



Mead Fermentation Process Monitoring by Using Analytical Semiobjective Techniques

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Mead is a wine-type alcoholic beverage obtained from honey fermentation, traditionally consumed in Eastern Europe and Africa. In this work mead was obtained by alcoholic fermentation over 21 d at 25 °C by using honey, pollen, and *Saccharomyces cerevisiae* subsp. *bayanus*, from a must of 24 °Brix. During the fermentation, different physicochemical indexes of the process were assessed, namely pH, titrable acidity, °Brix, density, and sugars' profile and ethanol concentration. The variation of these indexes, which allowed for determining productivity, conversion rate, yield, and other important variables of the process, did not show a clear relationship with the variation of sensory characteristics that the product undergoes as the fermentation process occurs. A new methodology to determine how organoleptic properties vary over time is proposed, considering their importance for the overall quality of this type of beverages. Conventionally, these determinations are made by sensory panels of trained people. In this work an electronic nose and an electronic tongue were used to evaluate the behaviour of sensory characteristics during fermentation. Such instrumental tools relate electrical signals obtained through different sensors, having different values of selectivity and sensitivity, to the presence of various chemicals responsible of the sensory profile of the samples. The dynamic electronic responses from these devices were recorded at different stages of the fermentation process and related to the physicochemical indexes. Multivariate statistical analysis permitted to find a clear correlation between the responses of electronic nose and tongue with fermentation time. These results confirm that such semiobjective techniques are adequate tools for online process monitoring, facilitating the sampling and data collection, and would eventually enable sensory-accurate online monitoring of mead fermentation processes.

1. Introduction

Mead is one of the oldest beverages and probably a precursor of beer. Its wide distribution was reported by the Egyptians, Greeks, Celts, Saxons and barbarians. In America this drink was developed by the Mayans, which dissolved the honey of stingless bees (*Melipona*) with water, macerated with pieces of a tree bark and fermented until to get a beverage used in their festivities (Berry, 2009). This beverage is obtained by fermentation of diluted honey, which reaches a content of 8 – 12 % v/v ethanol. Despite being one of the oldest alcoholic beverages, in the literature there are few studies taken as research problem relevant aspects to mead production.

Traditionally, monitoring of ethanolic fermentation at industrial level employ simple measurements as Brix degrees of alcohol (degrees Gay Lussac), percentage of volatile acidity, among others. At the end of the process, comparative sensory evaluations are done to determine whether the final product remains within the specified sensory parameters of a product defined as a pattern. Moreover, fermentation follow up can be performed by using techniques such as liquid chromatography for quantification of alcohols, organic acids and sugars, as well as gas chromatography to assess acids and alcohols (Buratti et al., 2011; Colombié, Latrille, & Sablayrolles, 2007).

Sensory analysis seeks to develop an objective methodology for determination of organoleptic parameters in food. In general, instrumental measurements are complementary to sensory evaluations conducted by humans. Among the instrumental methodologies considered to be objective, is colorimetric evaluation, which can be

performed instrumentally, more effectively than visually. There are other instrumental evaluations, called semiobjective techniques. Electrochemical instruments, such as electronic tongue and nose are included in this group. These are complementary techniques (Di Natale et al., 2000) and their employment has been demonstrated to yield a variety of useful relationships and conclusions regarding the product, the raw materials and the transformation processes (Kollmannsberger, Nitz, & Blank, 2007). Electronic noses consist of arrays of gas electrical sensors usually made of metal oxides, with different selectivity patterns, which produce a drive signal that can be recorded and analysed by a pattern recognition software. The principle is based on the fact that a large number of different compounds contribute to define a measured odour, the chemical relationship of the sensor array of the electronic nose provides a pattern output that represents a combination of all components. On the other hand, electronic tongues are based on the understanding of how taste perception works in humans, taking into account simple chemical principles, which are valid not only for food but for other types of matrices. The electronic tongues essentially comprise an array of electrochemical sensors wherein each measuring a particular property of the sample, providing a specific feature for each species tested, the sum of all these features in the sample allows for a pattern recognition each flavour. It is important to notice that the electronic tongues are effective tools for analysis, able to characterize samples through a nonspecific approach, which may provide useful information for many purposes, allowing both qualitative and quantitative applications (Oliveri, Casolino, & Forina, 2010). Since electronic tongue analysis does not require extensive sample pre-treatment, it is an easily implementable technique in production processes to perform online measurements. In the present work, an electronic nose and an electronic tongue were used to monitor ethanolic mead fermentation by using a commercial yeast strain. Their responses were correlated to conventional physicochemical parameters, such as Brix degrees, total acidity, alcohol concentration and sugars content, at the different stages of the fermentation process by means of multivariate statistical analysis.

2. Materials and Methods

2.1 Fermentation

Multifloral honey and corbicular pollen from Bolzano acquired from the local market were used for the fermentation substrate formulation. As the fermentation starter a white wine commercial yeast (*Saccharomyces cerevisiae* subsp *bayanus*) was used (Lallemand).

The fermentations experiments were performed at 25 °C for 504 h and samples were up taken nine times at different stages of the process.

2.2 Electronic nose

Analyses were performed with a portable electronic nose (PEN2) operating with the enrichment and desorption unit (EDU). The system was from WMA (Win Muster Airsense) Analytics Inc. (Germany). PEN2 consists of a sampling apparatus, a detector unit containing the array of sensors, and a pattern recognition software (Win Muster v.3.0) for data recording. The sensor array is composed of 10 metal oxide semiconductor (MOS) type chemical sensors: MOS1 (aromatic) MOS2 (broadrange) MOS3 (aromatic) MOS4 (hydrogen) MOS5 (arom-aliph) MOS6 (broad-methane) MOS7 (sulphur-organic) MOS8 (broad-alcohol) MOS9 (sulph-chlor) MOS10 (methanealiph). The sensor response is expressed as resistivity (Ohm). EDU is a microprocessor-controlled device capable of automatically trapping and thermally desorbing the samples. The adsorbent material is Tenax-TA® polymer, 150 mg.

Three samples, each of 1 mL, during different fermentation times were taken and placed in 30 mL Pyrex® vials provided with a pierceable Silicon Teflon disks in the cap. The analysis was done by using the methodology reported by (Buratti, Benedetti, Scampicchio, & Pangerod, 2004).

2.3 Electronic tongue

Analyses were performed with the commercially available Taste-Sensing System SA 402B (Intelligent Sensor Technology Co., Ltd., Japan). The detecting part of the system consists of detecting sensors whose surface is combined with artificial lipid membranes having different response properties to chemical substances on the basis of their taste. The measurement principle of the electronic tongue is based on the capability of tasty substances to change the potential of the detecting sensors through electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes.

Three musts samples of 60 mL were measured by using the same procedure reported by (Buratti et al., 2011), and the "taste values" were calculated by multiplying sensor outputs for appropriate coefficients based on Weber Fechner law, which gives the intensity of sensation considering the sensor properties for tastes.

2.4 Chemical analysis

Complete chemical analysis for each wine sample was performed in order to measure those features that are believed to be relevant for wine characterization, namely titratable acidity, density, °Brix, ethanol content and total extract were determined as reported by (Buratti et al., 2004).

Glucose, fructose, ethanol and glycerol were determined by high-performance liquid chromatography (HPLC) analysis, using a HPLC apparatus (Jasco 2000, Japan) equipped with a gradient pump (Jasco 2000, Japan), refractive index detector (RI- Jasco 2000, Japan). A SugarPak 1 column (300 mm × 76.5 mm, Waters, USA) was used.

2.5 Data analysis

A principal component analysis (PCA) of the different parameters assessed (including the electronic tongue and nose responses and the physicochemical indexes) was carried out with the Origin software. This multivariate statistical analysis permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables.

3. Results

The behaviour of different physicochemical variables at different fermentation times are presented in the Figures 1 and 2. It is clearly noticeable how overall sugars concentration decreases with time, while ethanol (Figure 1), glycerol and total acidity (Figure 2) increase, corresponding to the expected behaviour of an ethanolic fermentation (Ramalhosa, Gomes, Pereira, Dias, & Estevinho, 2011).

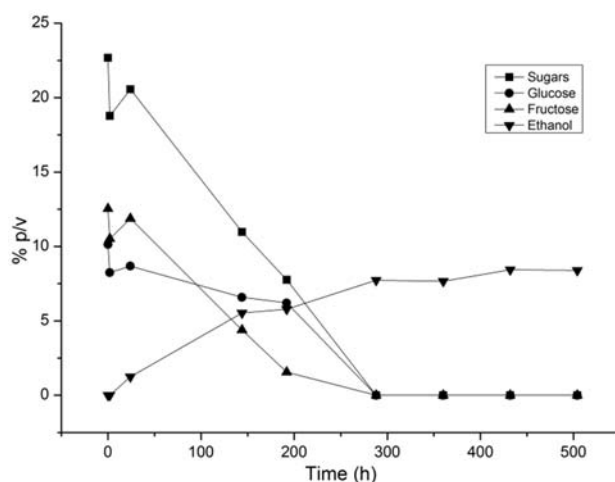


Figure 1: Sugars and ethanol profiles among time

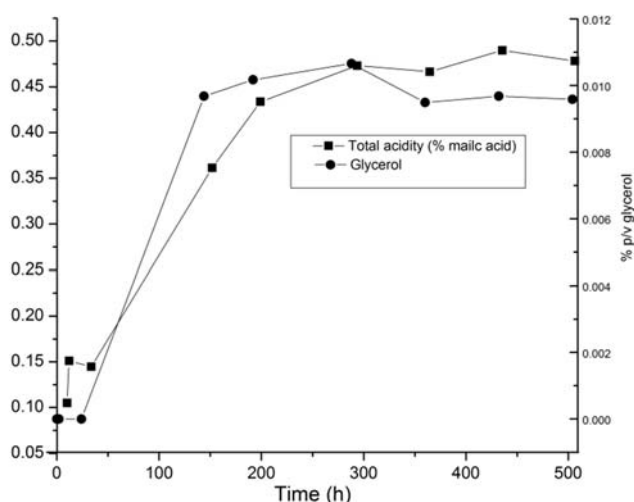


Figure 2: Total acidity and glycerol profile among time

The behaviour of some sensors of the electronic nose with fermentation time is presented in figure 3, whereas the behaviour of each sensory measurement made by using the electronic tongue is presented in figure 4. These results show that the signals of both electronic nose and tongue are related to the fermentation progress, as confirmed by doing a principal components analysis of all data. Figure 5 shows the result of the two principal components which explains the 92 % of total data variance, and it permits to affirm that fermentation time is related with all measurements performed. At the beginning of fermentation process, sugars consumption, sweetness perception, density, and sensors such as W1W, W2W, W3S and W6S are important. In the middle of the fermentation process, sensors W1C, W3C and W5C are related with the flavour that is developed by the yeast, and when time is finishing, total acidity, ethanol, glycerol content, acidity and astringency are much more important such as sensors W2S and W1S.

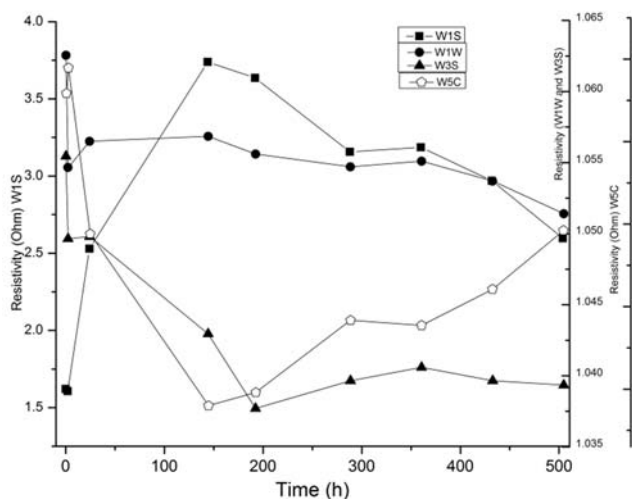


Figure 3: Signals obtained by some sensors of the electronic nose

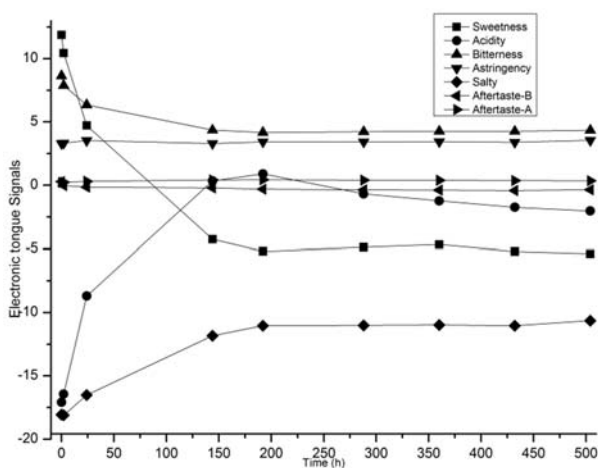


Figure 4: Electronic tongue sensory profile

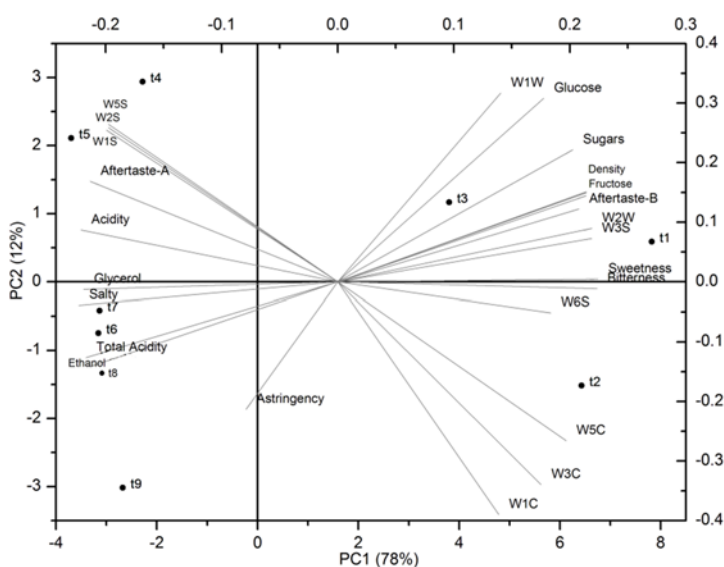


Figure 5: PCA analysis for all variables determined during time

4. Conclusions

It is possible to use electronic tongue and electronic nose to monitor a mead fermentation process as all signals determined vary with the process time. The fact that their signals are related to different physicochemical and sensory relevant characteristics, and that these analytical techniques require minimum sample treatment, thus reducing the measuring time, suggest that they are promising tools to be applied for online monitoring of fermentation processes as well as other types of transformation processes because there is not required any processing treatment for the sample, which reduces the measuring time.

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