



Interstitial concentrations of carbon dioxide and oxygen in stored canola, soybean, and wheat seeds under various conditions



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ARTICLE INFO

Article history:

Accepted 3 December 2013

Keywords:

Respiration
CO₂ concentration
Quotient of respiration
Stored crops
Daily CO₂ production

ABSTRACT

Interstitial concentrations of CO₂ and O₂ were measured to determine respiratory activities of microflora infecting stored canola, soybean, and wheat in different airtight storage times (1, 3 or 5 days) and at different moisture contents and temperatures (10, 15, 23, 25, 30, 35 and 40 °C). Canola seeds and ground canola with moisture contents (m.c. wet basis) of 8.0%, 10.0%, 12.0%, 13.6% and 14.0%, soybean with 23.0% m.c., and wheat with 20.3% m.c. were used. There were significant differences in CO₂ concentrations between 1-d airtight storage time and other airtight storage times except at 40 °C. The same moisture content canola at the same environmental condition but in different replicates accumulated different CO₂ concentrations at 10 °C and 40 °C but not at temperatures of 23 and 30 °C. Compared with the respiration of microflora, respiration by canola itself was negligible. There was no significant difference in concentration of CO₂ produced by microflora within different crops at 35 °C, while there was significant difference at 15 °C and 25 °C. Values of respiration quotients (RQ) were >1 at almost any testing condition with few exceptions. Sum of CO₂ and O₂ concentrations were close to 21%–22% at most airtight storage times and within any crop. There was a strong positive relationship between the sum and RQ values. The sum might be used to identify whether stored grain had high level of spoiled spots with high moisture content.

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1. Introduction

Interstitial air of stored grain usually consists of nitrogen (N₂), oxygen (O₂), carbon dioxide (CO₂), water vapour, trace gases and a small amount of volatile chemical compounds. During grain storage, gas composition of the interstitial air often changes. These changes can be caused by the respiration of stored grain kernels and infestation agents: microorganisms, insects, and mites. During this respiration process, O₂ is consumed and CO₂ is produced by living organisms (including the grain itself). This respiration process is usually mixed with other oxidative reactions (Reuss and Pratt, 2001) and there are various pathways of respiration (Wilks, 1959; Fischer and Lutge, 1978). Concentrations of CO₂ and O₂ also influence the pathways (Ellis et al., 1991). Under absence of O₂, anaerobic respiration and fermentation can occur and this metabolic process can produce trace amounts of chemical compounds such as carbon monoxide (CO), hydrogen, acetate, ethanol, acetic acid and butanol (Dufour et al., 2011). Less than 1% CO has been reported to accumulate in stored cereal grains and oilseed (Whittle

et al., 1994; Reuss and Pratt, 2001). The concentration of CO₂ inside a stored grain mass is related to grain deterioration and the amount of CO₂ produced is directly related to dry mass loss of stored products (White et al., 1982a,b; Ileleji et al., 2006; Maier et al., 2010).

The rates of CO₂ production and O₂ consumption are governed by non-living and living factors such as moisture content, temperature, O₂ concentration, mechanical damage to seeds, microbial contamination, the condition and period of previous storage and infestation history by insects and microorganisms. The main factors influencing this respiration process are temperature, moisture content of the grain, and respiration of the infesting insects and microflora. Mould-free wheat with lower than 0.9 water activity (about 23.7% moisture content) at 15–35 °C has a relatively low and constant level of respiration (Lacey et al., 1994). Wheat with high moisture contents and infected by moulds has a respiration rate that exceeds microbial respiration (Seitz et al., 1982). The high rate of respiration of high moisture grain may mostly be caused by the respiration of microorganisms (Magan et al., 2004). The same grain under different storage conditions can be infected by different microorganisms (Lacey et al., 1994; Pronyk et al., 2004). This explains differing respiration rates among different cultivars (Cantone et al., 1983), ages

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(Kittock and Law, 1968) and qualities of grains tested. Reuss and Pratt (2001) found the accumulation of CO and CO₂ in stored canola atmospheres under both laboratory and field conditions at far exceeding rates than in stored cereals and dry peas. However, Lacey et al. (1994) found the respiration differences between wheat, barley and oilseeds were smaller than between varieties of the same grain types. In the same study, Lacey et al. (1994) reported that oilseeds consumed significantly less O₂ than cereal grains. Pronyk et al. (2004) found wheat produced about 4-fold higher CO₂ than canola at 25 °C at the same moisture content. There is no clear explanation whether this difference is caused by the different grain types or testing conditions.

The respiration process consumes carbohydrates, lipids, proteins or a combination of these and results in different respiration quotients (Pomeranz, 1992). During respiration testing, the concentrations of CO₂ and O₂ are measured and quotient of respiration (RQ) is calculated ($RQ = \text{CO}_2 \text{ produced} / \text{O}_2 \text{ consumed}$). At the initiation of respiration, CO₂ and O₂ concentrations are similar to that of air (0.04% of CO₂ and 21% of O₂). As time progresses in an airtight storage environment, CO₂ concentration will increase with corresponding decrease in O₂. If fats, proteins or carbohydrate are used for energy, the RQ is about 0.7, 0.9 or 1.0; respectively. Respiration quotient >1.0 means microorganisms are relying on anaerobic respiration or fermentation to obtain energy. Stored seeds may absorb the produced CO₂ (Mitsuda et al., 1973; Cofie-Agblor et al., 1995, 1998). If there is no absorption or desorption of CO₂ and O₂ by the stored product, the sum of CO₂ and O₂ inside the airtight environment should be less than 21% if RQ is less than 1, or equal to 21% if RQ = 1. The CO₂ concentration could exceed 21% if the airtight storage time is long enough and anaerobic fermentation occurs. Studies reported RQ values of the stored wheat ≥ 1 (White et al., 1982a; Woodstock and Justice, 1967) or <1 (Lacey et al., 1994). Rapeseed has 0.7 to 0.8 RQ values when the moisture content is 11.3%, and >1 when moisture content is above 15.2% (White et al., 1982a). The sum of CO₂ and O₂ was reported about 15% by Reuss and Pratt (2001) and about 21% by Abalone et al. (2011a,b). The O₂ concentration reduces to <0.3%, and CO₂ concentration increases to 23% within 5.5 d inside a hermetically sealed container holding cocoa beans at 75% RH and 26 °C (Navarro et al., 2010). It is not known whether the sum of the CO₂ and O₂ concentrations inside the stored-grain mass is related to the condition of stored grain such as RQ value, anaerobic or aerobic respiration, and dry mass loss.

Respiration rates of various grains were measured in different airtight periods by different researchers (White et al., 1982a,b; Lacey et al., 1994). White et al. (1982a) found a reduced respiration rate than that reported in other studies (Seitz et al., 1982; Milner et al., 1947). White et al. (1982a) sampled three times weekly (airtight storage time was 2 or 3 d) at 20 °C and five times weekly at 30 °C; this sampling frequency was less than those reported in the other studies. In a longer sampling period, CO₂ may be accumulated and O₂ may be reduced. Accumulated CO₂ and reduced O₂ concentrations may inhibit respiration and direct it toward different pathways of respiration (Lacey et al., 1994). If sampling frequency influences the determined respiration rate and the accumulated CO₂ production, the effect of airtight duration on RQ values should be evaluated. In a stored-grain environment, the produced CO₂ may not diffuse away immediately after it was produced and CO₂ may accumulate around the respired seeds. The accumulated CO₂ and depleted O₂ may inhibit further aerobic respiration and stimulate anaerobic fermentation when wheat moisture content is $\geq 18\%$ (Weinberg et al., 2008). The influence of airtight storage time on respiration rate, maximum CO₂ concentration, and sum of CO₂ and O₂ concentrations in stored-grain bins is presently unknown.

To monitor stored grain condition, it is required to know: 1) whether CO₂ concentrations are different in different crop bins under similar storage conditions; 2) whether RQ values in different airtight storage times are different; 3) whether respiration pathways can be determined by measuring RQ values; 4) how much CO₂ is produced by different crops under similar conditions; and 5) whether sum of CO₂ and O₂ concentrations is different under different storage conditions. Therefore, the objectives of this study were to measure CO₂ and O₂ concentrations of interstitial air of canola, soybeans and wheat under similar storage conditions and measure respiration of canola with different moisture contents under different temperatures and airtight storage times. Based on these measured data, questions mentioned above were answered.

2. Materials and methods

2.1. Crops

Canola (Nex4 105 RR, Nexera canola Clearfield[®] and Roundup Ready[®] hybrid, oil content $45.4 \pm 0.4\%$), soybean (Pekko No. 1), and wheat (hard red spring No. 1) were stored at room condition inside polypropylene woven sacks (non-airtight) for about 3 months after the crops were purchased from Manitoba (Canada) farmers. After dockage was removed, moisture content (m.c., wet basis) of the purchased crops was determined by oven-drying triplicate samples at the appropriate temperatures and times for each crop (ASABE, 2009). The initial moisture contents of canola, soybean and wheat was $9.0 \pm 0.1\%$, $7.0 \pm 0.0\%$ and $13.0 \pm 0.1\%$ (standard errors provided after '±' in this article are with $n \geq 3$), respectively. The initial germination of canola, soybean, and wheat were $98.7 \pm 1.0\%$, $95.0 \pm 1.7\%$, and $97.3 \pm 2.7\%$, respectively. After the initial storage period and before testing, the grains were tempered to desired moisture contents by following the method reported by Jian et al. (2012). For example, to create 14% m.c. canola, 814 mL of distilled water and 10 kg of the 7.0% canola seeds were mixed inside a tumbler for 15 min. Seven hours later, this mixing process was repeated for another 15 min. The reconditioned grains were stored inside sealed plastic bags at 5 ± 1 °C for at least 14 d before use in experiments. The m.c. of the crops was determined after this 14 d storage (ASABE, 2009) and this measured m.c. was reported in this study.

The tempered crops were used to conduct three sets experiments: 1) interstitial CO₂ and O₂ concentration of 14% m.c. canola at 10, 23, 30 or 40 °C with 1, 3 or 5 d of airtight storage time (referred to as experiment DTightTime); 2) interstitial CO₂ concentration of canola with 8%, 10%, 12% or 14% m.c. and at 10, 23, 30 or 40 °C with 3 d of airtight storage time (referred to as experiment DMC&T); and 3) interstitial CO₂ and O₂ concentrations of different crops (canola, ground canola, soybean, or wheat) at about 90% relative humidity at 15, 25 or 35 °C with 3 d of airtight storage time (referred to as experiment DCrop).

2.2. Measurement procedure for experiment DTightTime

Methods reported by White et al. (1982a,b) were used to measure the interstitial CO₂ and O₂ concentrations of 14% m.c. canola at 10, 23, 30 or 40 °C with 1, 3 or 5 d of airtight storage time. Glass Erlenmeyer flasks (300 mL), each with a side-arm covered with a rubber septum and containing a plastic Nalgene tube (4 mm outer-diameter, 3 mm inner-diameter) extending from the septum to the bottom of the flask, were partially filled with 150 g canola with 14% m.c. To transfer canola into the flasks, a funnel (6.0-cm high and 6.0-cm diameter top opening and 0.8-cm outer-diameter spout) was inserted into the neck of the flask. After 150 g canola was slowly poured (pouring procedure took at least 10 s) into the flask,

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