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Review

A digital image-based method for determining of total acidity in red wines using acid–base titration without indicator

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ABSTRACT

This work proposes the use of digital image-based method for determination of total acidity in red wines by means of acid–base titration without using an external indicator or any pre-treatment of the sample. Digital images present the colour of the emergent radiation which is complementary to the radiation absorbed by anthocyanines present in wines. Anthocyanines change colour depending on the pH of the medium, and from the variation of colour in the images obtained during titration, the end point can be localized with accuracy and precision. RGB-based values were employed to build titration curves, and end points were localized by second derivative curves. The official method recommends potentiometric titration with a NaOH standard solution, and sample dilution until the pH reaches 8.2–8.4. In order to illustrate the feasibility of the proposed method, titrations of ten red wines were carried out. Results were compared with the reference method, and no statistically significant difference was observed between the results by applying the paired *t*-test at the 95% confidence level. The proposed method yielded more precise results than the official method. This is due to the trivariate nature of the measurements (RGB), associated with digital images.

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1. Introduction

Among the relevant properties of wine, pH or acidity contributes to fermentation and takes part in the sensorial characteristics of the wine, influencing the stability and colour.

The acidic characteristics of the must or wine are determined by relatively weak organic acids. Therefore, when must and wines are titrated against a strong base the equivalence point will be at a pH higher than 7.0, usually between 7.8 and 8.3 [1].

Wines contain a complex mixture of organic acids that include tartaric, malic, succinic, acetic, citric and lactic acids. Tartaric acid is the strongest, and influences the pH and sensorial characteristics of the wine proportionately [2]. For this reason the total acidity of wine is expressed in g L^{-1} of tartaric acid [3,4].

Analyzing coloured samples is problematic. Common indicators many times yield unsatisfactory results due to difficulties in titration end point determination. This is particularly true of red wine total acidity determinations. Various methodologies have been developed aimed at solving this problem, Runyan [5], Cardwell et al. [6], Rangel and Tòht [7], Gaião et al. [8], Honorato et al. [9] and Garcia and Reis [10]. The common element in all of these approaches is the addition of an external indicator and sometimes combining the choice of the indicator with the spectral window of the light source or LED being used. Wines absorb electromagnetic radiation in a wide range of the visible spectra due to anthocyanines present in grapes [11].

Anthocyanines (from greek: *anthos* = flower and *kianos* = blue) are pigments of the flavonoid class and are responsible for most of the blues, purples and all shades of red found in the flowers, fruits, some leaves, stems and roots of plants. According to Bordignon et al. [12], anthocyanines present different structures having different colours. Colour change depends on several factors, including temperature, pH, and copigment binding. However, colour changes depend principally on the acidity or alkalinity of the medium (pH).

Gaião et al. [13] exploited the potential of digital imaging to carry out quantitative analysis, and developed a digital image-based (DIB) titration method, which was successfully applied to the determination of total alkalinity in mineral and tap waters (using methyl orange as indicator).

The present work proposes a digital image-based methodology for determination of total acidity in red wines by using acid–base titration without the use of an indicator. In order to show the feasibility of the proposed method, ten samples of red wines were analysed and the results were statistically compared with those obtained by reference methods.

2. Experimental

2.1. Reagents, samples, and solutions

About 100.0 mmol L^{-1} of NaOH prepared from analytical grade chemical in freshly distilled-deionised water was used for the total acidity titration of the red wines. This titration solution was standardized by conventional methodology [14].

Ten brands of red wines were purchased from local supermarkets. All samples were directly analysed without any further treatment.

2.2. Apparatus

A schematic diagram and photograph of the system proposed for digital image-based total acidity titration of red wines are presented in Fig. 1. This system comprises a common CMOS model 11090-CL Clone WebCam (WC), which was positioned in front of the flow cell (FC) in order to capture the digital images. The FC consists of a glass tube with 4.5 cm of length and 4 mm of inner diameter [15], which was used to transport the acid–base titration products of the red wines. These reaction products were illuminated by a light from a 5 W Avante fluorescent lamp (L).

In order to avoid interferences of ambient light, the WebCam, flow cell and analytical system were put into a wood box measuring 32 cm × 54 cm × 36 cm. The walls of the box were also covered with white paper in order to provide uniform illumination and to reduce glare.

The WebCam was connected to the USB port of an Intel Core2Duo 2 Gigabytes notebook (PC) and configured to capture 24-bit digital images (16.7 million colours) at a rate of 30 frames s^{-1} and a 640 pixels × 480 pixels spatial resolution. The images were captured and stored as "jpeg" compressed files by using the software provided by the manufacturer of the WebCam.

A model 8453 Hewlett-Packard (HP) diode array UV–Vis spectrophotometer, equipped with a model QS 178.010 Hellma flow cell (inner volume 100 μL and optical path 1.0 cm) was used for spectrophotometric measurements.

A model 713 Metrohm pH meter was used to obtain the reference values (potentiometric titration) for the total acidity of the samples of red wines.

A model White IKA LabDisc magnetic stirrer (MS) driving a stirring bar (SB) inside the Erlenmeyer was used to uniform the solutions.

An 8 channel-12 rolls Ismatec, peristaltic pump (PP), model IPC, employing Tygon® pumping tubes of 1.14 mm inner diameter, and Teflon® line tubes of 0.8 mm inner diameter were used to propel the solutions.

Three three-way solenoid valves (V_1, V_2 and V_3) model LHDA0531415HA Lee, with 77 μL inner volumes, were used to add increments of the titration solution.

A NI USB-6008 National Instruments control interface (CI) was used to control the solenoid valve.

The control software of the digital image-based titration system was developed in Labview® 5.1.

The image treatment software was developed in Borland® Core Gear Rad Studio 2007. The procedure for RGB-based value calculation was the same as described by Gaião et al. [13], but the delimited region was about 30 pixels × 180 pixels.

2.3. The reference method

The determination of total acidity in red wines is carried out according to protocols established by official organs like the Association of Official Analytical chemists (AOAC) [3], Office International de la Vigne et du Vin (OIV) [16], Instituto Adolfo Lutz (IAL) [17], as well as the Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento-MAPA) [18].

For all these organs, the recommended methods are based on the neutralization titration of the acids against a standard solution of base using phenolphthalein as the indicator for clear solutions of wines or using a pH meter for dark solutions.

Their procedures consist in the titration of an aliquote of descarbonated sample against a standard solution of sodium hydroxide up to a pH of between 8.2 and 8.4.

2.4. DIB, spectrophotometric and potentiometric titrations

DIB titration is accomplished using a WebCam (Fig. 1(a)) and a standardized NaOH solution in four steps: (i) an aliquote of 10 mL of the sample is put into an erlenmyer, (ii) an aliquote of the titrant is added to the sample and the solution homogenized, (iii) the sample is pumped to the flow cell and a digital image is obtained, and (iv) the sample returns to the erlenmeyer. Steps (ii)–(iv) are repeated

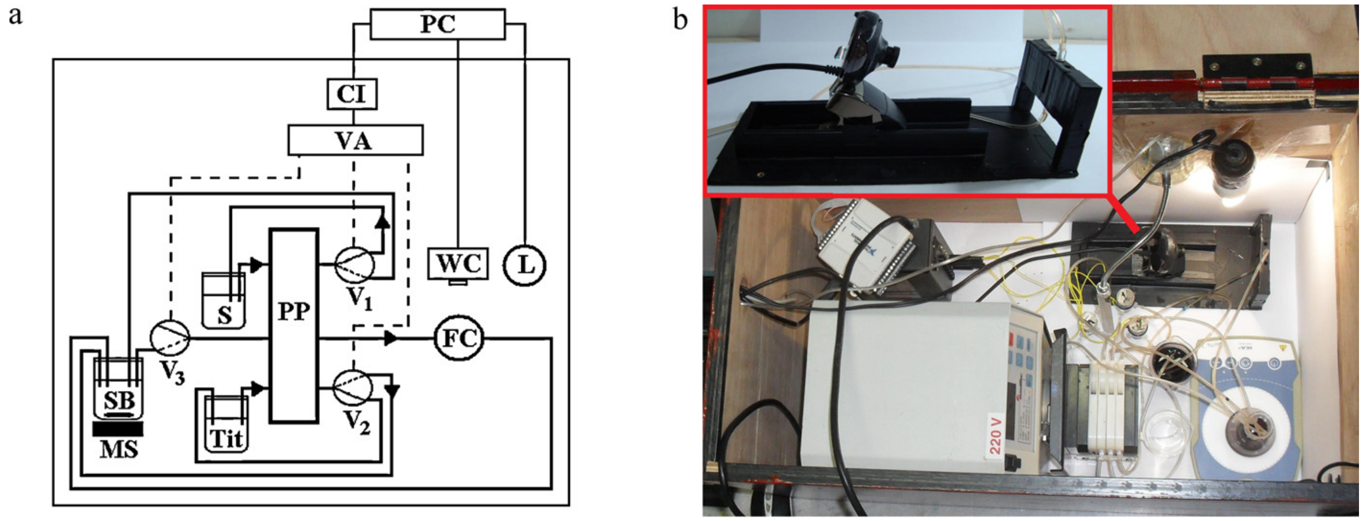


Fig. 1. Schematic diagram (a) and photograph (b) of the system proposed for determination of total acidity in red wines by using digital image-based titrations. WC, WebCam; V_1, V_2 and V_3, solenoid valves; CI, control interface; PP, peristaltic pump; SB, stirrer bar; MS, magnetic stirrer; S, sample; Tit, titrant; PC, personal computer; FC, flow cell; L, lamp; VA, valve actuator.

until reaching a greenish colour for the sample (the parameter used to finish the titration run).

The end point is approximated by comparing the relative colour for each of the sequence of digital images, and changing the volume increment of the titrant to 0.2 mL between the last volume increment (which corresponds to a reddish colour) and the first volume increment (which corresponds to a bluish colour).

The spectrophotometric and potentiometric titrations were simultaneously carried out using the spectrophotometer and pH meter respectively with an aliquote of 10 mL of the sample diluted with 100 mL of distilled water. The spectra obtained during the spectrophotometric titration were recorded from 400 to 700 nm.

3. Results and discussion

3.1. Study of the volume increments of the titrant

In weak acid titrations the pH value in the equivalence point depends on the concentration of the analyte (acid). For tartaric acid ($K_{a1} \approx 9.20 \times 10^{-4}$ and $K_{a2} \approx 4.31 \times 10^{-5}$) the ionizable hydrogens can not be separately determined in solution by titration until their respective equivalence points, because the ratio K_{a1}/K_{a2} is much smaller than 10^4 [14]. This calls for in practice, a potentiometric titration up to a fixed pH value (8.2–8.4) to be performed [19].

A titration having curves with subtle inflections, requires an experienced chemist to adequately perform the titrant additions. It usually necessitates pre-calculation as well [20]. Moreover, instrumental analysis techniques that employ measures based on the inflection point, can be compromised due to the need for signal differentiation, which is very sensitive to instrumental noise [21].

Studies made by Presteni et al. [22] showed that the several compounds in the wine increase the buffering effect of the matrix and an acid base titration shows an inflection point of around pH = 7.5 (a weak acid titration).

To avoid end point localization problems caused by the buffering effect, we studied the titrant increment using the second derivative method. Fig. 2 shows the titration curve (a) and its respective second derivative curve (b). Additions of 0.2 mL of titrant near the end point was the smallest titrant increment that ensured precise localization of the end point, and detectable colour variations by WebCam.

3.2. Digital images, absorption spectra and pH along the DIB titration

Fig. 3 shows the digital images and pH value (a) obtained after each titrant addition and the recorded spectra with the same additions, but in a 1:10 (v/v) dilution before (b1) and after (b2) the end point for a red wine sample.

Anthocyanines present in wines are capable of absorbing electromagnetic radiation in the visible region yielding various colours, as can be seen in Fig. 3(a), depending on the medium. The most important factor affecting the colour of the anthocyanines is pH, and depending on the acidity or alkalinity, anthocyanines exhibit different chemical structures in aqueous medium [23]. This can be observed in the recorded spectra of the titration in Fig. 3b1 and b2 that reveal several spectral features depending on pH, due to the predominance of different chemical structures for each medium [24].

By observing the images, one notes that in an acid medium the red colour predominates and the absorbance gently decreases. After the end point the colour changes to greenish blue and the absorbance increases in the alkaline medium [25].

In aqueous solutions, four species of anthocyanines molecules exist in equilibrium: flavinic cation (AH^+), quinoidal base (A), carbinol (B) and chalcone (C) [24]. The structures are shown in Fig. 4 and correspond to the different chemical structures of anthocyanines when the pH of the medium varies. They are responsible for the colour change and spectra presented by the red wine sample along the titration.

An increase in pH displaces the equilibrium among many chromophores and consequently the relative quantities of all these structures vary according to chemical equilibriums. At a pH >9.0 the anthocyanines mainly occur in ionized forms of the quinoidal base (A) and chalcone (C). The main factor in the colour fade of anthocyanines is the highly reactive flavinic cation facing nucleophilic reagents such as H_2O and OH^- [26].

3.3. Titration curves and localization of the end point

In order to locate the titration end point, DIB titration curves and their respective second derivatives were built as showed in Fig. 2. The equations for the first and second derivatives of the obtained curves are described in the literature [27].

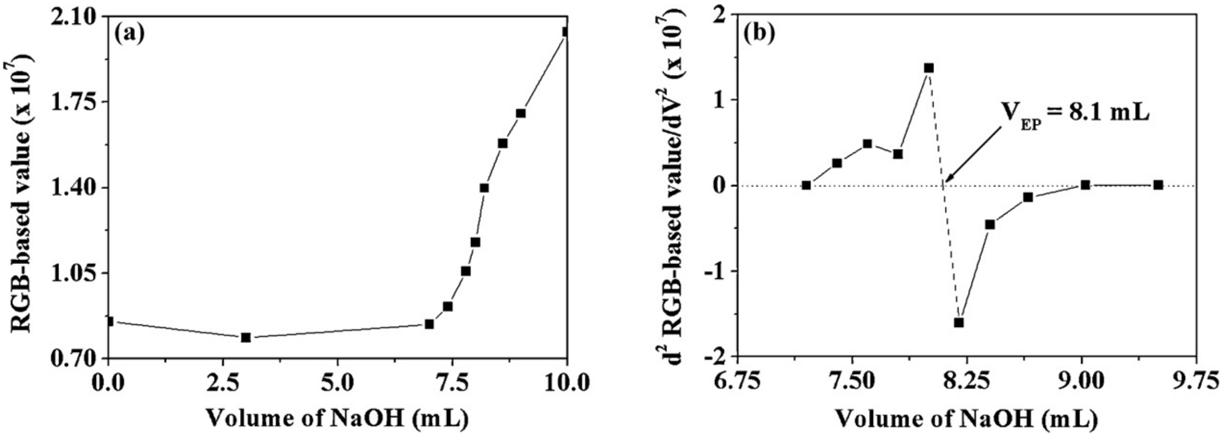


Fig. 2. DIB titration curve (a) and its respective second derivative curve (b).

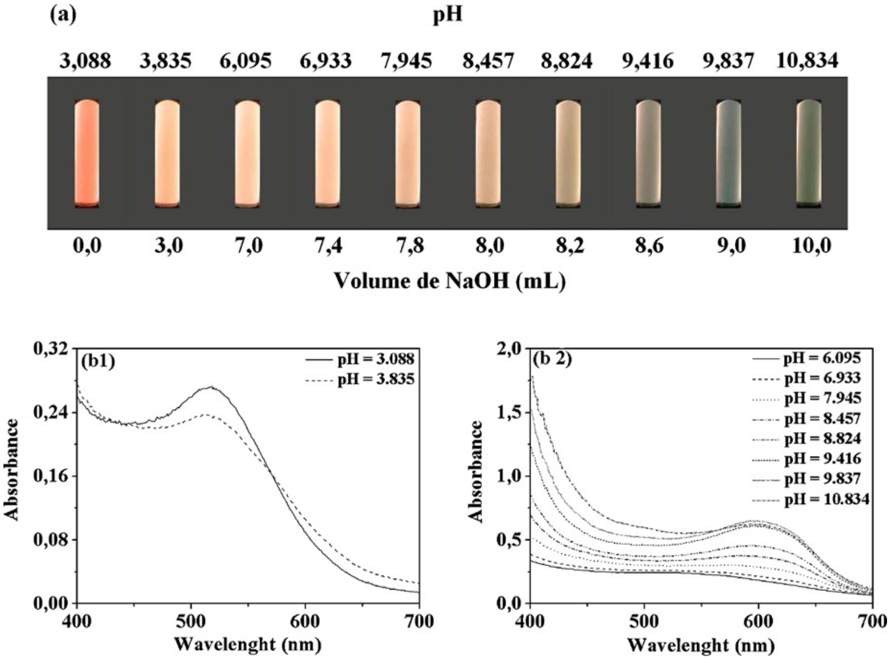


Fig. 3. (a) Digital images of an original sample of red wine and (b) visible absorption spectra of the same diluted sample 1:10 (v/v) obtained during the titration before (b1) and after (b2) the end point.

By analyzing Fig. 2 one perceives that the most pronounced colour modulation in DIB titration is due to the contribution of two components (green and blue), just before the end point because the red component remains constant. This allows viewing the red colour of the images. After the end point of the DIB titration, variations of the three components in different combinations and intensities lead to the colours displayed by red wine samples in an alkaline medium.

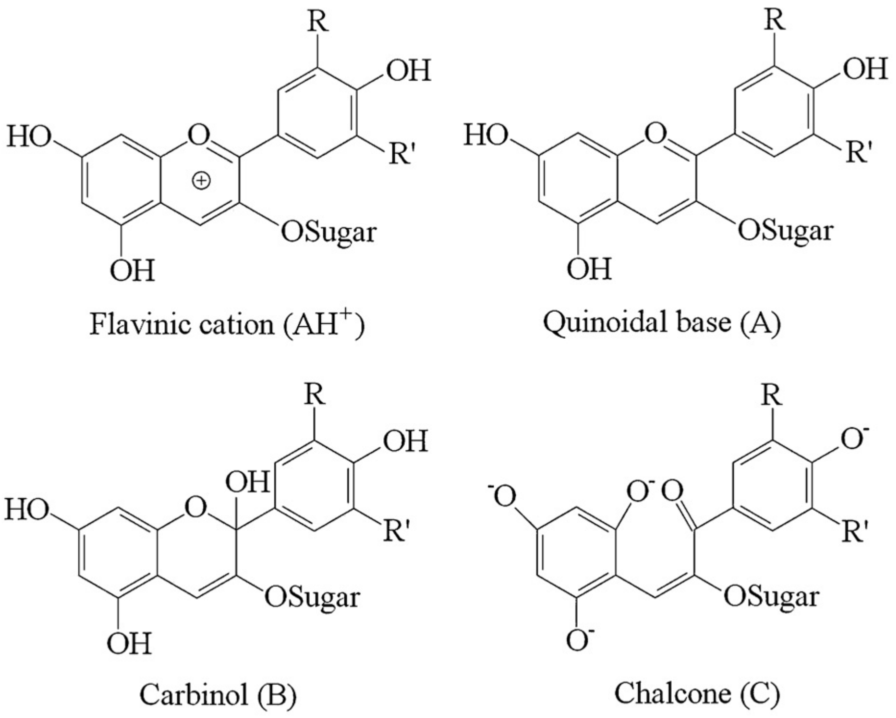
In Fig. 2(b) the zero crossing, indicated by an arrow in the second derivative curve of red wines provides the end point volume (V_{EP}). In the potentiometric titration of the same, (Fig. 2) the end point volume was also 8.1 mL, showing good agreement between the proposed and reference methods.

3.4. Analytical determinations and statistical validation of results

The proposed method was applied to determination of total acidity in ten wine samples (dry and sweet), and the results were compared to those obtained by the reference method. Table 1 shows the results in terms of $g\,L^{-1}$ tartaric acid for both methods.

Table 1
Average results (in terms of $g\,L^{-1}$ tartaric acid) of three determinations of total acidity in red wines obtained by using proposed DIB and potentiometric titrations.

Sample	Titration	
	DIB	Potentiometric
1	11.4 ± 0.2	11.4 ± 0.2
2	12.3 ± 0.2	12.2 ± 0.2
3	11.9 ± 0.0	12.4 ± 0.1
4	12.3 ± 0.2	12.1 ± 0.1
5	11.8 ± 0.1	12.0 ± 0.3
6	12.3 ± 0.2	12.6 ± 0.3
7	12.2 ± 0.1	12.3 ± 0.2
8	12.0 ± 0.1	11.7 ± 0.2
9	12.4 ± 0.1	12.2 ± 0.2
10	12.2 ± 0.1	12.1 ± 0.1
Overall R.S.D.	0.13	0.19



Flavinic cation (AH^+) Quinoidal base (A)

Carbinol (B) Chalcone (C)

Fig. 4. Molecular structures of anthocyanines in aqueous solution.

The results show good agreement. The application of the paired *t*-test, based on the hypothesis test, revealed no statistically significant difference between the results at a 95% confidence level. This inference is corroborated by the paired *t*-test based on a built confidence interval for the true average difference (Δ). At 95% confidence level, the limits of this interval are: $\Delta = [-0.4038, 0.3638]$.

The "zero" value is included in the interval. So there does not exist a statistically significant difference between the results at the adopted confidence level. Consequently, the null hypothesis of $\Delta = 0$ can not be rejected [28].

In terms of analytical performance, the proposed method presented better performance than the reference method, as revealed by the overall R.S.D. value.

4. Conclusions

This paper demonstrates that the use of digital images obtained with a simple WebCam can support a new strategy of acid–base titration for the determination of total acidity in red wines without the use of an external indicator.

The proposed methodology is based on the inherent absorbent properties of the red wine matrix. In fact, anthocyanines display different colours according to the pH of the medium, and from the titration RGB-based image values, the end point was accurately located without the use of an indicator or sample dilution.

The proposed method yielded more precise results than the reference method, which might be attributed to the trivariate nature of the measurements associated with digital images.

Another important point that must be taken into account is that there is no detector/sample contact, a great advantage over pH glass electrodes, because even with all care, they have a limited lifetime and need to be replaced. Moreover, as the proposed methodology uses an inexpensive WebCam as the detector, it offers an economically viable alternative to glass electrode titrations. In fact, the price of a pH glass electrode is expensive when compared with the price of a WebCam.

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References

[1] F.G. Barros, M. Tubino, J. Assoc. Off. Anal. Chem. 74 (1991) 346.
[2] L.A. Rizzon, A. Miele, Ciênc. Rural 31 (2001) 893.
[3] B.W. Zoecklein, K.C. Fugelsang, B.H. Gump, F.S. Nury, Wine Analysis and Production, first ed., Chapman & Hall, New York, 1995.
[4] J. Darias-Martin, A. Socas-Hernández, C. Díaz-Romero, E. Díaz-Díaz, J. Food Comp. Anal. 16 (2003) 555.
[5] E.G. Runyan, J. Am. Chem. Soc. 23 (1901) 402.
[6] T.J. Cardwell, R.W. Cattrall, G.J. Cross, G.R. O'Connell, J.D. Petty, G.R. Scollary, Analyst 116 (1991) 1051.
[7] A.O.S.S. Rangel, I.V. Tóth, Analyst 123 (1998) 661.
[8] E.N. Gaião, R.S. Honorato, S.R.B. Santos, M.C.U. Araújo, Analyst 124 (1999) 1727.
[9] R.S. Honorato, M.C.U. Araújo, R.A.C. Lima, E.A.G. Zagatto, R.A.S. Lapa, J.L.F.C. Lima, Anal. Chim. Acta 396 (1999) 91.
[10] A.J.C. Garcia, B.F. Reis, J. Automot. Methods Manage. Chem. 83247 (2006) 1.
[11] C.R. Malacrida, S. Motta, B. Ceppa 24 (2006) 59.
[12] C.L. Bordignon Jr., V. Francescatto, A.A. Neinow, E. Calvete, F.H. Reginatto, Ciênc. Tecnol. Alim. 29 (2009) 183.
[13] E.N. Gaião, V.L. Martins, W.S. Lyra, L.F. Almeida, E.C. Silva, M.C.U. Araújo, Anal. Chim. Acta 570 (2006) 283.
[14] G.H. Jeffery, J. Basset, J. Mendham, R.C. Denney, Vogel's Textbook of Quantitative Chemical Analysis, fifth ed., Longman Scientific and Technical, London, 1989.
[15] M.C.U. Araújo, R.S. Honorato, A.V. Santos, E.C. Silva, Quím. Nova 19 (1996) 86.
[16] Office International de la Vigne et du Vin, Recueil des Méthodes Internationales d'Analyse des Vins, OIV, 1969.
[17] Instituto Adolfo Lutz, Métodos Físico-Químicos para Análise de Alimentos, Digital, São Paulo, 2008.
[18] http://extranet.agricultura.gov.br/sislegis (accessed January 2011).
[19] N.N. Golovnev, O.S. Romanova, N.V. Busygina, J. Anal. Chem. 55 (2000) 457.
[20] J.A. Medeiros, M.L.C.P. Carvalho, Quím. Nova 14 (1991) 282.
[21] V.L. Martins, L.F. Almeida, S.L. Castro, R.K.H. Galvão, M.C.U. Araújo, E.C. Silva, J. Chem. Inf. Comp. Sci. 43 (2003) 1725.
[22] E. Presteni, S. Toso, P.G. Daniele, V. Zelano, M. Ginepro, Anal. Chim. Acta 507 (2004) 263.

[23] P.B.L. Constant, P.C. Stringheta, D. Sandi, B. Ceppa 20 (2002) 203.
[24] P.H. Março, R.J. Poppi, I.S. Scarmínio, Quím. Nova 31 (2008) 1218.
[25] M.S. Cortes, L.A. Ramos, E.T.G. Cavalheiro, Quím. Nova 30 (2007) 1014.
[26] P.H. Março, M.A.B. Levi, I.S. Scarmínio, R.J. Poppi, M.G. Trevisan, Anal. Sci. 21 (2005) 1523.
[27] D.A. Skoog, D.M. West, F.J. Holler, Fundamentals of Analytical Chemistry, Seventh ed., Saunders College Publishing, Philadelphia, 1996.
[28] R.E. Bruns, I.S. Scarmínio, B.B. Neto, Statistical Design – Chemometrics, Elsevier, Amsterdam, 2006.