Food Chemistry 82 (2003) 637–643

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Comparative spectrophotometric determination of the total iron content in various white and red Greek wines

Kyriakos A. Riganakos[a,*], Panayiotis G. Veltsistas[b]

[a]*Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece*
[b]*Laboratory of Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece*

Received 15 February 2002; received in revised form 6 January 2003; accepted 13 February 2003

Abstract

Iron content in a satisfactory number of bottled and non-bottled white, rosé and red Greek wines has been determined in this work. The wines analyzed were from various regions of Greece. Iron content was determined using two modified spectrophotometric methods. The classical method of Fe(III)-thiocyanate complex formation has been properly modified to work in a sulfuric acid medium, and in the presence of potassium persulfate ($K_2S_2O_8$) as an oxidant. The second method, involved the use of the compound 2-(5-nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)-amino]-phenol disodium salt (Nitro-PAPS, $C_{17}H_{19}O_6N_5SNa_2$), known for its highly sensitive reaction with Fe(II). The experimental results of the two methods were found to be in good agreement with those obtained using the Atomic Absorption Spectroscopy (AAS) method. The proposed methods are rapid, accurate, simple, relatively inexpensive and can be used for all types of wine (white, rosé and red). Therefore, they may be used for the iron assay in routine wine analyses, as an alternative to the AAS method. The values of iron content determined in the Greek white, rosé and red wines were in the range 1.00 to 10.00 mg/l.

Keywords: Wine; Total iron; Spectrophotometric method; Atomic Absorption Spectroscopic method

---

1. Introduction

The quantity of inorganic ions in wine is of great interest, because of their influence on wine quality as well as their toxic effects. Their presence is attributed, obviously, either to the raw material (must) and/or to the processing (Gonzalez-Hernandez, de la Torre, & Arias Leon, 1996).

The establishment of the cation profile of a wine may reveal the region and conditions of cultivation of grapes, as well as the manufacturing fabrications used. On the other hand, the excessive use of fertilizers with the new manufacturing and storage techniques of wines are two of the principal sources, which elevate contents of certain metallic species. Although at the end of alcoholic fermentation there is a significant reduction of mineral content, it may not be enough to prevent some problems related to wine stability, namely precipitation and changes in wine organoleptical properties (de Campos Costa, Cardoso, & Nova Araujo, 2000). Therefore, the determination of some critical metallic species, such as iron becomes important.

Iron is usually present in musts and wines at a concentration range varying from 0.5 up to 20 mg/l. Normally, iron levels in grapes are low, even when grown on high iron soils. If no contamination occurs, a typical must has from 1 to 5 mg/l of iron (Amerine & Ough, 1974). Evaluation of Fe content in wines is of major importance either due to the changes in stability it may cause and to its effects on the oxidation and wine aging. Iron concentrations above 10 mg/l result in turbidity and colour changes adversely affecting the organoleptic properties of the wine.

The natural content of iron in the must varies in relation to its quantities present in the soil and in the bloom, which usually covers the grapes. Other possible sources of iron contamination in wine include grape harvesting, transporting, fermenting, storing in tanks, etc. Thus iron content of wine may sometimes exceed 20 mg/l. On the other hand, there may be significant losses

---

* Corresponding author. Tel.: +30-651-98341; fax: +30-651-98795.

E-mail address: kriganak@cc.uoi.gr (K.A. Riganakos).

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00120-1

in iron content during the manufacturing process, as a result of its utilization by the yeasts. The importance of iron in wine production is largely due to the fact that at concentrations higher than 8–10 mg/l may result in fermentation failure or colour deteriorates (Garcia-Jahres, Lage-Yusty, & Simal-Lozano, 1990). Discrepancies in the iron content may be attributed to unpredictable technical vinification parameters, such as soil contamination, secondary fermentation processes and storage tanks. The highest permissible concentration of iron in table wines set by the official national legislation has been determined up to 10 mg/l.

Most of the iron content in wines is present in the ferrous state. The $[Fe^{3+}]$ to $[Fe^{2+}]$ ratio depends on the oxidation prehistory of the wine, with the ferrous form predominating at low oxygen levels (Zoecklein, Fugelsang, Gump, & Nury, 1995).

The determination of iron content in wines has so far relied on numerous Colorimetric, Spectrophotometric Methods (Amerine & Ough, 1974; Garcia-Jahres, Lage-Yusty, & Simal-Lozano, 1990; Lazaro, Luque de Castro, & Valcarel, 1989; Meredith, Baldwin, & Andreasen, 1970; Ribereau-Gayon, & Peynaud, 1962; Salinas, Galeano Diaz, & Jimenez Sanchez, 1987; Salinas, Jimenez Sanchez, & Galeano Diaz, 1986), AAS (AOAC, 1998; Lucan, Palic, Vahcic, & Gacic, 1992; Ough, Crowell, & Benz, 1982; Soulis, Arvanitoyannis, & Kavlentis, 1989), Adsorptive Stripping Voltametry (Mannino & Brambilla, 1992; Wang & Mannino, 1989), Differential-pulse Polarography (Vazquez Diaz, Jimenez Sanchez, Callejon Mochon, & Guiraum Perez, 1994), Ion-exchange chromatography-FAAS (Ajlec & Stupar, 1989), HPLC with Electrochemical and FAAS detection (Weber, 1991), SIA-FAAS (de Campos Costa, Cardoso, & Nova Araujo, 2000; de Campos Costa & Nova Araujo, 2001), etc.

In the present study the total iron content of a number of white, rosé and red Greek wines was determined using two modified spectrophotometric UV-Visible methods: (1) the extraction of iron (III)–thiocyanate complex from a medium of high acidity into ethyl acetate and (2) a novel sensitive method, based on the complexation reaction of Fe(II) with 2-(5-nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)-amino]-phenol disodium salt (Nitro-PAPS, $C_{17}H_{19}O_6N_5SNa_2$). Atomic absorption spectrophotometry (AAS) was used as a standard reference method for comparison purposes.

2. Materials and methods

2.1. Materials

The bottled wines (white, rosé and red) were purchased by local super-markets and wineries. The non-bottled wines were donated from small house wineries from various regions of Greece. All samples were stored in glass bottles and kept at room temperature until analyzed.

2.2. Reagents

All chemicals used were of analytical reagent grade. Nitro-PAPS was purchased from Dojindo, Japan. The water used throughout this work was doubly distilled water, obtained using a borosilicate glassware apparatus.

2.3. Stock solutions

An iron stock solution (1000 ppm) was prepared by dissolving 1.000 g of very pure iron metal wire (J.T. Baker, 99.90% purity) in aqua regia. The solution was then evaporated several times on a hot plate, adding small portions of H_2SO_4, until complete elimination of any trace of nitric and hydrochloric acids (white fumes). Sulfuric acid (5 ml) was then added and finally the solution made up to 1 l with water (DDW). From this stock solution, fresh working standards were prepared in the range 1–10 mg Fe/l, by proper dilutions for all the subsequent iron analyses.

2.4. Apparatus

Absorbance values were measured for all the reference standards and the unknown samples of white, rosé and red wines. Calibration graphs of absorbance versus iron concentration were then plotted and subsequently used for the determination of iron in wine samples. The following instruments were used for UV-Visible and AAS spectrophotometric analytical procedures: (1) UV-visible spectro-photometer, Secomam, model Anthelie Advanced, France, (2) Fiber Optic CCD spectrophotometer, model S-2000, Ocean Optics Inc., USA, and (3) Flame Atomic Absorption Spectrophotometer, Perkin-Elmer, model 560, USA.

2.5. Methods

2.5.1. Iron spectrophotometric assay using the modified thiocyanate method

Iron (III) reacts with excess of thiocyanates to give a series of soluble and intensively blood-red coloured complex compounds (Basset, Denney, Hjeffery, & Mendham, 1978). The characteristic red colour of these solutions is attributed to the formation of acidic form of the hexa-pseudohalogenic complex compound $[Fe(SCN)_6]H_3$.

This method permits the determination of the total Fe(II) and Fe(III), since $K_2S_2O_8$ which acts as an oxidant, quantitatively transforms all the iron to the Fe(III) state.

The benefits from the use of persulfates as an oxidant are well known and have been proposed (Lange, 1956). The use of HCl–H_2O_2 should be avoided, since the produced free halogen (Cl_2), oxidizes thiocyanate to sulfate ions and the red colour in the organic phase slowly deteriorates. Ethyl acetate has proven to be a suitable alternative extraction medium used for the determination of the total iron content in both white and red wines, among those tested in this study as well as those reported in the literature so far (Garcia-Jahres, Lage-Yusty & Simal-Lozano, 1990; Pehkonen, Erel, & Hoffmann, 1992; Ribereau-Gayon & Peynaud, 1962).

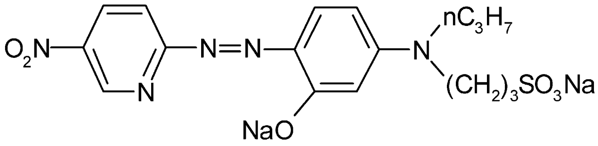
In a typical experimental procedure for the direct determination of total iron content in wines, a 5-ml sample was inserted in specially screw-stoppered vials and the following reagents were added in sequence: 1 ml H_2SO_4 (10% v/v), 1 ml solution of KSCN (50% w/v) and 1 ml of $K_2S_2O_8$ (1% w/v). Finally, 5.0 ml of ethyl acetate were added and the vials were vigorously shaken using a Vortex mixer for 1 min. The biphasic system was allowed to stand for 5 min, to separate the phases in two transparent layers. The red colour complex was entrained in the organic solvent. Portions of 3 ml of the upper organic layer were transferred into a measuring quartz cuvette (1 cm pathlength), after filtering through a Whatman No. 42 ashless paper. The filtrates were absolutely clear and transparent, since any aqueous drops were held on the porous paper. The absorbance was measured at 495 nm, against a reagent blank.

Vials were properly screw-cap stoppered to prevent the evaporation of the solvent due to vapour fugacity, especially during long storage.

No serious turbidity was observed during this procedure in the vials of the reference standards and also in the probes to be analyzed. This phenomenon (cloudiness) under other experimental conditions could result in significant drawbacks. It is mainly due to the partial separation of the two phases. A large number of small water droplets are slowly migrating downward to the layer interface.

2.5.2. *Iron spectrophotometric assay using the Nitro-PAPS method*

The Nitro-PAPS reagent (Makino, Kiyonaga & Kina, 1988), [2-(5-nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)-amino]-phenol, disodium salt, dihydrate, with the following structural formula,



has been recently introduced as a highly sensitive colorimetric reagent for Fe(II), by forming the 1:2 water-soluble complex $[Fe(C_{17}H_{19}N_5O_6S)_2]Na_4$ at a pH range 3.0–8.0, with $\varepsilon = 107.000$ at $\lambda_{max} = 582$ nm. The Fe(II)-Nitro-PAPS complexation reaction proceeds according to the following quantitative and stoichiometric reaction (1).

$$2C_{17}H_{19}O_6N_5SNa_2 + FeSO_4 \rightarrow [Fe(C_{17}H_{19}N_5O_6S)_2]Na_2 + Na_2SO_4 \qquad (1)$$

Wine samples (1.00 ml) were transferred to the reaction tube with a pipette, and the following reagents were also added in sequence: 5 ml of distilled water, 1 ml of NaN_3 solution (1.0 M), 1 ml of ascorbic acid (1.0 M) and 2 ml of Nitro-PAPS solution (1×10^{-4} M). The wine samples were then further diluted (by a factor of 10), in order to determine total iron content within the linear range 100–600 ppb, thus achieving simultaneously a prompt and quantitative reduction of any interference, caused by those cations, coexisting in the iron samples to be analyzed. After 10 min, the absorbance of the coloured complex was measured at 582 nm, against a reagent blank, by using a Fiber Optic CCD spectrophotometer set up.

2.5.3. *Atomic Absorption Spectrophotometry (AAS)*

For the iron determination using the Perkin-Elmer, model 560, Flame Atomic Absorption Spectrophotometer, the following settings were selected: absorption line at 248.0 nm, slit-width 0.2 nm, sensitivity 0.12 ppm ($A = 1\%$) and air-acetylene flame mode. Total iron content measurements were conducted by two different ways: (a) directly, by measuring iron in the red and white wine samples, without any preliminary pre-treatment and (b) after digestion with a $HClO_4$/HNO_3 mixture.

2.6. *Statistical analysis*

All determinations were carried out five times and reported values represent the mean average of these five values ($n = 5$).

Paired *t*-test and one-way ANOVA treatments were used to determine if there are any significant differences between the means of the three analytical methods.

3. Results and discussion

3.1. *Thiocyanate method*

A series of absorption spectra for various Fe(III) concentrations are presented (Fig. 1). Such spectra can be obtained for white, rosé and red wines, as only the iron complex is quantitatively extracted in the organic phase, while the natural wine red colour due to flavonoid anthocyanines, remains in the aqueous layer. This

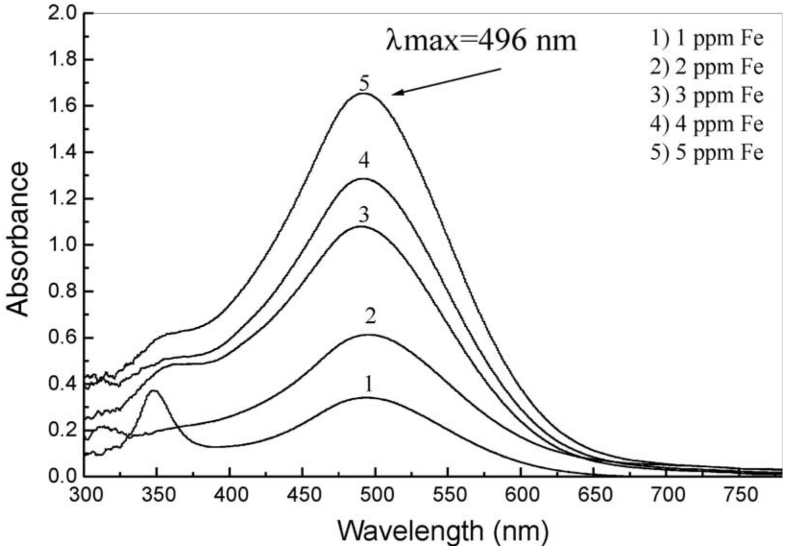


Fig. 1. Absorption spectra for the thiocyanate ethyl acetate extraction method for various iron concentrations.

enables a simple and convenient routine colorimetric determination of iron content.

The linear calibration of the proposed thiocyanate modified method, was obtained in the range 1–5 ppm Fe(III), following the equation: $A = 0.238(\pm 0.007) \times C(\text{ppm}) - 0.009(\pm 0.023)$, with a correlation coefficient $r^2 = 0.9996$ and reproducibility 0.50% ($n = 5$). A denotes absolute absorbance units and C the Fe(III) concentration in ppm.

The linear calibration represents the classical thiocyanate method in the absence of any extraction reagent medium. In this case the absorbance of the active species $[Fe(SCN)_6]H_3$ was measured at 480 nm. The calibration curve obtained is linear, obeying the equation $A = 0.112(\pm 0.005) \times C(\text{ppm}) - 0.008(\pm 0.020)$ with a correlation coefficient $r^2 = 0.9999$ and reproducibility 0.40% ($n = 5$). Therefore, the sensitivity of the modified extraction method is approximately three times higher than the classical method which operates exclusively in aqueous media. Nevertheless, the classical method remains in use, but is limited to iron determination in white wines, while the proposed modified thiocyanate method can be used for all types of wine.

3.2. Nitro-PAPS method

The spectra obtained with this method for various iron concentrations are shown (Fig. 2). Beer's law is obeyed in the range 100–600 ppb Fe(II), using the mathematical equation $A = 0.0015(\pm 0.0002) \times C(\text{ppb})$, with a correlation coefficient $r^2 = 1.0000$ and an R.S.D. = 0.38% ($n = 5$). Consequently, Nitro-PAPS proved to be a very suitable reagent for the micro-determination of iron in all types of wine (white, rosé and red tested).

The main interferences with this complex formation could arise from the presence of any of the following

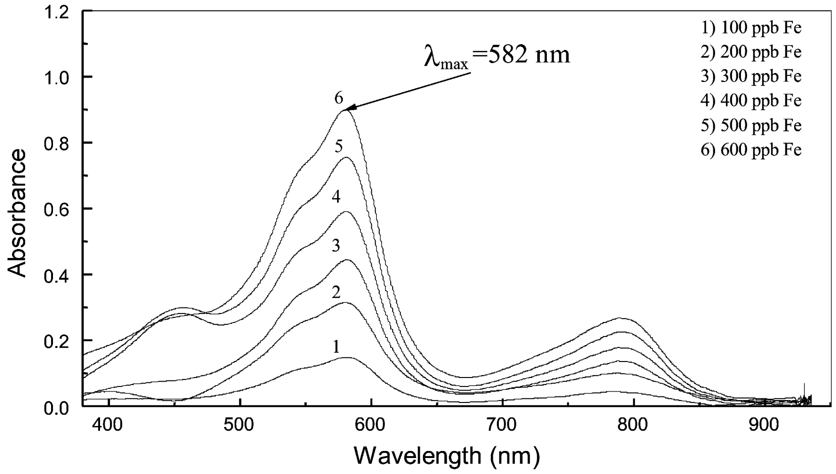


Fig. 2. Absorption spectra of the reaction product of Nitro-PAPS with Fe(II) for various iron concentrations.

metals in solution: Cu(II); (pH 2.0–5.0), Ni(II); (pH 4.5–9.0), Co(II); (pH 3.0–7.5) and Zn(II); (pH 7.5–9.0). The operational pH range for each metal is given in brackets.

Actually, in real wine samples Ni(II) and Co(II) cations are normally not found. Among several buffer solutions tested, the most suitable buffer-reducing agent was NaN_3-ascorbic acid (0.10 M each), prepared by mixing these two solutions at pH 6.0. Using this buffer system, the following benefits could be achieved simultaneously: (a) quantitative reduction of all the iron content to the Fe(II) state, (b) quantitative reduction of all the copper content to the inactive Cu(I) form, which gives no colour reaction with Nitro-PAPS and finally (c) complete inactivation of the Zn(II) cations, due to their strong complexation with the constituents of the buffer.

The iron concentrations determined for 36 bottled and non-bottled Greek wines, by applying the three described analytical approaches are tabulated along with their corresponding values of relative standard deviation in Table 1. Type, origin, colour and bottling year are also included in Table 1.

Values of the total iron content measured in the Greek wines are in good agreement and comply with iron levels, reported in the literature for similar wines produced in other countries (Cox, Eitenmiller, & Powers, 1977; Lazos & Alexakis, 1989; Meredith, Baldwin, & Andreasen 1970; Ough, Crowell, & Benz 1982; Siegmund & Bachmann, 1977).

It can be stated that the iron content in wines decreases according to the following order: red, rosé and white wines. This is probably due to the unique must fermentation of red wines, in which the grape skins, which contain large quantities of iron, are also present.

Table 1
Iron content in Greek wines, using three different analytical methods

Wine samples	FAAS method (ppm)	Thiocyanate method (ppm)	Nitro-PAPS method (ppm)
1. Attica (Savvatiano) (W) 1999	4.60±0.02[a]	4.75±0.08[a]	4.80±0.03[a]
2. Attica (Kourtakis) (W) 2000	6.20±0.05	6.12±0.10	6.16±0.11
3. Zitsa (Ambelones) (W) 1999	1.83±0.01	1.87±0.02	1.75±0.03
4. Zitsa (Balthasar) (W) 1999	4.71±0.02	4.75±0.04	4.62±0.06
5. Zitsa (Primus) (W) 1999	2.69±0.04	2.70±0.01	2.65±0.03
6. Zitsa (Zitsa) (W) 1999	4.17±0.02	3.99±0.08	4.00±0.03
7. Zitsa (1000 Pearls) (W) 1999	3.99±0.03	3.82±0.09	3.92±0.02
8. Halkidiki (Makedonikos) (W) 1998	1.55±0.07	1.61±0.08	1.51±0.04
9. Thessalia (Thessalikos) (W) 2000	0.73±0.01	0.84±0.04	0.76±0.05
10. Patra (Demestica) (W) 2000	8.50±0.06	8.74±0.08	8.65±0.10
11.Mantinia (Moschofélero) (W) 1999	2.09±0.03	2.17±0.03	1.97±0.01
12. Limnos (Limnos) (W) 1999	1.62±0.03	1.65±0.07	1.60±0.03
13. Santorini (Nichteri) (W) 1999	2.56±0.03	2.53±0.03	2.49±0.07
14. Rhodes (Ilios) (W) 1998	1.40±0.03	1.36±0.03	1.39±0.01
15. Crete (Kritikos) (W) 2000	1.62±0.02	1.65±0.04	1.60±0.04
16. Zakynthos (Verdea) (W) 1996	5.40±0.03	5.37±0.08	5.28±0.05
17. Attica (Kokkineli) (RO) 2000	7.93±0.08	7.98±0.10	8.01±0.10
18. Zitsa (Red Velvet) (R) 1997	4.30±0.08	4.40±0.03	4.27±0.04
19. Ioannina (Agiorgitikos) (R) 1998	1.37±0.04	1.40±0.03	1.36±0.04
20. Metsovo (Katogi) (R) 1997	5.36±0.02	5.29±0.05	5.20±0.03
21. Naoussa (Naoussa) (R) 1998	3.80±0.06	3.79±0.02	3.72±0.02
22. Goumenissa (Goumenissa) (R) 1998	2.17±0.02	2.20±0.08	2.20±0.04
23. Patra (Danielis) (R) 1996	9.68±0.08	9.38±0.06	9.62±0.02
24. Patra (Mavrodafni) (R) 1997	6.34±0.06	6.58±0.10	6.45±0.03
25. Nemea (Agiorgitikos) (R) 1997	5.14±0.06	5.04±0.07	5.00±0.07
26. Paros (Paros) (R) 1996	2.13±0.04	2.22±0.05	2.13±0.08
27. Crete (Kritikos) (R) 1999	3.87±0.03	4.20±0.12	3.96±0.04
28. Crete (Archanes) (R) 1998	6.20±0.04	6.38±0.12	6.24±0.03
29. Attica (Moschato) (W) 1999	3.35±0.03	3.38±0.03	3.28±0.08
30. Attica (Retsina) (W) 1999	7.18±0.12	7.14±0.05	7.24±0.05
31. Zitsa (Debina) (W) 1999	6.68±0.05	6.58±0.05	6.54±0.04
32. Zitsa (Local) (W) 1999	5.30±0.06	5.60±0.09	5.37±0.07
33. Attica (Roditis) (RO) 1999	4.04±0.04	4.01±0.04	4.10±0.06
34. Zitsa (Local) (RO) 1999	4.96±0.02	5.10±0.10	5.00±0.04
35. Attica (Brousko) (R) 1999	8.72±0.14	8.68±0.11	8.56±0.10
36. Zitsa (Local) (R) 1998	5.30±0.09	5.60±0.08	5.37±0.01

General description:(1–28) (B = Bottled), (29–36) (NB = Non-Bottled), (1–16, 29–32) (W = White), (17, 33–34) (RO = Rosé), (19–28, 35–36) (R = Red).

[a] Mean ± standard deviation (S.D., $n = 5$).

Paired t-test treatment between the standard method (FAAS) and the two proposed methods (thiocyanate and Nitro-PAPS) for a number of real samples $N=36$, showed no significant differences between them, at the confidence level 95%. In addition, paired t-test treatment revealed no statistical deviation between the mean values of the three methods, since the theoretical value of t is greater than the calculated value of t. Further confirmatory application of one-way ANOVA treatment between the three methods this time, revealed no significant differences between them, at the confidence level 95%.

Approximately 75% of the values determined, comply with the general limitations (maximum, 5 ppm), according to the criteria set by the official national legislation for table wines. Since the total iron content in the wines examined was found to be bellow 10 ppm, there should be no risk of any cloudiness, or lack of stability of the wines.

A general observation is that, the values of iron of bottled wines are significantly lower, compared with non-bottled ones. This is mainly attributed to the following reasons: (a) the removal of a part of iron during wine processing, following the alcoholic fermentation and (b) the special treatment that is applied during the vinification procedure.

4. Conclusions

Iron content of selected Greek wines obtained using the two proposed spectrophotometric methods compared satisfactorily to those received by the standard AAS method. These are rapid, accurate, simple, relatively inexpensive, can be used for all types of commercial wine (white, rosé and red), and most importantly do not require special equipment or highly trained personnel. Also, paired t-test one-way ANOVA treatments showed no significant differences among the three methods.

The modified thiocyanate extraction method can be successfully proposed for the total iron assay in routine wine analyses, such as local small capacity wineries as well as for educational and exhibitional purposes with satisfactory repeatability and accuracy.

The Nitro-PAPS method shows a very high sensitivity, accuracy and precision.

The modified thiocyanate and Nitro-PAPS methods may, therefore, be recommended as alternatives to the AAS method.

References

Ajlec, R., & Stupar, J. (1989). Determination of iron species in wine by ion-exchange chromatography-flame atomic absorption spectrometry. *Analyst*, *114*, 137–142.

Amerine, M. A., & Ough, C. S. (1974). *Wine and must analysis*. New York: Wiley.

AOAC. (1998). *Official methods of analysis, "Iron in distilled liquors"* (16th ed.). Maryland: Gaithersburg.

Basset, J., Denney, R. C., Hjeffery, G., Mendham, J. (Eds.). (1978). *Vogel's textbook of quantitative inorganic analysis,* 4th ed. London: Longman.

Cox, R. J., Eitenmiller, R. R., & Powers, J. J. (1977). Mineral contents of some California wines. *Journal of Food Science*, *42*(3), 849–850.

De Campos Costa, R. C., Cardoso, M. I., & Nova Araujo, A. (2000). Metals determination in wines by sequential injection analysis with flame atomic absorption spectrometry. *American Journal Enology and Viticulture*, *51*, 131–136.

De Campos Costa, R. C., & Nova Araujo, A. (2001). Determination of Fe(III) and total Fe in wines by sequential injection analysis and flame atomic absorption spectrometry. *Analytica Chimica Acta*, *438*, 227–233.

Garcia-Jahres, C. M., Lage-Yusty, M. A., & Simal-Lozano, J. (1990). Second derivative visible spectroscopic determination of iron and manganese in Galician wines. *Fresenius' Journal of Analytical Chemistry*, *338*, 703–706.

Gonzalez-Hernandez, G., Hardisson de la Torre, A., & Arias Leon, J. J. (1996). Quantity of K, Ca, Na, Mg, Fe, Cu, Pb, Zn and ashes in DOC Tacoronte-Acentejo (Canary Islands, Spain) musts and wines. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *203*, 517–521.

Lazaro, F., Luque de Castro, M. D., & Valcarel, M. (1989). Intergrated retention/spectro-photometric detection in flow-injection analysis. Determination of iron in water and wine. *Analytica Chimica Acta*, *219*, 231–238.

Lange, B. (1956). *Kolorimetrische Analyse, 5 auf*. Weinheim: Verlag Chemie.

Lazos, E. S., & Alexakis, A. (1989). Metal ion content of some Greek wines. *International Journal of Food Science and Technology*, *24*, 39–46.

Lucan, Z., Palic, A., Vahcic, N., & Gacic, M. (1992). Copper and iron content in wines. *Rivista di Viticoltura e di Enologia*, *4*, 29–33.

Makino, T., Kiyonaga, M., & Kina, K. (1988). A sensitive, direct colorimetric assay of serum iron using the chromogen, nitro-PAPS. *Clinica Chimica Acta*, *171*(1), 19–27.

Mannino, S., & Brambilla, M. (1992). Determination of iron(III) and total iron in wines by constant current potentiometric stripping analysis. *Italian Journal of Food Science*, *1*, 47–51.

Meredith, M. K., Baldwin, S., & Andreasen, A. A. (1970). Determination of iron in alcoholic beverages. *Journal of the Association of Official Analytical Chemists International*, *53*(1), 12–16.

Ough, C. S., Crowell, E. A., & Benz, J. (1982). Metal content of California wines. *Journal of Food Science*, *47*, 825–828.

Pehkonen, S., Erd, Y., & Hoffmann, M.R. (1992). Simultaneous spectrophotometric measurement of iron (II) and iron (III) in atmospheric water. *Environmental Science and Technology*, *26*(9), 1731–1736.

Ribereau-Gayon, J., & Peynaud, E. (1962). *Analisis de vinos*. Madrid: Aguilar.

Salinas, F., Jimenez Sanchez, J. C., & Galeano Diaz, T. (1986). Spectrophotometric determination of iron in wines, foods, and minerals with 5,5-dimethyl-1,2,3-cyclohenetrione 1,2-dioxime 3-thiosemicarbazone. *Analytical Chemistry*, *58*, 824–827.

Salinas, F., Galeano Diaz, T., & Jimenez Sanchez, J. C. (1987). Spectrophotometric determination of iron by extraction of the iron(II)-5,5-dimethyl-1,2,3-cyclohenetrione 1,2-dioxime 3-thiosemicarbazone complex. *Talanta*, *34*(7), 655–656.

Siegmund, H., & Bachmann, H. (1977). The assignment of wines to location by determination of trace element patterns. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *164*(1), 1–7.

Soulis, Th., Arvanitoyannis, I., & Kavlentis, E. (1989). Iron, copper, manganese and zinc contents of some bottled and non-bottled Greek wines. *Sciences des Alimentes*, *9*, 799–803.

Vazquez Diaz, M. E., Jimenez Sanchez, J. C., Callejon Mochon, X., & Guiraum Perez, M. A. (1994). Differential-pulse polarographic determination of iron in acids, waters, fruit juices and wines. *Analyst*, *119*, 1571–1574.

Wang, J., & Mannino, S. (1989). Application of adsorptive stripping voltametry to the speciation and determination or iron (III) and total iron in wines. *Analyst*, *114*, 643–645.

Weber, G. (1991). Speciation of iron using HPLC with electrochemical and flame-AAS detection. *Fresenius' Journal of Analytical Chemistry*, *340*, 161–165.

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., Nury, F. S. (Eds.). (1995). *Wine analysis and production*. New York: Chapman and Hall.