

DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v26n5p374-380>

Development of an ethylometer with an MQ-3 sensor for measuring ethanol in soybean seeds¹

Desenvolvimento de etilômetro com sensor MQ-3 para medição de etanol em sementes de soja

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HIGHLIGHTS:

More equipments and technologies needed for fast assessment of seed quality.

The equipment developed measures ethanol in 16 s.

The ethanol equipment developed is fast and practical for evaluating seed quality.

ABSTRACT: In the seed production sector, it is essential to use fast and practical equipment to help decision-making processes. Thus, the use of sensors that can capture information to assist in agriculture has contributed significantly to soybean cultivation worldwide, being used in several stages, whether pre-harvest or post-harvest. Thus, this study aimed to develop a prototype of ethylometer with an MQ-3 sensor and to evaluate its stabilization time when used in soybean seed lots submitted to hypoxia. The research was carried out with 10 lots of soybean seeds from five cultivars (NS 8383 RR, SOY Peso RR, M 8372 IPRO, M8644 IPRO and M8808 IPRO) and was divided into two stages. The first one corresponds to the development of the ethanol measuring equipment with an MQ-3 sensor, and the second one to measurement of the sensor's stabilization time (3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 s) in soybean seeds submitted to anaerobiosis. The equipment and the MQ-3 sensor showed the stability of average measurement at 16 s after activation. This time is recommended for use of the ethylometer in soybean seeds. Through the MQ-3 sensor, it is possible to efficiently detect ethanol present in the air released by soy seeds during the anaerobic process.

Key words: *Glycine max* L., anaerobiosis, alcohol measurement

RESUMO: No setor de produção de sementes, é imprescindível o uso de equipamentos rápidos e práticos que auxiliem nos processos de tomada de decisão. Assim, o uso de sensores que podem capturar informações para auxiliar na agricultura tem contribuído significativamente para o cultivo da soja em todo o mundo, sendo utilizado em diversas etapas, seja em pré ou pós-colheita. Objetivou-se desenvolver um protótipo de um etilômetro com sensor MQ-3 e avaliar seu tempo de estabilização quando utilizado em lotes de sementes de soja submetidos à hipóxia. A pesquisa foi realizada com 10 lotes de sementes de soja, oriundas de cinco cultivares (NS 8383 RR, SOY Peso RR, M 8372 IPRO, M8644 IPRO e M8808 IPRO), a qual foi dividida em duas etapas, a primeira correspondendo ao desenvolvimento do equipamento de aferição do etanol com o sensor MQ-3 e a segunda relacionada ao tempo de estabilização do sensor (3, 6, 9, 12, 15, 18, 21, 24, 27 e 30 s) em sementes de soja submetidas ao processo de anaerobiose. O equipamento, bem como o sensor MQ-3 apresentaram estabilidade de aferição média aos 16 s após seu acionamento, sendo recomendado esse tempo para o uso do etilômetro em sementes de soja. Por meio desse mesmo sensor MQ-3, é possível, com eficiência, a detecção de etanol presente no ar liberado por sementes de soja durante o processo de anaerobiose.

Palavras-chave: *Glycine max* L., anaerobiose, medição de álcool

• Ref. 256305 – Received 13 Sept, 2021

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• Accepted 14 Dec, 2021 • Published 28 Dec, 2021

Editors: Geovani Soares de Lima & Carlos Alberto Vieira de Azevedo

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INTRODUCTION

Brazil stands out as the world's largest producer of soybeans, with record grain production in the 2020/2021 harvest of 136 million tons (CONAB, 2021). To achieve results of this magnitude, more and more sensors have been used to maximize results in the field, whether pre-harvest or post-harvest.

Considering this, an alternative has opened for studies on new vigor tests on soybean seeds, which can benefit from electronic tools. For example, Cavalcante et al. (2017, 2019) subjected ryegrass seeds and bean seeds, respectively, to hypoxia and used an adapted ethylometer to measure the amount of ethanol released by the seeds. Subsequently, they correlated their results with traditional vigor tests.

To move forward with studies related to the production of ethanol in soybean seeds and its relationship with traditional vigor tests, that have a relatively long analysis time, it is necessary to design new equipment, specific for this test, which uses sensors that allow a reliable reading, in a fast, practical, and accurate way, and would be useful for the soybean seed sector. However, any type of sensor needs to be previously calibrated and adjusted according to the needs for which it will be used (González-Buesa & Salvador, 2019).

Among the most commonly used sensors for the detection of ethanol in the environment is the MQ-3 sensor, from the family of MQ sensors, manufactured by the Chinese company Hanwei Electronics Ltd. MQ-3 sensors have ideal characteristics for this application, such as high sensitivity to alcohol, fast response, long duration and stability of readings, a simple feeding circuit, and low cost (Assoni & Magro, 2017).

Thus, this study aimed to develop a prototype of ethylometer with an MQ-3 sensor and to evaluate its stabilization time when used in soybean seed lots submitted to hypoxia.

MATERIAL AND METHODS

The study was carried out at the Teaching Laboratory for Seed Analysis of the Graduate Program in Seed Science and Technology, and at the Agrotechnology Laboratory at the Engineering Center, both located on the Capão do Leão Campus of the Federal University of Pelotas, RS, Brazil. The research took place between August and November 2017. The research location has the following geographic coordinates: 31° 48' 03.8" S, 52° 24' 47.7" W, and an altitude of 21 m.

For development of the research, 20 lots of soybean seeds were used, from five cultivars (NS 8383 RR – lots 2 and 3, SOY Peso RR – lots 1 and 7, M 8372 IPRO – lots 4 and 6, M8644 IPRO – lots 5 and 8, and M8808 IPRO – lots 9 and 10), each cultivar containing four seed lots. From the 20 lots of soybean seeds, 10 were selected to continue the analyses: two lots from each cultivar that had germination (Brasil, 2009) equal to or greater than 80%, and that were statistically similar to the others.

Subsequently, the research was divided into two stages. The first corresponded to development of the ethanol measuring equipment and the second to measurement of the stabilization time of the sensor mounted in the equipment.

A series of electronic components for system development and various products were purchased to begin the development of the ethanol measuring equipment. The purchase of these components and products, and the assembly of the equipment, were divided into stages:

For the system, a source for external use, a Jack P4 connector, a 1N4007 diode, a 7805 voltage regulator, and a LED (light emitting diode) were purchased.

For this, it was purchased an ATmega 328P microcontroller with 14 digital input/output ports (d0, d1, d2, d3, d4, d5, d6, d7, d8, d9, d10, d11, d12, and d13), six of which could be pulse width modulation (PWM) outputs (d3, d5, d6, d9, d10, and d11), and six analog input ports (a0, a1, a2, a3, a4, and a5), a 10 kΩ pull-down resistor, a 16 MHz crystal oscillator, and 22 pF and 100 nF ceramic capacitors.

To build the equipment, a 16-pin 16 x 2 liquid crystal display (LCD) was used. It presents 16 columns every two rows, with a blue background and readings in white. For this system, only a DHT11 sensor was purchased, which can express temperature and humidity measurements in the environment while the equipment is in use.

As with the temperature and humidity sensor, only a 4-pin MQ-3 sensor (Figure 1) was acquired. The MQ-3 gas sensor is composed of a micro AL₂O₃ ceramic tube, tin dioxide (SnO₂) sensitive layer, measuring electrode, and heater fixed into a casing made of plastic and stainless steel net. The heater provides necessary working conditions for sensitive components.

Glass vials of 150 mL were purchased, with lids equipped with a sealing system, as well as pneumatic ampoules with a volume of 30 cm³.

The 4-pin MQ-3 sensor was integrated into an analog pin of the microcontroller, which converts analog signal to digital signal. Prior to the conversion process, the noise provided by the external environment was eliminated through low-pass filters in the converter, which converted the voltage variation signals into analog values. The data generated are processed in a microcontroller, which is connected to an output system, a display, so that the results can be written down manually.

To make handling the equipment and the test itself easier, a gas storage tube with a pneumatic ampoule implanted over the lid of the bottle was inserted to extract the ethanol incubated in the samples and transfer it to another storage bottle. The second bottle contains the MQ-3 Datasheet sensor and an exhaust valve.

The second stage consisted of an experiment to determine the stabilization time of the MQ-3 Datasheet sensor, already inserted into the electronic system and the equipment's gas retention bottle, so that there was a waiting time between one analysis and another.

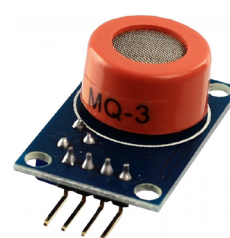


Figure 1. MQ-3 semiconductor sensor for ethanol

A completely randomized design was used for the ethanol measurements, to determine the stabilization time of the sensor, with five replicates of 25 visibly intact soybean seeds from each batch. The seeds were selected manually and placed in glass jars with a known volume (150 mL), containing 40 mL of distilled water (simulation of an anaerobic environment) (Cavalcante et al., 2019), and then submitted to a 2 hours rest period at a controlled temperature of 41 °C (Cavalcante et al., 2019). After this hydration period, the vials containing the seeds were carefully coupled to the other vials containing the MQ-3 sensor. Then, with the aid of the ampoule, a known volume of the air contained in the bottle in which the seeds were stored was transferred to the bottle containing the sensor. Subsequently, measurements were made at different time intervals (3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 s), in continuous readings.

During measurements with the equipment, the external environmental conditions (air temperature and relative air humidity) were controlled, to avoid environmental interference with the sensor's operation during the readings. The place where the research was carried out was maintained with an average air temperature of around 20 °C and relative air humidity of 70%.

The data obtained were subjected to analysis of variance at $p \leq 0.05$ by the F-test and, when significant, the exponential curve model was applied to determine the minimum stabilization time of the MQ-3 sensor. The results were evaluated using the statistical software R, version 3.1.1. (R Core Team, 2017), and the graphic software SigPlot, version 12.5.

RESULTS AND DISCUSSION

To begin assembling the equipment, a power system was connected to the electrical network through an external source

(Figure 2A), which is 7 to 20 V. The external source and the power system were connected through a Jack P4 connector (Figure 2A). Jack P4 provides the system with a positive tension called VDC (volt direct current) and a negative tension/zero logic level called GND (ground), or ground wire.

It is noteworthy that the GND was connected to a cable that will always receive excess energy in the electronic circuit, to avoid damage to the operating system of the board when it is subject to excessive loads, even when the electrical current is unstable (Mohd et al., 2018).

A 1N4007 diode was inserted next to the supply system to protect it, allowing the passage of current in a single direction (Hu & Gong, 2014). If there is an inversion between the VDC and the GND on the external source connector, and consequently on the Jack P4 connector, the diode will protect the system, as it will not allow current to flow in the opposite direction for which the system is designed. A 7805 voltage regulator was also inserted into this system, with the function of sending 5 V to the output terminal (terminal 3).

This component can receive voltages between 7 and 20 V. If it receives a signal of less than 7 V, it will not work correctly, as it will deliver a signal of less than 5 V to the output terminal. If the signal received is greater than 20 V, the component will be damaged. This component operates simply: the positive tension enters the voltage regulator through the VI terminal (voltage in, terminal 1), and the VO terminal (voltage out, terminal 3) provides the system with a positive 5 V tension. Terminal 2 of the component is connected to the GND system.

Still in the power system, there are two capacitors: an electrolytic capacitor and a polyester capacitor (Figure 2A). These components prevent external noise and oscillations from interfering with the power system and compromising the power supply (Keogh, 2010).

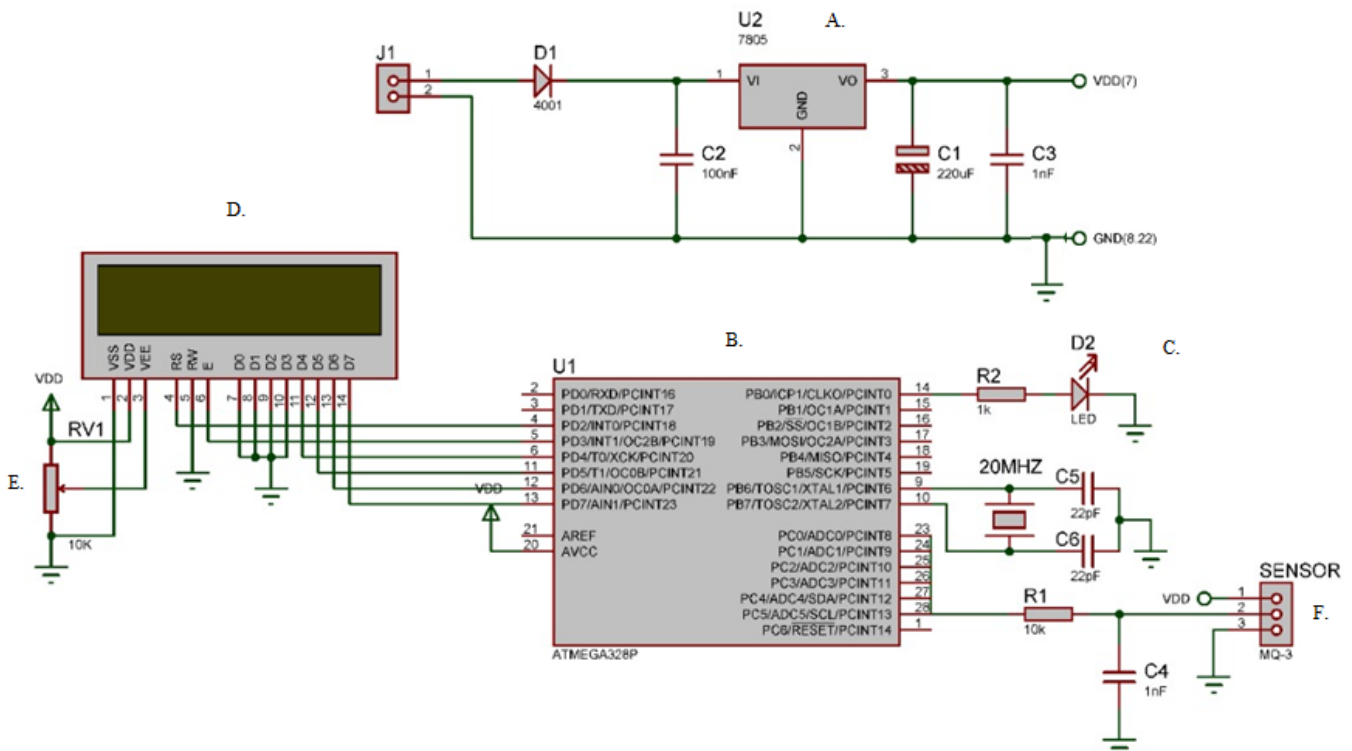


Figure 2. The electronic system of the ethylometer and its components. (A) Power system, (B) microcontroller, (C) 220 Ω resistor, (D) liquid crystal display, (E) on/off switch, and (F) MQ-3 sensor

The ATmega 328P microcontroller with 14 digital input/output ports (d0, d1, d2, d3, d4, d5, d6, d7, d8, d9, d10, d11, d12, and d13), six of which can be PWM outputs (d3, d5, d6, d9, d10, and d11), has a 8-bit resolution. The microcontroller also has six analog input ports (a0, a1, a2, a3, a4, and a5), each with a 10-bit resolution. The operating voltage of this component is 5 V, so the digital–analog conversion will have 1024 values, that is, the analog values read on the analog ports will be between 0 and 1023.

A 10 k Ω resistor (Figure 2B) was connected to terminal 1 of the ATmega 328P (reset terminal), to keep the microcontroller powered at all times, preventing the system from restarting periodically, especially with electrical load oscillations that can promote instabilities in the microcontroller (Prakash et al., 2018).

Likewise, a 100 nF ceramic capacitor was also added, to avoid oscillations in the system that could compromise the readings. A 16 MHz crystal oscillator (Figure 2B) was also mounted to the microprocessor to accurately determine the time synchronization (clock) of the operations carried out by the microcontroller. In other words, the oscillator ensures that each task is executed when proposed by the microcontroller programming. The 22 pF ceramic capacitors are part of the crystal oscillator circuit and guarantee it functions smoothly.

A 220 Ω resistor (Figure 2C) was connected to the LCD (Figure 2D). The resistor limits the current that passes through the LED. The LED serves as an indicator of the power system, indicating that it is powered (Beiranvand, 2016).

A 16-pin 16 x 2 LCD (Figure 2D) was used, which has 16 columns every two rows, with a blue background and readings in white. Pins 1 and 16 of the display were connected to the Graduated Neutral Density (GND) system. Pins 2 and 15 were connected to the system's VDC. Pins 4, 6, 11, 12, 13, and 14 were connected to the microcontroller, each one connected to a specific digital port, in which pin 4 was connected to digital port 2 (4-d2), and so on: 6-d3, 11-d4, 12-d5, 13-d6, 14-d7, respectively. Pins 7, 8, 9, and 10 of the display were not connected to any part of the system. Pin 5 is connected to a connector, which is connected to the GND through a wire; it is a resource used when defining the tracks and positioning the components on the printed circuit board (Naderi & Abbaszdeh, 2016).

Pin 3 was connected to a 10 k Ω multiturn trimpot. In the trimpot, pin 1 was connected to the system's VDC, and pin 3 was connected to the system's GND via a wire. This wire was used to connect the track from pin 3 of the trimpot to the GND. The trimpot is used to adjust the brightness of the display screen.

One of the terminals (1) of the on/off switch (Figure 2E) is connected to the system's VDC, and the other terminal (2) is connected to digital pin 8 of the microcontroller. Terminal 2 of the button is only powered when the button is pressed by the user. A 10 k Ω pull-down resistor was mounted to ensure that the logic level on digital pin 7 remains at zero when the button is not pressed, thus preventing the system from interpreting that the button is pressed when it is not.

Of the four pins of the relative air humidity and air temperature sensor, pin 1 was inserted into the printed circuit

board, connected via a wire to the VDC pin of the DHT11 sensor (Figure 1B). Pin 2 of the sensor (on the printed circuit board) was connected via a wire to the pin of the DHT11 sensor and the GND pin of the DHT11 sensor, respectively (Figure 1B). It should be noted that the DHT11 can measure air temperatures between 0 and 50 °C with an accuracy of ± 2 °C, and relative air humidity between 20 and 90%, with an accuracy of $\pm 5\%$.

The 4-pin MQ-3 sensor was mounted in the equipment module to complete the system (Figure 2F). Only three of the four pins were used in this project (VDC, GND, and analog output). The unused pin belongs to a digital output regulated by an internal trimpot on the sensor module.

Pins 1, 2, and 3 of the MQ-3 sensor (on the printed circuit board) were connected via a wire to the VDC pin and the AOUT (analog output) pin in the sensor module; finally, pin 3 of the sensor (on the printed circuit board) was connected via a wire to the GND of the sensor module. Note that pin 2 of the MQ-3 sensor transmits the information to the microcontroller. The 10 k Ω resistor and the 470 nF polyester capacitor make up the system's low-pass filter, filtering out noise from frequencies above 33.88 Hz because noises above this frequency do not interfere with the sensor readings (Yang et al., 2017).

Given what has already been described for assembly of the system as a whole, it should be noted that all connections between the printed circuit board and the display were made via wiring.

After the system and its components (Figure 2) had been assembled, the MQ-3 sensor (Figure 3D) was mounted on vial B (Figure 3B) for receiving air containing the ethanol released by the seeds (Figure 3H) submerged in water (Figure 3G), which had been placed in vial A (Figure 3A). The two vials are connected by a system of tubes and a central register (Figure 3F) with a diameter of about 5 mm, to transport the ethanol present in vial A to vial B. This transport is carried out with

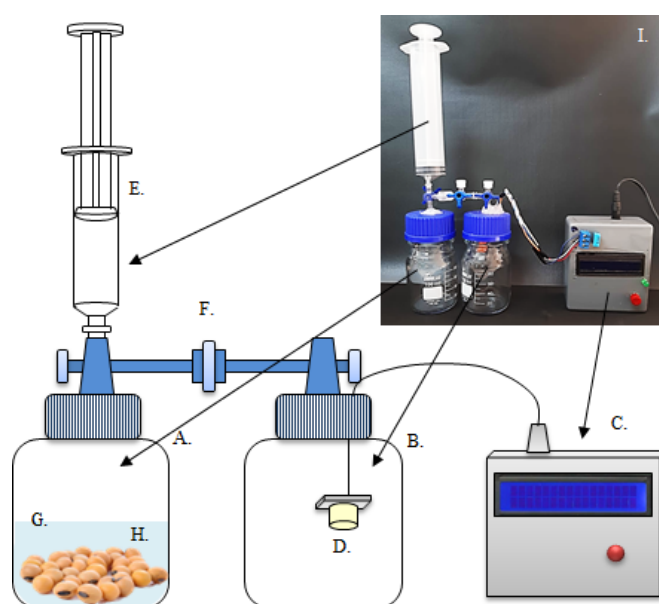


Figure 3. Sketch and photograph of ethylometer for analysis of soybean seeds. (A) Vial A, (B) vial B, (C) electronic system, (D) sensor, (E) ampoule, (F) central register, (G) water, (H) soybean seeds, and (I) prototype of the equipment

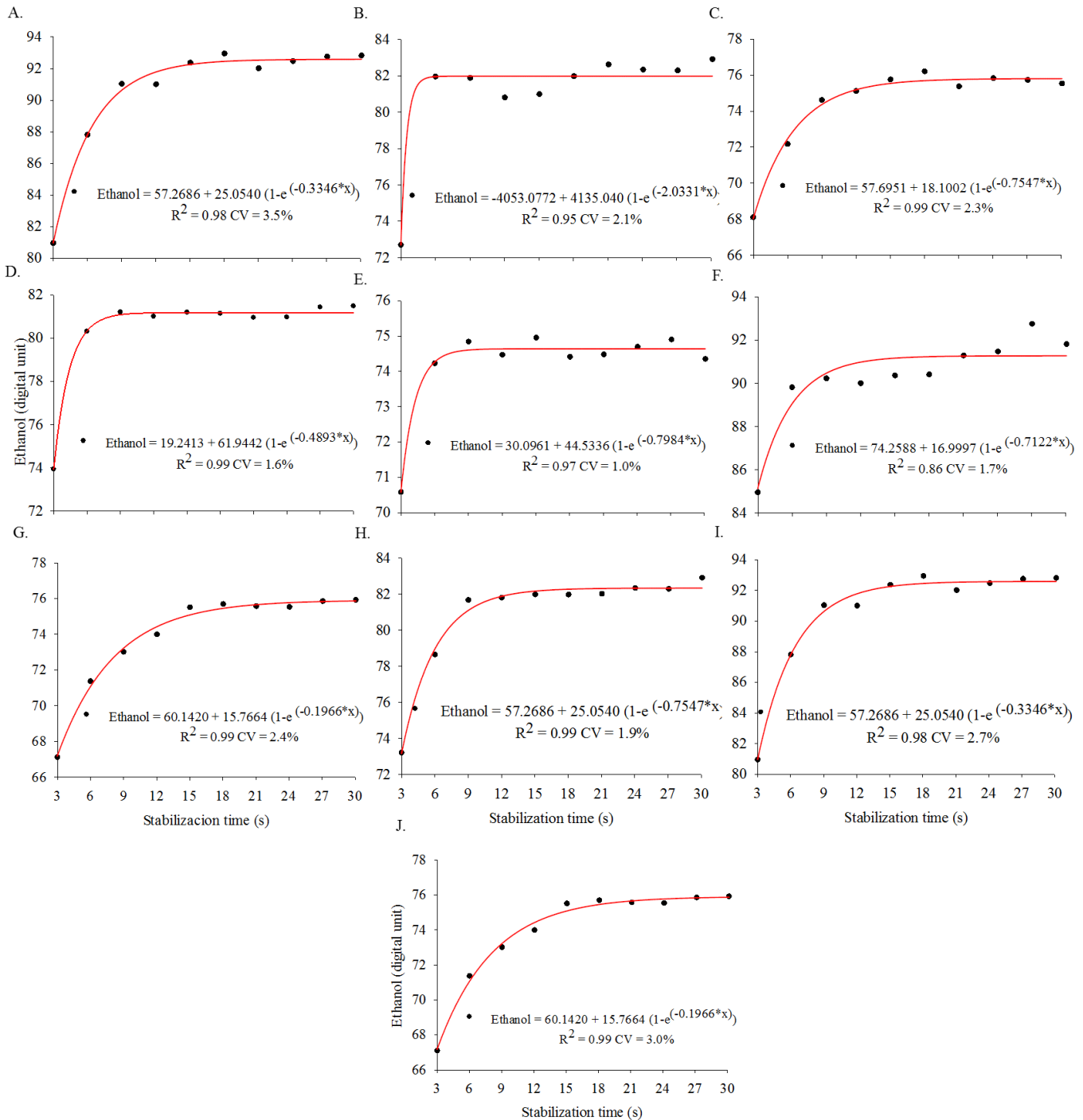
the aid of a pneumatic ampoule (Figure 3E) attached to the top of vial A.

Thus, the air is removed from vial A with the ampoule (Figure 3F), and a record is closed in vial A (Figure 3A) below the ampoule, which is pressed until the air containing ethanol is transferred to vial B, in which the sensor is mounted. Subsequently, the register of vial B is closed, and the reading begins. Thus, the electrical conductivity of the alcohol sensor changes when it is in an environment with higher concentrations of gaseous ethanol. That is, the higher the concentration of ethanol in the air, the greater the electrical conductivity of the sensor. Therefore, the results are shown

directly on the display in digital units (Figures 2 and 3C). Figure 3I shows the prototype in real scale.

When developing a prototype containing 13 gas sensors, including an MQ-3 sensor, to monitor volatile organic compounds in pre-harvest peach fruits, Voss et al. (2020) observed that the MQ-3 sensor was effective in detecting ethanol in peach fruits after physiological maturity.

Figure 4A shows that the minimum time for stabilization of the MQ-3 sensor in lot 1 was 24.34 s. In lot 2 (Figure 4B), different from what was observed in lot 1, the sensor had stable readings after 6.1 s. In both, the sensor remained stable until the last reading, which was at 30 s.



* - Significant at p ≤ 0.05 by F-test

Lots 1 and 7 – cultivar SOY Peso RR; lots 2 and 3 – cultivar NS 8383 RR; lots 4 and 6 – cultivar M 8372 IPRO; lots 5 and 8 – cultivar M8644 IPRO; lots 9 and 10 – Cultivar M8808 IPRO

Figure 4. Average stabilization time of the 4-pin MQ-3 Datasheet sensor for measuring ethanol in 10 lots of soybean seeds submitted to anaerobiosis. (A) lot 1, (B) lot 2, (C) lot 3, (D) lot 4, (E) lot 5, (F) lot 6, (G) lot 7, (H) lot 8, (I) lot 9, and (J) lot 10

These results show the practicality and speed with which these sensors can work, and that they can be used in a portable and inexpensive commercial device, capable of detecting volatile organic compounds (VOCs) (especially ethanol), with highly sensitive sensors (Röck et al., 2008). Several analytical techniques, such as liquid chromatography, infrared spectroscopy, and mass spectrometry, have been used to measure ethanol concentrations (Hamid et al., 2018). However, they are expensive and are not portable. Due to the high cost and complex processes, it is necessary to develop inexpensive, portable ethanol sensors with high sensitivity and a fast response.

Lots 3 and 4 (Figures 4C and D) had similar stabilization times, 18.4 s for lot 3 and 13.6 s for lot 4. Lots 5 and 6 (Figures 4E and F), on the other hand, had stabilization times of 11.2 and 10.2 s, respectively. For lots 7, 8, 9, and 10, stabilization times of 23.95, 16.15, 15.97, and 21.10 s (Figures 4G, H, I, and J), respectively, were observed. This demonstrates the efficiency of the MQ-3 sensor to determine the amount of ethanol released by the seeds in a short time, for all lots.

In a similar study, Silva et al. (2017), when assessing the stability of an MQ-3 sensor for beans, observed that the sensor became stable, considering the average among the evaluated samples, at around 23 s. The same authors stated that the MQ-3 sensor was efficient, practical, and fast in measuring ethanol in beans in anaerobiosis.

It was also found that all soybean lots evaluated were stable until the final measurement time (30 s), demonstrating the reliability of the results of the sensor, which could also be used for other species. In this perspective, Maciel et al. (2003) pointed out that the development of this type of sensor is extremely important for many applications, due to its advantages such as small size, high stability, sensitivity, and long service life.

After the injection of 100 ppm of ethanol, Anjum & Khainar (2016) found that the resistance of the sensor gradually increased and reached a constant value that provided the response time of the sensor, similar to the results found in this study. The same authors reported a response time of 180 s for ethanol, while the recovery time was 700 s. The present study found a minimum response time of 16 s and a recovery time of 30 s, corroborating these findings. The sensor developed by Anjum & Khainar (2016) is made of nanoparticles and therefore behaves like an electrochemical sensor, different from metal alloy sensors.

Based on the results (Figure 4), it is possible to state that the MQ-3 sensor could be used as a tool to control fermentation in soybean seeds, as a future vigor test. In tests that assess the amount of ethanol released by seeds, such as those conducted by Buckley & Buckley (2009) in canola seeds, Kodde et al. (2012) in cabbage seeds, Onwimol et al. (2019) in corn, and Cavalcante et al. (2019) in beans, the authors used an adapted ethylometer, specific for humans, and reported problems with the equipment at some moments of the analyses. It is worth noting that the ethylometer identifies, through a quick and adequate analysis of the air expelled from the lungs, the presence and quantity of alcohol in the body, providing results equivalent to the values found in alveolar air.

Also, this type of ethanol sensor can be used to monitor seeds stored in hermetically sealed packaging. It is known that a hermetic package imposes a physical barrier to gas exchange, with seed respiration capable of altering the concentration of CO₂ within the packaging over time, restricting aerobic respiration and favoring anaerobic respiration. According to Taiz et al. (2017), respiratory rates decrease if the atmospheric oxygen concentration is below 5% for whole organs or below 2 to 3% for tissue parts. These observations show that oxygen supply can impose a limitation on plant respiration.

CONCLUSIONS

1. The sensor had a satisfactory performance, presenting measurement stability, with average stabilization values of approximately 16 s for soybean seed lots. This initial time is recommended so that the results of the ethylometer are reliable.
2. Through the MQ-3 sensor, it is possible to efficiently detect ethanol present in the environment during anaerobic processing of soybean seeds.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES) (Finance Code 001), Conselho Nacional de Desenvolvimento e Tecnológico (425399/2016-8 and 306772/2017-5), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

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