A flow-based platform for measuring the acidity parameters in wine

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ABSTRACT

The present work describes a valuable tool for winemaking industry to measure the acidity parameters with rapid response, simple sample handling, with no or minimal pre-treatment. Thus, a sequential injection analysis (SIA) system with spectrophotometric detection was used as platform for the development of methodologies for the quantification of volatile and total acidity in wine samples. Both procedures make use of the same colour reagent, bromothymol blue (BTB) that changes its intrinsic colour in the presence of the acidity compounds. The volatile acidity value was attained with the separation of the volatile fraction of acids by means of a membrane separation technique, a gas-diffusion unit. For the total acidity value, the sample was merged with the colour reagent on the way towards detection. The fixed acidity is a result of the difference from the total and the volatile acidity.

The presented tool displayed a low sample and reagent consumption, 346 and 102 µL of sample and 37 and 32 µg of BTB, for the volatile acidity (VA) and for the total acidity (TA), respectively. The observed limits of detection and quantification were 0.03 and 0.01 g L⁻¹ (VA) and 0.09 and 0.02 g L⁻¹ (TA) with high determination rates, 35 (VA) and 62 (TA) determinations per hour. The proposed system was successfully applied to the quantification of volatile, total and fixed acidity in white table wine samples. The obtained results were in good agreement with the ones obtained by the reference methods.

1. Introduction

Grapes contain substantial quantities of several acids. The acids present in wines are a mixture of the acids of must and others formed during and after the alcoholic fermentation. The acidity in wine is usually divided in two categories: the volatile acidity, corresponding to the acids that can be removed by steam distillation, and the fixed acidity, referring to the poorly volatile ones. Total (titratable) acidity is the combination of both categories [1]. The acidity parameters must be evaluated in several steps of the wine production. In order to determine the appropriate quantity of sulphur dioxide to be added to the must, or if there is any need to carry out a correction of the acidity, it is necessary to determine the total acidity of the must. After fermentation and during aging, it is also important to follow the variation of the concentration of some fixed acids and the levels of volatile acids. The quantification of total acidity has to be performed in the final part of several steps to standardize the wine that is being produced. The volatile acidity quantification is one of the most important acidity parameters for the winemaker. Besides the specific regulations, high content of volatile acidity can be an indicator of microbiological contamination [2].

The official methods proposed by the Office International de la Vigne et du Vin (OIV) [3], are based on titration against a standard alkaline solution. In the case of the determination of total acidity in wine, the method describes a direct titration with comparison with an end-point colour standard; for the volatile acidity, the titration is carried out after steam distillation. The value of the fixed acidity is obtained by calculating the difference between the total and the volatile acidity values. In routine analysis, these parameters are presented in terms of milliequivalents per litre (meq L⁻¹); however, in his work, the acidity parameters will be expressed as g L⁻¹ of acetic acid to describe the volatile acidity and g L⁻¹ of tartaric acid to describe the total acidity. As the volatile acidity measurement implies a distillation of the sample prior to the titration, the method becomes complex, laborious and time consuming. Flow injection analysis (FIA) system [4] has been introduced as a versatile tool for the automation of wet chemical analysis. These systems are characterized by its low consumption of sample and reagents with easy handling. A precise and reproducible volume of sample is injected in a carrier stream and mixed with the reagent on the way to detection. Further automation was achieved when sequential injection analysis (SIA) was proposed [5]. These systems make use of programmable flow and comprise a multi-position valve, used to aspirate accurately solution.
Table 1

Comparison of some analytical figures of flow methodologies for the determination of the acidity parameters in wine.

| Acidity parameter | Matrix | Flow method | Separation step | Colour reagent | Detection system | Range of applicability | LOD | LOQ | Determination rate | RSD (%) | Ref |
|-------------------|--------|-------------|----------------|----------------|-----------------|------------------------|-----|-----|-------------------|---------|-----|
| Volatile acidity  | Wine   | FIA         | Pervaporation   | Bromocresol Purple (5×10⁻⁴ mol L⁻¹) | Spectrophotometry | 0.20–0.80 g L⁻¹        | 0.035 g L⁻¹ | –   | 10 h⁻¹            | –       | [8] |
| Volatile acidity  | Wine   | FIA         | Pervaporation   | –              | Electrophoresis | 0.1–0.9 g L⁻¹          | 5 µg/mL (0.005 g L⁻¹) | 16.5 µg/mL (0.01 g L⁻¹) | 10 h⁻¹ | –               | [9] |
| Volatile acidity  | Vinegar, wine and vegetable juice | FIA | GDU | – | Bulk acoustic wave impedance sensor | up to 10 mmol L⁻¹ (0.6 g L⁻¹) | 50 µmol L⁻¹ (0.003 g L⁻¹) | 72 h⁻¹ | 25% | 1% | [10] |
| Volatile acidity  | Red and white wine | FIA | GDU | – | Bromocresol Purple (1×10⁻⁴ mol L⁻¹) | Condutimetry Spectrophotometry | 0.02–0.1 g 100 mL⁻¹ (0.2–1 g L⁻¹) | – | 80 h⁻¹ | 60 h⁻¹ | – | [11] |
| Volatile acidity  | Wines and similar products | FIA | Microdistillation | – | – | Spectrophotometry | 0–1 g L⁻¹ | – | 12 h⁻¹ | 2.4% | [12] |
| Total acidity     | Table and port, red and white wine | FIA | No need | Bromothymol blue (1.28×10⁻⁴ mol L⁻¹) | Spectrophotometry | up to 0.16 g L⁻¹ | 0.01 g L⁻¹ | 0.05 g L⁻¹ | 48 h⁻¹ | 4.49% | [13] |
| Total acidity     | Red wine | MCFS | No need | Phenolphthalein (0.01% (w/v)) | Spectrophotometry | 5.70–8.50 g L⁻¹ | – | – | 22 h⁻¹ | 2% | [14] |
| Total acidity     | White and red wine | FIA | No need | m-Cresol (0.5 g L⁻¹) | Spectrophotometry | – | – | – | 72 h⁻¹ | 1.5% | [15] |
| Total acidity     | Table and port, red and white wine | FIA | No need | Phenolphthalein (30 mg L⁻¹) | Spectrophotometry | 1–10 g L⁻¹ | 1 g L⁻¹ | > 16 h⁻¹ | 2.6% | [16] |
| Total acidity     | Wine    | FIA | No need | – | Potentiometry | 5×10⁻⁴ to 12.5×10⁻² mol L⁻¹ (0.067–16.7 g L⁻¹) | 10.4 meq L⁻¹ (0.78 g L⁻¹) | – | 12 h⁻¹ | 1% | [17] |
| Total acidity     | White and red wines | SIA | Pervaporation | Bromothymol blue (1×10⁻⁴ mol L⁻¹) | Spectrophotometry | 20–80 meq L⁻¹ (1.5–6 g L⁻¹) | 0.02 g L⁻¹ | – | 2.7% | Present Work | [18] |
| Total acidity     | White table wine | SIA | GDU | – | Bromothymol blue (6.4×10⁻¹ mol L⁻¹) | Spectrophotometry | 0.01 g L⁻¹ | 0.02 g L⁻¹ | 62 h⁻¹ | 5.1% | [19] |

FIA – flow injection analysis; MCFS – multicommuted flow system; SIA – sequential injection analysis. Values in parentheses are the units. Conversion considered by the authors.
volumes and packing them in a holding coil. The mixture of the sample with the reagent is attained by reversing the flow towards detection. Therefore, reagents and carrier solutions are only aspirated when necessary, reducing significantly the assay consumables.

The use of flow based systems, namely sequential injection analysis (SIA) systems, for the automation of wine routine determinations, turns to be a good solution to overcome the disadvantages of the described reference procedures [6,7]. Some works have been presented for the quantification of acidity parameters using flow-based systems (Table 1).

Most of the proposed methods make use of FIA systems for the quantification of one of the acidity parameters, only SIA systems [19, present work] were used for the determination of both parameters. The methods were coupled to different detection systems based on spectroscopy, electrophoresis, impedance sensors, and electroanalytical techniques. For the determination of volatile acidity, a separation of the volatile fraction is required, thus techniques based on membrane separation and distillation were presented. The distillation [13] in the form of microdistillation was presented in a patent proposed by Universidad Politecnica de Valencia; the membrane separation procedures make use of pervaporation [6–8,17] or gas-diffusion units [11,12, present work]. When comparing the performance of gas-diffusion unit (GDU) with the pervaporation unit, the GDU presented higher throughput with similar low limits; however, GDU can be more prone to membrane contamination.

Taking into account the features described above, the aim of this work was the development of a system for the determination of total and volatile acidity with rapid response with minimum sample pre-treatment. Therefore, a SIA system was used as platform coupled to a GDU and a spectrophotometric detector. The developed methodology was based on the spectrophotometric monitoring of the change of the color solution of bromothymol blue (BTB) in the presence of the acidity compounds. The proposed volatile acidity assay was as follows: the aspirated sample was propelled towards the donor channel of the GDU while in the acceptor channel stream was the BTB solution. This step was performed at a low flow rate to increase the efficiency of the mass transfer through the membrane. The quantification was achieved as the reaction product formed in the acceptor channel of the GDU was propelled towards detection. Since the determination of total acidity does not require membrane separation, the proposed analytical procedure was simpler; the aspirated sample merged with the color reagent solution on the way to detection.

2. Materials and methods

2.1. Reagents, solutions and samples

Boiled deionised water was used throughout the work and all chemicals were of analytical grade.

For the volatile acidity quantification, working standard solutions of acetic acid (C4H6O2, Merck, 1.00063) were prepared daily in the range from 0.07 to 1.06 g L⁻¹ by rigorous dilution of the concentrated acid. Ethanol (C2H5OH, Panreac, 221086) was added to the standards to attain 10% (v/v) for analyses of table wines.

For the total acidity, working standard solution of tartaric acid (C4H6O6, Merck, 1.00804) were prepared in the range from 0.02 to 0.16 g L⁻¹ by rigorous dilution of a stock standard solution (1 g L⁻¹). These solutions were prepared every day.

The bromothymol blue (BTB, C27H28Br2O5S, Merck, M1.03026.0005) stock solution was prepared by rigorous weighing and dissolution in ethanol. The 40 mg L⁻¹ working reagent solution was prepared daily by proper dilution in water from the stock solution. A 70 mmol L⁻¹ of sodium hydroxide solution was used to adjust the pH value of this solution to 8.0.

To perform the interference studies, glucose (C6H12O6, Merck, 108337), fructose (C6H12O6, Merck, 104007), glycerol (C3H8O3, Sigma G-6279), magnesium chloride hexahydrate (MgCl2·6H2O, Merck, 5833) and calcium chloride dehydrate (CaCl2·2H2O, Merck, 2382) were used. A wine model solution (WMS) composed by: 3 g L⁻¹ of sugars (40:60; Glucose: Fructose), 7 g L⁻¹ of glycerol, 100 mg L⁻¹ of Mg²⁺, 100 mg L⁻¹ of Ca²⁺, 50 mg L⁻¹ of Na⁺ (Sodium chloride, NaCl, Merck, 1.06404), and 10% of ethanol, was used in the interference studies.

All the samples tested were commercially available and only a 50-fold dilution step was required prior to the total acidity analysis.

2.2. Apparatus and procedure

The flow manifold (Fig. 1) consisted of two Minipuls 3 peristaltic pumps (Gilson, Villiers le Bel, France), one connected to the central channel of an 8-port selection valve (VICI, Houston, Texas, USA), and the other connected to the acceptor channel of the gas-diffusion unit (GDU). The GDU, made of acrylic with a surface area of 140 mm² and a zig zag channel with a depth of 0.3 mm, was equipped with a Durapore® hydrophobic PVDF membrane with a 0.45 µm pore size.
reagent. Afterwards, the sample plug is propelled towards the donor acceptor channel of the GDU in order to sample or standard while the reagent solution is propelled towards the determination (step A to C) consisted in the aspiration of 346 µL of the sample or standard to the holding coil; afterwards, the sample was propelled towards detection by aspiration of 102 µL of the diluted sample or standard to the holding cell (internal volume of 18 µL), fibre optics (P400), and a UV/VIS/NIR light source, DH-2000-BAL Mikropack, OceanOptics (Winter Park, Florida, USA). The connections between all the components of the SIA system were made with PTFE tubing (Omnifit, Cambridge, UK) with 0.8 mm i.d.

A SpectraSuite software on a HP compact computer was used to data acquisition at 617 nm. A Samsung SD700 computer with a PCL818L interface card, running with Quick Basic 4.5 software controlled the selection valve (SV) position, the direction and speed of the peristaltic pump 1, and the start of pump 2. The speed of the pump 2 was set manually at 40, which corresponds to a flow rate of 40 µL s\(^{-1}\).

The flow protocol is summarized in Table 2. The volatile acidity determination (step A to C) consisted in the aspiration of 346 µL of sample or standard, while the reagent solution is propelled towards the acceptor channel of the GDU in order to fill the whole channel with the reagent. Afterwards, the sample plug is propelled towards the donor channel of the GDU at a low flow rate (12.5 µL s\(^{-1}\)) to improve the yield of the mass transfer through the membrane, while the donor stream is stopped in the GDU. For the quantification, the reaction product formed in the acceptor channel of the GDU, is pumped to the detector.

The total acidity quantification (step D to F) consisted in the aspiration of 102 µL of the diluted sample or standard to the holding coil; afterwards, the sample was propelled towards detection by reversing the flow at the same time as the reagent was pumped on the way to detection. These two solutions merged on the way towards detection.

The fixed acidity quantification was attained by calculus: difference between total and volatile acidity.

### 2.3. Reference methods

For the total acidity quantification, white table wine samples were analysed as described by OIV [3]: a potentiometric titration against a standard alkaline solution to a pH value of 7. The volatile acidity quantification, determined by the acetic acid content, was carried out by using an HPLC method where an Aminex HPX-97H 300 (BioRAD) column was coupled to an K-1001 Pump (KNAUER) and to a refractive index detection system K-2301 (KNAUER). The sample (20 µL) was injected in a mobile phase composed by sulphuric acid (2.5 mmol L\(^{-1}\)) at a flow rate of 0.6 mL min\(^{-1}\). The fixed acidity quantification [3] was calculated from the difference between total and volatile acidity.

### 3. Results and discussion

#### 3.1. Study of the manifold

The chemical and the physical conditions of the proposed method were studied using a univariate method in order to attain the necessary linear range with good sensitivity.

##### 3.1.1. Volatile acidity

Initially, the methodology for the volatile acidity was optimised and the preliminary conditions were set as: 500 µL of sample volume; 635 µL of carrier to propel the sample towards the donor channel of the GDU; pump 2 speed 40 (40 µL s\(^{-1}\)); 577 µL at a flow rate of 40 µL s\(^{-1}\) of reagent to propel the reaction product towards detection; 40 mg L\(^{-1}\) of BTB (pH 8.0) concentration on the reagent.

To begin the optimisation procedure, the influence of the concentration of the reagent (SBT) was studied; therefore, standards of acetic acid were prepared in a range from 0.13 to 1.06 g L\(^{-1}\). The BTB concentration was evaluated from 10 to 150 mg L\(^{-1}\) (Fig. 2). The increase on the concentration up to 40 mg L\(^{-1}\) resulted in an increase on the sensitivity of about 60%, with improvement of the linearity with good repeatability. Further increase to 75 mg L\(^{-1}\) presented a decrease on the sensitivity of about 25%, moreover a loss of repeatability was achieved. As a result, 40 mg L\(^{-1}\) of BTB was used in further studies. The pH value of the reagent was studied at the values 7.0, 7.5, 8.0 and 8.5. With the increase of the pH up to 8.0, an increase on the sensitivity was attained. Further increase resulted in the loss of the linearity. The adjustment of the pH value was carried out by adding some drops of 70 mmol L\(^{-1}\) of OH\(^-\) solution to the solution prepared by dilution in water from the stock solution, and by dilution of the stock solution in a 0.2 mol L\(^{-1}\) phosphate buffer solution (pH =8.0). By comparing the obtained results, higher sensitivity was achieved by using the OH\(^-\) solution to adjust the pH value.
Regarding the physical parameters, the volume of sample and the volume of carrier used to propel the sample towards the donor channel of the GDU, were aimed of study. The volume of the sample aspirated was appraised from 192 to 650 µL (Fig. 3.). The increase on the volume of sample aspirated in step A of the flow protocol to 346 µL produced an increase on the sensitivity of about 50%, with good linearity. Further increase did not produce a significant variation on the sensitivity, in fact, the volume of 650 µL, a loss on the linearity was observed. Therefore, for further studies, 346 µL of sample was used.

The volume of carrier used to propel the sample towards the donor channel of the GDU, was evaluated in a range from 444 to 1016 µL. The augment of the volume up to 889 µL increased the sensitivity; further increase did not produce a significant variation on the sensitivity of about 50%, with good linearity. Further increase did not produce a significant variation on the sensitivity of about 50%, with good linearity. Therefore, 102 µL of sample was used throughout the work. For the total acidity quantification, only the physical parameters were evaluated, since an option was made to use the same colour reagent solution for both determinations. Therefore, the volume of sample and the corresponding flow rate and volume of carrier used to propel the sample were studied. The optimisation studies were performed with tartaric acid standard solutions in a range from 0.02 to 0.16 g L⁻¹.

The influence of the volume of sample was evaluated from 50 to 125 µL (Fig. 4.). The increase of the volume of sample from 50 to 102 µL, resulted in an increase on the sensitivity of about 25%; a further increase presented a minor decrease on the sensitivity, with poor linearity. Thus 102 µL of sample was used throughout the work.

The flow rate used to propel the sample towards detection was assessed from 30 to 45 µL s⁻¹. In this study, the flow rate used in pump 2 was the same as the flow rate of pump 1. With the augment on the flow rate from 30 to 40 µL s⁻¹, no significant difference was found in terms of sensitivity; however, a better linearity was verified. With additional increase to 45 µL s⁻¹, besides presenting lower sensitivity (about 40%), a poorer repeatability was observed. Therefore, 40 µL s⁻¹ was the flow rate used to propel the sample towards detection. The volume used to propel the sample towards detection was also an evaluated parameter (from 192 to 769 µL). The chosen volume was 577 µL, since higher volumes did not show significant difference in terms of linearity and sensitivity. On the other hand, lower volume presented worse results in terms of repeatability.

### 3.2. Interference studies

Wine samples have a very complex matrix; therefore, the developed methodology may display unexpected results due to the presence of potential interfering species. Considering the typical composition of wine samples, the effect on the sensitivity caused by the presence of some major compounds was evaluated.

#### 3.2.1. Volatile acidity

The first expected interference would be from ethanol. Therefore, standard calibration curves were preformed and the results were compared with the results obtained by calibration curves performed with standards with the addition of ethanol, 10% (v/v) as final content. In terms of sensitivity of the method, some difference was found: −1.228 (± 0.019), aqueous standards; −1.388 (± 0.045) and standards with 10% ethanol. This interference was expected, as ethanol can migrate through the membrane causing schlieren effect [20]. Therefore, 10% (v/v) of ethanol was added to the standards in order to match the sample matrix. The addition of a wine model solution (WMS) to the standards did not show significant variation on the sensitivity: −1.398 (± 0.042); standards with 10% ethanol; −1.402 (± 0.050), standards with WMS and 10% ethanol.

Besides these species, sulphur dioxide (SO₂) and carbon dioxide (CO₂) were also tested for 126 mg L⁻¹ and 5.7 g L⁻¹, respectively. Considering the sensitivity obtained for the calibration curves with and without the addition of the interfering agent, no significant variation was found: −1.398 (± 0.042), standards with 10% ethanol; −1.462 (± 0.063), standards with 10% of ethanol and SO₂; −1.361 (± 0.049), standards with 10% of ethanol and CO₂.

All the values presented in parentheses, in this section, are the limits of the 95% confidence intervals [21].

#### 3.2.2. Total acidity

On the total acidity quantification, the possible interference from ethanol was evaluated as 10% (v/v), corresponding to table wines. Therefore, calibration curves were performed with standards with the addition of ethanol, to a final content of 0.2% (v/v), corresponding to the 50-fold dilution of the sample. The results obtained showed no significant difference from the calibration curve performed without ethanol, in terms of sensitivity: −8.341 (± 0.967), aqueous standards; and −8.485 (± 0.775), standards with 0.2% (v/v) of ethanol. The probable interference from other species was evaluated by comparing the results obtained using the aqueous standards and the results using a wine model solution (WMS). The results of the sensitivity obtained when the calibration curve was performed with and without WMS, did not present significant difference: −8.341 (± 0.967), aqueous standards; −8.402 (± 1.047), standards with WMS. All the values presented in parentheses, in this section, are the limits of the 95% confidence intervals [21].

### 3.3. Figures of merit

The performance of the developed system was assessed in terms of range of applicability, limits of detection and quantification, reproducibility, sample consumption and determination rate.

It was possible to establish a linear relationship up to 0.16 and up to 1.06 g L⁻¹ with low limits of detection and quantification [21], 0.01 and 0.03 g L⁻¹, and 0.02 and 0.09 g L⁻¹, for the total and the volatile acidity quantification, respectively. The presented methodologies have a low
reagent and sample consumption: 102 and 346 µL of sample and 32 and 37 µg of BTB per assay, for the total and the volatile acidity quantification, respectively; consequently, very a low production of waste is attained.

The methodologies operate at a high determination rate, 62 and 35 quantifications are completed within one hour for the total and the volatile acidity quantification, respectively. These results showed good repeatability showing RSD below 5.1% and 2.7% the total and the volatile acidity quantification, respectively.

3.4. Application to white table wine samples

A total of four white table wine samples, commercially available, were analysed in order to evaluate the accuracy of the developed methodologies; for that, the reference procedure was also performed using the content of the same bottle. The reference procedure for the total acidity quantification was carried out as described in Section 2.3 of this manuscript. For the volatile acidity, the reference protocol used was the one described by OIV that is based on the titration against alkaline solution after a steam distillation procedure. Unfortunately, this procedure has some drawbacks, besides being time consuming, and requiring a skilled operator, the distillation apparatus must be in the perfect conditions, otherwise some loss of the volatile fraction can occur. It is very difficult to collect all the volatile fraction, that were the difficulties that we encountered, so we had to select other methodology to use for comparison for the quantification of the volatile acidity. The comparison method chosen was an HPLC method (detailed in Section 2.3), so the quantification of this parameter was attained in terms of acetic acid, as the developed method.

As presented in Table 3, the results obtained by the developed methods are in good agreement with the ones obtained by the reference methods.

4. Conclusions

A system for the determination of total and volatile acidity in wines was assembled. Both methodologies were performed using the same platform and the same colour reagent. The authors have already developed a method for the quantification of total acidity [14] in wine; the present work shows a lower limit of quantification, with similar repeatability. At the same time, turns to be a more automatic system, applied for one more determination, volatile acidity, allowing the determination of an additional parameter, the fixed acidity.

In comparison with the reference procedure and other methodologies described in Table 1, namely the system proposed by Mataix and Luque de Castro [19], the present system exhibits higher throughput for both methodologies, with lower reagent consumption and similar limits of detection and quantification. There are other analytical techniques like the Fourier transform infrared (FTIR) spectroscopy, characterized by a large throughput of information in a very short analysis time, that are being used in the front line for wine analysis [22]. However, these make use of equipment of high cost for routine laboratories and, to obtain useful data, they demand calibration for each specific wine matrix. The proposed method presents a rapid response with a simple reading signal, which can be very important when it is necessary to assess the values of the acidity parameters during the winemaking process, turning to be a valuable instrument for winemaking industry.

Acknowledgements

Susana Vidigal acknowledges Fundação para a Ciência e a Tecnologia (FCT), Portugal, and Fundo Social Europeu (FSE), European Union, for the financial support through the Programa Operacional Capital Humano (POCH) via the grant number SFRH/BPD/78705/2011. Susana Vidigal also acknowledges the financial support in the framework of the project “Biological tools for adding and defending value in key agro-food chains (bio – n2 – value),” no NORTE-01–0145-FEDER-000030, funded by Fundo Europeu de Desenvolvimento Regional (FEDER), under Programa Operacional Regional do Norte - Norte2020. This work was supported by National Funds from FCT through project UID/Multi/50016/2013.

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