Food Research International 54 (2013) 1369–1375

Contents lists available at ScienceDirect

Food Research International

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

Monitoring grape ripeness using a voltammetric electronic tongue

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ARTICLE INFO

Article history:
Received 31 July 2013
Accepted 7 October 2013

Keywords:
Electronic tongue
Grape ripeness
Total Acidity
Brix

ABSTRACT

The use of a voltammetric electronic tongue as a tool to monitor grape ripeness is proposed herein. The electronic tongue consists of eight metallic electrodes housed inside a stainless steel cylinder. The study was carried out over a period of ca. 1 month (August 2012) on different grape varieties (*Macabeo*, *Chardonnay*, *Pinot Noir*, *Cabernet Sauvignon*, *Shyrah*, *Merlot* and *Bobal*) from various vineyards near Requena and Utiel (Valencia, Spain). Apart from the electrochemical studies, the physico-chemical parameters, such as, Total Acidity, pH and °Brix, were also determined in grapes. The PCA models, obtained using the physico-chemical or electrochemical data, showed variation of ripeness with time. Moreover the study was completed by using partial least squares (PLS) regression in an attempt to establish a correlation between the data collected from the electronic tongue and Total Acidity, pH and °Brix values. A good predictive model was obtained for the prediction of Total Acidity and °Brix. These results suggest the possibility of employing electronic tongues to monitor grape ripeness and of, therefore, evaluating the right time for harvesting.

1. Introduction

During wine production, the composition of grapes at the time of picking is an important parameter which may be considered the most crucial decision in winemaking. While grape ripens, some complex physicochemical and biochemical processes occur, such as the continuous rising and lowering of sugar concentrations and acid levels, respectively, which are also influenced by genetic, climatic and geographical factors, and by cultural practices (Andrades & González-Sanjosé, 1995; González-Sanjosé, Barrón, Junquera, & Robredo, 1991; Jones & Davis, 2000; Palacios, Cea, Cancer, Martínez, & Avenoza, 1986). All these defining the ripeness process determine grape quality and the time of harvesting for winemaking. Traditionally, harvesting date indicators have been determined by parameters such as berry weight, must density (Coombe, 1987) and the relation between sugar content and Total Acidity (King, Sims, Moore, & Bates, 1988; Peynaud, 1989; Robredo, Junquera, González-Sanjosé, & Barrón, 1991). Nowadays, Near Infrared Spectroscopy (NIRS) has also been reported as a suitable technique capable of measuring parameters such as sugar content (Fernández-Novales, López, Sánchez, Morales, & González Caballero, 2009), pH value (Larraín, Guesalaga, & Agosin, 2008) and acidity (Chauchard, Codgill, Roussel, Roger, & Bellon-Meurel, 2004) in grapes. All these procedures imply the use of instrumentation. In fact, even one of the simplest traditional systems to determine harvesting date, i.e., the determination of sugar content (Brix) and Total Acidity, requires the use of a refractometer and an automatic titration system (or manual titrations) which employs chemical products (Jackson, 2000). Moreover, other techniques, such as Near Infrared Spectroscopy (NIRS), require expensive instrumentation and complex calibration (Ferrer-Gallegoa, Hernández-Hierrob, Rivas-Gonzaloa, & Escribano-Bailóna, 2013). In this context, the possibility of designing alternative methods to monitor grape ripeness (an important factor to determine when to harvest) simply and rapidly may be of importance.

From another point of view, an electronic tongue is a device that produces a signal which can be correlated with taste when properly calibrated. Besides, there are many electronic tongues capable of identifying and classifying liquid samples, and of predicting or quantifying the concentration of particular substances or taste attributes (Ciosek & Wróblewski, 2007). In the last few years, electronic tongues have made the best of different measuring principles, including potentiometry, voltammetry and amperometry (Del Valle, 2010). They have also been constructed on the basis of optical and mass sensors (Zhang & Turner, 2005). Electronic tongues are usually composed of an array of chemical sensors coupled to a pattern recognition analysis. Another aim in electronic tongue development is to design easy-to-use reduced-in-size systems applicable for in-situ and

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http://dx.doi.org/10.1016/j.foodres.2013.10.011

at-site monitoring. A number of studies have described the use of such sensor arrays to analyze foodstuffs (Gallardo, Alegret, & Del Valle, 2005; Scampicchio, Ballabio, Arecchi, Cosio, & Mannino, 2008) pharmaceutical samples (Lorenz, Reo, Hendl, Worthington, & Petrossian, 2009; Wilson & Baietto, 2011), clinical samples (Gutierrez, Alegret, & Del Valle, 2007; Lvova et al., 2009) and environmental monitoring (Mimendia et al., 2010; Rudnitskaya, Ehlert, Legin, Vlasov, & Buttgenbach, 2001). Moreover, the literature includes some works which apply electronic tongues to grapes and to wine samples. For instance, electronic tongues have been used to monitor both post-harvest grape dehydration (Buratti et al., 2011; Santonico, Bellincontro, De Santis, Di Natale, & Mencarelli, 2010) and wine aging (Apetrei et al., 2012; Gay et al., 2010). Besides, attempts have also been made to correlate electronic tongue data with traditional human sensory perception of wine attributes (Legin et al., 2003) or to quantify the presence of grape varieties in wines (Buratti, Benedetti, Scampicchio, & Pangerod, 2004; Gutiérrez et al., 2011). Recently, electronic tongues have also been employed to determine concentrations of chemical compounds in wine, such as sulfites (Kirsanov, Mednova, Vietoris, Kilmartin, & Legin, 2012), polyphenols and different sugars (Cetó et al., 2012; Pigani et al., 2011). As far as we know, no studies on the potential use of electronic tongues for grape ripeness monitoring or for determining harvesting dates have been reported.

Following our interest in the development of sensing systems and probes (Martínez-Máñez & Sancenón, 2006), and given our experience in designing electronic tongue devices for several applications (Campos et al., 2010; Campos et al., 2012; Labrador, Olsson, Winquist, Martínez-Máñez, & Soto, 2009; Labrador, Soto, Martínez-Máñez, & Gil, 2009), we report herein the development of an electronic tongue based on simple metallic electrodes and voltammetry, and its use to characterize grape ripeness. This research aims to demonstrate the potential use of this electronic tongue to determine the Total Acidity and °Brix parameters that are related with grape ripeness, which is traditionally used to determine the harvesting date.

2. Experimental

2.1. Samples preparation

The study into the ripeness process was carried out on seven grape varieties (*Macabeo*, *Chardonnay*, *Pinot Noir*, *Cabernet Sauvignon*, *Shyrah*, *Merlot* and *Bobal*) over a period of ca. 1 month (August 2012), which were sampled every 2 days (11 sampling days in all) from different vineyards near Requena and Utiel (Valencia, Spain). The final number of samples was 68, which were less than the total expected samples (11 sampling days × 7 grape varieties) because the harvest of same varieties took place before the studies finished. After collection and crushing, and after waiting for 5 min, samples were decanted to separate the juice from the pulp. During the grape monitoring period, the average daily temperature was between 35 °C and 40 °C, and precipitation during this time was practically zero (ca. 0.5 l/m^2).

2.2. Physicochemical characterization

For the purpose of controlling grape ripeness, three chemical parameters, i.e., Total Acidity, pH and sugar content (°Brix), were determined. Total Acidity was calculated by titration with NaOH 0.5 N using a pH meter (Crison Titromatic 2S 3B, Spain). This equipment was also used to determine pH. Sugar content was determined with a manual refractometer (Zuzi 300, Spain).

2.3. The electronic tongue

2.3.1. The electronic system

The system used in the electronic tongue was developed by the Centre of Molecular Recognition and Technological Development (IDM) at the Polytechnic University of Valencia. The system consists of electronic equipment and a software application that runs on a PC. The system included a potentiostat which applies voltage to the electrochemical cell and measures both the voltage and current at the working electrodes. The potentiostat permits measurements with up to eight multiplexed working electrodes. Briefly, the electronic equipment included a 16-bit microcontroller (PIC24FJ256), a 12-bit Digital-to-Analog converter (DAC), two 12-bit Analog-to-Digital converters (ADC), a potentiostat that incorporated a current measurement circuit, a working electrode multiplexing block and a stabilization circuit. Some analog signal conditioning circuits were also used to adapt the signals connecting the potentiostat to the DAC and the ADC. The microcontroller received the data sent by the PC. After receiving all the data corresponding to a test, the microcontroller configured the current scale and the stabilization level of the potentiostat, and then selected the desired working electrode. Then it outputted the value corresponding to the temporal evolution of the signal to the DAC at a rate that fulfilled the signal timing requirements. In the same loop, the program of the microcontroller sampled the signals corresponding to the voltage and current flowing at the selected working electrode. The collected data were sent to the PC where they were processed and stored. Details of the characteristics of this electronic equipment have been published elsewhere (Alcañiz et al., 2012).

2.3.2. Preparation of an e-tongue

The electronic tongue device consisted of an array of eight working electrodes (Au, Pt, Ir, Rh, Ag, Cu, Ni, and Co) with a purity of 99.9% and a 2 mm diameter from GOODFELLOW, which were housed inside two homemade stainless steel cylinders that were used at the same time as both the body of the electronic tongue system and the counter electrode. The different wire electrodes were fixed inside the cylinder using an epoxy RS 199-1468 polymer. The generation of pulses and recording of current data were performed by the previously described electronic equipment. Traditionally, fouling is one of the major drawbacks that limits the application of electrochemical sensors. In this context, one advantage of using electronic tongues based on metallic electrodes is that it is quite simple to remove any accumulated unwanted material on the electrode by simply polishing the surface (Olsson, Winquist, & Lunsdtröm, 2004). This simple procedure helps obtain a clean new surface ready to be used after each, or after a certain number of, measurement(s). Before being used in this work, the electrode surface was prepared by mechanical polishing with emery paper and was rinsed with distilled water. Then the electrode was placed on a felt pad and was polished with 0.05 μm alumina polish from BAS, washed with distilled water and polished again on a nylon pad with 15 μm, 3 μm and 1 μm diamond polishes to produce a smooth, mirror-like electrode surface. Later while taking the series of measures, only simple diamond polishing was applied.

2.4. Measurement procedure

Every 2 days, samples of different grape varieties were collected early in the morning and were analyzed on the same day in the Torre Oria laboratories. For each sample, traditional determinations were made (Brix, pH and Total Acidity), whereas at the same time, the electrochemical response of the sample was analyzed with the electronic tongue. Before the electrodes were used, they were polished. Having completed the measurement, the electronic tongue was switched off until the next samples arrived 2 days later. All the samples were measured with the electronic tongue under thermostatted conditions (25.0 ± 0.1 °C).

In this study, we employed a Large Amplitude Pulse Voltammetry (LAPV) wave form (Gutés et al., 2006; Winquist, Lundström, & Wide, 1999). Fig. 1A shows the applied pulse pattern, which consists of 40 pulses in a similar configuration to a staircase voltammetry, but with