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Microcontroller based low cost device for determination of aerobic stability of silages

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Aerobic stability is one of the important factors affecting the nutritive value of silages for livestock and determined by various methods. The aim of the current experiment was to design a microcontroller based low cost device using CO_2 sensor for determination of aerobic stability of silage and compare with the conventional temperature measurement system. It was hypothesised that the time taken to increase the silage temperature by 2ºC above room temperature and produce 10 g CO_2 kg^{-1} DM might be similar or comparable for the same silages when the silage is exposed to air simultaneously. The microcontroller based low cost device was designed and allowed discrimination between silages in terms of aerobic stability as in the temperature measurement system. The data obtained by microcontroller based low cost device is consistent with data obtained by the temperature measurement system. The time taken to increase silage temperature by 2ºC above room temperature and produce 10 g CO_2 kg^{-1} dry matter (DM) of silage were similar in both measurement systems. It was also found that the high correlation coefficients between CO_2 production and temperature until reflection point of temperature obtained and ranged from 0.990 to 0.995 for sugar beet pulp silage (SNPS) and corn silage (CS). The CO_2 sensor has provided the opportunity in designing a simple and handy device with an affordable cost to measure the aerobic stability of silage as accurately as temperature measurement system.
Keywords: Aerobic stability, arduino, CO_2 sensor, silage, temperature.

INTRODUCTION

Silage is a feedstuff produced by partially fermenting green or succulent forages or other feed materials under anaerobic conditions in a structure called as silo and provide nutrients for livestocks in the most parts of wold throughout the year or in periods of restricted seasonal availability of forages (Wilkinson and Davies, 2013). Many factors such as water soluble carbohydrate of forage, buffering capacity of forage, moisture content of forage epiphytic bacteria content of forage, harvesting stage of forage, speed of fermentation and use of additives may affect the silage quality (Wilkinson and Davies, 2013, Ali and Tahir 2021). It was also reported that type of silo had a significant effect of chemical composition and physical quality of several silages due possibly to differences in wall material used in silos, packing and compaction of silage materials (Rafiuddin *et al*., 2017). Although ensiling is a common practice worldwide, enabling a moist forage to be conserved until fed to livestock, aerobic deterioration of silage results in is inevitable dry matter loss when exposed to air especially during feed out periods (Muck 2007, Wilkinson and Davies, 2013; Weiss *et al*., 2016). Biochemical, microbiological, physical and management factors may affect aerobic stability of silage. (Wilkinson and Davies, 2013). Various methods have been developed to measure the stability of silage against aerobic deterioration. Aerobic stability of silage is normally determined under laboratory conditions at constant ambient temperature by leaving the fresh silage exposed to air for several days to monitor the increase the silage temperature or accumulated CO_2 (Ashbell *et al.*, 1991, Weinberg *et al*., 2009, Wilkinson and Davies, 2013). Aerobic stability was regarded as the time taken to increase the silage temperature by 2 ºC above room temperature and produce 10 g CO_2 kg^{-1} DM (Ashbell *et al.*, 1991; Moran *et al*., 1996; Filya, 2003; Kung *et al*., 2007; Borreani, 2008; Weinberg *et al*., 2009; Koc, 2009; Nkosi *et al*., 2012; Canbolat, 2014; Nkosi *et al*., 2016, Mutavhatsindi *et al*., 2018, Besharati *et al*., 2021).

Although the increase in temperature and CO_2 production have been used as an indicator of aerobic deterioration of

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silages, there are limited researches in which temperature and CO_2 are monitored simultaneously. Moran *et al.* (1996) showed that there was some small agreement between accumulated CO_2 and the accumulated temperature. There is a correlation between the accumulated CO_2 and temperature when the silages are exposed to aerobic deterioration, even if the correlation coefficient (r) between the accumulated CO_2 and temperature was 0.44. Schmidt *et al*. (2010) also found that a positive correlation coefficient of 0.66 was observed between the number of hours to peak temperature and the number of hours to the peak of CO_2. The low correlation coefficient is possibly associated with monitoring CO_2 evolved during the aerobic exposure. It is inevitable that some of CO_2 evolved during the aerobic stability test is lost when simple polyethylene terephthalate bottles was used in the systems suggested by Ashbell *et al*. (1991). This drawback of system described by Ashbell *et al*. (1991) might result in underestimation of CO_2 evolved during the aerobic stability test.

It was hypothesised that time taken to increase the silage temperature by 2ºC above room temperature and to produce 10 g CO_2 kg^{-1} DM might be similar or comparable for a given silage. However, this hypothesis has not been tested so far.

The aerobic stabilities of silages based on monitoring the increase in silage temperature have been determined by using commercially available expensive temperature sensors and Hobo Data logger. The collection and determination of CO_2 in the method developed by Ashbell *et al*. (1991) was not easy and laborious, especially titration of KOH with HCl. It is evident that cheap determination systems for aerobic stability of silages are required to explore the relationship between temperature and CO_2 production. Recently it has been suggested that commercially available NDIR CO_2 gas sensors with high sensitivity, lower power consumption, long life, straightforward installation and low cost can be used to monitor the CO_2 in the buildings and environment (Müller *et al*., 2020, Ragnarsson *et al*., 2021; Chojer *et al*., 2022). Furthermore, Karadol and Kamalak (2022) suggested that NDIR CO_2 sensor can be used to monitor CO_2 evolved in biochemical process such as respiration and fermentation. Therefore, the aim of the current experiment was to design a microcontroller based low cost device using CO_2 sensor for determination of aerobic stabilities of silages and compare the aerobic stabilities with the conventional temperature measurement system.

MATERIALS AND METHODS

***Design of microcontroller based device*:** The device consists of accumulation chamber, CO_2 sensor, Arduino Mega 2560, converter and DC power supply. The accumulation chamber is made of airtight polyethylene bottle (20 L capacity) equipped with a screw-top lid (Figure 1). Non-dispersive infrared (MH-Z19B) CO_2 sensor was chosen due to an inexpensive candidate to measure the CO_2 concentration when compared with commercially available sensors. Non-dispersive infrared CO_2 sensor operates at 0-50 °C and 0-95% humidity. The measurement range was 0-10000 ppm with an accuracy of 5% of reading. It was powered at 3.6-5.5 V, average current consumption lower than 10 mA and it has UART and PWM output (Winsen, 2021). Non-dispersive infrared CO_2 sensor was placed on the left side of accumulation chamber. As can be seen from Figure 2, Non-dispersive infrared CO_2 sensor was connected to a digital input of Arduino Mega 2560 which is an open-source electronics platform based on easy-to-use hardware and software. Arduino boards are able to read sensors using UART (Universal Asynchronous Receiver Transmitter), I2C (Inter-Integrated Circuit) and SPI (Serial Peripheral Interface) communication peripherals that makers and electricians use in microcontroller development (Arduino, 2021).

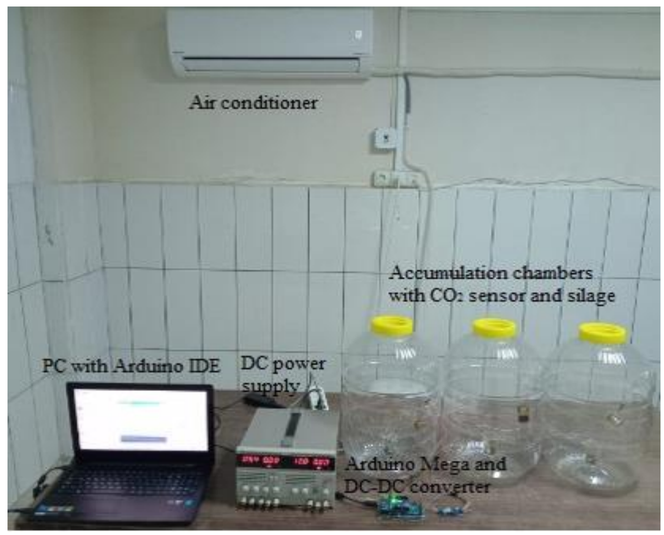

Figure 1. Temperature-controlled room consisting of accumulation chambers with CO_2 sensors, PC, DC power supply, Arduino microcontroller, DC-DC converter and air conditioner

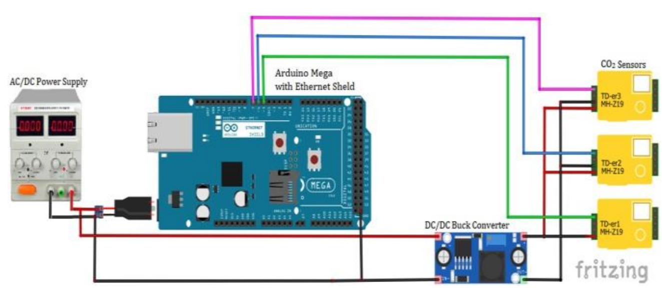

Figure 2. Connection of Non-dispersive infrared (MH-Z19B) CO_2 sensors to Arduino Mega using UART (Universal Asynchronous Receiver Transmitter)

The Arduino Mega 2560 is a microcontroller board based on the ATmega2560. It has 54 digital input/output pins (of which 15 can be used as PWM outputs), 16 analog inputs, 4 UARTs (hardware serial ports), a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It contains everything needed to support the microcontroller. The Mega 2560 board is compatible with most shields designed for the Uno and the former boards Duemilanove or Diecimila.

The reading values (ppm) of non-dispersive infrared CO_2 sensor are recorded to SD card at 5 minutes intervals through Ethernet shield card connected to Arduino Mega 2560.

***Determination of aerobic stability of silage*:** Two set of experiments were carried out to determine the aerobic stability of silage. First, CO_2 accumulation due to microbial activity of silages exposed to air was monitored by non-dispersive infrared CO_2 sensors connected to Arduino Mega 2560. Second, temperature increases due to microbial activity of silages exposed to air was monitored by heat sensor connected to Hobo Data logger.

***Determination of aerobic stability of silage using microcontroller based device*:** Sugar beet pulp silage and CS in plastic bag in triplicate were collected in January 2021 from a commercial farm in Kahramanmaras, Turkiye. Approximately 5 gram of fresh silage sample was selected to avoid excessive CO_2 accumulation (more than 10.000 ppm) in the accumulation chamber and weighed into Erlenmeyer flask and transferred into accumulation chamber to measure the aerobic stability of silage. The lid was placed on the accumulation chamber described in Figure 1. The evolved CO_2 in accumulation chambers for 5 days were recorded at 5 minutes intervals using non-dispersive infrared CO_2 sensor in a temperature-controlled room at 20 ºC. The first reading (R1) of CO_2 was recorded to determine background CO_2 concentration in accumulation chamber. The final reading (RF) of non-dispersive infrared CO_2 sensor was recorded to determine the evolved CO_2 in accumulation chamber at any time of reading. The CO_2 production (g kg^{-1} DM) of silage samples in accumulation chamber for 5 days were calculated using the equation suggested by Karadöl and Kamalak (2023).

$$CO_2 \text{ (g/kg DM)} = \frac{[(RF - R1) * V * dCO_2]/\text{DM of silage(g)}}{1000}$$

R1 = First reading of CO_2 concentration (ppm) of accumulation chamber (background CO_2 concentration); RF = Final reading of CO_2 concentration (ppm) of accumulation chamber at any time of reading.

dCO_2 (density of CO_2) = 1.819 g L^{-1} at 19.3 ºC

Aerobic stability was defined as the time taken to produce 10 g CO_2 kg^{-1} DM (Weinberg *et al*., 2009)

***Determination of aerobic stability of silage using temperature measurement system*:** Approximately 150 gram of silage sample in triplicate was weighed in a glass bottle of 1 litre capacity placed separately in insulated polystyrene foam boxes. The probe (TMC6-HD) connected to Hobo Data logger was inserted in the middle of the silage samples in each glass bottle, and also in the room where the silos were stored to simultaneously monitor the temperature of the silage and room for at 5-min intervals for 5 days (Figure 3). The aerobic stability tests were carried out in temperature-controlled room at 20 ºC, in triplicate as in CO_2 accumulation test. Aerobic stability was defined as the time necessary for the internal temperature of silage to increase 2°C above room temperature (Kung et al., 2007).

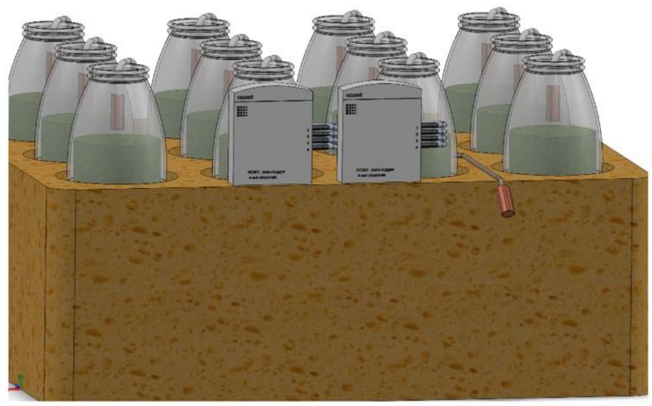

Figure 3. Determination of aerobic stability of CS and SBPS using the temperature sensors (TMC6-HD) connected to Hobo Data logger.

Regression analysis was used to determine the relationship between accumulated CO_2 and temperature of silage exposed to air. The accumulated temperature of silage material was dependent, accumulated CO_2 in the accumulation chamber was independent variable. Regression analysis was carried out using IBM SPSS Statistic Version 20.0 (2011).

RESUTS

The CO_2 productions (ppm) of SBPS and CS when incubated in the accumulation chamber were given in Figure 4. The CO_2 production (ppm) of silages increased with increasing of time of aerobic exposure. As can be seen from Figure 4, CO_2 production (ppm) of SBPS started to increase earlier than that of CS whereas the extent of CO_2 production for CS was higher than that of SBPS. The CO_2 production (ppm) of SBPS at 120 h of aerobic exposure was approximately 2870 ppm whereas CO_2 of CS at 120 h of aerobic exposure was approximately 4047 ppm at 20 ºC.

Temperature profile of SBPS and CS during exposure to air is given in Figure 5. SBPS was stable up to 8 h of aerobic exposure and peaked approximately at 24 ºC by 24 h of aerobic exposure. In contrast, corn silage was stable up to 20 h of aerobic exposure and peaked approximately at 27 ºC by 44 h of aerobic exposure.

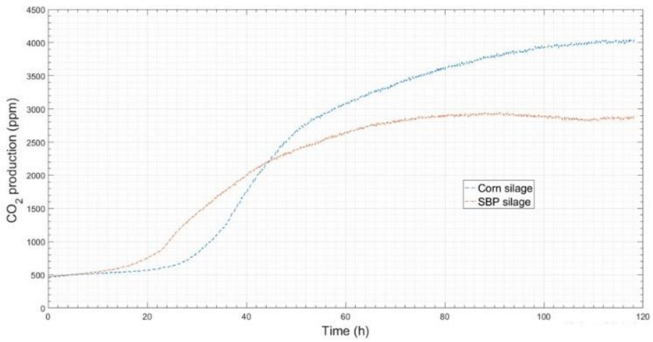

Figure 4. The CO_2 production (ppm) of corn and sugar beet pulp silages when incubated in the accumulation chamber.

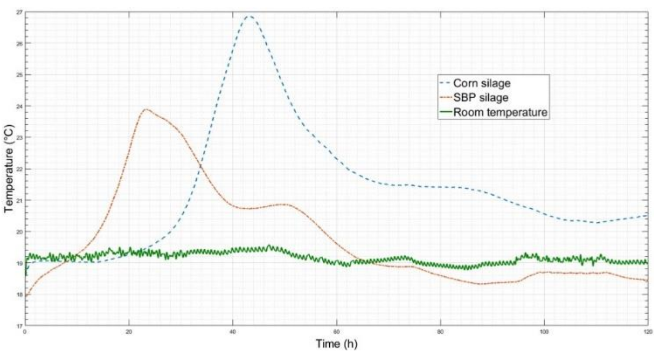

Figure 5. Temperature of corn and sugar beet pulp silage after exposure to air in the room**.**

The cumulative CO_2 production (g kg^{-1} DM) of SBPS and CS when incubated in the accumulation chamber is given in Figure 6. The CO_2 production of silages increased with increasing of time of aerobic exposure. As can be seen from Figure 6, cumulative CO_2 production (g kg^{-1} DM) of SBPS at all time of aerobic exposure was significantly higher than that of corn silage. The cumulative CO_2 of SBPS at 120 h of aerobic exposure was approximately 98 g kg^{-1} DM whereas cumulative CO_2 of corn silage at 120 h of aerobic exposure was approximately 79 g kg^{-1} DM.

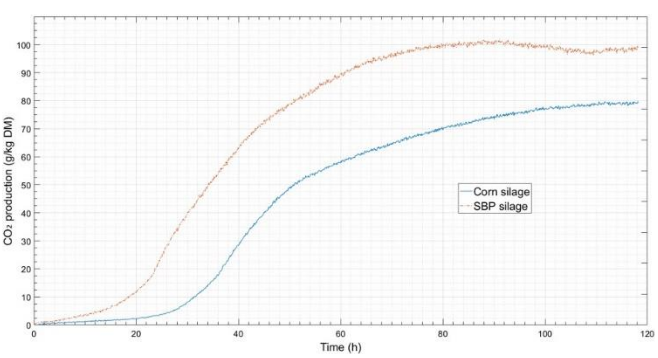

Figure 6. Cumulative CO_2 production of corn and sugar beet pulp silages when incubated in the accumulation chamber

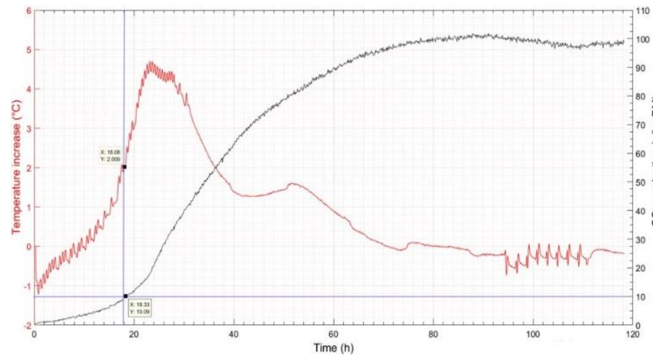

Figure 7.The temperature increase and CO_2 production of sugar beet pulp silage when incubated in the accumulation chamber. The x-axis is the exposure time of silage to air, the y-axis is the temperature increase (^{O}C) or cumulative CO2 production (g/kg DM) of sugar beet pulp silage measured by temperature sensor or CO_2 sensors. Aerobic stability was deemed to be the time taken to increase silage temperature by 2.009 ^{O}C above environment temperature and produce 10.09 g CO_2 kg^{-1} DM of silage

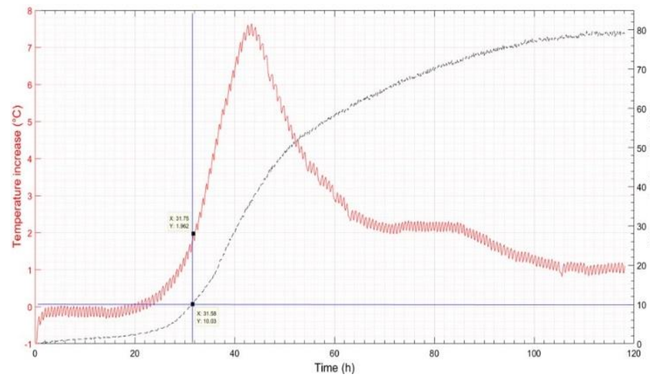

Figure 8. The temperature increase and CO_2 production of corn silage when incubated in the accumulation chamber. The x-axis is the exposure time of silage to air, the y-axis is the temperature increase (^{O}C) or cumulative CO2 production (g/kg DM) of corn silage measured by temperature sensor or CO_2 sensors. Aerobic stability was deemed to be the time taken to increase silage temperature by 1.952 ^{O}C above environment temperature and produce 10.03 g CO_2 kg^{-1} DM of silage.

The CO_2 production (g kg^{-1} DM) and temperature increase (^{o}C) of SBPS and CS when incubated in the accumulation chamber are given in Figure 7 and 8 respectively.

The relationship between CO_2 production and temperature of SBPS and CS is given in Figure 9 and 10 respectively.

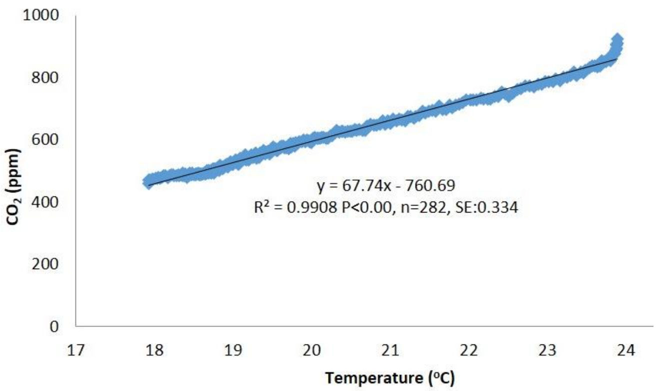

Figure 9. The relationship between CO_2 production and temperature of sugar beet pulp silages. The x-axis is the temperature (^{O}C) of sugar beet pulp silage after exposure to air measured by temperature sensor. The y-axis is the CO_2 production (ppm) of sugar beet pulp silage after exposure to air measured by CO_2 sensor.

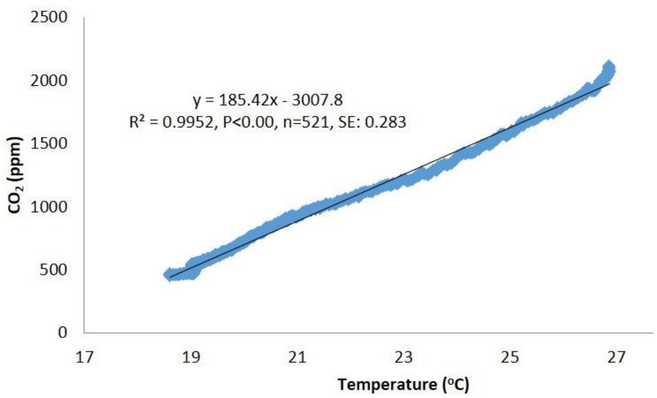

Figure 10. The relationship between CO_2 production and temperature of corn silage. The x-axis is the temperature (°C) of corn silage after exposure to air measured by temperature sensor. The y-axis is the CO_2 production (ppm) of corn silage after exposure to air measured by CO_2 sensor.

DISCUSSION

During the aerobic deterioration, water soluble carbohydrate and lactic acid are metabolised by yeast into CO_2, water and heat (Ranjit and Kung, 2000). The heat production results in increase of temperature of silages. Elevated temperatures up to 54.5 ^{o}C were recorded in the peripheral areas of maize silages sampled from fifty-four commercial farms in Italy (Borreani and Tabacco, 2010). The extent of increase in silage temperature depends on dry matter content and available substrate of silage for yeast (Wilkonson and Davies 2013). The rise in temperature in CS was higher than in SBPS. The higher increase in corn silage temperature obtained in the current experiment is possibly associated with higher dry matter content and available substrate of corn silage since more heat is needed to increase the temperature of wetter material than is required for drier material (Wilkinson and Davies 2013).

As can be seen from Figure 5, the temperatures of SBPS and CS increased with increasing of time of aerobic exposure and peaked approximately at 24 ^{o}C by 24 h of aerobic exposure and at 27 ^{o}C by 44 h of aerobic exposure respectively. Then, the temperature of silage started to decrease sharply. The reason of decrease in the temperature is likely associated with exhaustion of available substrate in silage for yeast and other aerobic micro-organism. The cease of heat production due to the exhaustion of available substrate and dissemination previous heat produced resulted in decrease in the temperature of silage with time.

As can be seen from Figure 5 there are two peaks on temperature line of SBPS and CS although second peaks are ambiguous. It was suggested that first peak on temperature line is possibly associated with development of yeasts and aerobic acetic acid bacteria and second temperature peak is a reflection of mould development (Wilkinson and Davies, 2013). The metabolisms of lactic acid and other acids result in increase in pH of silage, enabling the environment to be more favourable for yeasts, acetic acid bacteria, clostridia and moulds. (Tabacco et al. 2009; Borreani and Tabacco, 2010).

As can be seen from Figures 4 and 5 the CS was more stable than SBPS when exposed to air. This is in agreement with findings of Ashbell *et al*. (1990) who suggested that the aerobic deterioration depends on the the chemical composition and microbial compositions of silages, and CS is more stable than the other silages such as ryegrass and wheat.

As can be seen from Figures 4 and 5 the considerable amount of aerobic deteriotion occurred in both sialges, which indicated that both silages consisted of substrates for spoilage microorganisms. Although aerobic deterioration of SBPS started earlier than that of CS, the rise in the temperature of SBPS was lower than that corn silage. The reason why the aerobic deterioration of SBPS started earlier than that of CS is possibly associated with population and type of yeast present in SBPS (Abdah 2022).. In the current experiment the amount of dry matter in SBPS was aproximately 0.889 g whereas the amount of dry matter in CS was aproximately 1.633 g. Therefore the extent of aerobic deterioration of SBPS was lower than that of CS, which is reflected in Figs. 4 and 5. On the other hand, when CO_2 production was experessed on kg DM base, the cumulative CO2 production (g/kg DM) was considerably higher than that of CS. Ashbell *et al.* (1990) found significant positive correlation (P>0.05) between CO_2 production and dry matter losses of silages during aerobic exposure. Therefore dry matter losses from SBPS is likely to be higher than that for corn silage (Figure 6).

Aerobic deterioration results in not only dry matter loss but also production of toxins which are harmful to animal and human (Shimshoni *et al*., 2013; Ogunade *et al*., 2018). Therefore after opening the silage containers, SBPS and CS should be consumed by ruminant in 18 and 33 hours respectively to prevent possible dry matter losses and consumption of toxins in silages.

As can be seen from Figures 4 and 5, there is clear difference in the shape of lines for CO_2 productions of silages. Therefore, the device on the measurement of CO_2 production performed very well and the allowed discrimination between silages in terms of aerobic stability.

It can be reasonably expected that time taken to increase silage temperature by $2^{O}C$ above environment temperature and produce 10 g CO_2 kg^{-1} DM of silage be similar since both are used interchangeable for aerobic stability of silage. Therefore, temperature increase and CO_2 production of each silage are plotted against time in the same figure (Figures 7 and 8). It is interesting to notice that time taken to increase silage temperature by $2^{O}C$ above environment temperature and produce 10 g CO_2 kg^{-1} DM of silage was similar. The previously suggested hypothesis was supported by the fact that aerobic stability of silages obtained in both methods are similar.

The CO_2 production (g kg^{-1} DM) and temperature increase (^{o}C) of SBPS or CS when incubated in the accumulation chamber are given in Figures 7 and 8 respectively. Moran *et al*. (1996) suggested that the comparison of CO_2 production with temperature of silage is justifiable as both represent accumulated CO_2 and heat respectively over the course of exposure time. Moran *et al*. (1996) also suggested that correlation might be improved if CO_2 production and temperature were measured directly from sample using suitable analytical probes. This is the case in the current experiment in which CO_2 production and temperature were obtained from silage exposed to same air. Therefore the correlation coefficient between CO_2 production and temperature of silage was very high when compared with that indicated by Moran *et al*. (1996). The correlation coefficients (r) between CO_2 production and temperature until reflection point of temperature were 0.997 and 0.931. These correlation coefficients obtained in the current experiment were higher than that (r=0.44) found by Moran *et al*. (1996). The low correlation coefficient obtained by Moran *et al*. (1996) is possibly associated with monitoring the CO_2 evolved during the aerobic exposure. It is inevitable that some of CO_2 evolved during the aerobic stability test is lost when simple polyethylene terephthalate bottles was used in the systems suggested by Ashbell *et al*. (1991). This drawback of system described by Ashbell *et al*. (1991) might result in underestimation of CO_2 evolved during the aerobic stability test. The airtight accumulation system of CO_2 evolved during the aerobic stability test may increase the correlation coefficient between accumulate temperature and CO_2 during aerobic exposure of silage. These results clearly showed that the microcontroller based low cost device can be used to determine the aerobic stability of silages measuring accumulated CO_2 and produce consistent data when compared with temperature measurement system. The microcontroller based low cost device based on the measurement of CO_2 production allowed discrimination between silages in terms of aerobic stability as in the temperature measurement system. The data obtained by the method based on CO_2 accumulation is consistent with data obtained by the method based on temperature measurement. Time taken to increase silage temperature by $2^{o}C$ above environment temperature and produce 10 g CO_2 kg^{-1} DM of silage was similar in both methods based on CO_2 and temperature measurement.

The cost of microcontroller based device designed in the current experiment is approximately 100$ which is significantly lower than that of commercially available temperature sensors connected to Hobo Data logger (700$). It is likely that the cost of microcontroller based device designed in the current experiment may be further reduced when the cheap CO_2 sensors become available in the market in future.

Conclusion: On the basis of consistency with temperature measurement system, the microcontroller based device designed in the current experiment seems to be reliable and very useful to determinate the aerobic deterioration of silage in the laboratory. The CO_2 sensor has provided the opportunity in designing a simple and handy device with an affordable cost to measure the aerobic stability of silage as accurately as temperature measurement system. Two measurement systems used in the current experiment clearly showed that SBPS is more prone to aerobic deterioration when compared with CS. Therefore, care must be taken into consideration to improve the aerobic stability of SBPS and avoid possible losses under practical farm conditions.

***Authors' contributions*:** Karadöl H, designed the study and conducted data gathering. Kamalak A, performed statistical analyse and wrote the article.

Conflict of Interest Declaration: The author declare that I have no conflict of interest.

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