GRAIN STORAGE STUDIES

XXX. Chitin Content of Wheat as an Index of Mold Contamination and Wheat Deterioration[1]

MOISHE GOLUBCHUK,[2] L. S. CUENDET,[3] AND W. F. GEDDES

ABSTRACT

Chitin was determined on hydrolyzed crude fiber preparations from nine wheat samples with varying degrees of mold infestation by the colorimetric procedure of Elson and Morgan and a modification of Tracey's alkaline decomposition method. Both gave the same relative results, but the latter method gave higher values with a higher experimental error. The mean chitin values (expressed as glucosamine) for the two methods varied from 18 to 393 p.p.m. dry basis for the different samples. Wheats of low mold count, low fat acidity, and high viability gave glucosamine values of 18 to 55 p.p.m. Samples with mold counts of 8.8 and 400 million spores per g. with high fat acidity and zero viability contained 214 and 393 p.p.m. glucosamine. The chitin content of four samples from a previous storage experiment was more indicative of their fat acidity and viability values than mold spore count.

The determination of chitin content offers promise in evaluating incipient grain deterioration, but for routine use the analytical procedures would have to be simplified and their replicability improved.

Molds are a major factor in the deterioration of bulk grain in storage, and the extent of mold invasion is now frequently employed as one of the criteria for detecting and evaluating the extent of spoilage. Christensen and his associates (3,4,5) have developed microbiological procedures for measuring the external and internal inoculum as well as the number of seeds deeply invaded by molds. These methods all depend upon the production of spores and do not provide a direct measure of the abundance of mycelium. A method which takes into account nonviable spores as well as living, dormant, and dead mycelium would perhaps give a more accurate picture of the extent of mold infestation. A quantitative chemical method for determining total mold mycelium would be particularly valuable for laboratories which do not have facilities or personnel for carrying out microbiological assays.

Most fungi have thick chitinous cell walls, whereas none of the vascular plants have been found to synthesize chitin (14). The mycelium of molds has been reported to contain from 3 to 5.5% chitin on a dry-matter basis (7,12). Although the amount of chitin in mold my-

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[2] Present address: Portage La Prairie, Manitoba.

[3] Present address: Grands Moulins de Cossonay, Cossonay-Gare, Switzerland.

celium varies appreciably, its determination might serve as a useful chemical index of the extent of mold contamination in grain. This paper reports exploratory studies on the chitin content of wheat in relation to mold count, viability, and fat acidity.

Materials and Methods

Nine hard red spring wheat samples with varying degrees of mold infestation, as listed in Table I, were employed. Four samples of wheat from a previous storage study were included; three of these did not contain any viable mold spores, but fat acidity and viability tests indicated varying degrees of deterioration.

Separation of Chitin. Chitin has been quantitatively recovered from crawfish waste, rock lobster, and Penicillium molds by a procedure similar to that employed in obtaining the crude fiber in the proximate analysis of feeds (2,12). Successive digestion with acid and alkali concentrates the chitin and eliminates substances which would tend to interfere with the final assay.

TABLE I

MEAN CHITIN CONTENT OF WHEAT SAMPLES BY EACH OF THE TWO METHODS

Sample No.	Variety	Treatment [a]	Chitin Content as Glucosamine [b]	
			Elson and Morgan Method	Tracey Method
			ppm	*ppm*
1	Selkirk	none	32	39
2	Marquis	none, control for samples 8 and 9	15	21
3	Lee	none	48	62
4	Redman	surface-sterilized, stored 8 months at 24°C.	84	102
5	Redman	surface-sterilized, stored 8 months at 38°C.	59	72
6	Redman	mold-inoculated, stored 8 months at 38°C.	62	76
7	Redman	mold-inoculated, stored 12 months at 24°C.	75	98
8	Marquis	stored at about 25% moisture for 30 days at 24°C.	384	402
9	Marquis	stored at about 25% moisture for 30 days at 24°C.	200	227
		Mean	106.4	122.2
		Standard error (single determination)	7.1	10.1

[a] The Redman samples were either surface-sterilized or mold-inoculated as specified, conditioned to 18% moisture, and held for 7 days at 30°C., then dried to about 12% moisture and stored as indicated.

[b] Dry matter basis; each value is the mean of three determinations.

Summary of Variance Analysis

Variation due to	*DF*	*Mean Square*
Differences between methods, M	1	84058.7 **
Differences between samples, S	8	3329.2 **
Interaction, methods by samples, M × S	8	70.2
Experimental error	36	86.8

The wheat samples were ground in a micro Wiley mill to pass a 40-mesh sieve. Ten grams of the ground sample were extracted with ethyl ether to remove most of the lipids. The residue was refluxed with 1.0 liter of 1.25% by weight of sulfuric acid solution for 30 minutes, filtered through cloth, and washed free from acid. The residue was then refluxed with 1.0 liter of 1.25% by weight of sodium hydroxide solution according to the regular procedure for crude fiber (1). The residue was filtered and washed with several portions of hot water, followed by ethanol and ethyl ether to remove soluble coloring matter.

Methods of Assaying Crude Fiber for Chitin. According to Tracey (15), there are no quantitative methods for determining chitin which are beyond reproach. The most accurate assays are obtained by hydrolyzing the insoluble chitin[4] residue freed from fats, proteins, and soluble carbohydrates and then determining either the acetic acid or glucosamine content. Preliminary trials led to the use of the Elson and Morgan (6) and the Tracey alkaline decomposition method (14) for determining the chitin content of the "crude fiber." The Elson and Morgan method is based on the condensation of glucosamine with alkaline acetyl acetone, followed by reaction of the substituted pyrrole condensation product with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) to form a red-colored compound which is estimated colorimetrically. The Tracey method is based on the fact that glucosamine nitrogen is liberated as ammonia upon heating with trisodium phosphate.

The crude fiber from the wheat sample was placed in a hydrolysis tube and 2 ml. of 12*N* hydrochloric acid solution added. When most of the residue had dissolved, 2 ml. of distilled water were added, and the tubes were sealed and maintained in a boiling-water bath for 6 hours. The reaction mixture was filtered into a 10-ml. volumetric flask, and the filtrate was neutralized with sodium hydroxide solution and made up to 10 ml. Aliquots or appropriate dilutions were then assayed for glucosamine by the two methods.

For the Elson and Morgan method, the modification described by Südhof and Petrovic was used (13). Five milliliters of the glucosamine solution were placed in 50-ml. glass-stoppered hydrolysis tubes and treated with 2 ml. of 1.96% acetylacetone in 0.5*N* sodium carbonate solution. The tubes were tightly stoppered and placed in a boiling-water bath for 20 minutes. After cooling under tapwater, 12 ml. of 95% ethanol and 2 ml. of Ehrlich's reagent (1.6 g. of *p*-dimethylaminobenzaldehyde dissolved in 60 ml. of 95% ethanol and

[4] Chitin is composed of acetylated glucosamine units joined by beta-1,4- linkages to form long chains.

60 ml. of 12*N* hydrochloric acid) were added. The solutions were allowed to stand 45 minutes and their absorbance was read in a Coleman Junior spectrophotometer at 535 mμ after the instrument was set to zero absorbance with a reagent blank carried through with the samples. The standard curve prepared with known amounts of glucosamine hydrochloride showed a straight line relationship over the range of 25 to 125 γ in the 5-ml. aliquots.

In the alkaline decomposition method a micro-Kjeldahl steam distillation apparatus was substituted for the Markham still used by Tracey. A 2-ml. sample was placed in the steam distillation apparatus and washed down with 5 ml. of saturated trisodium phosphate solution saturated with sodium borate. The distillation was carried out at the rate of 3 ml. per minute and the distillate was collected in a test tube containing 1 ml. of 2% boric acid. The receiving tubes were graduated at 10 ml. and the distillation was stopped when the mark was reached. Several milliliters of concentrated sulfuric acid were added to the distilled water in the steam-producing unit in order to bind any traces of ammonia in the water. Blanks and standard solutions of glucosamine hydrochloride were also carried through the distillation. To each tube 1 ml. of Nessler's reagent was added and after 30 minutes' standing the absorbance was read at 490 mμ in a Coleman Junior spectrophotometer. A standard curve prepared with known amounts of glucosamine hydrochloride showed a straight-line relationship over the range of 30 to 280 γ ammonia per ml.

Other Analytical Procedures. Mold counts were made according to the method described by Christensen (4) for determining external infection of seeds.

Viability was determined by placing 50 seeds on moist blotting paper in a Petri dish maintained at 20°C. and counting the seeds which germinated after 7 days.

Fat acidity tests were performed on the diethyl ether extract of wheat according to the procedure of Hunter *et al.* (9). The fat acidity was expressed as mg. of potassium hydroxide required to neutralize the ether extract obtainable from 100 g. of wheat on a dry weight basis.

Results and Discussion

The mean results of determinations of chitin content made in triplicate on subsamples of each wheat by the two methods are recorded in Table I, together with a variance analysis of the data. The modified Tracey procedure gave significantly higher values than the method of Elson and Morgan and a significantly higher experimental error. The

TABLE II

RELATION BETWEEN FAT ACIDITY, CHITIN CONTENT, MOLD COUNT, AND VIABILITY OF THE WHEAT SAMPLES

SAMPLE No.	VARIETY	TREATMENT [a]	FAT ACIDITY [b]	MEAN CHITIN CONTENT [c]	MOLD COUNT [d]	VIABILITY
				ppm	*thousands*	%
1	Selkirk	none	17	36	5	96
2	Marquis	none; control for samples 8 and 9	18	18	1	92
3	Lee	none	22	55	18	93
4	Redman	surface-sterilized, stored 8 months at 24°C.	39	93	0	92
5	Redman	surface-sterilized, stored 8 months at 38°C.	62	66	0	3
6	Redman	mold-inoculated, stored 8 months at 38°C.	79	69	0	30
7	Redman	mold-inoculated, stored 12 months at 24°C.	92	86	2.8	61
8	Marquis	stored at about 25% moisture for 30 days at 24°C.	104	393	400,000	0
9	Marquis	stored at about 25% moisture for 30 days at 24°C.	129	214	8,800	0

[a] The Redman samples were either surface-sterilized or mold-inoculated as specified, conditioned to 18% moisture, and held for 7 days at 30°C., then dried to about 12% moisture and stored as indicated.

[b] Fat acidity is expressed as mg. potassium hydroxide required to neutralize the free fatty acids in the diethyl ether extract per 100 g. wheat (dry basis).

[c] Expressed as glucosamine, dry matter basis. Each value is the mean of six determinations, three by the Elson Morgan and three by the modified Tracey procedures.

[d] Mold count per g. wheat.

pooled standard errors for the nine samples of 10.1 and 7.1 p.p.m. respectively are 8.3 and 6.7% of the corresponding means. These percentage errors are considerably higher than those for protein and are somewhat higher than those for thiamine assays made on the same series of samples in different laboratories (10,11). This high relative error would be expected, since it includes those involved in the isolation of the crude fiber, hydrolysis of the chitin, and the assay of glucosamine. The two methods give the same relative results for the various samples, as the interaction, methods × samples, is not statistically significant.

The mean chitin values by the two procedures are summarized in Table II, together with the mold count, viability, and fat acidity of the wheat samples. The chitin contents of the heavily mold-infested samples (8 and 9) were markedly higher than those of the samples of sound wheat (1, 2, and 3) of high viability which had not been subjected to any special storage treatment. While the chitin contents of samples 8 and 9 place them in the same order as mold count, they are not in relative agreement with the fat acidity values. However, in a

previous study it was found that fat acidity sometimes decreased as mold growth became extensive, indicating the utilization of fatty acids by the fungi (3). The chitin values for the Redman samples from a previous storage experiment (8) are of particular interest. The prestorage treatment of the samples by either surface-sterilization or mold-inoculation, followed by holding the samples at 18% moisture for 7 days at 30°C., would permit viable molds to grow. After air-drying at room temperature (approx. 24°C.) to about 12% moisture, samples 4, 5, 6, and 7 gave mold counts of 0, 0, 31,000, and 31,000 respectively. During subsequent storage at 12% moisture the mold counts of samples 6 and 7 fell to 0 and 2,800 respectively. In this particular series the mold counts alone did not reflect accurately the storage condition of these samples. The chitin values, however, are in line with the fat acidity and viability data.

These limited experiments indicate that the chitin determination might be useful as a criterion for evaluating the extent of deterioration of wheat. Although the results indicate that the degree of mold infestation occurring in wheat is amenable to chemical analysis, the determination of chitin would have to be simplified and its replicability improved before it would be of any value in routine work.

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