



Original papers

An Arduino-based low cost device for the measurement of the respiration rates of fruits and vegetables

Jaime González-Buesa^{a,b}, María L. Salvador^{a,*}^a Plant Foods Research Group, Instituto Agroalimentario de Aragón-IA2-(Universidad de Zaragoza-CITA), Miguel Servet 177, 50013 Zaragoza, Spain^b Unidad de Hortofruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, 50059 Zaragoza, Spain

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ABSTRACT

A modular device based on open source software was developed for the determination of the respiration rates of produce in a closed system. The advantages of this system include simplicity, adaptability and low cost. The respirometer allowed the continuous measurement of CO₂ concentration, barometric and differential pressures, and temperature. With the data obtained it was possible to calculate the CO₂ production rate and to predict the O₂ consumption rate and the respiratory quotient of the product, without using an O₂ sensor. To assess the function of the device, the O₂ and CO₂ respiration rates of three products were determined. The good agreement observed between the respiratory quotient and the differential pressure suggests the possibility of using the differential pressure data as a direct indicator of the respiratory quotient evolution. This data could also be useful for monitoring metabolic shifts.

1. Introduction

Modified atmosphere packaging (MAP) is a useful technology for reducing quality loss and extending the shelf life of whole and fresh-cut fruits and vegetables. The respiration rate of the product and the gas transmission rates through the film are the key elements for successful MAP design (González-Buesa et al., 2009). The respiration rate depends on internal factors such as the cultivar in question, the growing season and region, and the maturity stage, together with external factors such as the temperature and oxygen and carbon dioxide concentrations inside the package, among others (Fonseca et al., 2002). Thus, the respiration process is complex, and the models used to predict the respiration rate have limitations, as Caleb et al. (2013) note while pointing out that the lack of suitable respiration rate data is a major problem. The respiration rate is usually determined from the experimental quantification of O₂ consumed and/or CO₂ produced by the product, frequently in the chamber headspace. For this reason, the respiration rate includes cellular respiration and other processes such as the skin resistance to gas diffusion, the solubility of gases, or the diffusion of gases inside the product (Fonseca et al., 2002).

In recent years, the methodology applied for the determination of the respiration rate has experienced significant changes. The closed system method continues to be more common than flushed or permeable systems, and gas monitoring is performed by O₂ and CO₂ gas

analysers using electrochemical sensors for O₂ and infrared sensors for CO₂, instead of gas chromatographs (Bhande et al., 2008; Iqbal et al., 2009; Alves et al., 2013; Banda et al., 2015). Gas analysers provide convenient measurements and have lower costs and maintenance requirements than gas chromatographs. However, these systems require a considerable amount of gas sample to perform each measurement. Therefore, the continuous monitoring of a small respiration chamber could be questionable since the pressure inside may be reduced. To avoid this disadvantage, sensors can be placed directly inside the respiration chamber, as done by Løkke et al. (2011) for oxygen or Mahajan et al. (2016) for oxygen and carbon dioxide monitoring. When the sensor is located on the internal side of the container lip (Caleb et al., 2013; Mahajan et al., 2016) there is no possibility of protection to avoid water condensation problems through water traps, filters and other accessories. This issue is solved in respiration systems that include an external closed loop to carry the gas sample to the sensor, as described by Acerbi et al. (2015) for assessing CO₂ respiration rates in cheese. However, these are not adapted to the requirements of produce respiration and MAP design.

Carbon dioxide sensors for gaseous samples are frequently based on the non-dispersive infrared detection method which is a cheap, precise, and reliable technology. For oxygen detection, there are many different sensor technologies, including electrochemical, infrared, ultrasonic, optical, and laser methods. Electrochemical sensors have been widely

* Corresponding author.

E-mail address: malsalva@unizar.es (M.L. Salvador).<https://doi.org/10.1016/j.compag.2019.03.029>

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used for the detection of oxygen in MAP systems (Fonseca et al., 2002), but they have a limited life expectancy. Ongoing improvements in optical detectors and the availability of integrated barometers suggest that luminescence quenching technology may offer a viable alternative to other oxygen sensors, but their longevity and robustness in industrial environments have not yet been demonstrated (Willet, 2014).

For sensor readout and computer interfacing, it is necessary to use a microcontroller. Since their launch in 2005, Arduino microcontrollers have become increasingly popular in the research community (Cressey, 2017). This open-source platform has been developed for prototyping purposes, and is based on easy-to-use hardware and software, providing researchers with key features that can translate into both economic and practical benefits. In recent years, numerous mentions of Arduino-based electronic control of biotechnology applications have appeared in research articles (Mathupala et al., 2016; Coronel-Reyes et al., 2018).

Linked to the appropriate sensors, this platform offers the possibility of monitoring fruit and vegetable O₂ and CO₂ concentrations in respiration chambers, MAP packages or controlled atmosphere containers. From these data, the CO₂ production rate and O₂ consumption rate can be determined. Therefore, the ratio between these parameters, known as the respiratory quotient (RQ), can also be calculated. This parameter provides important information about the metabolic routes of the product, and normally ranges between 0.7 and 1.3 (Kader, 1987). The RQ increases exponentially as the O₂ concentration approaches zero due to a change from aerobic respiration to fermentation (Fonseca et al., 2002). Recently, this parameter has gained relevance in the development of novel dynamic controlled atmospheres, since their control system is based on RQ measurements (Bessemans et al., 2016). The RQ is normally calculated from CO₂ and O₂ concentration data, but differential pressure sensors may offer an accurate and inexpensive alternative for measuring metabolic rates.

Thus, the first aim of the present work was to develop a device for the determination of the respiration rate of fruits and vegetables in a closed system with the following characteristics: it allows the continuous measurement of differential pressure and CO₂ concentration and it is low-cost, made with reliable components, fully configurable, flexible, based on open-source software, easily transformable into a gas analyser for MAP or for controlled atmosphere storage, and modular (the whole sensor module is located outside the respiration chamber and connected to it by a closed loop). Therefore, we used an Arduino UNO board that controlled, read, and logged the inputs of an infrared CO₂ and pressure sensors.

The second aim was to assess the functioning of the device and to explore the applications of the data obtained with it. For these purposes the respirometer was tested with three different products (apple, strawberry, and cauliflower florets) at 23 °C. The utility of the differential pressure data was examined for predicting changes in the RQ values and estimating the O₂ concentration. The predicted O₂ values were compared with the actual O₂ concentration inside the respiration chamber.

2. Materials and methods

2.1. Description of the respirometer

The respiration rate measurement system was composed of a respiration chamber connected to a pump system and a sensor module. The gas from the chamber was pumped to the oxygen and carbon dioxide sensors and then returned to the respiration chamber. A differential pressure sensor was directly connected to the respiration chamber. All sensors and devices were powered and controlled throughout by a microcontroller and data logger module. A general overview of the system is provided in Fig. 1, and the electronic connections and devices are detailed in Fig. 2.

2.1.1. Respiration chamber

The respiration chamber was a 1450 mL glass jar with a 110-mm wide mouth (Le Parfait, Auvergne, France) enabling easy access for the fruit and vegetables. The glass jar was sealed with a silicone-sealing capsule (Le Parfait, Auvergne, France) and a homemade press consisting of two acrylic plates and four wing nuts (Fig. 1). The connections of the respiration chamber with other modules were made with sealed barbed fittings and 4 × 2.5 mm flexible polyurethane tubes.

2.1.2. Pump

The pump system consisted of a small diaphragm pump (Gardner Denver Thomas GmbH, 2002VD/0.5/E/DC, Puchheim, Germany) and a motor microcontroller (Arduino motor shield rev3, Arduino SRL, Strambino, Italy). The selected pump delivers a maximum flow of 380 mL min⁻¹ at a normal pressure, and the frequency of the pump was set to 5 s every minute; the resultant flow for the system was approximately 32 mL min⁻¹.

2.1.3. Sensors

Carbon dioxide sensor: For measuring carbon dioxide, we used a non-dispersive infrared (NDIR) waveguide technology with an automatic background calibration (ABC) algorithm (CO₂ Engine BLG, Senseair AB, Delsbo, Sweden). The measurement range was 0 to 30% vol. with an accuracy of ± 0.2% vol. ± 3% of the reading. It was powered at 9 Vdc through the Arduino UNO. This sensor has dual I2C and UART communication, both performed at 3.3 V. In our case, we used the I2C connection.

Pressure sensors: The measurement of the relative pressure was carried out using a bidirectional pressure sensor (AMS 5915-0100-D-B, Analog Microelectronics GmbH, Mainz, Germany). One pressure port was connected to the respiration chamber, and the other was open to the atmosphere. The pressure outside the respiration chamber was measured using a AMS 5915-1200-B sensor (Analog Microelectronics GmbH, Mainz, Germany). Both sensors use a I2C communication protocol, and need to be powered from 3 to 3.6 V.

Prior to using the device as a respirometer, and to confirm that it was operating correctly, CO₂ and O₂ sensors were tested with calibrated gas mixtures (10 kPa O₂ – 20 kPa CO₂ and 20 kPa O₂ – 0 kPa CO₂) and pure nitrogen.

2.1.4. Microcontroller and data logger module

Board: The selected main board was an Arduino UNO (Arduino SRL, Strambino, Italy). It is based on an 8-bit microcontroller (ATMEGA328; Atmel Corp, San Jose, CA, USA) with 32 Kb flash memory. The board has 14 digital input/output pins and 6 analogue input pins. It has a USB that can act as a communication port for data transfer with the computer. The Arduino sketch for sensor control and data acquisition is available at the web page www.jaimegb.com.

Real time clock (RTC): An RTC module (Tiny RTC Elecrow, Shenzhen, China) based on the DS1307 clock chip (Maxim Integrated Products, California, USA) was used in order to time-stamp the values obtained from the different sensors. The RTC is powered by a battery and is connected to the Arduino through the I2C protocol.

Additional electronics: Since carbon dioxide and differential pressure sensor communication works at 3.3 V, and the digital and analogue ports on the Arduino perform at 5 V, we decided to include a bi-directional logic level converter compatible with the I2C protocol (BSS138, SparkFun Electronics, Colorado, USA). Some sensors have the same address, therefore it was necessary to include a breakout board (Adafruit Industries, New York, USA) with a TCA9548A multiplexer (Texas Instruments, Texas, USA) in order to avoid communication conflicts.

Data logger: All information gathered from the carbon dioxide sensor, the pressure sensors and the RTC was transmitted every 30 s through the Arduino USB port and recorded in a text file on a laptop (KIRA 10d, Toshiba, Japan) using freeware software CoolTermWin

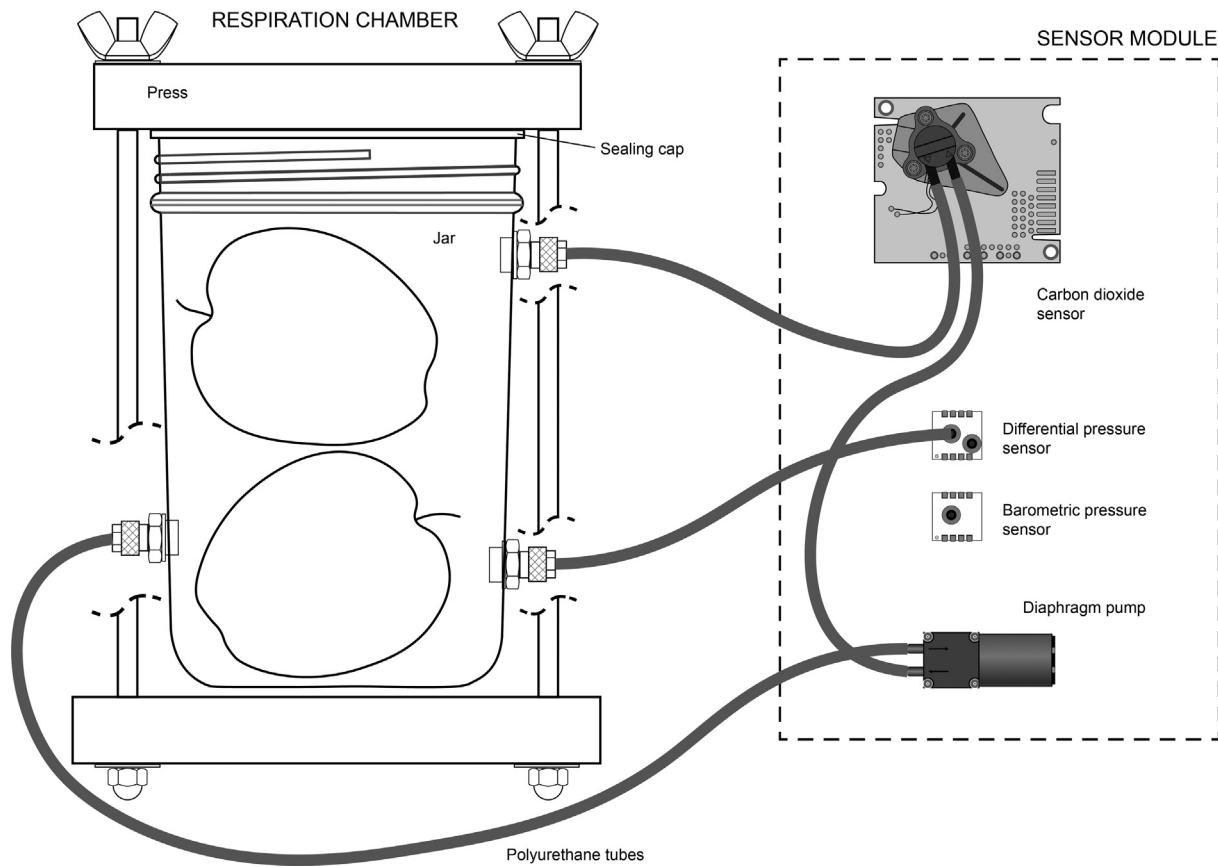


Fig. 1. Schematic of the respirometer.

(Roger Meier, CA, USA).

2.2. Respiration rate measurement

Prior to the respiration rate calculation, raw CO₂ concentration values were corrected following the manufacturer’s instructions taking into account the pressure dependence of the CO₂ sensor (1.6% reading per kPa deviation from normal pressure). The carbon dioxide production rate, R_{CO_2} (mL kg⁻¹ h⁻¹) was then calculated using the following expression (Fonseca et al., 2002):

$$R_{CO_2} = \frac{1}{W} \frac{([CO_2]_{t_2} - [CO_2]_{t_1})}{(t_2 - t_1)} \frac{1}{100 \cdot V} \quad (1)$$

where $[CO_2]_{t_2}$ and $[CO_2]_{t_1}$ (%) were the CO₂ concentrations for t_2 y t_1 (h), respectively, W (kg) was the product weight, and V (mL) was the free volume inside the respiration chamber. The R_{CO_2} data were smoothed using the Savitzky-Golay method (5 points, 2nd order polynomial).

$[O_2]$ was estimated from the values of $[CO_2]$ absolute and differential pressure, considering that changes in differential pressure were mainly attributable to the difference between the moles of CO₂ generated and those of O₂ consumed. Other factors, such as the partial water vapor pressure (close to the saturation values during almost the whole experiment) or the contribution of the pressure changes of the volatile compounds generated, were disregarded. Thus, the oxygen concentration inside the respiration chamber was calculated using the following expression:

$$[O_2]_{c,t_2} = [O_2]_{c,t_1} - ([CO_2]_{t_2} - [CO_2]_{t_1}) + \frac{(P_{dif,t_2} - P_{dif,t_1})}{P_2} \cdot 100 \quad (2)$$

where $[O_2]_{c,t_2}$ and $[O_2]_{c,t_1}$ (%) were the calculated O₂ concentration for certain t_2 and t_1 (s) times, respectively, $[CO_2]_{t_2}$, $[CO_2]_{t_1}$ (%) and P_{dif,t_2} , and P_{dif,t_1} (mbar) were the measured CO₂ and differential pressures for these

times, respectively, and P_2 was the measured absolute pressure for a certain time t_2 .

To verify the suitability of Eq. (2) to estimate the O₂ concentration, the actual values of the O₂ concentration inside the chamber were monitored. In order to do so, we integrated a LuminOx fluorescence quenching oxygen sensor into the system (UV Flux 25%, CO2meter Inc., Florida, USA). This sensor has a wide oxygen range (0–25% vol.) and good accuracy (< 2% full scale). It has an internal temperature and barometric pressure sensor (700–1200 mbar range and +/-5 mbar accuracy) for correcting measured values. It needs to be powered at 5 VdC, and it uses universally asynchronous receiver/transmitter (UART) communication to receive and transmit data serially at 3.3 V.

The oxygen consumption rate of the product, R_{O_2} (mL kg⁻¹ h⁻¹), was calculated using an expression similar to that for R_{CO_2} :

$$R_{O_2} = \frac{1}{W} \frac{([O_2]_{t_1} - [O_2]_{t_2})}{(t_2 - t_1)} \frac{1}{100 \cdot V} \quad (3)$$

where $[O_2]_{t_2}$ and $[O_2]_{t_1}$ (%) were the estimated O₂ concentrations for t_2 and t_1 (h), respectively, W (kg) was the product weight, and V (mL) was the free volume inside the respiration chamber.

The respiratory quotient, RQ , was also calculated using the experimentally obtained R_{CO_2} and estimated R_{O_2} values as:

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad (4)$$

2.3. Experimental procedure

The respiration rates were calculated for three products: cauliflower (*Brassica oleracea* var. *Botrytis* L., “Meridien” cultivar), strawberry (*Fragaria vesca* L., “Camarosa” cultivar), and apple (*Malus domestica* L. “Golden delicious” cultivar). All products were acquired in a local

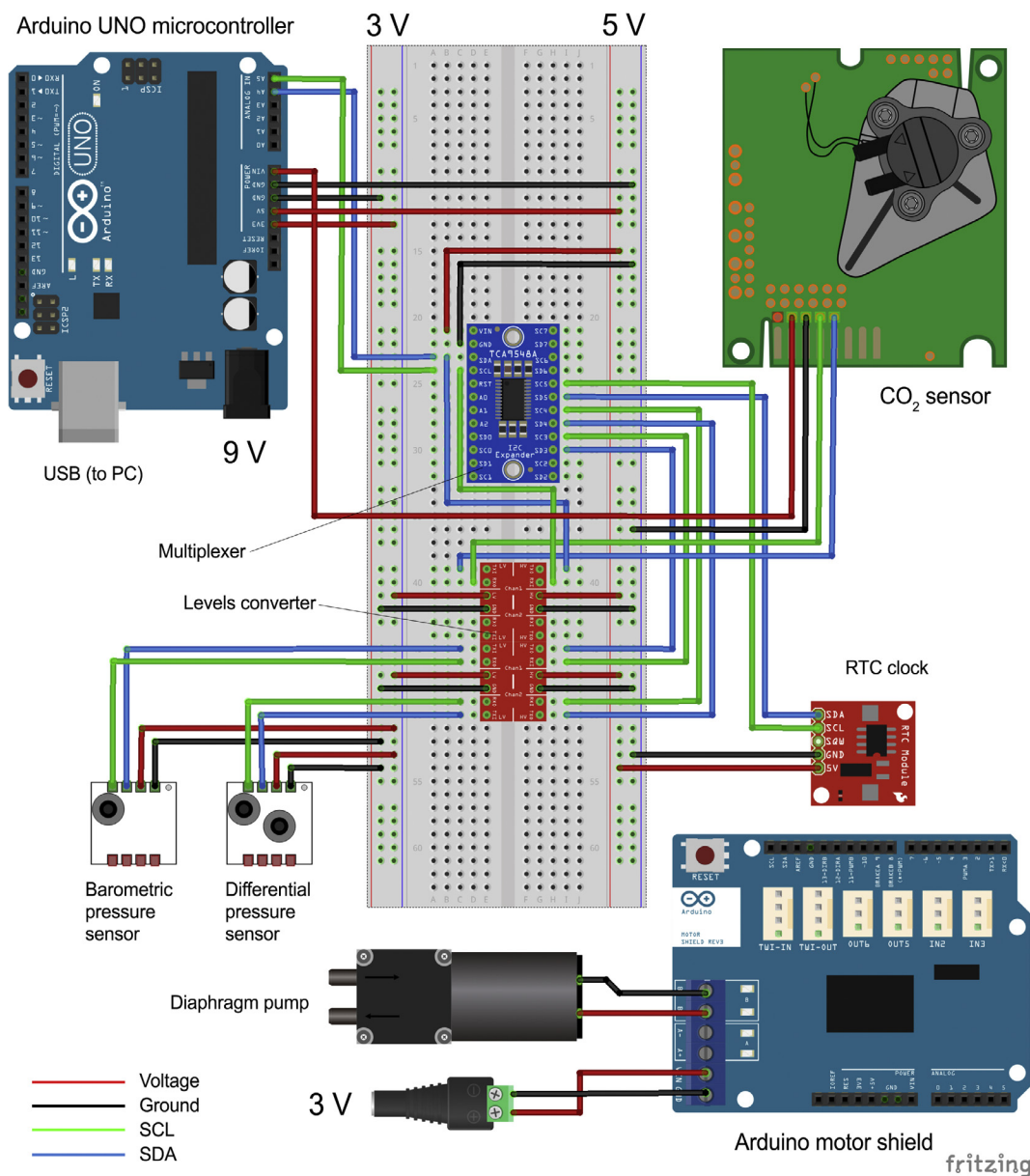


Fig. 2. Wiring diagram and sensors of the device.

market at commercial maturity. The weight of the product in each case was: 300 ± 5 g for cauliflower, 380 ± 5 g for strawberry, and 500 ± 25 g for apple. The system was kept at 23 °C in a temperature-controlled Sanyo MIR153 incubator (Sanyo Electric Co., Ltd., Mariguchi, Japan). The respiration chamber was closed, and the pump, sensors, and clock were plugged in to start the data logging. Three replicates were performed for each product, and data were expressed as the mean values.

3. Results and discussion

The evolution of the carbon dioxide concentration, $[CO_2]$ (%), and the differential pressure, P_{diff} (mbar), for the three selected products are shown in Fig. 3. The cauliflower florets had more intense respiratory activity than the strawberries or apples given that they produced faster $[CO_2]$ accumulation inside the respiration chamber, even when the amount introduced into the system was smaller compared with the other two products. The strawberries also showed high respiration rates while the apples showed slower $[CO_2]$ accumulation.

For all the products, the differential pressure showed negative values from the start of the experiment (Fig. 3), indicating that the O₂ consumption was greater than the CO₂ production. This trend was maintained during a wide range of gas compositions for the three products. However, after a certain time the differential pressure started to increase. The evolution of oxygen concentration is shown in Fig. 4. The values of $[O_2]$ predicted from Eq. (2) and those experimentally measured were similar for the interval in which the differential pressure evolution against time is linear (maximum differences were below 4% for the three products). From the point at which the linearity ceases, the differences between the calculated and experimental oxygen concentrations tended to be higher, probably caused by volatile compounds such as ethanol or acetaldehyde released via anaerobic metabolic routes. For the cauliflower, the differences between the measured and predicted oxygen concentrations were slightly higher, maybe due to its higher metabolic activity. The O₂ concentrations at which a shift in the differential pressure trend occurred were 3.57%, 2.24%, and 2.62%, for the cauliflower, strawberry, and apples, respectively. These values are in general higher than the minimum 2% O₂ tolerated by most apple

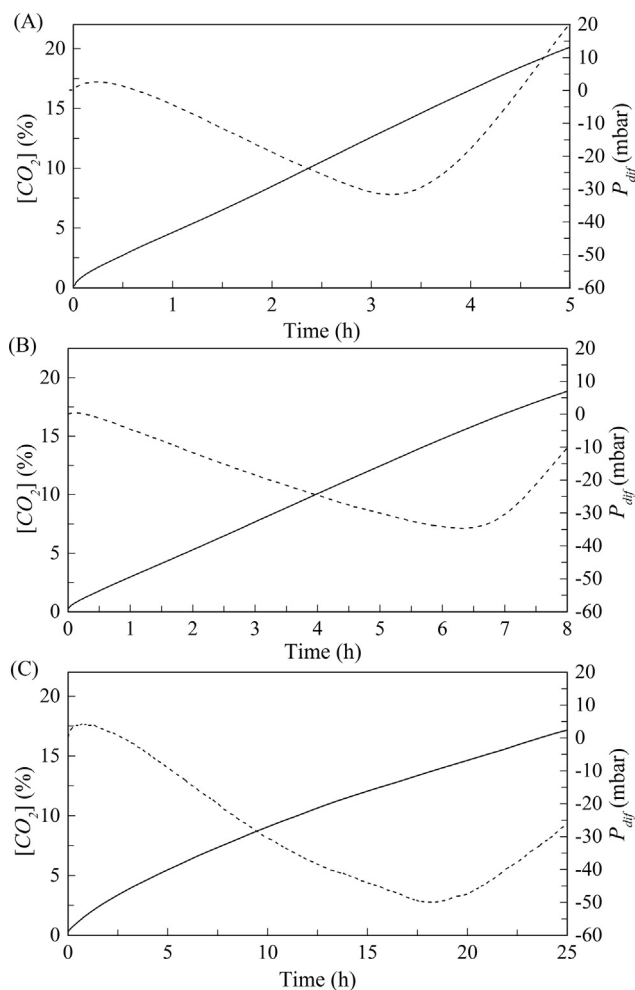


Fig. 3. Data transmitted by the sensors: CO₂ concentration, [CO₂] (solid line) and differential pressure, P_{diff}, (dashed line) for cauliflower (A), strawberry (B), and apple (C).

cultivars, strawberry and cauliflower (Kader et al., 1989). However, this minimum may increase with temperature in several products (Beaudry et al., 1992; Lakakul et al., 1999). Beaudry suggested that limited skin permeability to O₂ at higher temperatures could be involved in this increase, while Boersig et al. (1988) attributed these changes to an increased O₂ requirement for aerobic respiration at higher temperatures. The actual minimum [O₂] values may differ from those reported in the literature since the proposed system accumulates CO₂ continuously, and CO₂ concentration may also play a role (Beaudry, 1993).

Fig. 5 shows predicted R_{O₂} and R_{CO₂} values against predicted oxygen concentration. The strawberry and cauliflower florets showed a similar trend. Initially, the respiration rates were almost constant: 150 mL O₂ kg⁻¹ h⁻¹ and 200–220 mL CO₂ kg⁻¹ h⁻¹ for cauliflower florets and 80 mL O₂ kg⁻¹ h⁻¹ and 70 mL CO₂ kg⁻¹ h⁻¹ for strawberries. These results are in agreement with those obtained by Ratti et al. (1996) for cauliflower at similar temperatures; they observed that the highest CO₂ production was reached at high O₂ concentrations, and this value was maintained over a wide range of [O₂] levels. Barrios et al. (2014) measured respiration rates for strawberries at 23 °C, obtaining 70 mL O₂ kg⁻¹ h⁻¹ over a wide [O₂] range (21–8%). These values are similar to those obtained in our study. However, the apple O₂ respiration rates seem to be influenced by the gas composition, starting at 24 mL O₂ kg⁻¹ h⁻¹ and 25 mL CO₂ kg⁻¹ h⁻¹ at the beginning of the experiment (air) and decreasing to 12 mL O₂ kg⁻¹ h⁻¹ and 8 mL CO₂ kg⁻¹ h⁻¹ when the gas composition was 4% O₂ and 13% CO₂, respectively. The

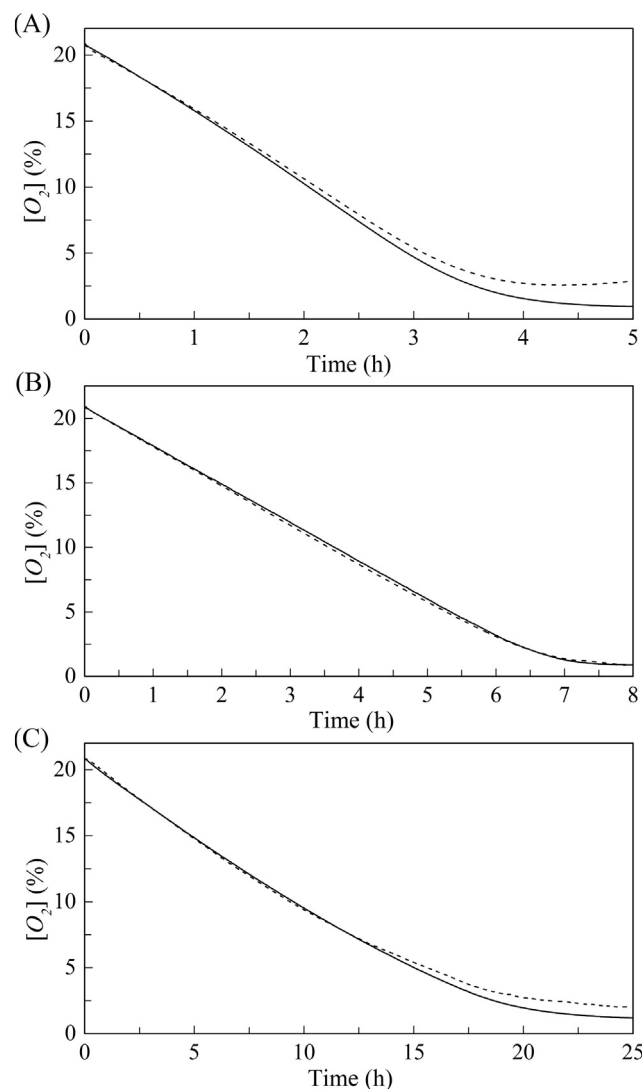


Fig. 4. O₂ concentration, [O₂], predicted from Eq. (2) (dashed line) and experimentally measured (solid line) for cauliflower (A), strawberry (B), and apple (C).

reduction of the O₂ respiration rate as a function of [O₂] in apple at 21 °C has been described by other authors (Andrich et al., 2006), ranging from 18.6 mL kg⁻¹ h⁻¹ in air to 12.9 mL kg⁻¹ h⁻¹ in a 10% O₂-10% CO₂ atmosphere. This is slightly lower than our values, but in our case the temperature was two degrees lower.

For all three products, the oxygen respiration rate experienced a strong reduction at low [O₂] while the carbon dioxide respiration rate was maintained at the same level. This respiration rate trend had a noticeable effect on the evolution of the respiratory quotient. The RQ calculated as the quotient between R_{CO₂} and the predicted R_{O₂} is included in Fig. 6. Initially, the RQ values remained below 1 (approximately 0.7 for cauliflower florets, 0.8 for strawberries, and between 1 and 0.6 for apples), but at low [O₂] these values began to increase up to values beyond 1. This behaviour was similar to that described previously for cauliflower florets at 4 °C (González-Buesa et al., 2009). The lowest [O₂] levels for which the RQ remained close to 1 were: 3.85, 2.63, and 2.70% for cauliflower, strawberry, and apple, respectively. These values were close to the O₂ values at which the differential pressure trend suffered a marked change. Therefore, we suggest the possibility of using the differential pressure values to identify the metabolic change and the initiation of anaerobic routes, thus providing additional information to that provided by the RQ. The fermentative

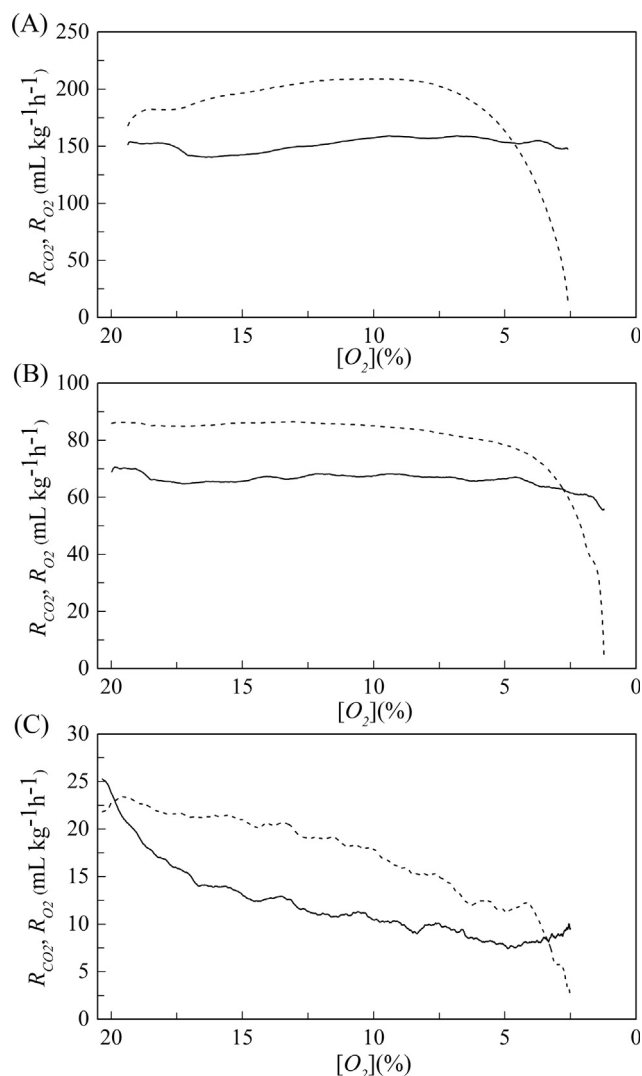


Fig. 5. Evolution of the respiration rates with the O_2 concentration for cauliflower (A), strawberry (B), and apple (C): carbon dioxide production rate, R_{CO_2} , (solid line) and oxygen consumption rate, R_{O_2} , (dashed line).

metabolic routes involve the production of ethanol and acetaldehyde, among other volatile compounds, and these compounds may have an effect on the internal pressure of the system. Furthermore, ethanol easily accumulates in tissues and may be involved in re-metabolism (Pesis, 2005). Thus, differential pressure measurements could be applied as a supplementary indirect tool for identifying changes in metabolic routes.

4. Conclusions

A novel low-cost respirometer was developed based on a modular design in order to obtain a fully configurable and flexible system based on open source software. The system allows the continuous measurement of CO_2 concentration, barometric and differential pressures, and temperature. It was possible to estimate the O_2 concentration and O_2 consumption rate from the CO_2 concentration and differential pressure, since changes in differential pressure could be due to the respiration of fruit or vegetable products. The good agreement between the estimated O_2 and measured O_2 in a wide range of conditions validated this procedure in tests performed with apple, strawberry, and cauliflower. The results obtained demonstrated the potential of using differential pressure measurement to predict metabolic changes and respiratory quotient changes in fruit and vegetable produce placed in the system. The

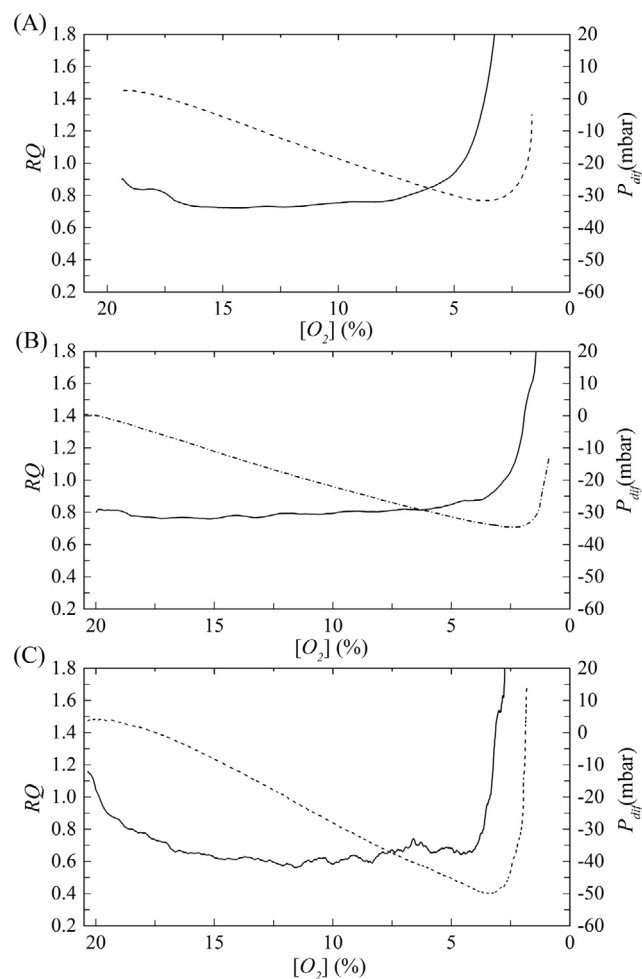


Fig. 6. Evolution of the respiratory quotient, RQ , (solid line) and the differential pressure data, P_{diff} , (dashed line) with the O_2 concentration for cauliflower (A), strawberry (B), and apple (C).

pressure differential measurement for determining the RQ shift may have relevance in produce characterisation and other applications where RQ determination plays an important role, as in novel dynamic controlled atmospheres.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.compag.2019.03.029>.

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