Piezoelectric MEMS resonators for monitoring grape must fermentation

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Abstract. The traditional procedure followed by winemakers for monitoring grape must fermentation is not automated, has not enough accuracy or has only been tested in discrete must samples. In order to contribute to the automation and improvement of the wine fermentation process, we have designed an AlN-based piezoelectric microresonator, serving as a density sensor and being excited in the 4th-order roof tile-shaped vibration mode. Furthermore, conditioning circuits were designed to convert the one-port impedance of the resonator into a resonant two-port transfer function. This allowed us to design a Phase Locked Loop-based oscillator circuit, implemented with a commercial lock-in amplifier with an oscillation frequency determined by the vibrating mode. We were capable of measuring the fermentation kinetics by both tracking the resonance frequency and by determining the quality factor measurements of the microresonator. Moreover, the resonator was calibrated with an artificial model solution of grape must and then applied for the monitoring of real grape must fermentation. Our results demonstrate the high potential of MEMS resonators to detect the decrease in sugar and the increase in ethanol concentrations during the grape must fermentation with a resolution of 100 µg/ml and a sensitivity of 0.16 Hz/µg/ml as upper limits.

1. Introduction

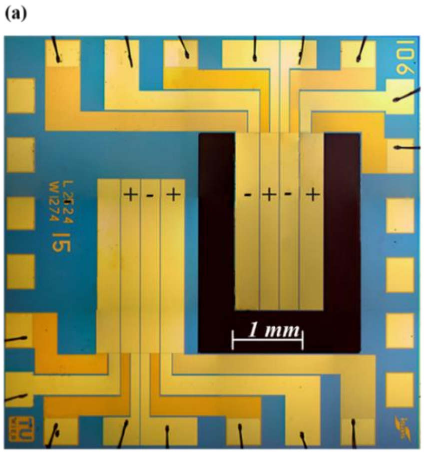
The fermentation of grape must involves the interaction between yeasts, bacteria, fungi and viruses. A correct bio-chemical process is a necessary condition but not sufficient by itself to determine the final quality of the wine, whose assessment relies on a comprehensive analysis of its chemical components, as the basic flavour of wine depends on at least 20 compounds [1]. Therefore, winemakers must carefully supervise the wine fermentation process to ensure a wine of the expected quality. One of the key parameters monitored during wine fermentation is the fermentation kinetics. This provides essential information about the steady transformation of grape must into wine due to the decrease of glucose and fructose that leads to the formation of ethanol, glycerol and carbon dioxide along with biomass, as a result of yeast metabolism [2]. This process is traditionally monitored by enologists, who manually extract and analyse discrete samples at least twice a day using an aerometer, spectrophotometer or a colorimeter. They essentially determine the density and its rate of change since these parameters provide information about the evolution of the fermentation as a result of yeast metabolism.

However, there are different ways for monitoring the grape must fermentation: (1) density determination, based either on differential pressure measurements [3] or on the use of flexural oscillators [4]; (2) monitoring of CO_2 released during the process due to the gradual loss of mass [3,5]; (3) determination of yeast cell population evolution by means of impedance techniques [6] and turbidity measurements [7]; (4) ultrasound measurements conducted to determine the propagation velocity in grape musts [8,9]; (5) refractive techniques based on fiber optics [10]; (6) optoelectronic device based on measurements of the refractive index [11,12].

Overall, these different approaches have not enough resolution or have only been tested in discrete must samples. In this context, the simultaneous determination of the density and viscosity of liquids, through measurement of the resonant frequency (f_r) and quality factor (*Q-factor*) of a mechanical resonator, has already been reported [13–15]. This approach presents several advantages with respect to traditional methods, such as real-time analysis, on-line configuration and low liquid volumes. Piezoelectric resonators with in-plane vibration modes [16], flexural and torsional modes [17] have already been tested. However, it is well-established that a different family, such as the roof tile-shape modes, present better quality factors at moderate frequencies [18,19]. The present work evaluates the performance of this vibration mode, within micromachined self-actuated and self-sensing aluminium nitride (AlN)-based cantilever sensor for the monitoring of grape must fermentation.

2. Resonator design and characterization

The resonator was designed for the 4th order roof-tile shaped vibration mode. It resulted in a cantilever resonator with optimized electrode layout featuring five nodal lines in one direction, and 1 nodal line in the perpendicular direction. Considering Leissa's nomenclature, the vibration mode is named as 15-mode [20]. The resonator was designed with a length of L=2524 µm, width of W=1274 µm and thickness T of about 20 µm. It was fabricated from a SOI wafer with a 20 µm-thick device layer covered with a 650 nm-thin AlN piezoelectric film [21]. The top metallization has four striped electrodes that allow a selective excitation of the vibration modes and act as a filter for higher modes [17]. The considered modal shape and the fabricated resonator are shown in figure 1.



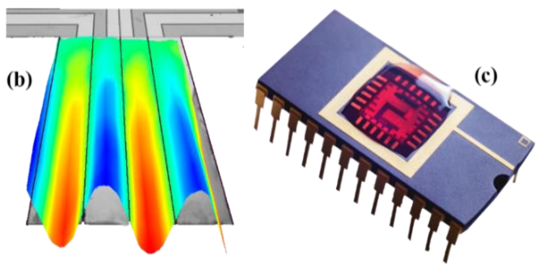


Figure 1. (a) Top-view micrograph of the resonator. (b) Modal shape measured (15-mode) with a laser Doppler vibrometer. (c) The resonator is packaged, wire bonded in a 24-pin DIP (dual inline package) and immersed in grape must.

3. PLL-Based oscillator

The simultaneous determination of density and viscosity of liquids, through measurement of the resonant frequency and the quality factor of a mechanical resonator, is challenging due to the low quality factors and parasitic effects present in liquid media [22]. For this reason, our approach is based on roof tile-shaped resonators in two-port configuration and a parasitic compensating device.

In the two-port scheme, one of the electrodes was used for actuation (+) and the other for sensing (-) (see Figure 1). However, our results show that a capacitive crosstalk across the actuation and sensing ports has a significant contribution to the output current.

To minimize this parasitic component, a compensating device was introduced. This dummy device reproduces the structure of the resonator, but without the backside release, thus preventing any vibration. For this reason, we designed an interface circuit, used in a previous work [15], to subtract the dummy response (v_{dum}) from that of the resonator (v_{res}). Since the materials and dimensions in the dummy and resonator devices are the same, they are expected to show identical electrical behaviour with respect to their parasitic effects. This results in a clear resonance, with low baseline (see Figure 2). However, a phase shift (about -12º) is introduced by the instrumentation amplifier, representing the differences in electrical performance between the dummy, the resonator device and C_{ft}.

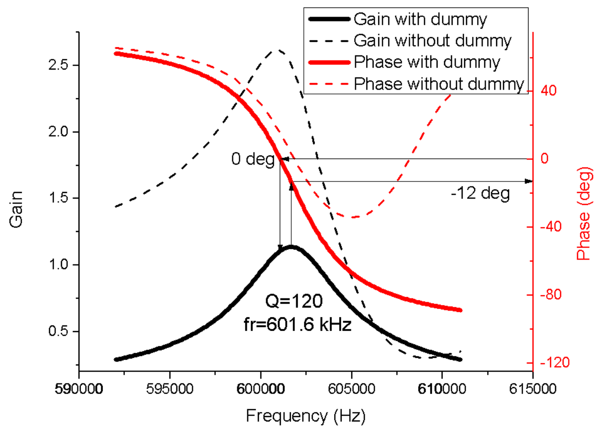


Figure 2. Open loop response for the 15-mode measured in a liquid test (2-Propanol) with and without the dummy compensation.

In order to avoid this issue, the resonators were finally included in an oscillator based on a Phase Locked Loop (*PLL*) instrument, instead of an oscillator circuit based on discrete components [15]. The phase shift was compensated by the *PLL* configuration, so that the oscillation frequency and the natural frequency of the resonator are approximately equal. The *PLL* instrument used (*Zurich HF2LI*) provides software-based voltage controlled oscillator (*VCO*), proportional-integral controller (*PI*) and phase detector (*PD*) blocks that form a control system able to track the resonant frequency.

4. Experimental details

With the goal of estimating the density (ρ) and viscosity (μ), thus being able to monitor the fermentation process, two variables were measured from our interface circuit: the oscillation frequency (f_{osc}) and the gain of the interface circuit module at operation (G_{osc}), given by the ratio V_{out}/V_{in}. Furthermore, for the application of the resonator as a density-viscosity sensor, a calibration procedure was developed with a model solution of artificial grape must [19] and finally applied to the monitoring of one model sluggish fermentation and other real grape must fermentation process. This calibration process was carried out in two steps, each with adjustable parameters [15].

4.1. Preparation of the model solutions of grape must

In order to confirm that piezoelectric resonators are valid for the monitoring of grape must fermentation, two different sets of model solutions were used for pre-investigation purposes. The first set represents a normal fermentation (N_1:N_9) and the second represents a sluggish fermentation (S_1:S_9). A variety of reasons may be attributable to this kind of failed or sluggish fermentations, which occasionally occur: the lack of dissolved oxygen, an unbalanced ratio between sugar and nitrogen, a low fermentation temperature, an inadequate rehydration, a thermal shock of yeast, etc. [23]. These model solutions represent different stages of the corresponding fermentation process according to their particular mixture of glucose, fructose, ethanol and glycerol dissolved in water. The composition of the prepared model solutions for a normal and sluggish fermentation is shown in a previous work [11]. In the normal model solution, a decrease in fructose and glucose concentration from 110&100 g/l (N_1) to 2&1 g/l (N_9) occurs. Similarly, the glycerol and ethanol concentration are increasing from zero to 9 g/l and 14 % v/v. In the sluggish fermentation the decrease in fructose and glucose concentration stops prematurely and settles around 55 and 20 g/l. Along with this development the increase in the ethanol concentration stops and does not exceed the value of 8 % v/v.

4.2. Calibration process with model solutions of grape must

With the aim of monitoring one real grape must fermentation, two different sets of model solutions were prepared in the previous section. The first set represents a normal fermentation (N_1:N_9) and was used as a model for the calibration process. The validation of this process was confirmed with a model sluggish fermentation (S_1:S_9) and finally with a real grape must fermentation (Section 4.4).

In the first step of the calibration process a constant K_{ic}, which is not affected by the loading conditions of the resonator, was used to transform the circuit outputs f_{osc} and G_{osc} into the key parameters of the resonator, namely the resonant frequency (f_r) and the quality factor (*Q-factor)*.

Due to the high sensitivity of the density and viscosity to the temperature and electronic noise from surrounding equipment at different frequencies, low, but measurable drift effects are present when determining f_r and *Q-factor*. The random uncertainty of f_r was obtained directly through the Allan deviation of f_{osc} [24]. However, the random uncertainty of G_{osc} was translated into random uncertainty of *Q-factor* by uncertainty propagation [25]. The random uncertainties or resolutions obtained for the model solutions of grape must were below 100 mHz for f_r and 0.7 for *Q-factor*. Figure 4 shows the mean values of f_r and *Q-factor* when the resonator is immersed in the model solutions of grape must: normal and sluggish fermentation process. The measurements of f_r and *Q-factor* were performed with a temperature control unit [15]. Nevertheless, the impact of temperature fluctuations in the resolution of the sensor during the measurement process is minimal compared with the continuous evolution of the real grape must fermentation. As can be seen in figure 3, the significant changes in the composition of the model solutions affects the f_r and the *Q-factor* of the resonator. The combination of unfermented sugars and less than expected ethanol concentration, resulted into a flat curve of f_r for the sluggish fermentation.

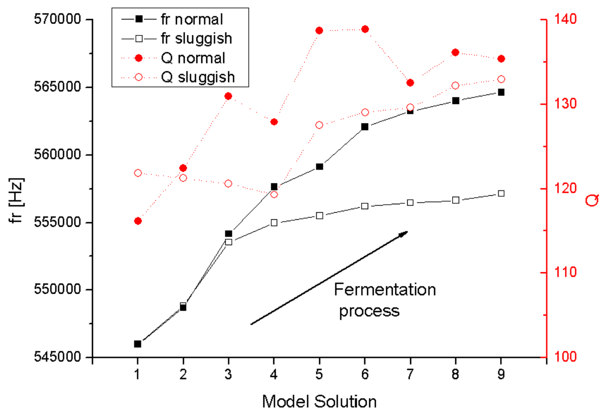


Figure 3. Resonant frequency and quality factor of the resonator immersed in two model solutions of grape must at 20°C.

In the second step of the calibration procedure, the physical properties of the liquid, density and viscosity, were obtained from the key parameters of the resonator, f_r and *Q-factor*. These are related to the mechanical properties of the resonators, i.e. resonator equivalent mass (m) and natural frequency in vacuum ($f_{0,vac}$), and the fluid conditions, i.e. distributed damping associated to the liquid

(g_1) and the distributed equivalent added mass (g_2), both per unit length (L). A model defined in previous works [26–28] allows the determination of these variables separately. Nevertheless, the dependence of g_1 and g_2 on the viscosity and density of the fluid, as a closed-form analytical expression, is only available for simple geometries and particular cases [29]. For this reason, we used a Taylor series expansion of g_1 and g_2 in terms of the viscosity, density and their product [30,31]. The model was solved iteratively to obtain the values of the coefficients (Table 1) that fit best to the experimental data for the normal model solution. The results of the density estimated values (*MEMS*) are compared to those measured from the density-viscosity meter *Anton Paar DMA4100M* (*viscometer*) at 20ºC in figure 4.

Table 1. Values of the coefficients obtained in the calibration process for the normal model solution.

	K_{ic}	C_1	C_2	C_3	C_4
Normal model solution	$3.58\cdot10^{-10}$	0.12	-20.99	2.11	-103.22

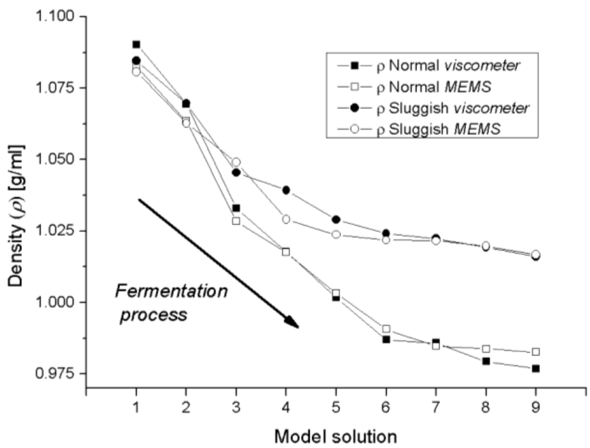


Figure 4. Density values estimated from our resonator (*MEMS*) and measured with a commercial density-viscosity meter (*viscometer*) at 20ºC for the two model solutions: normal and sluggish fermentation.

As can be seen in figure 4, an almost linear decrease in the density occurs for the normal model solution and a premature stop in the density decrease for the sluggish solution. These results show the possibility to distinguish between ordinary and sluggish fermentations at an early stage of the fermentation process in artificial grape must.

Once the density and viscosity for each liquid is known, both the error associated with the calibration process and the resolution can be determined. The error terms, are calculated as the deviation between the values of viscosity and density estimated from our calibration (*MEMS*) and the values measured with the commercial instrument (*viscometer*), obtaining a mean error term around 8.3% for the viscosity and 0.38% for the density. The resolutions were obtained from uncertainty propagation from f_r and *Q-factor* to density and viscosity, obtaining a value below 500 µg/ml and 0.017 mPa·s. As the results show, the resolution and the calibration error obtained are better in density than in viscosity. This occurs because the *Q-factor* measurement, which corresponds to the measurement of G_{osc} in the interface circuit, presents a lower resolution.

4.3. Winemaking samples

In the previous section, the theoretical background for using the density and viscosity as valid parameters of the fermentation process was checked. In addition, the performance of the resonator was verified and validated with two model solutions of grape must. Nevertheless, our main goal is the monitoring of a real red grape must fermentation process. In this section, there is a brief description of the winemaking of *Cencibel* grape must variety.

The *Cencibel* grape must was completed using 25% (w/v) of red grape skin. Afterwards, the must was inoculated with 0.2 g/ml of Saccharomyces cerevisiae strain (UCLM S325, Fould-Springer) previously rehydrated following the supplier's guidelines. A cylindrical fermenter (*mini-Bioreactor Applikon*) was filled with 3 L of inoculated must and grape skins, where the temperature was controlled at 28ºC.

The fermentation monitoring was carried out by means of the extraction and analysis of 7 mL-samples every 5 hours approximately during 6 days. After extraction the samples were centrifuged for one minute at 1000 rpm (*Universal 32R Hettich*) and kept refrigerated until analysis. Once the measurements were completed, the samples were kept frozen at -20ºC, in order to stop the evolution of the fermentation process.

In an industrial fermentation process temperature fluctuations would influence to some extent the fermentation process and thus the density. Nevertheless, it is the variations that help enologists determine the current fermentation status, and whether or not corrective measures are required as it progresses.

4.4. Monitoring grape must fermentation

In order to monitoring one real grape must fermentation, we applied the same procedure that was developed for the model sluggish solution. In this case, through the measurements of f_{osc} and G_{osc} in the interface circuit and using the coefficients obtained for the normal model solution (see Table 1), we were able to obtain the density and viscosity of the grape must.

Figure 5 represents the evolution in terms of density and viscosity of one real red wine fermentation, measured in the same way as in the previous section. Both density and viscosity follow a rather similar trend, as observed previously with the normal model solution. Therefore, it was confirmed that the calibration process performed for the normal model solution is also valid for other liquids in a similar range of density and viscosity.

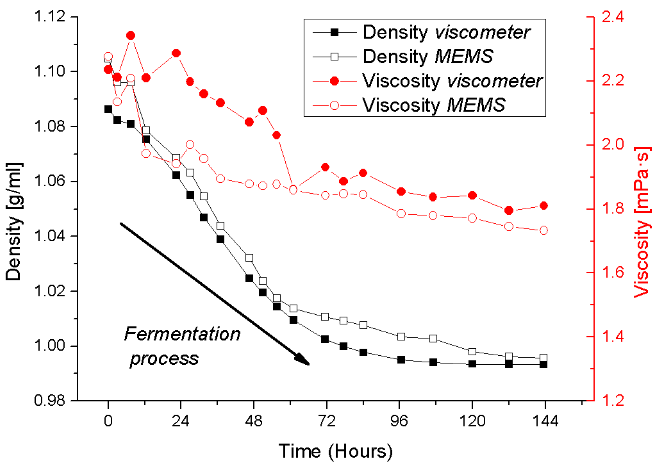


Figure 5. Evolution of density and viscosity values estimated from our resonator (*MEMS*) and measured with the commercial density-viscosity meter (*viscometer*) at 20ºC for a real fermentation process.

The error terms and resolutions were calculated as in the previous section. The mean error term obtained was around 6% for the viscosity and 0.7% for the density being the viscosity resolution below 0.006 mPa·s. In the case of the real grape must the obtained density resolution was below 100 µg/ml instead of the 1200 µg/ml value obtained in some previous works using a high-resolution low-cost optoelectronic instrument [11,12].

5. Conclusion

This work presents a novel approach for the monitoring of a grape must fermentation process with a piezoelectric MEMS resonator excited in the 4th order of the roof tile-shaped mode. Two important parameters of the resonator were measured using a PLL-based oscillator circuit: the quality factor and the resonant frequency. Once these two parameters are known, the viscosity and density of the liquid can be determined, requiring only a small amount of test liquid of about 100 µl. In order to measure the viscosity and density of a real grape must, a calibration procedure of the resonator was performed using model solutions of artificial grape must representing an ordinary (for calibration) and a stuck or sluggish (for validation) fermentation process and a commercial density-viscosity meter. Our results demonstrate the high performance of MEMS resonators to detect the decrease in sugar and the increase in ethanol concentrations during the grape must fermentation with a resolution of 100 µg/ml and a sensitivity of 0.16 Hz/µg/ml as upper limits.

Acknowledgments

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