

Carbohydrate and Protein Contents of Grain Dusts in Relation to Dust Morphology

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Grain dusts contain a variety of materials which are potentially hazardous to the health of workers in the grain industry. Because the characterization of grain dusts is incomplete, we are defining the botanical, chemical, and microbial contents of several grain dusts collected from grain elevators in the Duluth-Superior regions of the U.S. Here, we report certain of the carbohydrate and protein contents of dusts in relation to dust morphology. Examination of the gross morphologies of the dusts revealed that, except for corn, each dust contained either husk or pericarp (seed coat in the case of flax) fragments in addition to respirable particles. When viewed with the light microscope, the fragments appeared as elongated, pointed structures. The possibility that certain of the fragments within corn, settled, and spring wheat were derived from cell walls was suggested by the detection of pentoses following colorimetric assay of neutralized 2 N trifluoroacetic acid hydrolyzates of these dusts. The presence of pentoses together with the occurrence of proteins within water washings of grain dusts suggests that glycoproteins may be present within the dusts. With scanning electron microscopy, each dust was found to consist of a distinct assortment of particles in addition to respirable particles. Small husk fragments and "trichome-like" objects were common to all but corn dust.

Introduction

Recent investigations into the health of grain elevator workers revealed pulmonary function changes resulting from exposure to airborne grain dusts (1,2). Other investigations showed that inhalation provocation with airborne grain dusts resulted in the grain fever syndrome (3) and that grain storage employees are at risk of developing fumigant-induced neuropsychiatric changes (4). Because of the deleterious effects of airborne grain dust upon lung function for exposed workers, it has been suggested that grain dust should be regarded as more than merely a nuisance dust (5); on the contrary, these dusts pose a potentially serious threat to the health of a significant portion of the working population.

Because of the importance of grain dust to occupational health, we have initiated a study of both the chemistry and morphology of representative grain dusts. Such information is valuable in designing biochemical, immunological, and physiological experiments using these

dusts, and the data will serve as a basis with which we can compare dusts collected at various locations and during diverse growing seasons. In addition, attempts to isolate, identify, and characterize the biologically active agent(s) within these dusts could lead to either removal or control measures which could benefit the health of exposed individuals.

Although a general, systematic description of grain dust chemical contents does not exist, considerable information has accumulated concerning the occurrence of certain substances of special interest within the dusts. Airborne grain dusts consist of a heterogeneous mixture of constituents that may vary with encountered geographic, climatic, and handling differences (6). Both harvest and storage grain dusts contain a myriad of bacteria and fungi (7). For example, gram-negative bacterial endotoxins have been found within grain dusts (8) as have the toxic, fungal products aflatoxin B₁ (9), secalonic acid D (10), and zearalenone (11). Both the geographical growth regions and the storage conditions of the grains may affect the microbial flora and, therefore, the occurrence of such toxic materials as aflatoxins (9,12,13).

The purpose of this paper is to extend the characterization of airborne, grain dusts by reporting certain of the dusts' carbohydrate and protein contents emphasizing the botanical elements. Certain results presented within

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this paper have been communicated in preliminary form (14-16).

Materials and Methods

Collection and Storage of Dusts

Airborne dusts of barley, corn, flax, oats, as well as durum and spring wheats, were collected from the Superior-Duluth regions of the United States during the fall of 1977. The dusts were gathered with an industrial vacuum cleaner (17,18) during transportation of whole grains upon conveyor belts within active terminal grain elevators. Subsequent to their collection, the dusts were stored within sealed plastic bottles at 4°C.

Settled grain dusts were obtained from beams, rafters, and ledges within the same elevators (19). Dusts, which ranged from 8 to 30 cm in depth (ca. 20-year accumulation based upon eyewitness testimony), were scooped, packaged, and returned to the laboratory where

they were stored under the same condition as the airborne dusts.

Dry Weight

Fresh 100-mg lots of thoroughly mixed grain dusts were added to preweighed aluminum foil boats and then dried at 60°C for 24, 48, 72, and 96 hr. At these times, the boats containing the dried grain samples were reweighed to 0.1 mg. To verify the validity of this dry weight determination procedure, 1000 mg fresh weight lots of dusts were placed into preweighed aluminum foil boats, which were then sealed and subjected to 105°C for 24 hr and 96 hr.

Fractionation of Dusts for Carbohydrate Analyses

The procedures that were utilized to obtain, quantify and partially identify dust monosaccharides are depicted

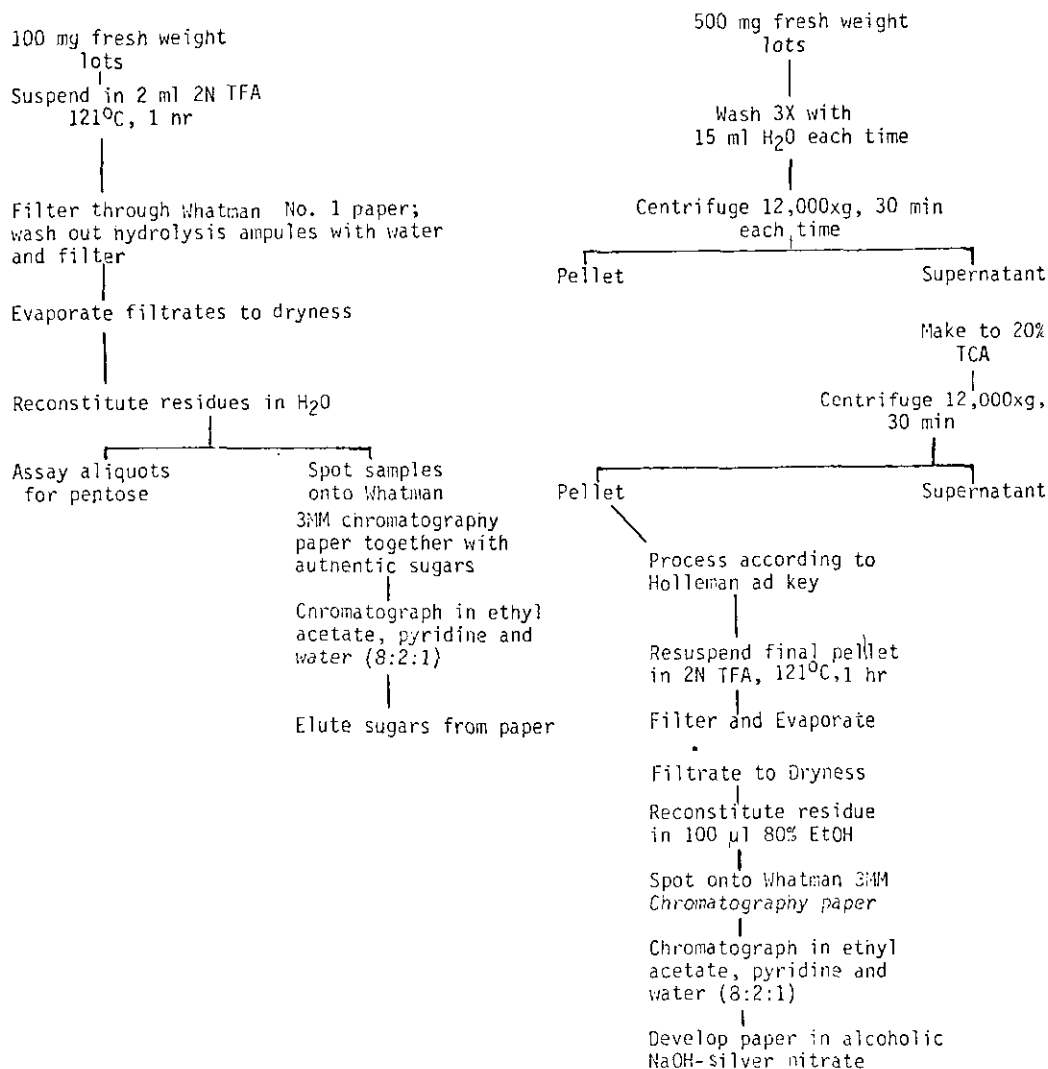


FIGURE 1. Flow chart for the preparation, quantification, and tentative identification of dust sugars.

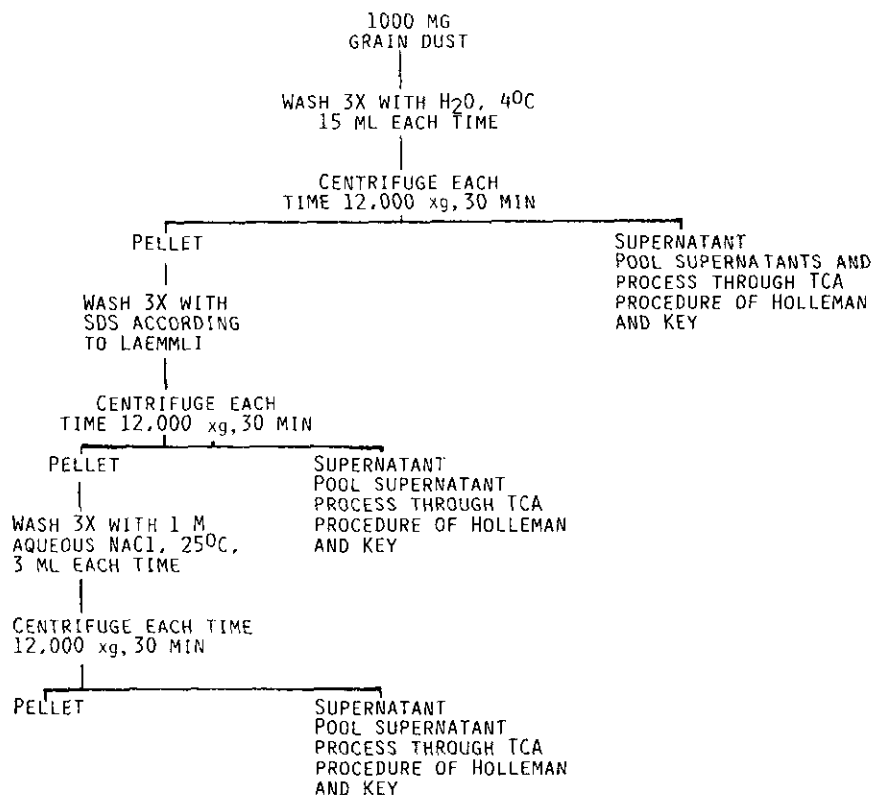


FIGURE 2. Flow chart for the release of protein by sequential washing of the dusts with water, SDS, and NaCl.

in Figure 1. Fresh 100 mg weight lots of thoroughly mixed grain dusts were autoclaved 1 hr in sealed hydrolysis ampules containing 2 mL 2 N trifluoroacetic acid (TFA) (20). Following autoclaving, the samples were filtered through a single layer of Whatman No. 1 filter paper which was washed with 5 mL H₂O. The filtrates were evaporated to dryness and the residues reconstituted in 200 μ L H₂O and spotted onto Whatman 3 MM chro-

matography paper which was developed for 8 hr in ethyl acetate, pyridine, and H₂O (8:2:1).

Following chromatography, the presumed pentoses were eluted from the paper with 50 mL 80% EtOH that was concentrated to 2 mL. Then 10, 20 and 50 μ L aliquots were withdrawn and assayed for pentose according to the procedure of Dische (21). To verify the orcinol-positive substances were indeed pentoses, absorption spectra of

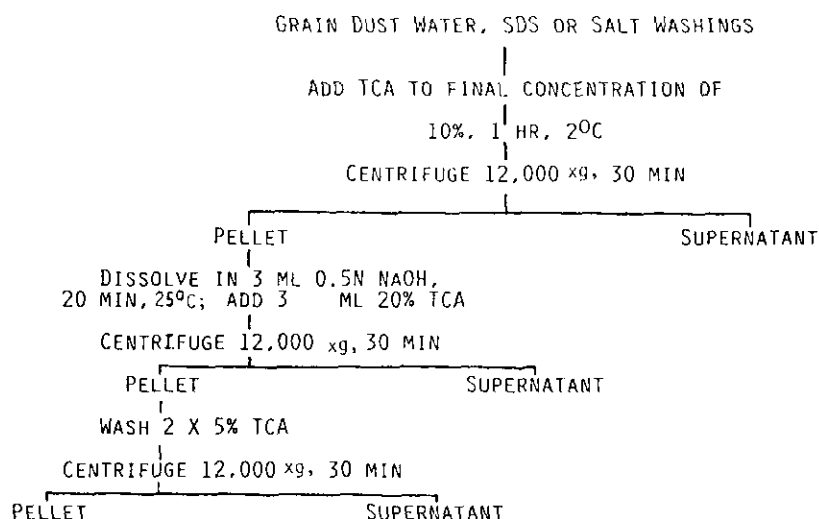


FIGURE 3. Flow chart for the precipitation of proteins from water, SDS, and NaCl washings of dusts.

authentic arabinose and presumed grain dust pentoses were performed. To insure that the presumed pentoses were eluted from the paper, the latter was dipped in acetone-saturated AgNO_3 (100 mL:6 drops) followed by NaOH/EtOH (0.5 g in 0.5 mL H_2O plus 9.5 mL EtOH).

In other instances, the water washings from 500-mg fresh weight lots of dusts were subjected to a trichloroacetic acid (TCA) precipitation procedure (Figs. 1 and 2) and the TCA-precipitable material assayed for pentose.

Fractionation of Dusts for Protein Analyses

Lots of 100, 500, and 1000 mg of dusts were washed sequentially with distilled H_2O , SDS, and NaCl (Fig 2). The washings from each were precipitated with TCA (Fig. 3) and processed according to Holleman and Key (24) with the final pellets being resuspended in 2 mL of 0.15 M NaCl for protein assay. Protein was quantified by the colorimetric Coomassie blue procedure of Bradford (25).

Amino Acid Identification and Quantification

Fresh 100 mg samples were suspended in 5 mL 6 N HCl and then hydrolyzed for 18 hr at 105°C . Next, the hydrolyzed samples were transferred to test tubes and evaporated to dryness with the resultant residue being reconstituted in 200 μL H_2O . Either 25 μL or 50 μL aliquots were spotted onto 250 μm thick Merck Darmstadt plates which were developed in chloroform:methanol:17% NH_3 (2:2:1, v/v) and 75 phenol:25 water (w/v) for direction 2 (22). The separated amino acids were detected by spraying the plate with 100 mL acetone, 10 mL H_2O , and 5 mL glacial acetic acid containing 100 mg CdCl_2 and 1 g ninhydrin (23) and its subsequent heating for 15 min at 100°C . The visualized amino acids were scraped from the plate, eluted with methanol, and quantified by spectrophotometry at 510 nm by using a standard amino acid mixture (Calbiochem, LaJolla, CA) as a reference which was subjected to the

same chromatographic, spraying, elution, and quantification procedures as the acid hydrolyzates were.

Unfractionated Dusts Proline. Fresh 100 mg dust lots were suspended in 5 mL of 6 N HCl and hydrolyzed within sealed ampules for 18 hr at 105°C .

Proline within acid hydrolyzates was quantified according to Hanson et al. (26) following filtration of the hydrolyzates, evaporation, and reconstitution in 2 mL of 0.05 N HCl . To insure the absence of interfering substances, absorption spectra were performed and compared to those for authentic proline.

Fractionated Dusts Proline. To assay TCA-soluble proline within H_2O , SDS, and salt washes, 1000 mg amounts of dusts were washed sequentially as in Figure 2. Following precipitation of the H_2O , SDS, and salt washings with TCA (Fig. 3), the resultant TCA-soluble fractions were evaporated to dryness with gentle heat and reconstituted in 100 to 300 μL H_2O prior to spotting onto Whatman 3 MM chromatography paper. A 50 μg portion of authentic proline was spotted onto the papers as a marker. The papers were developed in ethanol:ammonium hydroxide: H_2O (18:1:1) for 8 hr in an equilibrated chamber. Following chromatography, the papers were air-dried, and that portion of the paper containing the proline markers was separated from the paper and developed in a ninhydrin reagent (23). To establish the position of the presumed dust proline, the strip containing the localized proline was aligned with that portion of the paper containing the chromatographed hydrolyzate. Following the removal of those parts of the chromatogram which lacked proline, the chromatograms were eluted with 30 to 40 mL 80% ethanol and the eluates taken to dryness by gentle heat. The residues were reconstituted in 2 mL 0.05 N HCl , and 50, 100, and 200 μL aliquots were assayed for proline colorimetrically.

Scanning Electron Microscopy

Dusts were loosely distributed about 1 cm deep in small weighing boats. Scanning electron microscope specimen studs were coated with a thin layer of conducting paint (Cooper Print, B.C. Electronics, Rockford, IL). The freshly painted studs were lightly pressed into

Table 1. Moisture contents of various grain dust samples.^a

Dust	Moisture, % ^b			
	24 hr	48 hr	72 hr	96 hr
Barley	7.47 ± 1.00 ^p	7.10 ± 0.56	8.33 ± 0.73	8.50 ± 0.62
Corn	14.10 ± 0.95	13.10 ± 1.69	13.20 ± 0.90	14.70 ± 1.02
Flax	15.16 ± 0.38	16.40 ± 0.60	16.60 ± 0.15	17.30 ± 0.47
Oat	9.23 ± 2.28	10.90 ± 0.05	10.60 ± 0.30	14.60 ± 3.08
Settled	9.50 ± 1.05	9.00 ± 2.10	9.17 ± 0.06	11.30 ± 0.83
Wheat				
Durum	12.56 ± 1.51	14.07 ± 1.86	13.50 ± 2.02	16.40 ± 2.47
Spring	4.97 ± 0.64	6.33 ± 1.20	7.93 ± 0.65	8.03 ± 0.85

^a Fresh 100 mg lots of thoroughly mixed grain dusts were added to preweighed aluminum foil boats and then dried at 60°C for 24, 48, 72, and 96 hr. At these times, the boats containing the dried grain samples were reweighed to 0.1 mg. In other instances 1000 mg fresh weight lots of dusts were placed into preweighed aluminum foil boats which were then sealed and subjected to 105°C for 24 and 96 hr. The moisture contents at 105°C and 96 hr were: 8.2 (barley), 9.3 (corn), 8.4 (flax), 6.7 (settled), 8.8 (durum wheat), and 9.8 (spring wheat).

^b Mean ± SD; $N = 3$.

Table 2. Pentose content of grain dust samples following chromatography of TFA hydrolyzates.^a

Dust	Pentose, $\mu\text{g}/\text{mg}$ dry dust ^b
Barley	ND ^c
Corn	19.1 ± 9.4
Flax	ND
Oat	ND
Settled	28.1 ± 18.1
Wheat	
Durum	ND
Spring	22.4 ± 9.6

^a Fresh 100 mg samples were suspended in 2 mL 2 N TFA and subsequently autoclaved in sealed hydrolysis ampules for 1 hr at 121°C; the hydrolysis ampules were washed out with 3.5 mL H₂O and the washings filtered. The filtrates were taken to dryness by gentle heating; the resultant residues were reconstituted in 200 μL H₂O and 20 and 40 μL were withdrawn for pentose assay and the residues were again taken to dryness and reconstituted in 360 μL H₂O, and 20 and 40 μL aliquots used for pentose assay. Absorption spectra were determined on the chromogen-sugar complexes; the residues were again taken to dryness and reconstituted in 200 μL H₂O and spotted onto Whatman 3 MM chromatography paper; 10 μg each of D-(-) arabinose and xylose were spotted as markers. The papers were developed by descending chromatography for 8 hr in ethyl acetate: pyridine: H₂O (8:2:1); following chromatography that portion of the chromatogram which contained the markers was cut from the chromatogram and dipped in acetone/saturated AgNO₃ (100 mL: 6 drops) followed by NaOH/EtOH (0.5 g in 0.5 mL H₂O plus 9.5 mL EtOH); the chromatogram was cut into lengthwise strips corresponding to the width of each spotted sample and the origins removed since the markers moved from the origin; presumed pentoses were eluted from the strips by allowing 50 mL of 80% EtOH in small volumes to drip down the chromatogram. The ethanol was removed by evaporation and the resultant residues reconstituted in variable volumes of H₂O (usually 2 mL); 25, 50, 100 and 200 μL aliquots were removed for pentose assay. Absorption spectra were performed on the chromogen-dye complexes. Following elution of presumed pentoses, the strips were dipped as above to insure that the pentoses had been eluted (no pentoses were detected).

^b Mean \pm SD, $N = 18-27$.

^c ND = not detected.

the dust samples and then stored upon an open shelf. The following day, excess dust was removed with a dry camel hair brush and the specimens were coated with gold in a Tousimis Samsputter 2 A. Electron micrographs were prepared with an ISI Super Mini-SEM in the SE mode with all samples at a 20° angle from normal to the beam.

On other occasions dusts were highly dispersed in the weighing boats and processed as above in order to differentiate the respirable particles.

Results

Moisture Content

The time-dependent changes at 60°C in moisture contents of various grain dust samples are shown in Table 1. It is apparent that increasing the drying time from 24 to 96 hr resulted in greater moisture content. The moisture contents of the dusts ranged from $8.03 \pm 0.85\%$ (spring wheat) to $17.30 \pm 0.47\%$ (flax) at 96 hr.

Carbohydrate Content

The 2N TFA hydrolyzates of settled, corn, and spring wheat dusts contained 28.1 ± 18.1 , 19.1 ± 9.4 , and 22.4 ± 9.6 μg pentose/mg dry weight, respectively (Table 2). Hydrolyzates of barley, flax, oats, and durum wheat appeared to lack pentose. Paper chromatography of 2N TFA hydrolyzates did not reveal the types of pentoses within the hydrolyzates.

Preliminary data (single experiment) regarding the occurrence of possible sugars within the hydrolyzed water washings of grain dusts are presented in Table 3. Each of the dusts appears to contain galactose, but the occurrence of the other sugars varies with the dust in question. In contrast, sugars were not found in the TCA-precipitable fraction of water washings of grain dusts.

Protein Content

The total protein contents of the water-washings were 0.76 ± 0.10 , 0.00 , 0.00 , 0.81 ± 0.12 , 0.57 ± 0.06 , 1.42 ± 0.45 and 1.00 ± 0.19 $\mu\text{g}/\text{mg}$ dry weight for barley, corn, flax, oat, settled, durum wheat, and spring wheat, respectively. In contrast, the contents for the SDS washings were 0.91 ± 0.49 (barley), 0.83 ± 0.58 (oats), 0.65 ± 0.34 (settled grain) and 1.16 ± 0.46 (durum wheat). The detergent did not solubilize protein from water-washed corn, flax or spring wheat. Little protein (0.01 ± 0.02 , settled grain to 0.18 ± 0.27 , oats) was released by 1 M NaCl from both the H₂O- and SDS-washed dusts.

Amino Acid Content

Two-dimensional thin-layer chromatography of grain dust hydrolyzates followed by spraying of plates with ninhydrin did not yield reproducible R_f values. In addition, quantification of separated amino acids through

Table 3. R_f of possible sugars present within unhydrolyzed water washings of grain dusts.

Reference standards	Paper I			Reference standards	Paper II					
	Flax	Oat	Durum wheat		Barley	Corn	Settled	Spring wheat		
Galactose	0.19	0.19	0.20	0.19	Galactose	0.17	0.10	0.18	0.11	0.13
Glucose	0.23	—	—	—	Glucose	0.21	—	—	—	—
Mannose	0.31	0.32	0.34	0.39	Mannose	0.27	—	—	—	—
Arabinose	—	—	—	—	Arabinose	0.36	0.36	—	0.30	0.39
Xylose	0.53	0.55	0.52	0.55	Xylose	0.44	0.52	—	0.55	0.51
Ribose	0.73	0.75	0.73	0.74	Ribose	0.62	—	—	—	—
Rhamnose	0.82	0.86	0.86	0.88	Rhamnose	0.68	—	—	—	—

Table 4. Summary of protein contents of H₂O, SDS, and NaCl washings of dusts.^a

Dust	Protein, $\mu\text{g}/\text{mg}$ dry weight		
	H ₂ O ^b	SDS ^c	NaCl ^d
Barley	0.76 \pm 0.10	0.91 \pm 0.49	0.04 \pm 0.08
Corn	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Flax	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Oat	0.81 \pm 0.12	0.83 \pm 0.58	0.18 \pm 0.27
Settled	0.57 \pm 0.06	0.65 \pm 0.34	0.01 \pm 0.02
Wheats			
Durum	1.42 \pm 0.45	1.16 \pm 0.46	0.14 \pm 0.25
Spring	1.00 \pm 0.19	1.06 \pm 0.40	0.12 \pm 0.14

^a Fresh 1000 mg lots of dusts were washed sequentially with water, SDS, and NaCl (Fig. 2); these washings were precipitated with TCA (Fig. 3). The final TCA pellet was resuspended in 0.15 M NaCl and the protein contents quantified colorimetrically with Coomassie Blue utilizing BSA for standard curved construction.

^b $N = 5$ determinations for five replicate experiments.

^c $N = 6$ determinations for three replicate experiments (two determinations per experiment) except in the case of spring wheat where $N = 8$.

^d $N = 6$ determinations for three replicate experiments (two determinations per experiment).

combined spraying of plates with ninhydrin followed by both elution and spectrophotometric analyses of the visualized amino acids did not produce consistent absorbances. However, Table 4 reveals that reliable proline data were obtained via spectrophotometric analyses of chromatographed acid hydrolyzates. The proline contents were 1.21 \pm 0.11 (barley), 1.57 \pm 0.72 (corn), 1.15 \pm 0.12 (durum wheat), 1.45 \pm 0.37 (flax), 0.79 \pm 0.07 (oat), 1.21 \pm 0.23 (settled), and 1.40 \pm 0.47 (spring wheat). Little proline ($< 0.010 \mu\text{g}/\text{mg}$ dry weight dust) occurred within the TCA-soluble fraction of water washings of both durum and spring wheats (data not shown).

Gross Morphology

Examination of the gross morphologies of the dusts revealed that except for corn, each dust contained either husk or pericarp (seed coat in case of flax) fragments in addition to respirable particles.

Scanning Electron Microscopy

Figure 4 presents scanning electron micrographs of concentrated dusts. Each dust consisted of a distinct assortment of particles. Small husk fragments and "trichome-like" objects were common (Fig. 4A, 4C, 4D, 4E, 4F, and 4G) in all but corn dust (Fig. 4B). Scanning electron micrographs of highly dispersed dusts revealed the occurrence of respirable particles within each dust (Fig. 5).

Discussion

Organic dusts, in particular grain dusts, present workers with airborne exposures to a variety of known, suspected, or clearly unknown materials. The heterogeneous nature of the dusts (6,27) and the unique atmospheres

of the port-grain terminals provide an intriguing scientific problem in the search for the etiologic agent(s) in grain dust-induced lung diseases. In an attempt to define these agents within grain dusts, we investigated the moisture, pentose and protein contents as well as the microscopic appearance of airborne dusts from six grains and in aged, settled, rafter dust.

What is the significance of the pentose and low moisture contents? The low moisture as well as the husk and pericarp/seed coat contents of the dusts (Table 1) indicate that the dusts contain considerable cell wall debris. Cer-

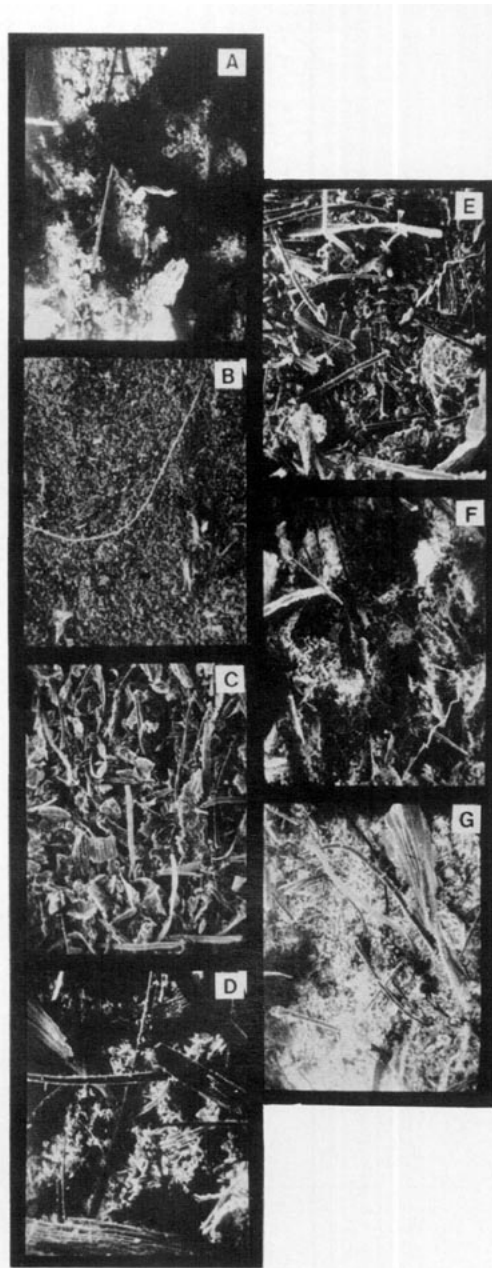


FIGURE 4. Scanning electron micrographs of concentrated dusts: (A) barley; (B) corn; (C) flax; (D) oats; (E) settled grain; (F) durum wheat; (G) spring wheat. $\times 800$.

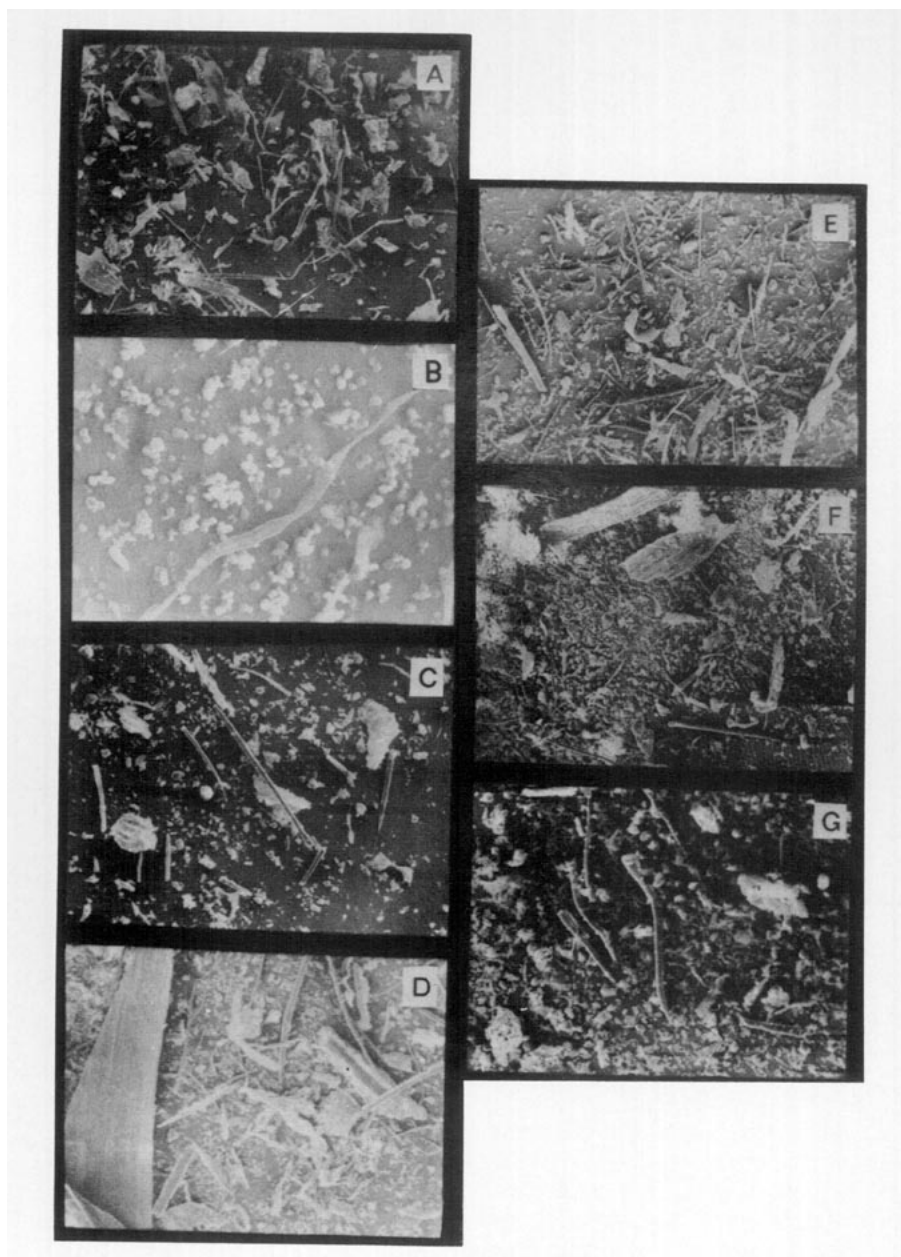


FIGURE 5. Scanning electron micrographs of dispersed dusts: (A) barley; (B) corn; (C) flax; (D) oats; (E) settled grain; (F) durum wheat; (G) spring wheat. $\times 800$.

tainly, this appears to be the case for corn, settled grain, and spring wheat which contain pentoses (Table 2). Thus, at least certain grain dusts represent a novel source of cell wall material for those investigators interested in defining the molecular structure of plant cell walls.

Is the Coomassie blue-positive material within certain of the dust washes proteinaceous? The answer appears to be affirmative since analysis of 6 N HCl hydrolyzates revealed the occurrence of ninhydrin-positive spots upon thin-layer chromatoplates which had been subjected to two-dimensional chromatography in those solvents which are commonly employed to separate amino acids (22).

The presence of protein together with carbohydrate within the water washings of the dusts suggests that the

dusts may contain glycoproteins. It is generally accepted that plant cell walls contain extensin, a hydroxyproline-containing glycoprotein (28). While our amino acid profiles were not consistent enough to warrant identification of amino acids within the dusts, colorimetry of dust acid hydrolyzates did reveal the presence of proline, a hydroxyproline precursor.

It is readily apparent from our findings that grain dusts differ among themselves both in moisture and pentose contents as well as microscopic appearances. This is noticeable especially when one compares the two wheat dusts, durum wheat and spring wheat. The former contains approximately twice the moisture level of the latter. However, an opposite relationship is found when the pen-

tose data are compared. Spring wheat dust contained 22.4 μg pentose/mg dry weight of dust, while pentoses were not detected in durum wheat dust. These results of both marked chemical and physical distinctions are analogous to the biological differences that were reported for these same dusts (18). In that study, it was presumed that differences in chemical compositions of the dusts because of growth, handling, or storage resulted in the complement toxicity range of 30 to 33 μg of dust/0.5 mL of human serum (18).

Limitations

Moisture Content. The moisture content should be placed into perspective of the ash content of the dusts. A step in this direction has been taken by Wirtz and Olenchock (29), who carried out an elemental analysis of airborne grain dusts. In this connection, biologics and inorganics present in the dusts might be identified by the energy-dispersive X-ray analyses similar to those carried out by Fleming et al. (31) and Fornes et al. (32). Finally, it may be useful to perform moisture contents upon respirable and nonrespirable fractions of the dusts. In this way it may be possible to rule out the dry weight contamination by material other than the dust.

Carbohydrate Analyses. Paper chromatography did not reveal the identities of the sugars within acid hydrolyzates of the dusts. These identities may be revealed partially by derivatization of the sugars and subsequent gas chromatography provided that appropriate standards are utilized. Vigorous identification of sugars tentatively identified by retention time might be accomplished by melting point.

Protein Analyses. The employment of H_2O to extract proteins rather than a buffer may seem questionable. However, maximum complement activation is achieved by H_2O -washing grain dusts (18). Perhaps, a comparative investigation of the protein contents of buffer and water-washed dusts should be undertaken. Characterization of the proteins within the water and SDS washings of the dusts should be performed. In this connection, a beginning attempt (14) has been made to isolate, partially characterize, and purify a phosphatase within the dusts. Certain of the phosphatase within each dust appears to possess both an acidic pH optimum and an elevated temperature optimum. The phosphatase activity, which is destroyed by boiling, is linear with both time and enzyme concentration, and can be eluted within the void volume of a G-100 column. However, both acidic and alkaline phosphatases occur within plants. The elevated temperature of this suggests the occurrence of a thermophilic microorganism within the dusts, thereby enhancing the usefulness of the phosphatase activity determinations.

As in the case of moisture content, it may be advantageous to separate dusts into respirable and nonrespirable particles in an effort to remove dust contaminations. The present protein data may reflect the protein contents of the dusts themselves and any contaminating microorganisms.

Scanning Electron Microscopy. A thorough transmission EM investigation of grain dusts should be performed in order to augment the SEM study. Such an investigation may be useful in establishing whether there are microorganisms within the dusts.

Finally, we suggest that further definition of the chemical and microbial compositions of the airborne dusts may hold the key to finding their biological activities.

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