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EARLY DETECTION OF FUNGI IN GRAIN USING CO_2 LEVEL MEASUREMENT

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Abstract – Experiments were performed for a possible application of carbon dioxide (CO_2) measurements for early detection of a fungi spoilage in grains. Grain model systems were prepared with different quantities of fungal pathogen *Fusarium culmorum*. The study found that the contaminated grain samples' CO_2 respiration rate was higher compared with the uncontaminated grain at the same moisture content levels. In addition, it was observed that the *Fusarium* spp. contaminated grains' CO_2 respiration rate increased and remained steady after the initial grain moisture and contamination level increase.

Keywords: Carbon dioxide, measurement, grain storage, fungi

1. INTRODUCTION

Grains are a good medium for the development of various microorganisms. Cereal grains just after being harvested usually contain microbial contamination coming from several sources, such as dust, water, plants, insects, soil, fertilisers and animal feces [1]. The mold fungi begin to actively develop in 15 to 18.5 % grain moisture, bacteria - at grain moisture greater than 20%. Bacteria develops extremely fast when the grain surface is covered with condensed moisture from the ambient air [2].

A knowledge of deterioration rate of wheat under various storage conditions would help farmers and grain storage managers to timely adjust the grain storage conditions to prevent further quality loss [3].

Utilizing thermal cables in a storage bin for temperature monitoring has been a typical method for detecting grain spoilage, since microorganisms produce a large amount of heat in the spoilage location. However, temperature monitoring is usually not sensitive enough due to the low grain's thermal conductivity in bulk grain. Furthermore, measured temperature cannot be easily interpreted due to the influence of the ambient air temperature fluctuation [4].

In recent years, CO_2 measurement, as the conditions of the stored grain indicator has attracted more and more attention due to its sensitivity and reliability.

Previous studies have shown that CO_2 sensors can be effectively used for monitoring early detection of spoilage during storage [4, 5]. The goal of our work was to apply the CO_2 level measurement techniques for possible detection of biological contamination in initial grain spoilage stages instead to spoilage detection by the traditional methods such as visual inspection, smell and temperature measurement.

2. CO_2 SENSORS

In recent years CO_2 sensors were technologically enhanced what significantly improved their parameters and expanded usage possibilities [6]. That made it possible to use CO_2 sensors for agricultural applications.

At least several methods can be used to measure the concentration of CO_2. Gas chromatography, mass spectrometry, electrochemical or optical sensors are often used in various CO_2 concentration measurement devices [5, 7, 8].

Some of the most reliable and cheapest CO_2 sensors are optical non-dispersive IR (NDIR) type sensors. The principle of operation is based on the CO_2 absorption in the specific IR wavelength range. IR gas sensors stands out from other analytical measurement devices because of their lower cost, small size, simple process management, possible continuous measurements.

The NDIR CO_2 concentration measurement sensors were used in this study.

3. MATERIALS AND METHODS

3.1. Preparation of Grain Samples

The research was carried out on wheat from a single source (2013 harvest spring wheat grains, obtained from the JSC "Kauno grudai"). The initial parameters of wheat grains used in all experiments were: moisture content (12 %), microbiological contamination- DON concentration (800 μg/kg).

During the preparation of the experimental samples, a required grain moisture content (MC) was achieved by adding certain amount of distilled water. The water amount, needed to wet the grains for the desired moisture content, was calculated according to the following formula:

$$m_{H_20} = \left[\frac{\left(G_1 \left(100 - w_1\right)\right)}{\left(100 - w_2\right)} \right] - G_1 , \quad (1)$$

where m_{H_20} is the amount of water (kg) required to wet the grain to the desired moisture content, G_I is the mass of dry wheat grains (kg), w_1 is the moisture content of dry grains (%), w_2 is the desired grain moisture content (%).

The relative gas respiration rate (RR) of grains was calculated for comparison of different experiment results evaluating the grain mass, the container volume, and the CO_2 concentration. Wheat respiration rates were expressed as mg of CO_2 produced per kg of wheat per hour, and were calculated by the following equation [4, 9]:

$$RR = \frac{\Delta c_{CO_2} \times M_{CO_2} \times V_h}{V_m \times m \times \Delta t} \quad , \quad (2)$$

where Δc_{CO_2} is the change in a CO_2 volumetric concentration in ppm (10^{-6} L/L), M_{CO_2} is the molecular weight of the CO_2 gas 44.01 (g/mol), V_h is the volume of the headspace in the container (L), V_m is the molar volume of a gas (L/mol), m is the mass of the grain sample (kg), Δt is the duration (h) for Δc_{CO_2}.

The molar volume of a gas:

$$V_m = \frac{R \times T}{P} \quad , \quad (3)$$

where R is the gas constant (0.08206 L mol^{-1} K^{-1}), T is the temperature (K), P is the pressure (bar).

The moisture contents were measured according to the ISO 712:2009 standard. Moisture content values were determined from the average of three replicate measurements by applying the oven drying technique.

The mycotoxin deoxynivalenol (DON) amount in grains was measured using an acoustic method. This quick and non-invasive method is in correlation with conventional results of the immunoenzymatic ELISA method and is comprehensively analysed and presented in the literature [10].

During the preparation of the fungi solution fungi were grown in the liquid MRS Broth medium with added 5 g/l yeast for 3 days (72 hours).

3.2. Measuring system and experiments

The experiments were conducted on the grain samples (200 g) of the specific moisture content (12; 15; 17; 19 and 21% in uncontaminated grain samples) and of the different contamination levels (3; 5 and 7 ml of the added fungi solution and the grain moisture content kept at 19%) which were placed in the sealed 800 mL containers (Fig. 1).

Each of the hermetic vessel had the installed measurement module which was able to measure the CO_2 concentration, the relative humidity (RH) and the temperature (T).

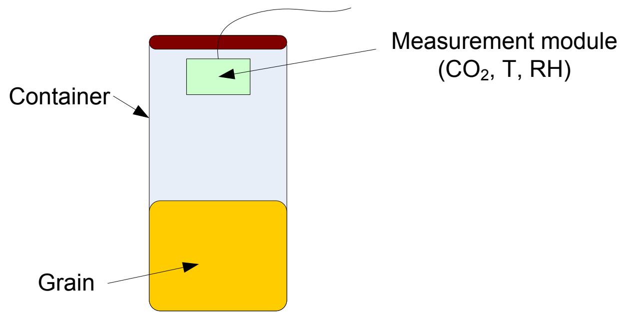

Fig. 1. The hermetic container filled with the grain sample and the measurement module.

The measuring modules (CO_2 Engine K33 LP T/RH, SensAir AB, Delsbo, Sweden) were used to measure the CO2 concentration, the temperature and the relative humidity (RH). The measurement range of the CO_2 concentration sensors was 5000 ppm (parts per million volumetric) (accuracy ±30 ppm ±3% of reading) and up to 10000 ppm in the extended measurement range (not specified accuracy). The response time of the sensors was 30 s. The temperature and the relative humidity measurement range of the sensors were 0 to 50 °C (accuracy ±0,4°C) and 0 to 80 % RH (accuracy ±3 %) up to 100% RH in the extended range (non-condensing environment), respectively.

Measurement modules were protected from grain dust by enclosing each of the modules inside a porous polymer textile. The whole measurement system consisted of 8 containers.

All measurement modules, used in the measuring system, had unique addresses and were connected to a common data bus (Fig. 2). A PC with the dedicated software was used to read the data at 1 minute intervals from each module and log this data for the further analysis.

Prepared grain samples were placed in the sealed containers with the measuring modules. The containers were stored in the environmental chamber ("Sheldon Manufacturing") (Fig. 3) with a controlled temperature at 25±1 °C during the experiments.

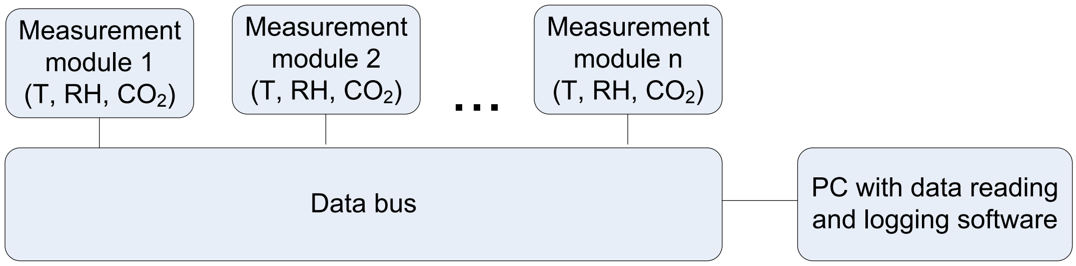

Fig. 2. The structure of the CO_2, temperature and RH measuring system.

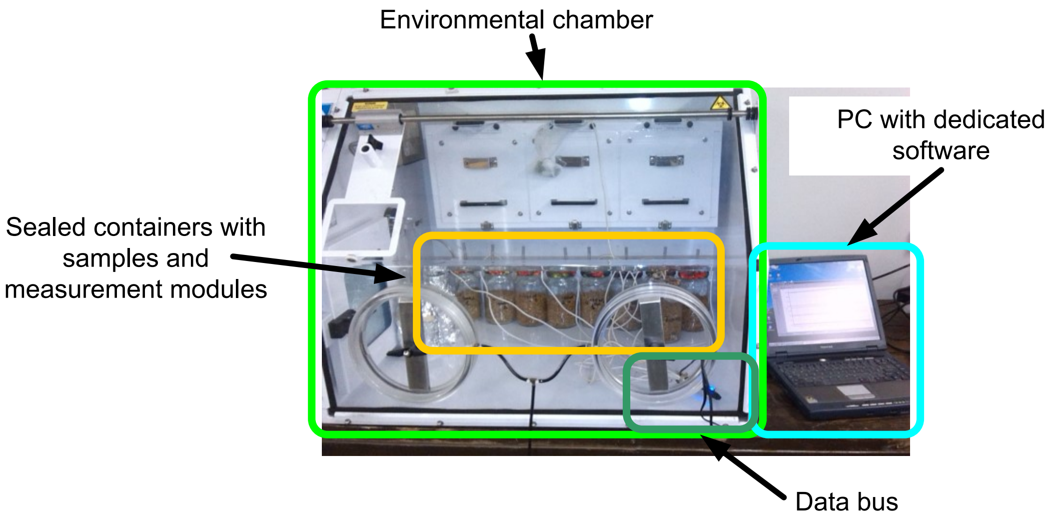

Fig. 3. View of the measuring system

4. RESULTS

The CO_2 sensors used in measuring modules had upper CO_2 concentration measurement limit at 10,000 ppm (parts per million, volumetric). In order to avoid saturation of the CO_2 sensors the periodical aeration process was made- the hermetic containers were opened, the grain samples were stirred, the containers were ventilated and closed, CO_2 concentration was further measured. The CO_2 respiration rate was calculated from the measurement results according to the equation (1).

The initial experiments were carried out by measuring the amount of produced CO_2 in uncontaminated wheat samples at different moisture contents (Fig. 4). Differences were found in the respiration rates of wheat grains at different moisture contents. The grain RR increased when the grain moisture content increased. The minimum grain respiration rate was observed in the control grain sample with the 12% moisture content.

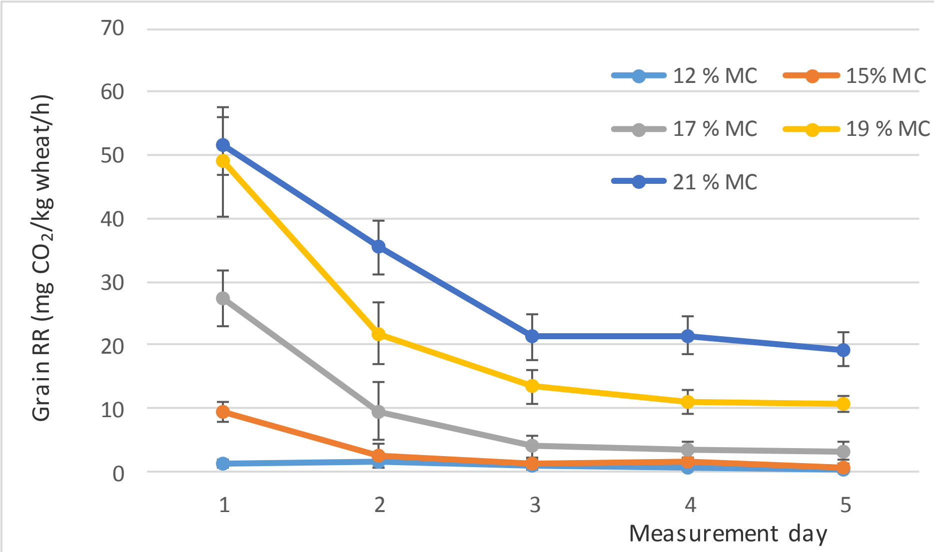

Fig.4. The CO_2 respiration rates of wheat grains at different moisture content levels without the additional fungal contamination (T=25 °C).

As an optimal medium for the development of fungi further experiments were performed at the 19% grain moisture content and T=25 °C. The wheat grain samples were contaminated with different amounts of fungi (F.culmorum). The measured moisture content of all samples was equal to 19 ± 0.3%. Added fungi quantity in the prepared grain samples are presented in the Table 3.

Table 3. Grain samples with differently added fungi.

Sample No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Added fungi quantity, mL	0	0	3	3	5	5	7	7

The figures 5 and figure 6 shows the raw data (CO_2 concentration and RH) during this experiment. The CO_2 respiration rates of wheat with different initial fungi quantity levels are shown in the figure 7.

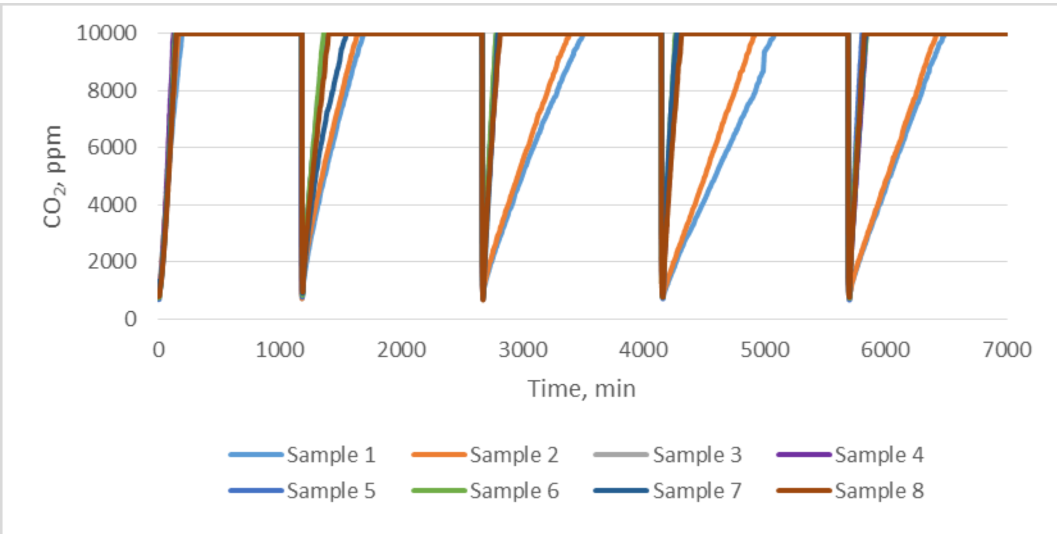

Fig. 5. The raw data of CO2 concentration measurements during one experiment.

Depending on the added fungi quantity, the measured CO_2 concentration dynamics had different trends (Fig. 5). The air RH values in each chamber increases at the start of experiments and later stabilizes.

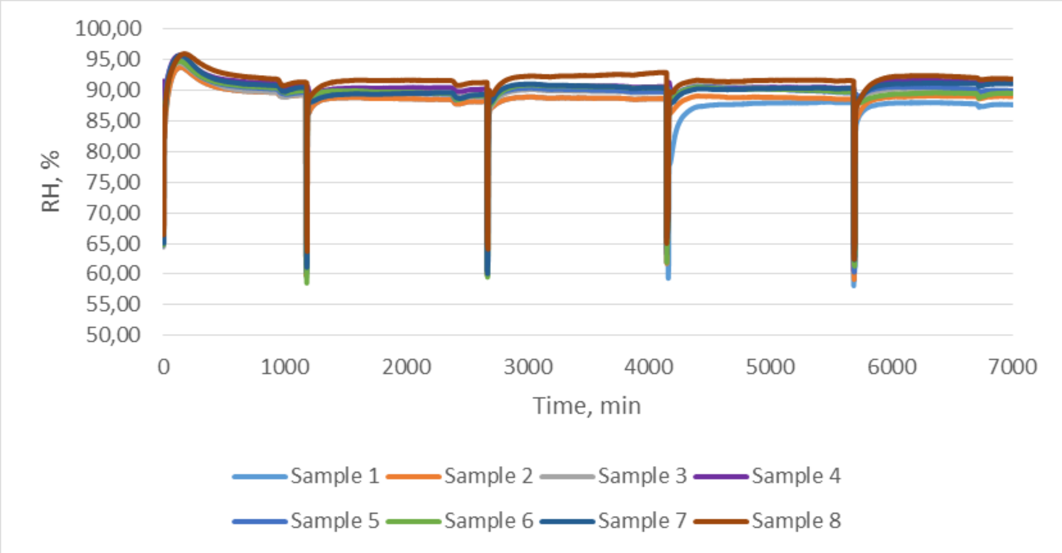

Fig. 6. The measured RH values during one experiment.

The RH value drops almost to environment RH value during the aeration process and reaches the previous RH value (Fig. 6) when the container is closed after the aeration.

Additionally, the separate experiment was performed to determine how much the ventilation process affects the grain moisture content. It was found, that the moisture content doesn't decrease significantly due to the ventilation process, even if grains were ventilated for a few hours.

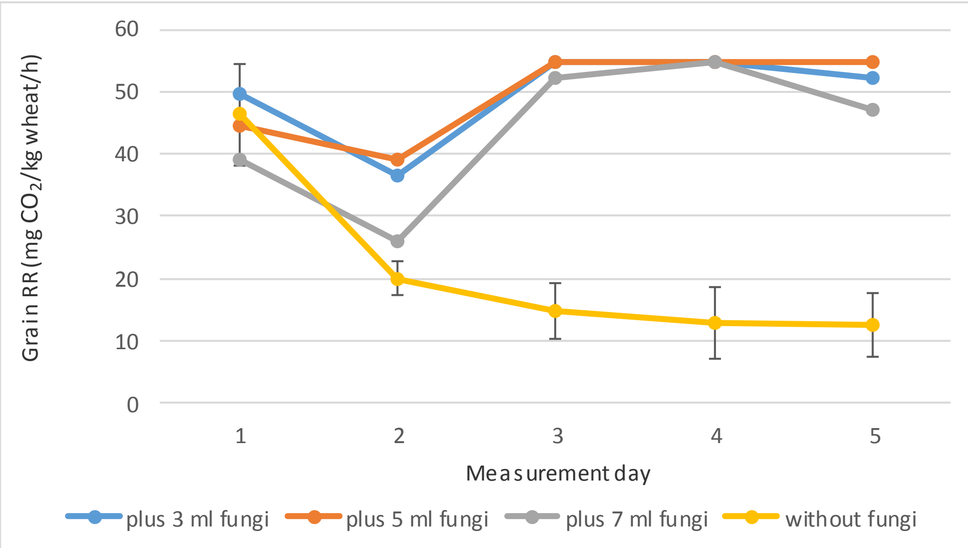

Fig. 7. The respiration rates of wheat at various initially added fungi quantities (T=25 °C, MC=19%).

The figure 7 shows that in the contaminated grain samples CO_2 respiration rate was higher compared with the uncontaminated grain. It was observed that *Fusarium* spp. contaminated grains CO_2 respiration rate remained stable at least for 3 days after the RR increase. In contrast, the fungi-free grains' CO_2 RR decreases irrespective of the grain moisture content (Fig. 4).

5. CONCLUSIONS

The experiments were performed using one of the dominant aggressiveness fungal pathogen *Fusarium culmorum*.

The study found that the fungi contaminated wheat grain CO_2 respiration rates are higher compared with uncontaminated grain CO_2 respiration rates.

It was observed that the *Fusarium* spp. contaminated grain CO_2 respiration rates remained stable at relatively high levels (approximately 55 mg of CO_2 per kilogram of wheat per hour) and were not significantly dependant on the initially added fungi quantities (3; 5 or 7 ml of added fungi solution) for at least 3 days after the initial RR increase while the RR of the uncontaminated grain samples decreased or remained constant at lower RR levels.

Measurement of wheat grain CO_2 respiration rates can provide valuable information about initial spoilage stages in stored grains in addition to conventional techniques such as the temperature measurement.

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