



## Analytical Methods

# Rapid microplate, green method for high-throughput evaluation of vinegar acidity using thermal infrared enthalpimetry



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## ARTICLE INFO

## Article history:

Received 4 May 2016

Received in revised form 21 July 2016

Accepted 21 July 2016

Available online 25 July 2016

## Keywords:

Food

Infrared thermography

Imaging

Calorimetry

Enthalpimetric analysis

Green analytical chemistry

## ABSTRACT

Infrared thermal imaging was combined with disposable microplates to perform enthalpimetric analysis using an infrared camera to monitor temperature without contact. The proposed thermal infrared enthalpimetry (TIE) method was used to determine the total, fixed and volatile acidities of vinegars. Sample preparation and analysis were performed in the same vessel, avoiding excessive sample handling and reducing energy expenditure by more than ten times. The results agreed with those of the conventional method for different kinds of vinegars, with values of 1.7%, and 2.3% for repeatability and intermediate precision, respectively. A linear calibration curve was obtained from 0.040 to 1.30 mol L<sup>-1</sup>. The proposed method provided rapid results (within 10 s) for four samples simultaneously, a sample throughput of up to 480 samples per hour. In addition, the method complies with at least eight of twelve recommendations for green analytical chemistry, making TIE a promising tool for routine vinegar analysis.

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## 1. Introduction

Vinegar is produced by fermentation, a process which yields organic acids, primarily acetic acid (Moros, Iñón, Garrigues, & de la Guardia, 2008; Ozturk et al., 2015; Thomas & Jamin, 2009). Vinegar may be classified according to the raw material used for its fermentation, whether from wine (red or white), cereals, vegetables, fruits, honey or hydroalcoholic mixtures (Brasil, 2012; Dong, Zheng, Jiao, Lang, & Zhao, 2016). Considering vinegar's high acidity, it is unsurprising that acidity has been used as a standard parameter to assure the quality of the product (Brasil, 1985; Guerreiro, Oliveira, Ferreira, & Catharino, 2014). In general, three types of acidity are considered to measure its quality: total, fixed and volatile. Volatile acidity represents the organic acids found in vinegars that are more easily vaporized than the non-volatile (fixed) acids. Volatile acidity is often obtained by calculating the difference between measured total and fixed acidities (Ubeda et al., 2016). The Brazilian official compendium for food quality control has established that the total acid content of vinegar should be between 4.0 and 6.0% (m/v), with a minimum of 4.0% of volatile acidity, expressed as acetic acid (Brasil, 2012).

Conventional protocols recommended for and used to determine the acidity of vinegars require a long time and high consumption of energy to perform evaporation and concentration before titration. These time-consuming methods require the attention of an analyst throughout the process, leading to low sample throughput and affecting their suitability for routine applications (Masino, Chinnici, Franchini, Ulrici, & Antonelli, 2005; Su & Chien, 2010; Zeng, Cao, Liu, Chen, & Ren, 2015). Therefore, alternative methods have been proposed in the literature to determine the acidity of vinegars, namely spectrophotometry (Kurauchi, Ogata, Egashira, & Ohga, 1996), capillary electrophoresis and ion-exchange chromatography (Castro et al., 2002), gas chromatography mass spectrometry (Marrufo-Curtido et al., 2012) and infrared spectroscopy (Moros et al., 2008). These alternative techniques generally fulfil some of the requirements of green chemistry, as they use fewer reagents and residues that are hazardous to human health and/or the environment, with resulting improvements in the safety of analytical operations compared to conventional methods (de la Guardia & Armenta, 2011; Gałuszka, Migaszewski, & Namieśnik, 2013; Melchert, Reis, & Rocha, 2012). However, obtaining results requires several steps, which, combined with the need to use certain sophisticated instrumental methods, impairs the widespread use of such methods in routine laboratories.

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Recently, a new analytical technique combining infrared thermal imaging with enthalpimetric analysis, called thermal infrared enthalpimetry (TIE), was proposed as a method for simple, fast and high-throughput analysis (Barin et al., 2015). This technique was used in titration applications, using neutralization, redox, precipitation and complexation reactions. In TIE, disposable devices (e.g., microplates) contain the reactions, while an infrared camera, without contact, simultaneously monitors the temperature of multiple reactions. Reagents were introduced by direct injection, which means that only one aliquot of reagent (e.g., sodium hydroxide for determining acidity) in stoichiometric excess is necessary, unlike the dropwise procedure used in titrations. Analytical signals were the differences in temperature before and after reagent addition. The use of multiple dispensing devices (e.g., multichannel pipettes) allowed simultaneous reactions in several wells, leading to high throughput that possibly reaches thousands of measurements per hour (Barin et al., 2015).

Therefore, an easy-to-use, rapid method was proposed for determining the acidity (total, fixed and volatile) of different types of vinegars (red and white wine, alcohol, apple, balsamic and rice) by TIE. Sample preparation and analysis were performed in the same vessel (microplates), avoiding excessive sample handling and improving throughput. Samples were inserted in wells followed by addition of excess reagents by means of multichannel pipettes, and the wells' temperatures (before, during and after injections) were recorded as an infrared thermal imaging video, through which the analytical signals were obtained. The dispensing speed, total well volume and the ratios of reagents were evaluated in order to obtain the lowest deviation between measurements. The results obtained by TIE were compared with those obtained by conventional analytical titration methods. The parameters of merit (including green ones) were presented.

## 2. Experimental

### 2.1. Samples, standards and reagents

Samples of alcohol, apple, balsamic, rice and wine (red and white) vinegars from different manufacturers were obtained from the local market of Santa Maria (Brazil). Acetic acid was used as a reference solution to construct the calibration curve (Vetec, Duque de Caxias, Brazil). Sodium hydroxide (Dinâmica, Diadema, Brazil) was used to determine the acidity. Standardization of acetic acid was performed using sodium hydroxide previously standardized with potassium biphthalate (Vetec, Duque de Caxias, Brazil; (Morita & Assumpção, 2007). Deionized water was further purified in a Milli-Q system (18.2 M $\Omega$  cm, Millipore Corp., Bedford, MA, USA), and this was used to prepare all solutions and reagents.

### 2.2. Instrumentation

A long wave infrared camera was used (8.5–13.0  $\mu$ m, FLIR E60 model, FLIR, Wilsonville, OR, USA), which provided images of 320  $\times$  240 pixels at a frame rate of 30 Hz. Images were processed using ResearchIR software (FLIR). The reactions were carried out in disposable polystyrene 24-well microplates, with each well having an internal volume of 3.0 mL (Nest Biotechnology, China). An electronic multichannel pipette (eight channels, 0.050–1.2 mL, Pro Research 1200, Eppendorf, Hamburg, Germany) was used to simultaneously inject reagents, and a magnetic stirrer (Centauro, Atuba, Brazil) was used to homogenize solutions. For evaporation of the samples in order to determine fixed acidity, a water bath (Solab, Brazil) was used. Energy consumption was measured by a power meter (Fluke 43B model, Fluke Corporation, Everett, WA, USA).

### 2.3. Analysis by conventional methods

Total acidity was determined by titration with 0.1 mol L<sup>-1</sup> sodium hydroxide solution and phenolphthalein as indicator, using 10 mL of sample and 50 mL of distilled water (Brasil, 1985). To determine fixed acidity, 10 mL of each sample were added in porcelain capsules, followed by heating in a boiling water bath. The evaporation procedure was performed three times, each sample reaching a total volume of 30 mL. After cooling, 50 mL of water was added, and the titration was performed in the same manner as for the determination of total acidity. Volatile acidity was obtained as the difference between total and fixed acidities. Results were expressed in grams of acetic acid per 100 mL of sample. All determinations were performed in triplicate.

### 2.4. TIE analysis

Acetic acid (0.04 to 1.3 mol L<sup>-1</sup>, 1.2 mL) or sample (1.2 mL) solutions were added to the wells, with further addition of sodium hydroxide (2 mol L<sup>-1</sup>, 1.2 mL). A polytetrafluoroethylene-covered magnetic stir bar (1.5  $\times$  3.5 mm) was added to each well to improve homogenization. Reaction temperatures were monitored before, during and after injection of NaOH. For all measurements, temperatures were corrected in software for ambient temperature and relative humidity using values obtained from a thermohygrometer. Using a tripod, the infrared camera was positioned 40 cm above the microplates. Using the camera software, a circle of 180 pixels was used to monitor the temperature of each well, and the average of these temperatures was plotted, forming an enthalpogram (Barin et al., 2015). The difference in temperature ( $\Delta T$ ) was obtained using the equation  $\Delta T = T_f - T_i$ , where  $T_f$  and  $T_i$  are the final and initial temperatures, respectively. These temperatures were calculated as the mean of temperature values obtained from 2 s before and after the reaction. Afterwards, a least-squares calibration curve was constructed using reference solutions, and the concentrations of analytes were obtained using this curve. The 24 wells of the microplate were used for each concentration of reference solutions or samples in all experiments ( $n = 24$ ). In all reactions, water was used as a blank.

The fixed acidities of the vinegar samples were determined using the microplate wells as vessels for the sample preparation (evaporation/concentration) and quantification steps. Samples (up to 3 mL each time) were added to the microplate, which was heated in a boiling water bath. For apple, balsamic, red and white wine vinegars, a total of 7 mL were evaporated, while 20 mL of alcohol and rice vinegars were evaporated due to those samples' lower concentrations of acetic acid. After evaporation, the plates were cooled at room temperature and the residues were dissolved in 1.2 mL of distilled water, followed by addition of excess of NaOH in the same way as the determination of total acidity.

### 2.5. Optimization of reactions for TIE analysis

Before determining total acidity by TIE, a reaction optimization was performed by considering the total volume of solution in each well, the proportion of reactants (acid/base) and dispensing speed. The optimization was carried out using 0.8 mol L<sup>-1</sup> acetic acid and 2 mol L<sup>-1</sup> sodium hydroxide solutions. The total volume in each well was evaluated using a 1:1 proportion of acid and base, ranging from 1.2 to 2.4 mL. To evaluate the proportion of reactants, which ranged from 1:1 to 5:1 (ratio of acetic acid to sodium hydroxide solutions), dispensing speed was 0.57 mL s<sup>-1</sup> under stirring. After optimizing the total volume and proportion of reagents, the dispensing speed of the multichannel pipette was evaluated from 0.2 to 0.71 mL s<sup>-1</sup>. Results were evaluated using the relative standard deviation (RSD) of measurements. The method was validated

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