

INTERGRANULAR CARBON DIOXIDE AS AN INDICATOR OF BIOLOGICAL ACTIVITY ASSOCIATED WITH THE SPOILAGE OF STORED WHEAT

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Intergranular carbon dioxide may be used as an indicator of incipient grain spoilage. Carbon dioxide and oxygen levels were measured and respiratory quotients were determined in wheat stored in 300-mL flasks for 3-5 wk at eight moisture contents between 14 and 25% at 10, 20, 30, and 40°C. Equations are given for rate of carbon dioxide production predicted from temperature, moisture content of seed, and days in storage; and cumulative carbon dioxide production from the beginning of storage at each temperature is predicted from the moisture content of the seed, and days in storage. Cumulative carbon dioxide production was related to physical and biotic variables associated with grain spoilage and also used as a basis for developing a storability index. The production of about 655 mg carbon dioxide/kg wheat was related to the beginning of loss in seed germination.

INTRODUCTION

The deterioration of stored cereals and oilseeds, caused by fungi, insects, and mites, is an economic problem in western Canada. In years such as 1968-1969, with damp grain harvests, 55% of the primary grain elevators in the prairie provinces reported grain spoilage (Sinha 1972). In a questionnaire survey of 2919 grain elevator managers, who were responsible for taking deliveries of farm-stored grain in 1970-1971, reports were made of 11 289 hot spots on farms and 929 in elevators; and 12 956 moldy grain bulks on farms and 570 in elevators (Sinha 1973).

Systems presently available to monitor deterioration of stored bulk grain on farms and elevators take point measurements of temperature. Because the thermal diffusivity of grain is low, temperature must be measured within 0.5 m of an active spoilage spot to detect deterioration (Sinha and Wallace 1965). Spoilage is not necessarily indicated by the measurement of grain temperatures that are well above the ambient temperature. The temperature of dry, undeteriorated wheat at the center of a 6-m-diameter bin in Winnipeg can be above 25°C in January when the outside ambient temperature is below -20°C (Yaciuk et al. 1975).

Increases in CO₂ concentration and decreases in O₂ concentration appear to be reliable indicators of incipient grain spoilage. A study on the spoilage of wheat in polyethylene, steel and wooden bins, and an open pile demonstrated high CO₂ levels considerable distances from the points of spoilage (Muir et al. 1980).

Cumulative production of CO₂ has been used to measure deterioration of shelled corn in the laboratory (Steele et al. 1969) and was related to theoretical indices of safe grain storage by Hall and Dean (1978).

The intensity of respiration in stored grain is determined by moisture content (MC) of the seed, temperature, mechanical damage, and type and degree of microfloral infection (Bailey 1940) as well as insects, if present. High moisture levels in stored grain are often related to dampness during harvest, moisture migration caused by convection currents in the bulk seed, or the entrance of snow or water through the bin structure. Above a moisture content in equilibrium with 75% RH, respiration increases sharply (Hummel et al. 1954) with microflora under the seed coat being the primary cause (Carter 1950; Hyde 1950).

The overall objective of the research project was to develop a scientifically valid and commercially feasible monitoring system to detect deterioration of cereal grains and oilseeds during storage in large bulks.

The objective of this phase of the study was to determine rates and quantities of CO₂ produced and O₂ consumed in aerobically stored wheat at various combinations of temperature and moisture, and to relate the values to selected variables affecting respiration within the grain.

MATERIALS AND METHODS

Grain and Treatments

Hard red spring wheat (*Triticum aesti-*

vum L. 'Neepawa', #2 Canada Western Red Spring, <0.6% dockage (Anonymous 1975a) harvested dry in September 1979 and stored dry for 6 mo, was adjusted in 1-kg lots to eight levels of moisture ranging between 14 and 25% MC (wet mass basis). Sterile water and an electric mixer were used for moisture conditioning. The conditioned wheat was placed in sealed plastic bags and held at 2.5°C for 2 days to allow the moisture to equilibrate. This period also permitted the evolution of CO₂ gas which accompanies the addition of water to grain (Yamamoto and Mitsuda 1980). The moisture content of each parcel of wheat was determined by oven-drying 10-g samples, in triplicate, at 130°C for 19 h (Anonymous 1975b) and expressed on a wet mass basis throughout the paper. Glass Erlenmeyer flasks (300 mL), each with a side-arm covered with a rubber septum and containing a plastic Nalgene tube (4 mm o.d., 3 mm i.d.) extending from the septum to the bottom of the flask, were partially filled with wheat calculated to equal a dry mass of 150 g.

The wheat in the flasks was flushed with compressed air which was moisturized by passing it through two gas-washing bottles containing water, at ca. 20 mL/sec for 1-5 min. Flushing of the flasks created an aerobic environment simulating conditions in granaries. Each flask was then sealed with a rubber stopper. Carbon dioxide production and O₂ consumption were measured at four temperatures with four replicates of wheat at each of eight moisture contents for a given temperature. The initial moisture levels of the wheat (mean

\pm SE) were 14.4 ± 0.09 , 15.3 ± 0.08 , 16.6 ± 0.05 , 17.6 ± 0.08 , 18.5 ± 0.05 , 19.1 ± 0.20 , 20.5 ± 0.21 and $24.2 \pm 0.14\%$. Experiments at each temperature were conducted separately with 32 flasks.

Measurement Methods

Levels of CO_2 and O_2 were determined with a Perkin-Elmer Sigma 3B gas chromatograph with a thermal conductivity detector. The carrier gas was helium, inlet pressure was 207 kPa, the detector temperature was 150°C , and the oven was held at 45°C . Carbon dioxide was separated from other gases by a 1.8-m column packed with Porapak N; and O_2 with Molecular Sieve 5A (mesh 60/80). A 1-mL fixed-volume gas sampling valve, at room temperature, was connected to each column to standardize the volume of injected samples. Maximum resolution was 25 ppm of CO_2 . The gas chromatograph was regularly calibrated with a series of commercially prepared high purity mixtures of CO_2 or O_2 in He of specified concentrations (Altech Assoc., Arlington Heights, Ill. 60004).

Gases from the flasks containing the wheat were analyzed three times a week (Monday, Wednesday, Friday) at 10°C and 20°C for 5 wk, and five times a week (Monday-Friday) at 30°C and 40°C for 3 wk. There were 15 sampling dates at each temperature and the length of time between dates was determined according to the expected rate of respiration. Temperatures were maintained at $\pm 1^\circ\text{C}$ in environmental chambers.

Intergranular CO_2 and O_2 levels were measured at the outset after wheat was initially placed in the flasks and flushed. Before sampling on subsequent days, the flasks were inverted for 15 min, then the gas was mixed by withdrawing and returning it through the septum four times using an evacuated 50-mL syringe as a precaution against CO_2 stratification. Two 4-mL samples were taken from each flask with a 10-mL gas-tight syringe; the levels of CO_2 and O_2 were determined by subtracting the corresponding values obtained on the previous sampling date. The rubber stoppers were removed from the flasks which were then flushed with compressed air as previously outlined. The levels of CO_2 and O_2 after flushing were determined from 4-mL samples injected into the gas chromatograph; the rubber stopper was replaced and the flasks were returned to the environmental chamber. The postflushing levels of CO_2 were generally less than 1%, which is well below values that inhibit fungal growth (Tabak and Cooke 1968).

Atmospheric pressure was noted after flushing and prior to sealing the flasks on each sampling date, and was used with gas temperature at the time of analysis to calculate the density (mg/L) of CO_2 and O_2 in the gas sample. The volume of intergranular air in each flask for wheat at each moisture content was initially determined by a water displacement procedure. All calculations were standardized to the mass of CO_2 produced or O_2 consumed by 1 kg (dry mass) of wheat.

Both the cumulative quantities of CO_2 produced and O_2 consumed since the start of the experiment, and the mean rates of change between sampling dates were determined. Odors and visible moldiness of seeds were also noted throughout the study.

At the beginning and end of each experiment the following variables were measured to determine whether they were related to cumulative respiration: fat acidity values (FAV), seed-borne microflora, and seed germination. Fat acidity values were determined on the four replicates for each moisture content using the American Association of Cereal Chemists' method 02-01 (Anonymous 1962). Seed-borne microflora were observed by placing 25 unsterilized seeds on sterile filter paper saturated with 4.5 mL of sterile water in a petri dish (three replicates per flask), and 25 unsterilized seeds on sterile filter paper saturated with 4.5 mL of sterile 7.5% NaCl solution in a petri dish (three replicates per flask) (Mills et al. 1978). The plates were then stacked, sealed in plastic bags, and held at $22 \pm 1^\circ\text{C}$ for 7 days (Wallace and Sinha 1962). Six replicates per flask (150 seeds), 24 replicates per moisture level (600 seeds) were used. The rate of seed germination was determined by the filter paper method (Wallace and Sinha 1962), using 4.5 mL of sterile water, for three replicates of 25 seeds per flask. Seeds were rated for germination after 1, 3, 5 and 7 days. The final moisture content of the wheat in each flask was determined by oven-drying four replicate samples per flask.

Statistical Analyses

A regression of milligrams of CO_2 produced by 1 kg of wheat in 24 h on three independent variables was done using the University of California BMDP1R computer program. The dependent variable of CO_2 was transformed using \log_{10} ; the independent variables were temperature ($^\circ\text{C}$), storage time (days), and moisture content (%).

Respiratory quotients, or the ratio of volumes of CO_2 produced to O_2 consumed

in 24 h, were calculated for each temperature and moisture content and averaged over the period of study.

Stepwise multiple linear regressions were calculated based on abiotic and biotic variables using the BMDP2R program, with the total cumulative CO_2 (transformed using \log_{10}) as the dependent variable. The independent variables were temperature ($^\circ\text{C}$), storage time (days), moisture content (%), FAV (mg KOH/100 g dry grain), germination, and microflora (number of seeds infected with bacteria or specific fungi out of 75 seeds) which included *Aspergillus glaucus* group, *A. candidus* Link ex. Fr., *A. flavus* Link ex Fr., *Alternaria alternata* (Fr.) Keissler, *Cladosporium cladosporioides* (Fr.) Saccardo, *Penicillium* spp., and bacteria.

A prediction model for the cumulative mg CO_2 produced by 1 kg of dry wheat was developed relating adjusted moisture content (actual - 14%) and time (days in storage) at 10, 20, 30 and 40°C . Moisture content was adjusted by subtracting 14% because little CO_2 is produced by dry grain and lower values tend to skew the results of the models. Values from the first sampling date were not included to compensate for CO_2 released following moisture conditioning of the wheat. The predicted and observed values for cumulative CO_2 were compared for each temperature and moisture content on days 7, 14, and 21.

RESULTS AND DISCUSSION

Rate of CO_2 Production

The rates and cumulative values of CO_2 output were determined and related to the condition of wheat in storage. The mean rates of CO_2 production throughout the study at most temperature and moisture conditions were found to fit parabolic regression curves best. Wheat at 10, 20 and 30°C generally changed in moisture content by less than 1% during the experiments. The wheat at 40°C , however, was partially dried with a maximum of 2.8% decrease at 24.2% moisture content. The drying was attributed to flushing of the samples with air which was needed for removal of the CO_2 produced.

Analysis of all data resulted in the following prediction equation (variables in all equations were significant at $P < 0.01$):

$$\log_{10} (R \text{ CO}_2) = -4.054 + 0.0406 (T) - 0.0165 (\theta) + 0.0001 (\theta)^2 + 0.2389 (M) \quad (1)$$

where

$R\text{CO}_2$ = Rate of CO_2 production (mg/kg in 24 h)

T = temperature ($^\circ\text{C}$)

θ = time (days in storage)

M = moisture content of seeds (%)

The coefficient of determination (R^2) of the equation is 0.794, the standard error is 0.437, and the standardized regression coefficients for each variable are temperature, 0.479; time, -0.162; time², 0.048; moisture, 0.719. The standardized regression coefficients indicate that moisture, which is about 1.5 times as important as temperature in predicting CO₂ rates, is the most important independent variable. Days in storage have a smaller effect and initially have a negative effect on CO₂ rates. This effect was not caused by drying of the samples since it occurred uniformly throughout the treatments from the beginning of storage and may be the result of quantitative changes in seed infection by various species of microflora. The rates of CO₂ production at 40°C were of the same magnitude as those observed by Bailey (1940) for wheat at 37.5°C, 15–17% MC.

The rate of CO₂ production from 1 kg of wheat in 24 h was calculated for 14–24% MC, 10–40°C, at maximum safe storage time based on the initial 5–10% seed germination loss in wheat (Fig. 1). The values for safe storage time were taken from Fraser and Muir (1981) who gave the formulae:

$$\log_{10}\theta = 6.234 - 0.219 M - 0.053 T \quad (2)$$

when $12\% < M < 19\%$, and

$$\log_{10}\theta = 4.129 - 0.010 M - 0.058 T \quad (3)$$

when $19\% \leq M \leq 24\%$.

θ = estimated maximum storage time (days)

M = moisture content (%)

T = temperature (°C).

A negligible amount of CO₂ is produced in 24 h by 1 kg of wheat of 14% MC or lower at 10, 20, and 30°C, although about 5 mg of CO₂ are produced in similar dry wheat at 40°C (Fig. 1). About 1 mg of CO₂/24 h indicates wheat is damp (>17% MC) at 10°C or tough (14.6–17% MC) at 20°C. Howe and Oxley (1952) reported that incubation of 1 kg of wheat at 25°C yielded up to 5 mg of CO₂ in 24 h when wheat was less than 15% MC. They found that CO₂ levels of 5 mg or lower were produced in insect-free dry grain, while between 5 and 9 mg indicated a slight insect infestation or active microbial respiration and they suggested that grain should not be stored for more than 1–2 mo. Carbon dioxide levels between 9 and 16 mg indicated accelerated respiration, and levels greater than 18 mg indicated that the grain was unsuitable for safe storage. These values are slightly higher than those estimated from Fig. 1 at 25°C.

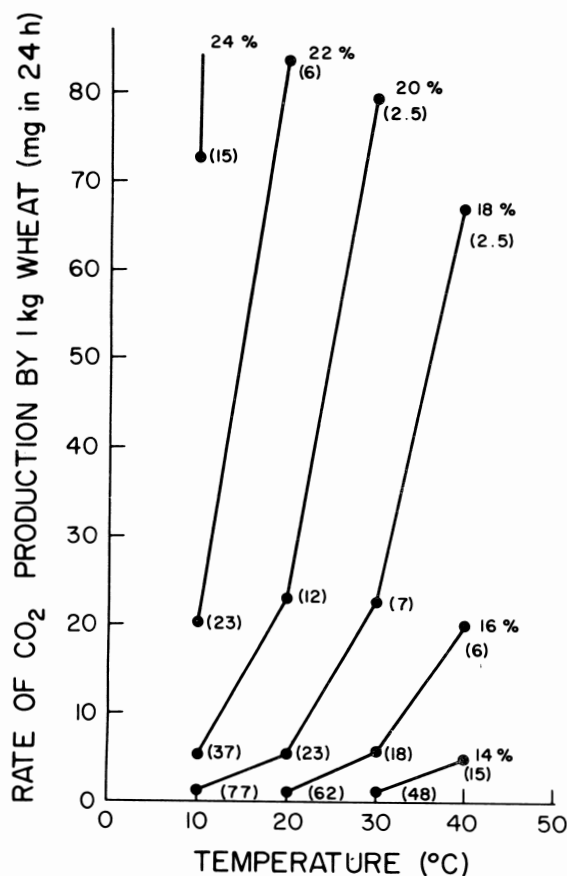


Figure 1. CO₂ rates (mg produced by 1 kg wheat in 24 h) predicted from regression equation (1) for 14–22% moisture content at the time when 5–10% germination loss occurs; days in parentheses.

Respiratory Quotients

Respiratory quotients calculated for each moisture-temperature treatment on each sampling date were averaged and most were found to lie between 1 and 2 (Table I). With few exceptions, increasing temperatures were accompanied by higher respiratory quotients at each moisture content. Trisvyatskii (1966) reported respiratory quotients of 1 to 2 for grain below 17% MC and calculations from data given by White and Sinha (1980), for wheat stored in 157-kg lots at 15.5–18.0% MC for 60 wk, were in the same range. When CO₂ production is greater than expected, it is possible that carbohydrates are being transformed into fats and the O₂ released is directly used in respiration thereby decreasing the amount taken from the atmosphere. Nonetheless, when the respiratory quotient is greater than 1.0, it is probable that some anaerobic fermentation is occurring and increasing the CO₂ output. It is possible that anaerobic microenvironments are present even within a mass of partially-aerated stored grain as numerous species of the genera *Aspergillus* and *Penicillium* are capable of fermentation, resulting in respiratory quotients greater than 1.0 (Foster 1949).

Visible Mold Infection

No external mold was present on the seeds by 35 days at 10°C, but it was observed by 23 days at 20°C on seed ≥18.4% MC, and by 35 days at 17.4% MC. The appearance of mold coincided with the safe-storage time-limit stated by Fraser and Muir (1981). Mold was seen on seeds by 11 days at 30°C and ≥17.8% MC with a safe storage limit of 7 days, but none was observed at 40°C by 21 days, although grain at 19.7% MC or higher was a darker brown color than dry seed. Carbon dioxide production in storage is directly related to microfloral infection of seed (L'vova 1968) and initial seed size and seed damage (Bailey 1940).

Cumulative CO₂

Cumulative CO₂ production increased sharply between 20 and 30°C when the moisture content was between 15.3 and 19.1% and was probably related to a corresponding increase in microfloral respiration (Fig. 2). These CO₂ values were related to seed germination, moisture content, microfloral infection, and fat acidity values, which were monitored at the end of 3 or 5 wk (Figs. 3, 4). Because seed germinations at 1, 3, 5, and 7 days incubation were highly correlated to one another, only germination data at 7 days were included in the analyses. Stepwise

TABLE I. RESPIRATORY QUOTIENTS (MEANS \pm SE) IN WHEAT STORED AT VARIOUS TEMPERATURES AND MOISTURE CONTENTS IN 300-ML FLASKS UNDER AEROBIC CONDITIONS

Temperature (°C)	No. of samplings	Total storage time (days)	Moisture content (%)							
			14.4	15.3	16.6	17.6	18.5	19.1	20.5	24.2
10	16	35	-	-	0.89 \pm 0.20	1.37 \pm 0.11	1.71 \pm 0.16	1.36 \pm 0.08	1.15 \pm 0.06	0.91 \pm 0.06
20	16	35	0.74 \pm 0.27	1.14 \pm 0.36	0.97 \pm 0.19	1.37 \pm 0.09	1.97 \pm 0.16	1.83 \pm 0.16	1.59 \pm 0.11	1.35 \pm 0.10
30	15	21	0.73 \pm 0.08	1.21 \pm 0.31	1.47 \pm 0.12	1.85 \pm 0.30	1.45 \pm 0.12	1.28 \pm 0.06	1.26 \pm 0.04	1.51 \pm 0.10
40	15	21	1.16 \pm 0.16	1.62 \pm 0.20	1.78 \pm 0.20	1.55 \pm 0.09	1.39 \pm 0.06	1.75 \pm 0.16	1.83 \pm 0.18	1.50 \pm 0.07

multiple linear regression resulted in the following prediction equations where

CO₂ = cumulative CO₂ (mg/kg wheat)

M = moisture content of seed (%)

A.g. = *Aspergillus glaucus* gr. (no. seeds infected/75 seeds)

A.f. = *Aspergillus flavus* (no. seeds infected/75 seeds)

P = *Penicillium* spp. (no. seeds infected/75 seeds)

C = *Cladosporium cladosporoides* (no. seeds infected/75 seeds)

Alt. = *Alternaria alternata* (no. of seeds infected/75 seeds)

B = bacteria (no. seeds infected/75 seeds)

G = germination (no. seeds germinating/75 seeds in 7 days)

FAV = fat acidity values (mg KOH/100 g wheat).

(a) 10°C, 35 days:

$$\text{Log}_{10} \text{CO}_2 = -5.537 + 0.292 (M) + 0.009 (A.g.) + 0.030 (G) - 0.069 (C). \quad (4)$$

Multiple $R^2 = 0.979$; standardized regression coefficients: moisture, 1.046; *A. glaucus* gr., 0.155; germination, 0.193; *Cladosporium*, -0.987.

(b) 20°C, 35 days:

$$\text{Log}_{10} \text{CO}_2 = -1.933 + 0.027 (M) + 0.012 (P) + 0.008 (A.g.) - 0.023 (A.f.) - 0.044 (Alt.). \quad (5)$$

Multiple $R^2 = 0.975$; standardized regression coefficients: moisture, 0.660; *Penicillium*, 0.400; *A. glaucus* gr., 0.243; *A. flavus*, -0.133; *Alternaria*, -0.114.

(c) 30°C, 21 days:

$$\text{Log}_{10} \text{CO}_2 = -2.828 + 0.295 (M) - 0.035 (B) + 0.009 (A.g.). \quad (6)$$

Multiple $R^2 = 0.934$; standardized regression coefficients: moisture, 1.204; bacteria, -0.566; *A. glaucus* gr., 0.250.

(d) 40°C, 21 days:

$$\text{Log}_{10} \text{CO}_2 = 4.236 - 0.051 (FAV) - 0.011 (G) - 0.038 (C). \quad (7)$$

Multiple $R^2 = 0.962$; standardized regression coefficients: FAV, -0.608; Germination, -0.041; *Cladosporium*, -0.100.

Moisture content is the predominant positive predictor of cumulative CO₂ at 10, 20, and 30°C although the storage fungi of *Aspergillus glaucus* gr. also play a significant positive role. Infection of seeds by *A. glaucus* gr. is low in dry grain but increases with moisture content (Figs. 3A, 4A); it decreases as *Penicillium* infection increases at high moisture contents (Figs. 3B, 4B). *Penicillium* was scarce at all moisture levels at 40°C. The field fungi, *Cladosporium* and *Alternaria*, are negatively correlated with cumulative CO₂. Both fungi infected few seeds (<5%) and were generally present on seeds at low moisture levels. *Aspergillus flavus* is negatively correlated to CO₂ at 20°C occurring only on seeds below 19% MC with a maximum of 9.3% infection at 14.7% MC. Bacterial infection of seed was common at 40°C for most moisture levels; but at

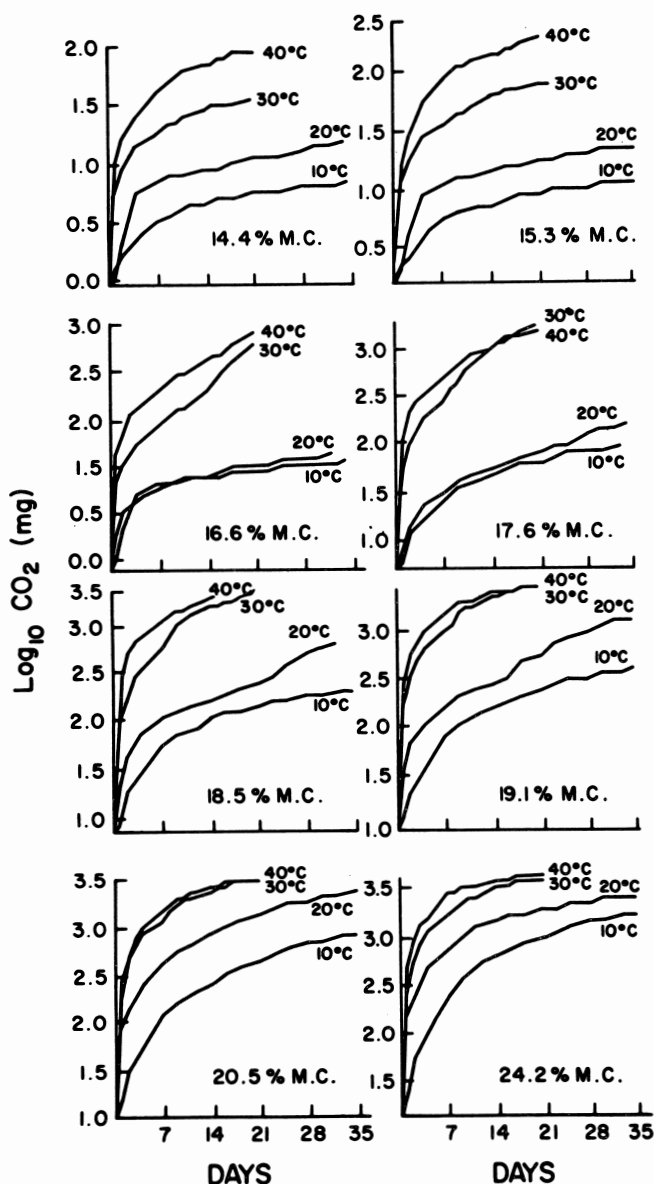


Figure 2. Observed cumulative CO₂ (mg/kg wheat) for 3-5 wk.

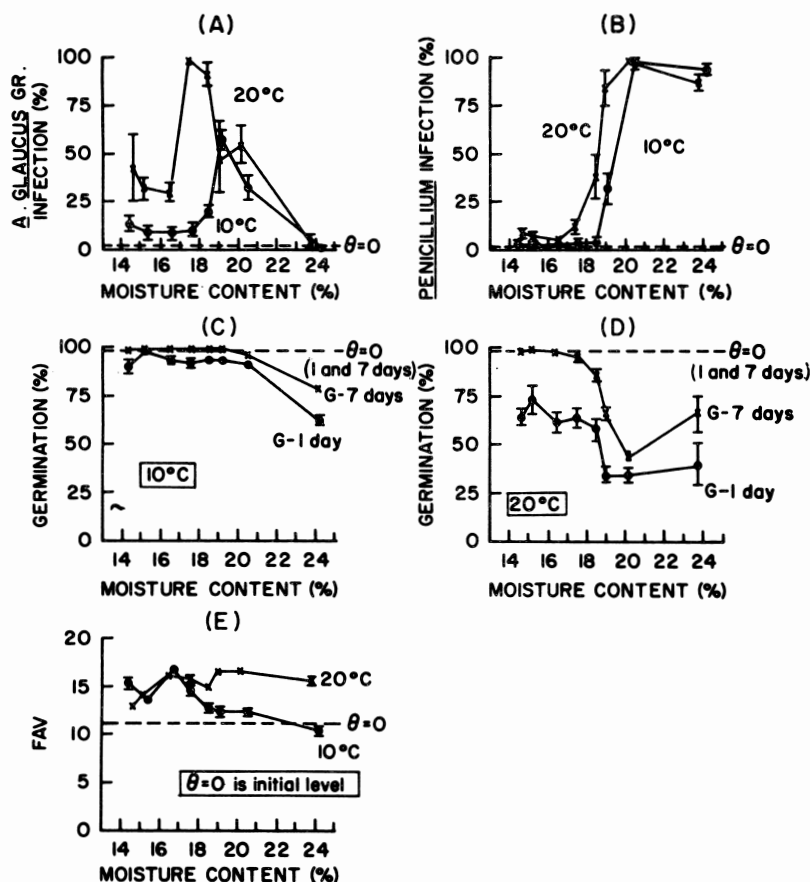


Figure 3. Microfloral infection, seed germination after 1 and 7 days incubation (G-1, G-7), and fat acidity values (FAV), mean \pm SE, of stored wheat at various moisture contents after 35 days at 10 and 20°C.

30°C, was common only for 24.5% MC (Fig. 4C). The negative regression coefficient for bacteria at 30°C indicates that high bacterial infection is related to lower CO_2 production and this may be caused by reduction of fungal infection by competition. Germination of seeds generally decreased as moisture content and temperature increased (Figs. 3C, D, 4D, E). High germination percentages were maintained at 10°C even after high total CO_2 production. At 40°C, high germinability was maintained only at the lowest moisture content, where CO_2 output was relatively low. Increased FAV levels may reflect the degradation of fats by lipolytic enzymes secreted by fungi (Hummel et al. 1954). FAV levels were highest at lower moisture contents (Fig. 4F) at 40°C. The negative regression coefficient for FAV at 40°C indicates that higher values are related to lower cumulative levels of CO_2 . Lower FAV levels at high moisture contents and at high temperatures seem to indicate that bacteria inhibit the formation of free fatty acids either indirectly by competing with fungi, or possibly directly by chemical action.

Prediction models for the total amount of CO_2 produced since the beginning of storage are needed to calculate loss in seed mass or to monitor CO_2 levels in granaries. Analyses of data at each of the four temperatures studied resulted in the following equations, where:

CO_2 = cumulative CO_2 (mg/kg wheat)
 W = moisture content - 14 (%)
 θ = time (days in storage)

(a) 10°C, $R^2 = 0.988$:

$$\text{CO}_2 = 53 - 3.934 (\theta) - 14.183 (W) + 0.533 (\theta \times W) + 0.515 (\theta \times W^2) \quad (8)$$

(b) 20°C, $R^2 = 0.908$:

$$\text{CO}_2 = -160 - 12.991 (\theta) + 36.650 (W) + 8.059 (\theta \times W) + 0.187 (\theta \times W^2) \quad (9)$$

(c) 30°C, $R^2 = 0.971$:

$$\text{CO}_2 = -345 - 58.608 (\theta) + 76.296 (W) + 45.965 (\theta \times W) - 2.076 (\theta \times W^2) \quad (10)$$

(d) 40°C, $R^2 = 0.960$:

$$\text{CO}_2 = -420 - 51.366 (\theta) + 175.904 (W) + 42.978 (\theta \times W) - 1.981 (\theta \times W^2) \quad (11)$$

These models result from observations that are serially correlated or dependent on previous observations. The equation for

40°C is an underestimate of CO_2 production because some wheat lost moisture during the experiment. A comparison of predicted and observed values indicated that the models produce good estimates at moisture contents above 16% as illustrated for storage times of 7 and 21 days listed in Table II.

Allowable Safe Storage Time

Steele et al. (1969) used cumulative CO_2 production as an indicator for calculating allowable safe storage time for shelled corn in the laboratory. Chemical reactions resulting in the complete combustion of 1 kg of dry carbohydrate liberate 1470 g of CO_2 , whereas anaerobic fermentation results in the liberation of about 493 g of CO_2 . Since carbohydrates are the predominant component of cereals, calculations are based on the assumption that metabolism of lipids and proteins does not play a large role and that anaerobic fermentation is negligible. If fermentation occurs, an underestimate in the calculation of mass loss is unavoidable. Steele et al. (1969) used a 0.5% dry matter loss (7350 mg CO_2 /kg) as an allowable limit during storage. A 1.0% dry matter loss (14 700 mg CO_2 /kg) in cereals is often considered acceptable over 1 yr of storage (Hall and Dean 1978). Although the production of 7350 mg CO_2 /kg may be acceptable for corn, the present study indicates that an arbitrary value of 1470 mg CO_2 /kg or a calculated 0.1% dry matter loss is often unacceptable for wheat. However, a far greater mass loss may occur at a slow rate without visible sign of grain deterioration when microfloral activity is low. Table III indicates the time required for the production of 1470 mg CO_2 /kg wheat as calculated from Eqs. 8-11.

Visible, external mold was present on wheat at 20°C, 18.4% MC by 23 days while a 0.1% mass loss is not predicted to occur until around 55 days in storage. At 30°C, visible mold was found on seed of 17.8% MC by 11 days while a 0.1% mass loss is predicted to occur after 16 days.

If stored wheat is to be used as seed, the approximate mean limiting range for safe storage is 655 mg CO_2 (Table IV) or a calculated dry mass loss of approximately 0.04%. Cumulative CO_2 values calculated from Eqs. 8-11 were related to allowable safe storage time, based on a 5-10% germination loss, at different temperatures and moisture contents (Fraser and Muir 1980).

Storability Index

Hall and Dean (1978) have postulated the use of a storability index to determine

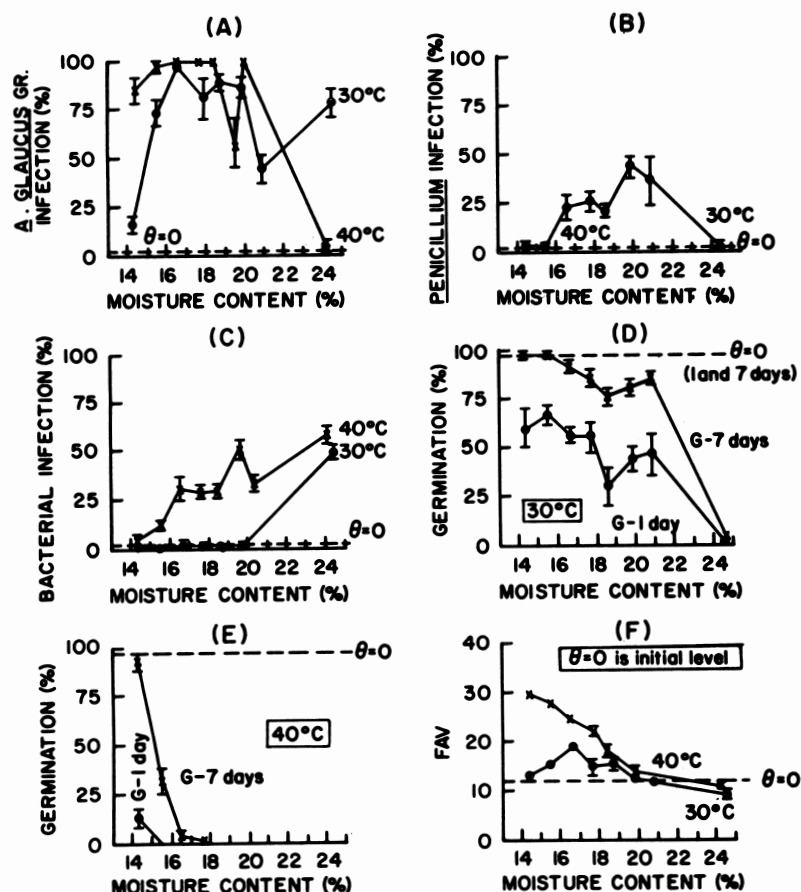


Figure 4. Microfloral infection, seed germination after 1 and 7 days incubation (G-1, G-7), and fat acidity values (FAV), mean \pm SE, of stored wheat at various moisture contents after 21 days at 30 and 40°C.

TABLE II. PREDICTED AND OBSERVED VALUES† (MEAN \pm SE) FOR CUMULATIVE CO₂ (mg) PRODUCED FROM 1 kg WHEAT BY 7 AND 21 DAYS IN STORAGE

Temperature (°C)	Moisture content (%)	Storage time (days)			
		7		21	
		Predicted	Observed	Predicted	Observed
10	16.6	23	18 \pm 4	36	28 \pm 5
	17.6	35	29 \pm 4	100	67 \pm 4
	18.5	52	57 \pm 3	176	149 \pm 4
	19.1	67	82 \pm 7	237	265 \pm 11
	20.5	110	121 \pm 2	408	431 \pm 13
	24.2	294	249 \pm 14	1065	1048 \pm 16
20	16.5	-	20 \pm 1	106	32 \pm 2
	17.4	80	34 \pm 2	312	81 \pm 4
	18.4	183	108 \pm 3	548	268 \pm 8
	19.0	246	171 \pm 9	694	612 \pm 48
	20.2	376	416 \pm 10	994	1272 \pm 49
	23.8	786	769 \pm 33	1961	1892 \pm 28
30	16.7	213	84 \pm 3	918	538 \pm 30
	17.8	547	298 \pm 15	1752	1669 \pm 53
	18.6	768	581 \pm 20	2293	2423 \pm 66
	19.8	1064	942 \pm 3	2999	3026 \pm 22
	20.8	1280	1175 \pm 12	3491	3186 \pm 85
	24.5	1822	1703 \pm 10	4555	4206 \pm 34
40	16.5	325	198 \pm 18	937	793 \pm 81
	17.7	794	515 \pm 17	1921	1602 \pm 59
	18.4	1049	1110 \pm 26	2440	2693 \pm 45
	19.7	1487	1503 \pm 22	3296	2921 \pm 69
	19.8	1518	1491 \pm 47	3356	3144 \pm 143
	24.1	2620	2879 \pm 50	5148	4560 \pm 139

†Observed values based on four replicates.

TABLE III. PREDICTED STORAGE TIME (DAYS) REQUIRED FOR 1 kg WHEAT TO PRODUCE 1470 mg CO₂ (ESTIMATED 0.1% DRY MATTER LOSS)

Moisture content (%)	Temperature (°C)			
	10	20	30	40
17	695	117	26	23
18	242	66	16	13
19	134	45	12	9
20	88	33	10	6
21	63	26	8	4
22	48	21	7	3
23	39	20	6	2
24	30	15	5	<1
25	25	12	5	<1

the keeping quality of stored products. This index is the ratio of a grain-quality variable under acceptable conditions (i.e. dry grain) to the same variable at different conditions. For example, a set of storability indices based on cumulative CO₂ production can be determined (Table V). The basis for acceptable long-term storage in this case is the cumulative CO₂ produced by 1 kg of wheat at 14.4% MC stored at 10°C for 7 days. The length of storage does not make an appreciable difference as long as it is the same for all calculations. Calculations of storability indices based on rate of CO₂ production are similar to those obtained using cumulative CO₂. Equation 2 indicates that wheat at 14.4% MC and 10°C can be stored safely for 355 days. Using this baseline and Eqs. 8-11, storability indices were calculated for wheat at various moisture contents and temperatures at 7 days in storage with values for 14.4 and 15.3% MC taken from Fig. 2. The square root of the ratios was used since the CO₂ values are exponentially related to moisture levels. The storability indices give an estimate of the relationship among temperature, grain moisture content, and other physical and biological factors. Using the base of CO₂ production at 14.4% MC and 10°C, even dry grain will undergo considerable biological activity at 30 and 40°C; wheat stored at 10°C and 18% MC has an index close to that for wheat stored at 40°C and 14.4% MC.

Various factors besides temperature and moisture can affect the rate of CO₂ production in stored seed. Some of these factors are condition of seed, and the species and the sexual stage of the invading microflora (Carter 1950). Any attempt to quantify CO₂ output further over a range of abiotic conditions would also require monitoring biotic variables. Cumulative CO₂ is a good indicator of respiratory activity, but calculation of mass loss in grain is less precise. When used in conjunction

TABLE IV. CUMULATIVE CO₂ PRODUCTION BY 1 kg STORED WHEAT (DRY MASS) IN RELATION TO ALLOWABLE STORAGE TIME

Temperature (°C)	Moisture content (%)	Approx. allowable storage time† (days)	Predicted cumulative CO ₂ (mg/kg)
10	18	77	500
	20	37	636
	22	23	713
	24	15	713
	26	9	580
	28	6	489
	30	3	240
20	18	23	823
	20	12	890
	22	6	838
	24	4	875
	26	3	936
30	18	7	604
	20	3	540
	22	1	441
40	18	3	550
	20	1	770

†Fraser and Muir (1981).

TABLE V. EFFECTS OF MOISTURE CONTENT AND TEMPERATURE ON THE STORABILITY INDEX (SI)† OF WHEAT AFTER 7 DAYS STORAGE

Moisture content (%)	Temperature (°C)							
	10		20		30		40	
	CO ₂ (mg/kg)	SI	CO ₂ (mg/kg)	SI	CO ₂ (mg/kg)	SI	CO ₂ (mg/kg)	SI
14.4	3	1.00	7	0.65	18	0.41	40	0.27
15.3	6	0.71	11	0.52	36	0.29	89	0.18
17.0	28	0.33	40	0.27	309	0.10	526	0.08
18.0	43	0.26	142	0.15	604	0.07	905	0.06
19.0	65	0.21	247	0.11	871	0.06	1257	0.05
20.0	94	0.18	355	0.09	1109	0.05	1582	0.04
21.0	131	0.15	465	0.08	1318	0.05	1878	0.04
22.0	175	0.13	578	0.07	1498	0.04	2147	0.04
23.0	227	0.11	694	0.07	1648	0.04	2388	0.04
24.0	285	0.10	812	0.06	1770	0.04	2602	0.03
25.0	351	0.09	931	0.06	1865	0.04	2787	0.03

$$\dagger SI = \sqrt{\frac{\text{cumulative CO}_2 \text{ (mg/kg wheat) at 14.4\% MC, } 10^\circ\text{C}}{\text{cumulative CO}_2 \text{ (mg/kg wheat) at } X\% \text{ MC, } Y^\circ\text{C}}}$$

with on-going studies of CO₂ diffusion through grain, and gas movement within granaries, the measurement of CO₂ may become a practical method of monitoring grain condition and storage potential at the farm level.

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