Talanta 96 (2012) 102–106

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of total protein content in white wines by solid phase spectrometry in a SI–LOV system

Susana S.M.P. Vidigal[a], Ildikó V. Tóth[b], António O.S.S. Rangel[a,*]

[a] CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[b] REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4090-030 Porto, Portugal

ARTICLE INFO

Article history:
Received 23 September 2011
Received in revised form 9 December 2011
Accepted 13 December 2011
Available online 19 December 2011

Keywords:
Solid phase spectrometry
Sequential injection lab-on-valve
Bead injection
Total protein content
White wine

ABSTRACT

Although present at low concentration in wine samples, proteins, have considerable technological importance, due to their capability of haze formation. The present work presents a methodology for the quantification of total protein in white wine in a sequential injection lab-on-valve system, exploiting the bead injection concept for solid phase extraction with spectrophotometric detection. The method is based on the retention of the proteins in the solid support, NTA (nitrilotriacetic acid) superflow beads, charged by Cu^{2+}. The change in the absorbance is monitored at 500 nm at the surface of the beads after addition of the Folin–Ciocalteu's reagent (FCr).

The developed method presented a sample consumption of 400 μL per assay and a consumption of FCr and Cu^{2+} solution of 25 μL and 100 μL per assay, respectively. It was possible to achieve a linear range up to 0.30 g/L with a limit of detection and quantification of 0.03 and 0.10 g/L, respectively. The proposed method was successfully applied to white wine samples.

1. Introduction

Quality is the main concern in winemaking, and is dependent upon various organoleptic properties, including also colour and clarity. These characteristics are extremely important as they can influence the product's image, consequently its acceptance by the consumer. In this context, proteins have an important role. The total protein content has been considered as a mixture of grape proteins and proteins from autolysed yeast. These proteins can show a wide range of characteristics: have molecular mass ranging from 9 to 66 kDa and isoelectric points ranging from 3 to 9 [1] including thaumatin-like proteins (TLP) and chitinases as predominant proteins of white wine. Therefore, the total protein content alone can be considered as a poor index of the tendency for the wine to become cloudy; on the other hand, the nature of the proteins actually responsible for the turbidity in wine remains uncertain, even though their presence is a prerequisite for haze formation. Several studies consider that if the total protein content is high, the tendency for the wine to become unstable also increases [2,3]. However, other authors [1,3,4] reported that the haze formation depends on the presence of specific proteins, TLP and chitinases.

Proteins are usually present in low concentrations in finished wines and their levels differ by variety. For total protein in white wine, Food Composition and Nutrition Tables [5] present a range from 0.08 to 0.29 g/L; other authors present a wider range from 0.01 to 0.50 g/L [6]. The reference method is based on the total nitrogen measurement. The recommended Kjeldahl method measures the total nitrogen in the sample, not only the protein nitrogen; it is time consuming, involves corrosive reagents and needs a relatively large amount of protein [7]. Therefore, the protein content is not obtained directly but rather obtained by calculations, leading to frequent overestimation [8]. Other methods used for wine protein quantification can be based on spectrophotometric detection such as the Bradford method [9,10]. These methods are simple and fast but do not provide an accurate result, considering the response variation to different proteins and due to the absence of a commercial standard of wine proteins. To cope with this problem bovine serum albumin (BSA) is accepted as an alternative standard and is usually used for total protein studies, since it presents a haze formation capacity similar to the major wine proteins (TLP and chitinase) [3]. Other colorimetric methods such as the Lowry [11], Biuret [12] or the Smith [13] test can provide good results; however, the results obtained are not always comparable within these methods due to the presence of a large number of wine components; in this particular determination, it is important to consider the possible interference of phenolic compounds [11,14–16]. Phenolic compounds are crucial components in wine that can react with the Folin–Ciocalteu's reagent (FCr) at alkaline pH, resulting in a false positive response [14,16]. Nevertheless, since this method is about 100 times more sensitive than the Buriet test, and about 5

* Corresponding author. Tel.: +351 225580064; fax: +351 225090351.
E-mail address: arangel@porto.ucp.pt (A.O.S.S. Rangel).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.12.028

times more sensitive than the Bradford test [17], it can be carried out if the interference of the phenolic compounds is neutralised [7]. With the aim of reducing the interferences, some protein quantification methods are based on a prior isolation, usually by gel filtration (Sephadex G-25) [8].

Solid phase extraction (SPE) can be an attractive alternative to the above-mentioned problem, because it might discard interfering agents. In the traditional SPE concept, the detection occurs in the eluted phase; however there is a partial loss of the preconcentration capabilities gained in the sorption step. To enhance sensitivity, solid phase spectrometry (SPS) [18] was developed; the target analyte is sorbed on the surface of the solid phase and the light attenuation of the adsorbent particles packed in the optical cell is measured without elution step. This procedure can be carried out by bead injection (BI) approach in flow-based system. It is well known that BI can be easily performed in the SI–LOV format in an efficient way. In this scenario, a methodology for the quantification of total protein content in white wine using a SI–LOV system in a BI mode was developed. In this work, the beads chosen as solid support were the commercially available NTA (nitrilotriacetic acid) superflow resin. This resin comprises nitrilotriacetic acid covalently bound to a highly crossed-linked agarose surface, that can be charged with several metal ions (e.g., Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}), and is applicable in a wide pH range (3–12). This resin was originally designed for high throughput sample clean-up procedures based on the affinity chromatography concept, and was recently applied for the preconcentration of total iron in wine samples [19] and sea [20,21] and river [22] water samples. Taking into consideration that NTA is potentially a tetradentate ligand, it might bind metal ions more tightly than tridentate ligands used for the same purposes, which can be advantageous to employ in the Lowry method. The Lowry method can be divided [7] in two steps: protein reaction with copper followed by the reduction of FCr. However, the exact role of the copper is not very clear. Some authors reported that Cu^{2+} binds the protein and this process results in the reduction to Cu(I) that then reacts with the FCr [23]; others claim that the proteins reduce the reagent, whereby copper chelates the peptides structure and facilitates electron transfer from the protein to the reagent [7].

In the present work, the classical Lowry method was applied to the bead injection SI–LOV format. First the Cu^{2+} is bound by the NTA [24] at the surface of the beads. The beads charged by Cu^{2+}retain the protein in the form of a chelate, as the sample solution passes though the beads column. When the FCr is added to the copper-protein retained on the solid support, the reduction of the FCr occurs and an increase of the colour intensity can be spectrophotometrically monitored at 500 nm.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared from analytical grade reagents, and deionised water (conductivity <0.1 μS cm^{-1}) was used throughout the work.

The 100 mM copper solution was prepared from $CuSO_4{\cdot}5H_2O$. The FCr solution was a dilution of 1/10 (v/v) from the commercial product (Folin–Ciocalteu's phenol reagent, 47641, Fluka) in deionised water. To daily prepare the working standards solutions, 1 g/L of BSA (fraction V, 05484, Fluka) was prepared in NaCl 0.15 M, and this solution was further diluted in water.

The bead suspension used was a dilution in water to half (w/w) of the commercial stock solution (NTA Superflow resin, highly cross-linked 6% agarose, 60–160 μm of bead diameter, 50% suspension in 30% ethanol, 30510, Qiagen).

Table 1
Flow protocol sequence of the developed SI–BI–LOV method.

Step	Description	Volume (μL)	Flow rate (μL/s)	Selection valve position
A	Aspirate carrier to SP from reservoir	1500	250	–
B	Aspirate beads suspension to HC	40	20	5
C	Propel beads suspension to FC	80	10	2
D	Aspirate Cu^{2+} solution to HC	100	50	3
E	Propel Cu^{2+}solution to FC	500	9	2
F	Aspirate protein sample to HC	400	50	4
G	Propel protein sample to FC	700	9	2
H	Aspirate FCr to HC	25	50	6
I	Reference scan, Absorbance scanning	–	–	–
J	Propel FCr to FC	250	6	2
K	Aspirate beads from FC, by reversed flow	200	300	2
L	Dispense beads to waste	Empty SP	300	1

2.2. Samples

The analysed samples, white wine, sparkling wine and beer were purchased in a local market. Before its introduction in the system, the samples pH was adjusted to 5.5. No other pre-treatment was required with the exception of the beer sample that was 5 times diluted.

2.3. Apparatus

The SI–LOV system (FIAlab-3500, FIAlab Instruments, Medina, WA, USA) presented in Fig. 1, consisting of a bi-directional syringe pump (2500 μL of volume), a holding coil, a bi-directional variable speed peristaltic pump, and a lab-on-valve manifold mounted on the top of a six-port multi-position valve, was used. As detection system, an USB 2000 Ocean Optics, charge coupled device (CCD) spectrophotometer equipped with fibre optics (FIA-P200-SR; id: 200 μm), and a DH-2000-BAL Mikropack, UV/vis/NIR light source, was used. FIAlab for windows 5.0 software on a personal computer was used for flow programming and data acquisition. The bead column was built between the two optical fibres (Fig. 1b) and a plug (small piece of yellow PEEK tubing, # 1536, Upchurch Scientific).

2.4. Flow procedure

The initial steps of A to C consisted in the preparation of the bead column in the flow cell, where 40 μL of the bead suspension was packed in the flow cell by 80 μL of carrier at 10 μL/s. Afterwards, 100 μL of the Cu^{2+} solution was propelled to the flow cell with 500 μL of carrier at 9 μL/s, followed by 400 μL of sample solution with 700 μL of carrier solution at a flow rate of 9 μL/s. The change in the absorbance was monitored at 500 nm while 25 μL of the diluted FCr drove though the bead column at 6 μL/s. After the measurement, the physical regeneration of the flow-through sensor was carried out by reversed flow; the beads were aspirated to the holding coil and subsequently discharged to waste. The SI–BI–LOV flow procedure is summarised in Table 1.

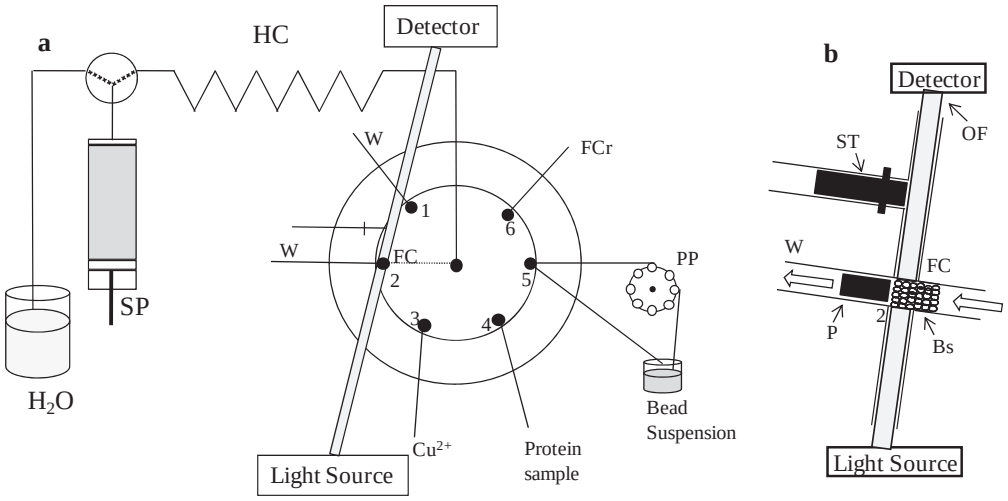

Fig. 1. (a) Configuration of the SI–BI–LOV manifold for the determination of total protein content in white wine samples. (b) Position of the bead column in the flow cell. SP, syringe pump; HC, holding coil; FC, flow cell; FCr, 25 μL; Cu^{2+}, 100 mM; W, waste; ST, PTFE stopper; P, PEEK plug; Bs, beads; OF, optical fibre.

3. Results and discussion

3.1. Study of the flow system

The preparation of the flow-through sensor was accomplished as described in the previous work [19]. Following the packing of the column, it was necessary to charge the beads present in the sensor with the copper ions in order to occupy all the possible binding sites (the breakthrough point has to be achieved). The concentration of the metal solution chosen for this study was 100 mM of Cu^{2+}, since it is the concentration recommended for this purpose by the bead manufacturer. The volume of Cu^{2+} solution was tested from 50 to 400 μL. It was possible to obtain an increase of 24% on the analytical signal with the increase of the volume from 50 to 100 μL. A further increase in the volume did not reveal a significant difference, therefore 100 μL was the chosen volume; it proved to be sufficient to charge all the beads of the flow-through sensor. The flow rate of this step was also aim of study to correlate the residence time with the efficiency of the process. Flow rates tested were 2, 4, 6 and 9 μL/s. As the results obtained were similar, 9 μL/s was the flow rate used to charge the beads with the Cu^{2+} solution.

The dilution factor of the FCr was the subsequent step of the optimisation study. The volume of FCr was set as 50 μL, and the dilution factor was varied from 0 to 20 (in deionised water). This study was carried out with 75 μL of BSA standard solution 1 g/L and the effect on the analytical signal caused by the increase on the dilution factor of the FCr was monitored at 500 nm. A dilution of 10 times of FCr was chosen since there was no loss on the repeatability and reproducibility of the results.

Afterwards, the effect of the volume and the flow rate used to propel the FCr and the protein solution on the sensitivity were studied simultaneously using a 2^4 randomised experimental design. The lowest and highest values studied were: 25 or 125 μL of FCr propelled at 4 or 9 μL/s, and 50 or 250 μL of protein solution propelled at 2 or 9 μL/s. With the results obtained for this experiment it was possible to verify that 25 μL of FCr was propelled at 9 μL/s. The volume of protein solution was the most important parameter to influence the analytical signal; therefore, this was subject of further study. The volume and the flow rate of FCr defined, the volume of protein was studied in a range from 200 to 500 μL, and the selected volume was 400 μL, since an increase of 30% on the analytical signal was found. With a further increase, no significant difference was found.

As described by Lowry et al. [11], the measurement of the reduction of the Folin–Ciocalteu's reagent by the copper-treated protein can be performed at 500 or at 750 nm. Therefore, a figure (Fig. S1) was included in the ESI where the spectral changes at the different analytical steps are recorded. It allows concluding that with the conditions defined, the sensitivity obtained at 500 nm was about 60% higher in comparison with the sensitivity obtained at 750 nm. For that reason, 500 nm was the wavelength used to register the absorbance variation throughout the analytical cycle.

The developed methodology comprises four steps: (i) load of bead column; (ii) charging the bead column with Cu^{2+}; (iii) load of protein sample; and (iv) reaction with the FCr. The next figure represents the change in the absorbance throughout these different steps. For the determination of total protein content in white wine, only the change in the absorbance of the fourth step is monitored. A reference scan is performed after loading of the protein sample and the analytical signal corresponds to the effect of the FCr in the presence of the protein. Fig. 2b also illustrates the increase of the analytical signal with the increase of the concentration of BSA.

3.2. Study of interfering species

The effect of possible interferences present in wine was evaluated by preparing solutions presenting a fixed concentration of BSA (0.20 g/L) and displaying a concentration of the suspected interferent compound normally found in finished white whites. These solutions were tested without any sample pre-treatment. The results obtained were compared using a *t*-test for two samples assuming equal variances [25], and the obtained values for t_{calc} and $t_{critical}$ are summarised in Table 2. Organic acids, sugars, glyc-

Table 2
Interfering species on the determination of total protein content in white wine.

Interfering species	Concentration tested	t_{calc}	$t_{critical}$
L(+)-Tartaric acid	4 g/L	2.73	2.77
Gallic acid	3 mg/L	0.08	2.57
Caffeic acid	2 mg/L	1.55	2.77
p-Coumaric acid	1 mg/L	0.77	2.57
Ascorcic acid	1.5 mg/L	0.10	2.77
Glycerol	10 g/L	0.18	2.77
Sugars (glucose:fructose; 40:60%)	3 g/L	2.09	2.77
Ethanol	10% (v/v)	0.63	2.45
Ca^{2+}	100 mg/L	1.07	2.77
Mg^{2+}	100 mg/L	2.56	2.77

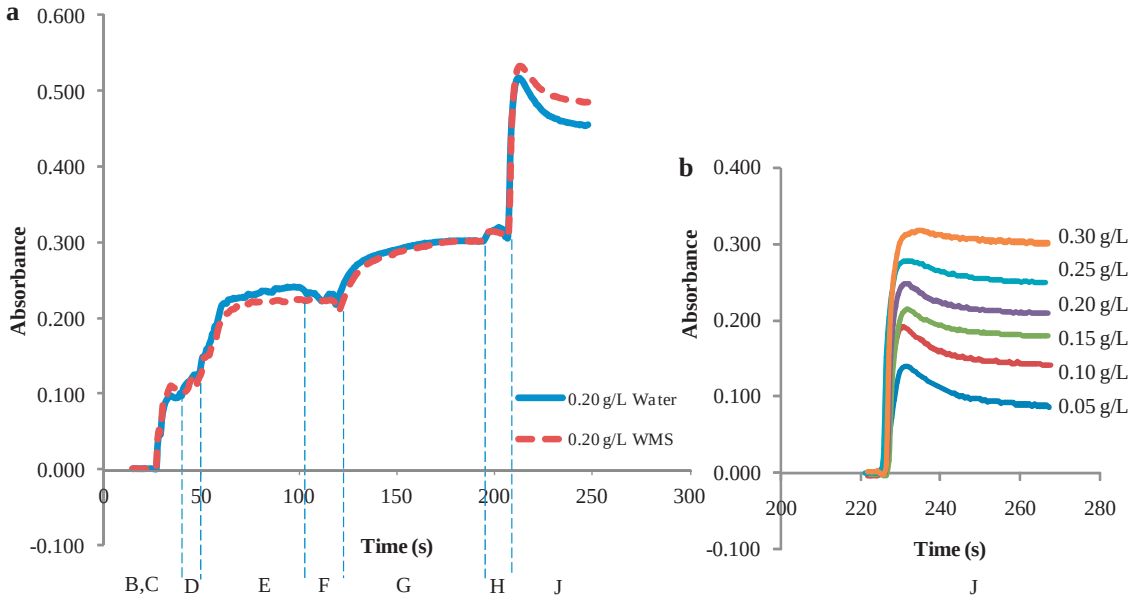

Fig. 2. Flow register of the determination of the total protein content. (a) Protein standard 0.20 g/L prepared in water and in a wine model solution, (b) and variation of the absorbance with the increase of the concentration of BSA. The different steps of the analytical cycle are indicated on the lower part of the figure with upper case letters corresponding to the ones in Table 1.

erol and ethanol, all main components in finished wine, did not interfere in the determination; the same occurred for other ions normally presented in wine as Ca^{2+} and Mg^{2+}.

Although there was no observed interference when the potential interfering species were separately measured, a wine model solution (WMS) containing 10% (v/v) of ethanol, 3 g/L of sugars (glucose:fructose; 40:60%), 7 g/L of glycerol, 100 mg/L of Mg^{2+}, 100 mg/L of Ca^{2+}, 50 mg/L of Na^{+} and 4 g/L of tartaric acid, was prepared and analysed to evaluate the effect of matrix interference in the determination. The results showed a decrease of 50% on the sensitivity of the calibration curve performed with the standards prepared in WMS compared to the sensitivity of the calibration curve with aqueous standards. To tackle this problem and considering that the majority of wine proteins have an isoelectric point (pI) in a range from 4.1 to 5.8 [2], and the BSA has a pI of 4.7 [26] a new calibration curve was performed using standards with a pH adjusted to 5.5. In these conditions, there was no significant difference on the sensitivity obtained in the analysis of standards prepared in water and for the ones prepared in WMS, as it can be concluded from the values presented in Table 3 for the slopes and intercepts (±standard errors) from each regression lines [25]. To verify the selectivity of the proposed method, 250 mg/L of gallic acid was added to the WMS prepared (WMS + G). The aim of this study was to revisit the effect of the phenolic compounds (normally present in the matrix) on the method. The authors Winters and Minchin [23] stated that accurate protein measurements could only be made in the presence of phenolic compounds up to a concentration of 40 mg/L. Once again there was no significant difference found, as it can be confirmed on Fig. 3.

Table 3
Figures of merit of the developed SI–BI–LOV method.

Parameter	Value
Solutions consumption per assay	
Sample	400 μL
FCr (1:10) (v/v)	25 μL
Cu^{2+} solution	10 μmol
Waste production per assay	4 mL
Determination rate	9 det./h
Dynamic range	Up to 0.30 g/L
LOD	0.03 g/L
LOQ	0.10 g/L
Repeatability (RSD)	4.9% (0.10 g/L) (n = 4) 4.4% (0.12 g/L) (n = 5) 3.9% (0.16 g/L) (n = 4) 1.9% (0.22 g/L) (n = 4)

3.3. Figures of merit of the method

The performance of the developed method was evaluated in terms of reagent and sample consumption, determination rate and dynamic range. The method presented a sample consumption of 400 μL per assay and a consumption of FCr and Cu^{2+} solution of 25 μL and 100 μL per assay, respectively. It was possible to achieve a linear range up to 0.30 g/L with a limit of detection and quantification [25] of 0.03 and 0.10 g/L, respectively. As for the determination rate, it was possible to perform 9 determinations per hour, which is satisfactory for the Lowry method [27]. These figures of merit of the developed method are summarised in Table 3.

The reproducibility of the system was assessed by performing the calibration procedure under identical physical and chemical conditions between six different working days (between 24 of March and 01 of April of 2011). The calibration curves were performed using 6 standard solutions; the slope of the calibration curve obtained was 0.847 ± 0.180 corresponding to the mean and to the 95% confidence interval of the estimate. Stability can be considered satisfactory, as this data reflects different batches of beads and reagents and daily preparation of standard solutions.

Within day the flow system presents an acceptable repeatability obtained with a RSD <5% as reported in Table 3.

3.4. Sample analysis

The developed method was applied to white table wines, to a sparkling wine and to a beer sample. The results obtained are summarised in Table 4. The values of the recovery test were calculated as reported by IUPAC [28].

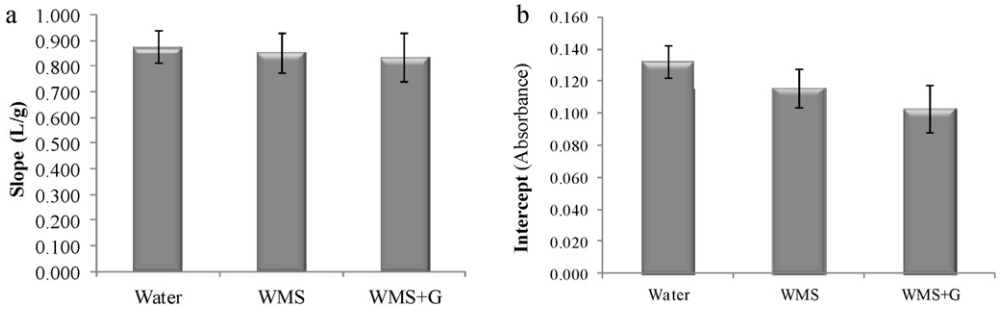

Fig. 3. Comparison of equation parameters for calibration curves for different matrices. (a) Slope and (b) intercept. Error bars represent the standard error of the parameters.

Table 4
Results obtained for the recovery tests for the SI–BI–LOV determination of total protein content.

Sample	pH_i[a]	Recovery (%)		
		[BSA] added		
		0.10 g/L	0.15 g/L	0.20 g/L
White table wine 1	3.1	85.7 ± 4.1	–	105.5 ± 11.8
White table wine 2	2.9	96.3 ± 4.2	–	100.8 ± 7.0
White table wine 3	3.2	107.0 ± 6.2	–	–
White table wine 4	3.3	98.4 ± 4.8	95.0 ± 4.4	87.7 ± 2.6
White table wine 5	2.6	75.7 ± 4.5	104.7 ± 3.9	110.0 ± 1.9
Sparkling wine	3.1	102.7 ± 13.6	104.1 ± 3.5	99.0 ± 9.0
Beer	4.2	102 ± 3.8	100.1 ± 7.0	95.3 ± 5.1

[a] Original pH of the sample.

A significant test was used to evaluate if the mean percentages obtained for the recovery test in the chosen ranges were statistically different from 100%. The values of $|t_{calc}|$ for the different concentrations, 0.10, 0.15, and 0.20 g/L, were 1.10, 0.44, and 0.09, respectively lower than their corresponding value of $|t_{critical}|$ 2.45, 3.18, and 2.57. Therefore, the recoveries were no statistically different from 100%, within a confidence interval of 95%.

4. Conclusions

SI–LOV platform proved to be a valuable tool for the development of a methodology for total protein content in white wines and also in a sparkling wine and a beer. When compared with other methodology for this quantification [29] using the same platform (SI–LOV) some similarities can be found: both present a linear range in the same order of magnitude. On the other hand, the method was applied to samples of different nature (human serum, urine, milk and yogurt). Although these samples present a complex matrix, they were subject to a high dilution factor which reduces significantly any interference with the matrix of the sample. In the developed methodology the possible interfering species were excluded from the determination by means of SPS.

Acknowledgements

Susana Vidigal thanks Fundação para a Ciência e a Tecnologia (FCT) and FSE (III Quadro Comunitário) for the grant SFRH/BD/23040/2005 and SFRH/BPD/78705/2011. I.V. Tóth thanks FSE and MCTES (Ministério da Ciência, Tecnologia e Ensino Superior) for the financial support through the POPH-QREN program and through grant PEst-C/EQB/LA0006/2011.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.12.028.

References

[1] F. Sauvage, B. Bach, M. Mouitounet, A. Vernhet, Food Chem. 118 (2010) 26–34.
[2] E.J. Waters, C.B. Colby, in: M.V. Moreno-Arribas, M.C. Polo (Eds.), Wine Chemistry and Biochemistry, Springer, New York, 2009, pp. 213–230.
[3] B.R. Ferreira, M.A. Piçarra-Ferreira, S. Monteiro, V.B. Loureiro, A.R. Teixeira, Trends Food Sci. Technol. 12 (2002) 230–239.
[4] M. Fusi, F. Mainente, C. Rizzi, G. Zoccatelli, B. Simonato, Food Control 21 (2010) 830–834.
[5] S.W. Souci, W. Fachmann, H. Kraut, Food Composition and Nutrition Tables, 7th ed., CRC Press, Germany, 2000.
[6] M. Marangon, S.C. Van Sluyter, K.A. Neilson, C. Chan, P.A Haynes, E.J. Waters, R.J. Falconer, J. Agric. Food Chem. 59 (2011) 733–740.
[7] N. Lindeboom, P.K.J.P.D. Wanasundara, Food Chem. 104 (2007) 30–38.
[8] M.A. Amerie, C.S. Ough, Methods for Analysis of Musts and Wines, Wiley, New York, 1980.
[9] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[10] D. Le Bourse, S. Jégou, A. Conreux, S. Villaume, P. Jeandet, Anal. Chim. Acta 667 (2010) 33–42.
[11] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randal, J. Biol. Chem. 193 (1951) 265–275.
[12] A.G. Gornall, C.J. Bardawill, M.M. David, J. Biol. Chem. 177 (1949) 751–766.
[13] P.K. Smith, R.I. Krohn, G.T. Hernmanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Anal. Biochem. 150 (1985) 76–85.
[14] M.V. Moreno-Arribas, E. Pueyo, M.C. Polo, Anal. Chim. Acta 458 (2002) 63–75.
[15] C.V. Sapan, R.L. Lundblad, N.C. Price, Biotechnol. Appl. Biochem. 29 (1999) 99–108.
[16] D.A.M. Zaia, C.T.B.V. Zaia, J. Lichtig, Química Nova 21 (1998) 787–793.
[17] A.C. Lucarini, B.V. Kilikian, Biotechnol. Tech. 13 (1999) 149–154.
[18] S. Matsuoka, K. Yoshimura, Anal. Chim. Acta 664 (2010) 1–18.
[19] S.S.M.P. Vidigal, I.V. Tóth, A.O.S.S. Rangel, Talanta 84 (2011) 1298–1303.
[20] M.C. Lohan, A.M. Aguilar-Islas, R.P. Franks, K.W. Bruland, Anal. Chim. Acta 530 (2005) 121–129.
[21] J. de Jong, V. Schoemann, D. Lannuzel, J.L. Tison, N. Matielli, Anal. Chim. Acta 623 (2008) 126–139.
[22] R.N.M.J. Páscoa, I.V. Tóth, A.O.S.S. Rangel, Microchem. J. 93 (2009) 153–158.
[23] A.L. Winters, F.R. Minchin, Anal. Biochem. 364 (2005) 43–48.
[24] G. Anderegg, Pure Appl. Chem. 54 (1982) 2693–2758.
[25] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed., Ellis Horwood, New York, 1993.
[26] C. Chaiyasut, T. Tsuda, Chromatography 22 (2001) 91–95.
[27] C.M. Pomory, Anal. Biochem. 378 (2008) 216–217.
[28] D.T. Burns, K. Dnazer, A. Townshend, Pure Appl. Chem. 74 (2002) 2201–2205.
[29] X.W. Chen, J. Jiao, J.H. Wang, Chin. J. Anal. Chem. 36 (2008) 1601–1605.