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Speciation analysis based on digital image colorimetry: Iron (II/III) in white wine

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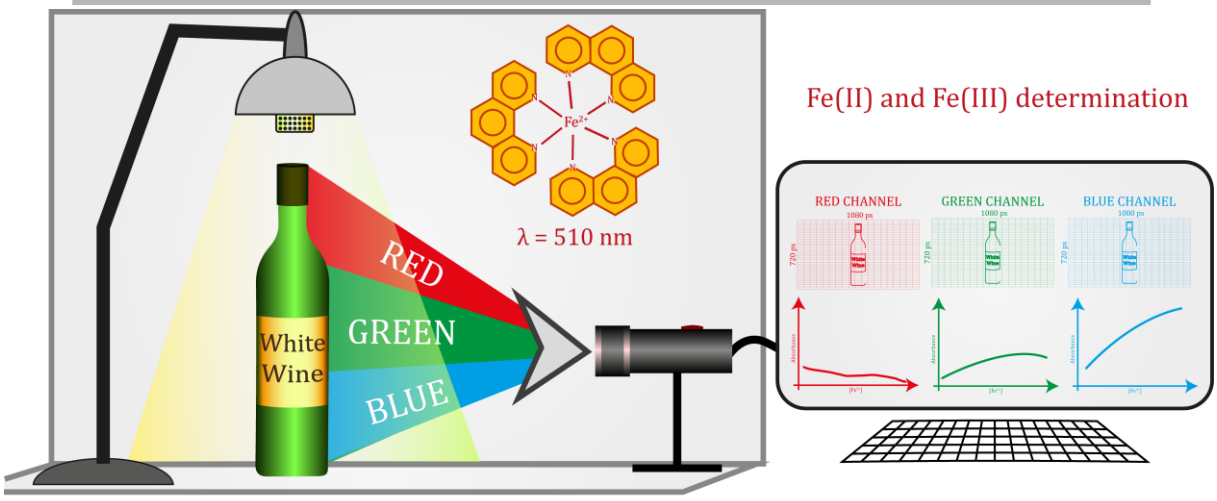
ABSTRACT

This work proposes an analytical strategy utilizing digital images (DI) for the iron inorganic speciation in white wine. The method was established by the reaction of iron(II) ions with 1,2 ortho-phenanthroline as a chromogenic reagent. Total iron was determined using the same reagent after the addition of hydroxyl ammonium chloride as a reducing agent. In both cases, digital images of the standards/chromogenic reagent and samples were acquired and stored in JPEG format. The region of interest (ROI) was determined with a constant square shape for all images. The ROI was submitted to decomposition in color values according to the RGB additive color model. However, the data obtained by the blue channel was the one used in the construction of the analytical curves because it presented the highest sensitivity. The optimization of the experimental conditions of the procedure was performed by employing multivariate techniques. The precision was evaluated using a wine sample with iron (II) and total iron contents of 0.41 and 0.69 mg L^{-1}, respectively. The results expressed as relative standard deviations were 3.57% for iron (II) and 4.76% for total iron contents. A comparison between the results obtained for total iron by the DI method with the results found using flame atomic absorption spectrometry confirmed the method accuracy. The DI procedure was applied for speciation analysis in six white wine samples and the contents found varied from 0.41 to 1.67 mg L^{-1} for iron (II) and from 0.69 to 1.71 mg L^{-1} for total iron. These results are in agreement with those found for speciation analysis of iron in wine samples. Iron (III) contents can be found by the difference between the total iron and iron (II) contents.

Graphical asbtract



Keywords: Iron speciation; Wine samples; Digital image; 1,2 Ortho-phenanthroline.

1. Introduction

The iron inorganic speciation in wine is an important analysis because the iron (III) compounds in this matrix are insoluble and undesirable. Therefore, the iron (III) content is a parameter of technological control in the wineries, which can guarantee the quality and the price of the wine after bottling [1-3]. In this sense, several methods have been proposed for the iron speciation analysis in wine [4-10]. Rousseva et al. [4] used a solid phase extraction procedure to evaluate the presence of the hydrophobic, cationic and residual forms of copper and iron in wine samples. Costa and Araujo [5] proposed a method for the determination of iron(III) and total iron in wine matrices. For iron(III), the procedure was based on the liquid extraction of the iron(III) thiocyanate complex and quantification, employing sequential injection analysis and molecular absorption spectrophotometry (MAS). Total iron was determined using flame atomic absorption spectrometry (FAAS). Another method also used MAS for the determination of iron(II) and total iron in wine. In this method, iron(II) was determined using 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (Br-PADAP). Total iron was quantified utilizing the same reagent after a reduction step using ascorbic acid [6]. Lopez et al. [7] proposed a procedure using 2,2'-dipyridylketone picolinoylhydrazone (DPKPH) in the absence and presence of ascorbic acid for the quantification of iron (II) and total iron in wine by MAS. Camara et al. [8] proposed a disposable biosensor for the detection of iron (III) in wines. Mitreva et al. [9] developed an ion-imprinted polymer for quantification of iron(II) in wine samples. Recently, Lao et al.[10] employed the Donnan membrane technique for the determination of free species of zinc(II), iron(III), calcium(II) and magnesium(II) in red wine.

In recent times, many analytical methods conventionally established by molecular absorption spectrophotometry have been adapted using computer vision-based methods, [11] such as employing a scanner [12, 13], digital camera, smartphone [14], tablet computer and webcam [11, 14-16]. These new approaches have allowed the development of fast analytical methodologies that are low-cost, accurate, with portability potential and the ability to remotely send the results via technologies such as Wi-Fi or Bluetooth, representing a true democratization of the sciences of analytical measures [11].

In methods that are digital image based, one crucial step is to convert the image into numerical information. The capture of RGB digital image can be employed to describe a

phenomenon involving the appearance or disappearance of color. Briefly, the RBG color space mimics the human visual system, and color value in a specific pixel is the weighted sum of contributions of the primary colors R, G, and B (additive color model). In this sense, RBG provides an orthogonal vector space in R3 [11].

In this context, several analytical methods have been proposed and successfully applied for iron quantitation in complex matrices by means of digital image [17-21]. Firdaus et al. [17] developed a procedure for the determination of chromium and iron in synthetic solutions using a formed complex of iron(III) ions and thiocyanate. The data processing was performed employing the simple linear regression (SLR) of individual color R, G or B, and also the partial least square method of those three colors. The results obtained were compared and discussed. Igoe and Parisi [18] proposed an analytical method for the evaluation of the corrosion of iron using a smartphone camera. Barros et al. [19] employed a webcam to acquire the digital images during the determination of aluminum, total iron, nitrite and soluble phosphorus in waters. The red, green, and blue intensities were converted into absorbances to establish the external calibration technique. The chromogenic reagent used for iron determination was 1,10 ortho-phenanthroline. Masawat et al. [20] also used 1,10 ortho-phenanthroline as the complexing reagent in Digital Image Colorimetry for determination of total iron in natural water. The authors conducted a deep study on the relationship between iron(II) concentration and the seven image parameters (red, green and blue values, as well as hue, saturation, brightness and gray, which are stored specifically for analysis). Cardoso et al. [21] developed a digital image system to study the corrosion of steel plates using a polyester laser printed device. The colored species were formed by the reaction of iron(III) with thiocyanate.

The two-level full factorial design has often been employed for preliminary evaluation of the experimental variables of the processes. This chemometric tool establishes linear models that allow the calculation of the effects of the factors [22-25].

In this work, an analytical methodology based on the digital image for speciation analysis of iron in white wine is presented for the first time. This new approach combines the well-established colorimetric reaction between iron (II) and 1,2 ortho-phenanthroline (as the chromogenic reagent) and the advantages of using a computer vision based analytical measure.

2. Experimental

2.1. Reagents and samples

All the solutions used were prepared using high-purity water with the resistivity of 18.2 MΩ cm obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA). Also, the reagents used in all experiments were of Analytical-grade, Merck (Darmstadt, Germany).

2.2. Equipment

The determination of total iron in wine samples was performed using a model ContrAA 700 high-resolution continuum source flame absorption atomic spectrometer from Analytik Jena AG (Jena, Germany), using the primary atomic absorption line of iron (248.327 nm).

2.3. RGB data acquisition and evaluation

The capture of the digital images was done in a closed system consisting of a wooden box (BB x BB x BB cm), with an aperture on its upper part, and a light control in the internal compartment in order to guarantee the reproducibility of the images during the measures. The use of a LED lamp eliminates the need of flash to capture the image. Also, the wood used to make the box was white to avoid reflection effects. The digital images of the chromogenic systems were recorded employing a Microsoft 720p HD video chat webcam.

For all experiments, digital images were stored in JPG format and a square region of interest (ROI) was defined in a fixed position using the *ImageJ* computer program. This program allows the RGB data acquisition for all pixels in ROI. This information is arranged in a color histogram and the mean value of each color channel, red (R), green (G) and blue (B), is computed. The definition of the analytical signals based on the color value in compatible compliance with Bee's law were defined as –log(P/Po), where P is the R, G and B value (mean or mode) from standard or sample solution and Po is the R, G and B value for analytical blank.

2.4. Optimization of the experimental conditions of the analytical method

During the optimization of the experimental conditions for the speciation analysis of iron in wine using digital imaging, previous experiments were performed using data from papers published in the literature involving the determination of iron in wine using molecular absorption spectrophotometry. The results of these tests were promising and the optimization of the parameters for digital measurements was done using experimental design techniques. Firstly, a two-level full factorial design was performed involving the pH and 1,10 ortho-phenanthroline volume factors to optimize the determination of iron(II). Another experiment was also conducted for the factors pH, 0.1% (w/v) 1,10 ortho-phenanthroline volume and 10% (w/v) hydroxylammonium chloride volume for optimization of the method of quantification of total iron. RGB data were extracted in the two experiments and the blue data were employed as the response due to their higher sensitivity. Triplicates of the central points were performed to determine the curvature test and the experimental error. The experimental domains were established considering the data reported in the literature for the determination of iron using 1,10 ortho-phenanthroline.

2.5. General procedure

2.5.1. Speciation analysis of iron in wine samples

For the determination of iron (II), 15.0 mL of wine sample were transferred to a volumetric flask of 25.0 mL. Then, 2.5 mL of 0.1% 1,10 ortho-phenanthroline solution was added. After shaking, 2 mL of an acetate buffer with pH of 3.88 was also added. Finally, the end volume was completed with demineralized water and the solutions were processed using the digital image system proposed. The procedure for the determination of total iron

was the almost the same, with the exception that a hydroxylammonium chloride volume of 2.0 mL was added before of the 1,10 ortho-phenanthroline solution.

2.5.2. Determination of total iron in wine samples using HR-CS FAAS

The wine samples were digested in a heating plate using 30% hydrogen peroxide and concentrated hydrochloric acid. Total iron was determined by employing a high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) in the 248.327 nm line using air-acetylene flame.

3. Results and discussions

3.1. Optimization of the procedure for speciation analysis of iron in wine samples

The optimization of the experimental conditions for the determination of iron(II) and total iron was made in two steps. First, a two-level full factorial design was performed to establish the procedure for the quantification of iron(II). The factors studied were the pH of the buffer solution and the 1,2 ortho-phenanthroline volume. All experiments were performed utilizing a constant wine volume. Table 1 shows the experimental domains of the factors as coded and real values, the digital image analysis in the RGB channels, and the absorbances, which were obtained using data of the B channel, which had the highest sensitivity. Triplicates of the central point were performed to determine the experimental error and the curvature test.

The evaluation of the factorial design is presented in Figure 1. It shows that the interaction between the two factors is not statistically significant. This means that the analytical signal obtained in the method is statistically constant for the experimental domains of the factors.

Another two-level full factorial design was also performed for the optimization of the determination of the total iron method. The factors involved were: pH of the buffer solution, 1,2 ortho-phenanthroline volume, and reducer volume. Table 2 shows the factors and the data obtained similarly to Table 1. The results of this factorial, as seen in Figure 2, demonstrated that the three factors and their interactions are not significant.

Considering the results obtained in the two factorial designs, the experimental conditions of the central point were chosen for the general procedures of the methods for the iron(II) and total iron determinations.

3.2. Analytical characteristics of the method for the iron speciation analysis in wine

Calibration curves were built for iron(II) determination using eight standard solutions of iron in a concentration range from 0.25 to 2.00 mg L-1. The digital image data were processed and the values obtained in the three RGB channels are shown in Table 3.

The Blue channel data showed greater sensitivity and therefore were processed for obtaining the analytical signals. The equation of the calibration curve was obtained using the linear regression method and it is shown in Table 4. Figure 3 shows the calibration curves and equations obtained with the data from the three RGB channels.

The limits of detection and quantification were calculated using the expression LOD = 3δ/s and LOQ = 10δ/s, where δ is the deviation standard of ten measurements of the analytical blank and s is the slope of the analytical curve. The LOD´s and LOQ´s for the two methods are also presented in Table 4. The method precision was expressed as the relative standard deviations (RSD) and it was determined using a wine sample with iron(II) and total iron contents of 0.41 and 0.69 mg L^{-1}, respectively. The RSD´s obtained are shown in Table 4.

3.3. Application – Iron speciation analysis in wine samples

The method proposed was applied for speciation analysis of inorganic iron in six wine samples. The iron(II) and total iron contents obtained varied from 0.41 to 1.67 mg L^{-1} and from 0.69 to 1.71 mg L^{-1}, respectively. The results expressed as confidence interval at 95% level are shown in Table 5. The iron contents found are in agreement with those found during the determination of iron(II) and total iron in wine samples [26]. The iron (III) contents can be found by calculating the difference between the contents of total iron and iron (II). Due to the reducing characteristics of wine, the iron (II) concentrations are always higher than the ones of iron (III), as it can also be observed in table 5.

4. Conclusion

In this work, for the first time, the efficiency of an optimized analytical methodology for quantitation and speciation of the inorganic iron in white wine samples was demonstrated. This methodology successfully combined a well-known colorimetric reaction and a computer vision-based approach to generate a method that fulfills the requirements of fastness, robustness, low-cost and accuracy. Going beyond analytical aspects, the present proposal has potential as a useful wine quality control technology, making it an effective tool for wine producer cooperatives. This type of low-cost technology will contribute to the

development of high-quality wines at competitive prices in the consumer market. The method sensitivity is perfectly compatible with the concentrations of iron (II) and total iron in wine matrices.

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Table 1. Optimization of the factors for the determination of iron(II) in wine

Exp.	pH	Or-fen (ml)	R	G	B	B (S_{Bl}/S_{Ex})	B-Log (S_{Bl}/S_{Ex})
Blank			176	181	170		
1	- (3.75)	- (2.0)	179	173	153	1.111	0.046
2	+ (4.00)	- (2.0)	176	170	151	1.126	0.051
3	- (3.75)	+ (6.0)	178	170	151	1.126	0.051
4	+ (4.00)	+ (6.0)	179	173	155	1.097	0.040
CP	0 (3.88)	0 (4.0)	175	170	152	1.118	0.049
CP	0 (3.88)	0 (4.0)	179	169	150	1.133	0.054
CP	0 (3.88)	0 (4.0)	178	170	151	1.126	0.051

Or-fen: 1,10 ortho-phenanthroline.

Table 2. Optimization of the factors for the determination of total iron in wine

Exp.	Reducer (mL)	Or-fen (mL)	pH	R	G	B	B (S_{Bl}/S_{Ex})	B-Log (S_{Bl}/S_{Ex})
Blank				179	181	169		
1	- (2.0)	- (2.0)	- (3.75)	178	172	151	1.119	0.049
2	+ (6.0)	- (2.0)	- (3.75)	180	174	153	1.105	0.043
3	- (2.0)	+ (6.0)	- (3.75)	181	175	154	1.097	0.040
4	+ (6.0)	+ (6.0)	- (3.75)	180	175	157	1.076	0.032
5	- (2.0)	- (2.0)	+ (4.00)	180	174	155	1.090	0.038
6	+ (6.0)	- (2.0)	+ (4.00)	181	174	152	1.112	0.046
7	- (2.0)	+ (6.0)	+ (4.00)	182	174	152	1.112	0.046
8	+ (6.0)	+ (6.0)	+ (4.00)	179	174	150	1.127	0.052

CP	0 (4.0)	0 (4.0)	0 (3.88)	178	171	147	1.150	0.061
CP	0 (4.0)	0 (4.0)	0 (3.88)	181	171	148	1.142	0.058
CP	0 (4.0)	0 (4.0)	0 (3.88)	182	178	149	1.134	0.055

Or-fen: 1,10 ortho-phenanthroline.

Reducer: hydroxylammonium chloride.

Table 3. Calibration curve for the determination of iron(II)

Exp.	Fe(II) (mg L^{-1})	R	G	B	B (S_{Bl}/S_{Ex})	B-Log (S_{Bl}/S_{Ex})
1	0.00	173.174	175.473	163.049	1.0000	0.0000
2	0.25	173.804	171.336	155.878	1.0460	0.0195
3	0.50	176.465	167.444	148.042	1.1014	0.0419
4	0.75	175.692	162.496	137.632	1.1847	0.0736
5	1.00	176.293	157.051	127.347	1.2804	0.1073
6	1.25	174.805	149.832	118.685	1.3738	0.1379
7	1.50	176.771	145.581	112.741	1.4462	0.1602
8	1.75	178.200	138.410	103.153	1.5807	0.1988
9	2.00	180.327	137.054	98.594	1.6537	0.2185

Table 4. Analytical characteristics of the method proposed

Parameters	Iron(II)
Equation curve (Blue channel)	Analytica signal = $0.1142 \times C_{(m/L)} + 0.0078$
Limit of detection (mg mL^{-1})	0.042
Limit of quantification (mg mL^{-1})	0.141
Precision as RSD% for iron(II) 0.412 mg mL^{-1} total iron 0.633 mg mL^{-1}.	3.57% for iron(II) and 4.76% for total iron.

Table 5. Speciation analysis of inorganic iron in white wine samples

Sample	Iron (II)* ($mg\ L^{-1}$)	Total iron* ($mg\ L^{-1}$)	Iron (III) ($mg\ L^{-1}$)	Total iron* by FAAS ($mg\ L^{-1}$)
1	0.41 ± 0.02	0.69 ± 0.02	0.28	0.73 ± 0.06
2	0.90 ± 0.07	1.04 ± 0.04	0.14	0.96 ± 0.10
3	1.09 ± 0.07	1.16 ± 0.09	0.07	1.13 ± 0.10
4	0.99 ± 0.03	1.10 ± 0.06	0.11	1.19 ± 0.11
5	1.67 ± 0.08	1.71 ± 0.09	0.04	***
6	0.68 ± 0.07	0.74± 0.09	0.06	***

*- Concentration expressed as confidence interval at 95% level.

Highlights

- A method for speciation analysis of iron in white wine has been proposed
- The iron speciation in wine has been proposed using digital image colorimetry
- The optimization step was performed employing multivariate techniques
- The method proposed has sensitivity compatible with the iron contents in white wine
- The digital images were processed using the RGB color system