reduction of a Fe (III) complex.

Table 1. Results of recovery obtained for sulfite determination in different beverage samples

S[a]	Added (mg L^{-1})	Measured (mg L^{-1})	Recovery (%)
A	32.0	32 ± 1	100
	64.0	55 ± 2	85.9
B	32.0	27 ± 2	84.4
	64.0	58 ± 3	90.6
C	32.0	30 ± 2	93.7
	64.0	63 ± 3	98.4
D	32.0	33 ± 2	103
	64.0	57 ± 2	89.1
E	32.0	32 ± 2	100
	64.0	60 ± 2	93.8
F	32.0	28 ± 2	87.5
	64.0	60 ± 2	93.8

[a] Sample from A to F: white wine, vinegar, coconut water, rosé wine, cashew juice 1 (more concentrated) and cashew juice 2, respectively. The samples of rosé wine, cashew juice 1 and cashew juice 2 were diluted to fit within the linear range of the digital image analysis method.

Table 2. Results for the sulfite determination in various commercial beverage samples ($n = 3$)

S[a]	Comparative method (mg L^{-1})	Proposed method (mg L^{-1})	Relative error (%)
A	111 ± 1	106 ± 1	− 4.5
B	65 ± 1	69 ± 1	+ 6.2
C	86 ± 1	88 ± 1	+2.3
D	233 ± 4	242 ± 1	+3.9
E	317 ± 8	309 ± 6	− 2.6
F	160 ± 1	158 ± 5	− 1.3

[a] Sample from A to F: white wine, vinegar, coconut water, rosé wine, cashew juice 1 and cashew juice 2.The samples of rosé wine, cashew juice 1 and cashew juice 2 were diluted to fit within the linear range of the digital image analysis method.