Journal of Food Engineering 149 (2015) 61–69

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Ultrasonic velocity of water–ethanol–malic acid–lactic acid mixtures during the malolactic fermentation process

M.A. Amer [a,*], D. Novoa-Díaz [b], A. Puig-Pujol [c], J. Capdevila [c], J.A. Chávez [b], A. Turó [b], M.J. García-Hernández [b], J. Salazar [b]

[a] Escola Universitària Salesiana de Sarrià, Passeig Sant Joan Bosco 74, 08017 Barcelona, Spain
[b] Sensor Systems Group, Department of Electronic Engineering, Universitat Politècnica de Catalunya, Jordi Girona 1-3, 08034 Barcelona, Spain
[c] Secció d'Investigació Enològica, Estació de Viticultura i Enologia, Institut Català de la Vinya i el Vi (INCAVI), Plaça Àgora, 2, Polígon Industrial Domenys II, 08720 Vilafranca del Penedès, Spain

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form 23 September 2014
Accepted 25 September 2014
Available online 7 October 2014

Keywords:
Malolactic fermentation
Ultrasound
Process monitoring
Lactic acid
Malic acid

ABSTRACT

During malolactic fermentation in wines, malic acid is transformed into lactic acid by the action of lactic acid bacteria. This process can be monitored on-line by measuring the velocity of a low intensity ultrasonic wave propagating through the medium. In this work, an experimental study of ultrasonic propagation velocity in laboratory mixtures of water–ethanol–malic acid and lactic acid is presented. A good correlation was found between the ultrasonic velocity and malic and lactic acid concentrations. These results could be used to predict the end-point of the malolactic fermentation process and show the great potential of this ultrasonic technique to determine malic and lactic acid concentrations during the malolactic fermentation process.

1. Introduction

Malolactic fermentation (MLF) is a process that consists in the transformation of malic acid into both lactic acid and carbon dioxide. This process, caused by lactic acid bacteria (LAB), takes place during the production of the majority of red wines as well as when producing ceratin types of white wines. The contribution of MLF is vital to the development of the sensory characteristics of wine: it reduces acidity, it adds microbiological stability and it improves the organoleptic profile by producing a wide range of colours, flavors and aromas (Wibowo et al., 1985; Maicas et al., 1999; Liu, 2002; Lerm et al., 2010).

During the winemaking process, MLF may be produced spontaneously due to the presence of lactic acid bacteria on the surface of the grapes. This may result in a lack of control over the malolactic stage and interferes with other stages, with uncertain results in the wine characteristics.

In order to obtain a correct, controlled malolactic fermentation process, a method known as "induced MLF" was recently introduced, consisting in the systematic inoculation of natural strains of LAB and the efficient monitoring of the MLF that ensues. However, the success of such an induced MLF is not guaranteed in all cases. The task of monitoring the progress of MLF is mostly carried out by measuring the concentration of malic and lactic acids in wine samples. Several measurement methods such as Paper Chromatography (PC), Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), enzymatic analysis, Fourier-transform Infrared Spectroscopy (FT-IR) and reflectance are described in the literature (Lerm et al., 2010). Most of these methods, however, share the fact that they are both destructive and, rather complex. Moreover, when these methods are used, obtaining accurate results tends to be a rather time consuming process. On top of all of that, the methods themselves are, generally speaking, not affordable to small wineries.

Ultrasound is an emerging and promising technology for both wine processing and property sensing, at present mostly limited to research activities within a laboratory environment (Cortada et al., 2011; Jiranek et al., 2008; Lamberti et al., 2009; Salazar et al., 2009). As a novelty, an ultrasonic technique is proposed here to be used as an in-situ method for the on-line monitoring of the MLF progress. Unlike the conventional methods above, ultrasonic techniques are non-invasive, non-destructive, accurate, rapid, non-expensive, on-line and suitable for process automation (McClements, 1997). Having said that, these techniques are known to be highly sensitive to physical parameters such as temperature, an aspect that can sometimes act as a disadvantage.

* Corresponding author.
E-mail address: maamer@euss.es (M.A. Amer).

http://dx.doi.org/10.1016/j.jfoodeng.2014.09.042
0260-8774/

The purpose of this paper is to study the ultrasonic propagation velocity of a 1 MHz sine-wave tone burst in laboratory mixtures of water–ethanol–malic acid and lactic acid, and the interactions between malic and lactic acids concentrations. A change in concentration of any of these components is seen to result in a change in the ultrasonic propagation velocity.

Experimental results show a good correlation between ultrasonic propagation velocity and the concentration of malic and lactic acids. Considering the overall costs of wine production management and control in terms of manpower, sampling and chemical analyses, the proposed system could represent an attractive solution for the on-line monitoring of malolactic fermentation processes. In addition, the ultrasonic velocity could also be used to predict the end of the malolactic fermentation process. The experimental method used, the difficulties encountered along the way as well as the results obtained are also discussed in this paper.

2. Materials and methods

2.1. Malolactic fermentation (MLF)

2.1.1. Stoichiometry

As shown in Eq. (1), during MLF malic acid ($C_4H_6O_5$) is transformed to lactic acid ($C_3H_6O_3$) and carbon dioxide (CO_2). This process is catalysed by a highly specialized enzyme (the "malolactic enzyme") and carried out by the LAB, mainly those of *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* strains (Wibowo et al., 1985).

$$C_4H_6O_5 \rightarrow C_3H_6O_3 + CO_2 \tag{1}$$

Stoichiometrically, 1 mol of malic acid produces 1 mol of lactic acid and 1 mol of carbon dioxide. But if mass concentrations are considered, the relationship between malic acid and lactic acid in an MLF process is described by Eq. (2)

$$x_{\text{lactic acid}} = x^{o}_{\text{lactic acid}} - \frac{M_{\text{lactic acid}}}{M_{\text{malic acid}}}\left(x_{\text{malic acid}} - x^{o}_{\text{malic acid}}\right) \tag{2}$$

In Eq. (2), x_i refers to mass concentration of component i, superscript ^{o} refers to the beginning of the fermentation and M_i represents the molar mass of component i.

The molar masses of lactic acid and malic acid are 90.08 g/mole and 134.09 g/mole, respectively. According to this, the ratio of these molar masses is approximately 0.67. So, considering Eq. (2), during the MLF process, a 3 g/l reduction of malic acid equals to an increase of about 2 g/l of lactic acid.

2.1.2. MLF process

Three steps are defined in MLF, correlative in time: (i) bacterial growth phase, (ii) stationary phase I and (iii) stationary phase II (Krieger, 2006).

(i) Bacterial growth phase:

This phase starts when lactic acid bacteria (LAB) are inoculated. LAB growth takes place during this phase. This results in a consumption of sugars that were no fermented during the alcoholic fermentation phase. A slight amount of acetic acid is also produced. No malic acid is metabolized, so malic acid and lactic acid concentrations are stable.

(ii) Stationary phase I:

This phase starts when the bacterial growth phase is finalized. During this phase, the amount of LAB is stable and malic acid is transformed to lactic acid. No sugar consumption is produced (LAB prefer malic acid).

(iii) Stationary phase II:

This is the last phase. During this third phase, no more malic acid is transformed to lactic acid, but citric acid is degraded and acetic acid is produced. Also, the amount of LAB is reduced. This phase should be avoided in wineries, because wine characteristics are degraded.

So, it is important to determine the end point of phase (ii), in order to prevent phase (iii) from happening.

2.1.3. Control of MLF

Decarboxylation of the malic acid in wine is the most obvious action of the MLF. The easiest way to monitor the progress of the MLF is to chemically analyze the disappearance of malic acid and the formation of lactic acid. The most commonly used quantitative analytical method for monitoring MLF is the enzymatic determination of L-malic acid. This method uses an enzyme that specifically reacts with L-malic acid and a UV–visible spectrophotometer to monitor the progress of the analytical reaction. Kits from manufacturers that contain all the reagents, enzymes and procedures required for L-malic acid determinations are readily available. For this study a multiparametric analyzer Lisa 200 (Hycel diagnostics, TDI Tecnología Difusión Ibérica, S.L., Spain) was used. In addition, two separate kits were used, one for each reagent: an L-Malic Acid Enzymatic Kit (Boehringer Mannheim-Roche, Spain) and an L-Lactic Acid Enzymatic Kit (Boehringer Mannheim-Roche, Spain). The detection of L-malic acid requires two enzyme reactions. In the first reaction, malic acid (L-malate) is oxidized to oxaloacetate by nicotinamide-adenine dinucleotide (NAD) in the presence of L-malate dehydrogenase (L-MDH):

$$\text{L-Malate} + NAD^{+} \xleftrightarrow{\text{L-MDH}} \text{oxaloacetate} + NADH + H^{+} \tag{3}$$

However, since the equilibrium of reaction (Eq. (3)) lies firmly in the favor of L-malate and NAD^{+}, a further reaction is required to trap the NADH product, and this is achieved by the conversion of oxaloacetate to L-aspartate and 2-oxoglutarate, in the presence of a large excess of L-glutamate, by glutamate–oxaloacetate transaminase (GOT):

$$\text{Oxaloacetate} + \text{L-glutamate} \xleftrightarrow{\text{GOT}} \text{L-aspartate} + \text{2-oxoglutarate} \tag{4}$$

The amount of NADH formed is stoichiometric to the amount of L-malate. The increase in NADH is measured through the measurement of its light absorbance at 334, 340 or 365 nm.

2.2. Ultrasonic velocity in liquid media

When the distance travelled by an ultrasonic wave through a liquid medium is a known constant, the wave's velocity can be calculated using Eq. (5)

$$v = \frac{d_{\text{travelled}}}{TOF} \tag{5}$$

where TOF corresponds to the time of flight, which is the time taken by a wave to travel a given distance ($d_{\text{travelled}}$). A series of practical methods to measure TOF were described and analyzed in a previous paper (Novoa-Díaz et al., 2012), as was the method for determining ultrasonic velocity.

Generally, TOF varies in accordance with the physical and chemical changes in the medium. Given this, it is reasonable to assume that variations of lactic and malic acid concentrations in the liquid mixture will cause changes to the TOF, and