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Electroanalysis Applied to Monitor Mead Fermentations

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Electroanalysis is applied to study reactions that involves oxidations and reductions. Currently, there are different studies that report electroanalysis used to determine food quality, classification, and evaluation of different types of contaminants or adulterants as well as to quantify different nutrients and functional compounds of interest; however, there are few applications of these techniques for the evaluation of productive processes over time. Mead is one of the oldest and most traditional alcoholic beverages and its production is regulated according to the initial must (water content, honey and other raw materials) and final product physicochemical characteristics. Normally alcoholic honey must fermentation is usually monitored by using traditional techniques such as pH, acidity, Brix, density, and others such as liquid and gas chromatography, Proton Transfer Reaction – Mass Spectrometry as well as electronic nose and tongue. There are different studies related to mead fermentation that report yeast selection, effect of nitrogen sources, immobilization, addition of different flavour enhancers such as spices and fruits, among others. In this work some of traditional techniques and electroanalysis were applied to evaluate mead fermentation process during time for mead production at 25°C for 30 days. It was found that it is possible to follow during time alcoholic fermentation by using commercial sensors and techniques such as cyclic and square wave voltammetry, because there is a relationship to sugars consumption during fermentation, as well as to organic acids generation, converting electroanalysis in a useful tool to evaluate fermentative processes.

1. Introduction

Mead is a traditional drink in some countries of Africa and Eastern Europe. It is obtained by alcoholic fermentation of diluted honey, which reaches a content of 8 to 12% v/v of ethanol. It was used *Saccharomyces cerevisiae* an ethanol tolerant which presents a high rate substrate conversion and growth at pH among 4.0 and 5.0.(Ramalhosa et al. 2011).
Simple measurements such as Brix degrees, alcohol degrees (Gay Lussac degrees), percentage of volatile acidity, and others have been used to follow industrial ethanolic fermentations. At the end of the process, comparative sensory assessments are made to determine if the final product is within the sensory parameters specified in a known standard product. Also, at research studies, this fermentation has been monitored by using techniques such as liquid chromatography for alcohols, organic acids and sugars quantification, as well as gas chromatography for esters and alcohols (Roldán et al. 2011).
When culture medium has a simple composition, it is easy to follow biomass growth or substrate consumption by using different techniques such as Kejhdal, dry weight, spectrophotometry, electrical conductivity, turbidity, among others. But since honey contains different substances such as aminoacids, organic acids and other nutrients, those techniques recommended for a large number of fermentations cannot be used to track both substrate consumption and biomass production, and therefore, it is necessary to find tools that allow to carry out these determinations in a simple way (Zaunmüller et al. 2006).
Voltammetry is an electroanalysis method where a potential is applied to the surface of an electrode and the resulting current is measured with a three-electrode system. It can be a method for determining reduction potential of an analyte and its electrochemical reactivity. It is considered a non-destructive method since only a small amount of sample is used without any treatment.

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There are a lot of studies which report voltammetric sensors applied to alcoholic beverages characterization (Novakowski et al. 2011; Wei et al. 2011; Prieto et al. 2011; Pigani et al. 2011; Gil-Sánchez et al. 2011; Gutiérrez et al. 2011).
Square wave voltammetry (SWV) allows to perform a linear sweep in time of the current on a working electrode while measuring the potential between it and a reference electrode, as well as a potential stair sweep. It is obtained a potential function of the current. This is one of the most sensitive techniques, whose main advantage is the nanomolar concentrations detection (Wang 2000).
Despite mead is an ancient beverage, there are no reports in which electroanalysis is used to follow its fermentation process. The objective of this work was to use square wave voltammetry as an electrochemical technique to monitor the progress of the alcoholic fermentation of honey.

2. Materials and methods

2.1 Mead fermentation

Traditional mead was fermented for 306 hours at 25°C in triplicate at the Instituto de Ciencia y Tecnología de Alimentos by using the methodologies reported by (Cuenca et al. 2015). Commercial yeast of *Saccharomyces Cerevisiae subsp bayanus,* tap water from Bogota's aqueduct network, Colombian multifloral honey and bee pollen from Boyacá, Colombia were used. Samples were taken at five different times (0, 48, 140, 242 and 306 hours) in triplicate.

2.2 Physicochemical mead characterization

Meads were characterized by determinations of different parameters: titratable acidity, density, Brix degree and ethanol content as reported by (Hernández et al. 2015). It was determined also product/substrate yield. Glucose, fructose and ethanol content were determined by high-performance liquid chromatography (HPLC) analysis, with a HPLC apparatus (Jasco 2000, Japan) equipped with a gradient pump (Jasco 2000, Japan), refractive index detector (RI- Jasco 2000, Japan) and a SugarPak 1 column (300 mm × 76.5 mm, Waters, USA).

2.3 Electrochemical characterization

A multi-potentiostat (Palmsens, the Netherlands) with two different voltammetric commercial microsensors (BVT Technologies, Czech Republic) with copper and platinum as working electrodes, silver/silver chloride reference electrodes and platinum counting electrodes were used. Methodologies reported by Marioli & Kuwana, 1992, Novakowski et al., 2011 were used. Each measurement was done in triplicate. Copper working electrode and 0.1M sodium hydroxide as an electrolytic solution for mead fermentation monitoring. Platinum working electrode and 0.01M perchloric acid with 0.1 M of potassium chloride as an electrolytic solution were used.

2.4 Data analysis

Relationship between electrochemical sensors signals and the carbohydrate content was established by using multivariate statistical technique of Ordinary Least Squares (OLS), in which a function was obtained to characterize and predict the behavior of a set of samples belonging to the same class, and being used as a prediction tool. In this case, a cross-validation data was performed, with correlation coefficient R and mean calibration error RMSEC, being reported for both training and model validation. It is said that a model has robustness and adequate predictive capacity, when both R and R_{cv} are close to 1 and the difference $|R - R_{cv}|$ close to 0. Regression models were performed by using Matlab software (The Mathworks, USA).

3. Results and analysis

Figure 1 presents the results obtained for glucose and fructose consumption and ethanol production profiles obtained by HPLC. As observed, at 242 hours almost all sugars were consumed by yeast and converted into ethanol, confirming the typical behavior of an ethanolic fermentation as reported by (Ramalhosa et al. 2011; Gupta & Sharma 2009; Cuenca et al. 2015). These results are correlated to current trends obtained for copper and platinum sensors shown in Figures 2.

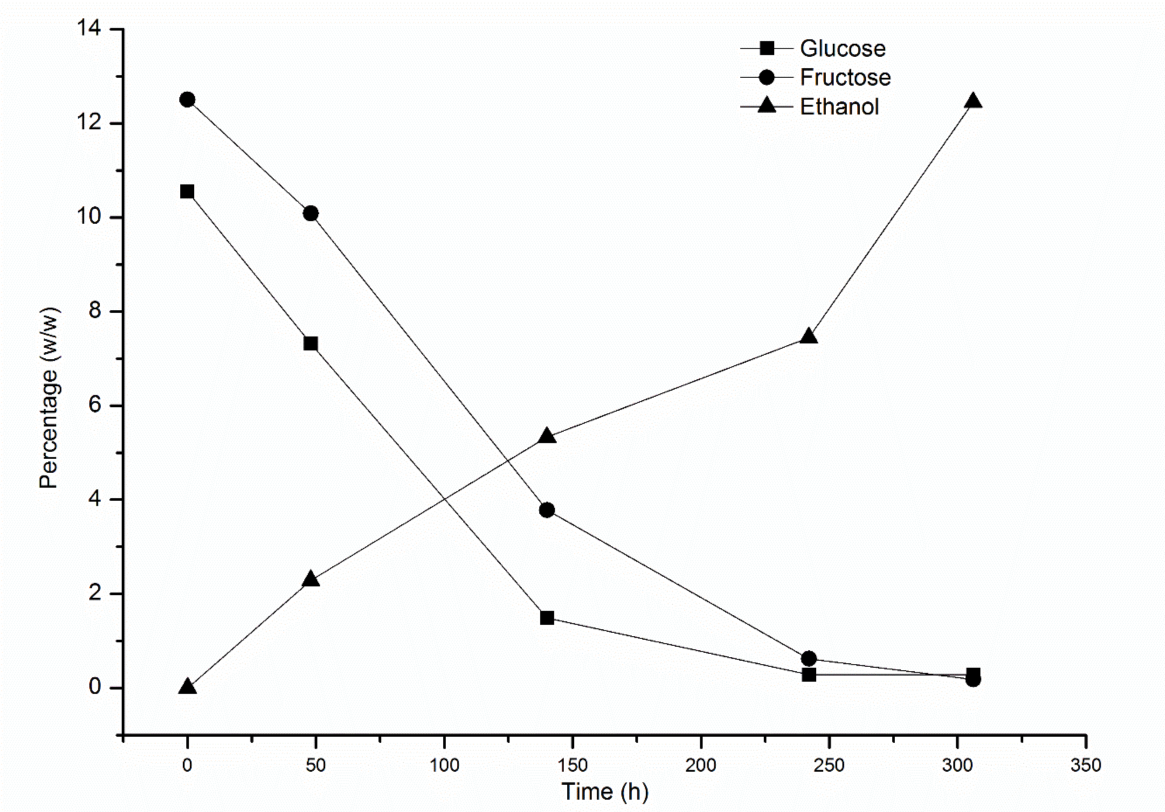

Figure 1: Glucose, fructose and ethanol behaviour during mead fermentation time

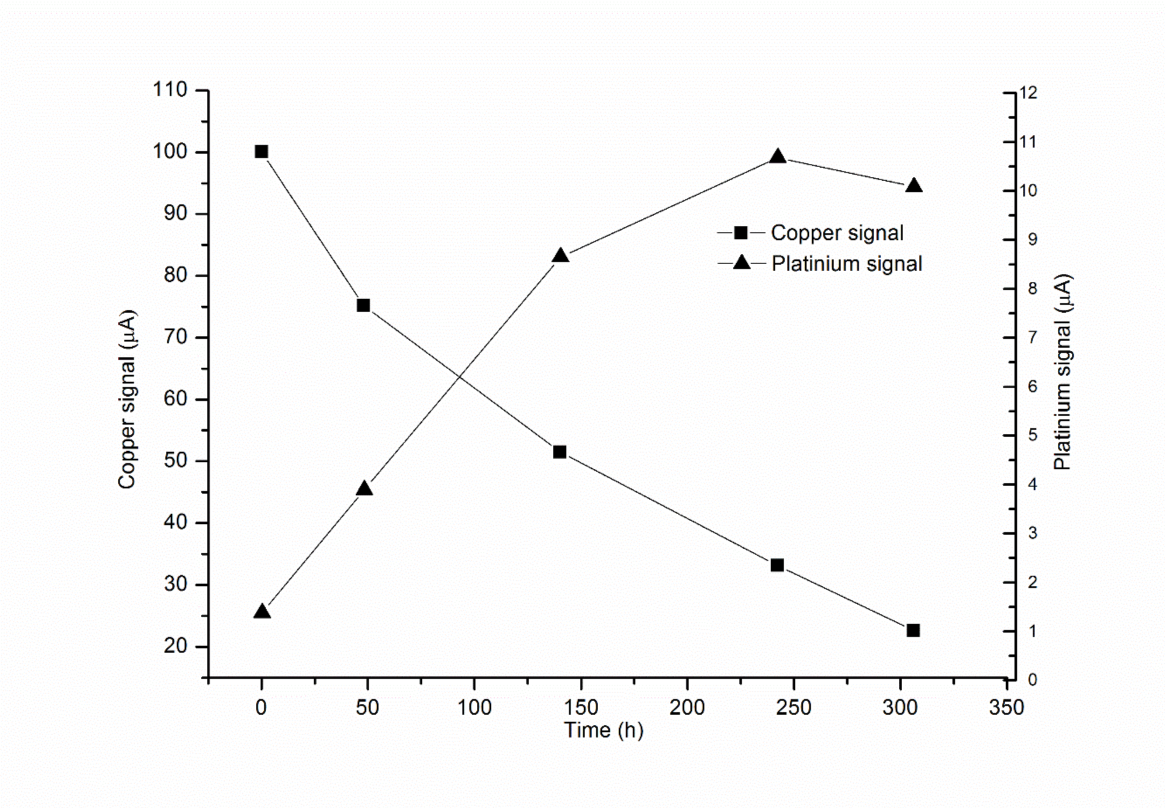

Figure 2: Copper and platinum sensors current signals during mead fermentation time

Figures 3, 4 and 5 show the results of the principal component analysis (PCA) applied to this fermentation. It is observed that main changes occur around the 140 hours. In addition, a higher correlation between the first days of the fermentation (0 - 50 hours) and copper electrode signals and sugar content is evident, whereas for the end (242 to 306 hours), the correlation is given by the results of the platinum electrodes, total acidity and product/substrate yield.

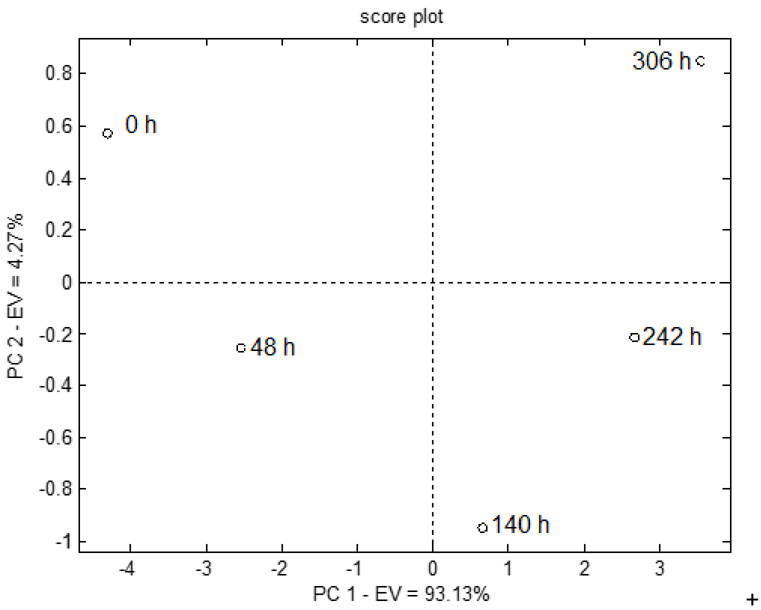

Figure 3: Score plot for PCA analysis during mead fermentation

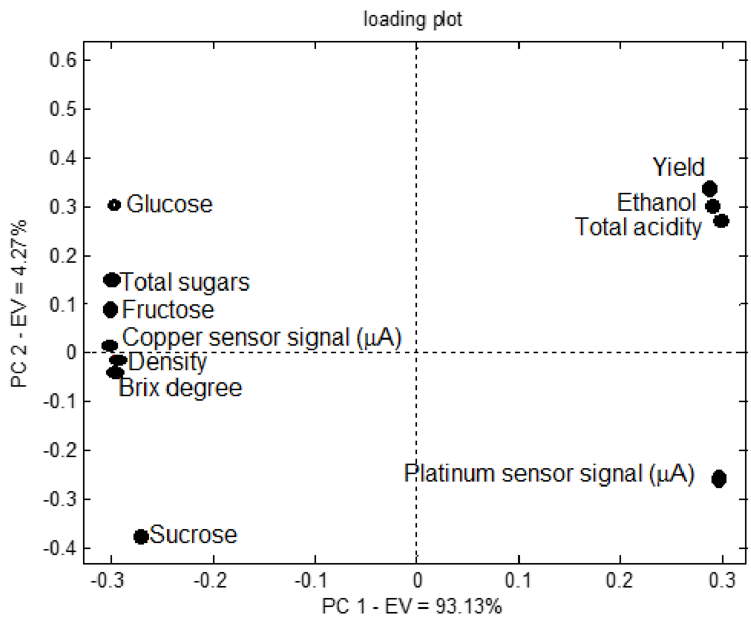

Figure 4: Loading plot for PCA analysis during mead fermentation

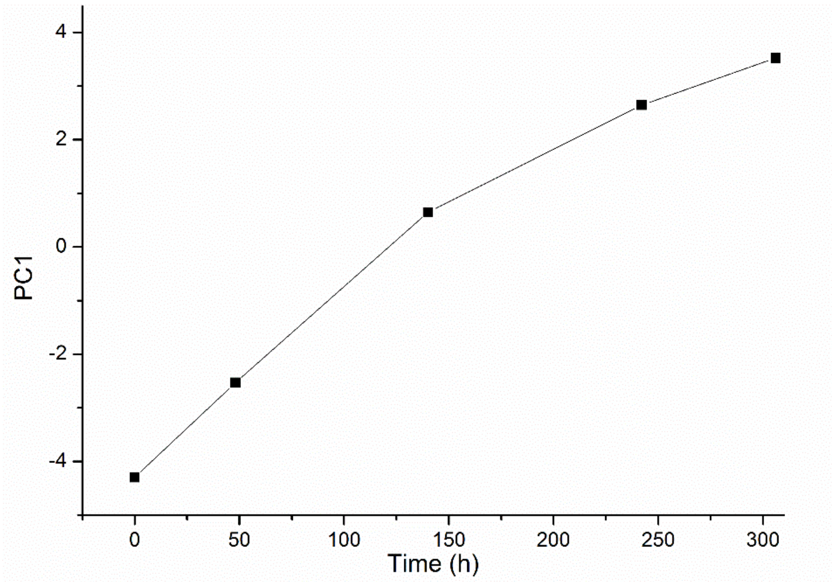

Figure 5: PC1 eigenvalues obtained from PCA analysis during mead fermentation

An ordinary least squares regression was performed by using copper and platinum current signals. Carbohydrates and ethanol concentration were used as responses, and current was used as independent variable. Table 1 shows the results, showing a low R for ethanol, but high for the other variables. In Table 1, Pt and Cu represent the currents determined with these sensors.

Table 1: Ordinary Least Squares regression results for copper and platinum current signals

Variable	R	RMSEC	Rcv	RMSEC cv	Equation
Total sugars	0.995	0.669	0.946	2.186	Y = 16.3583 + 0.1078*Cu - 1.7609*Pt
Fructose	0.991	0.469	0.919	1.427	Y = 7.8712 + 0.0643*Cu - 0.8745*Pt
Glucose	0.992	0.373	0.959	0.852	Y = 11.6369 + 0.0024*Cu - 1.1227*Pt
Ethanol	0.966	0.796	0.627	2.628	Y = 30.1001 - 0.2929*Cu - 1.1588*Pt

4. Conclusion

Square wave voltammetry by using copper and platinum commercial sensors allows to establish a relationship between generated currents with the behavior of glucose, fructose and ethanol concentration over time by using the square wave voltammetry technique. This fact is confirmed by the accuracy of the linear correlation obtained between these parameters within the models obtained, allowing to generate a tool not only for alcoholic beverage industry but also for food sector, due to its simplicity and speed, since it is possible to follow alcoholic mead fermentations by using these types of techniques.

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