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ANALYTICAL PROCEDURES AND EQUIPMENT REQUIREMENTS

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At a setting on the hygrometer controller to maintain a relative humidity (R.H.) of 46% in the cabinet one can maintain ground wheat at a moisture content of 11.7-11.9 at approximately 21-22 C. Depending on the moisture content of the cereals when placed in the cabinet, constant moistures are obtained in 3-5 days for ground material and 21 days for whole kernels of wheat. Various cereals come to their own specific moisture levels at the constant condition in the equilibrating cabinet. Differing protein levels and previous drying treatments may influence final moisture slightly. The moisture equilibrating cabinet is supplied with 12 small doors. Normally we place 9 or 10 shelves in the unit. Each shelf contains 9 drawers, each drawer holds 45 polystyrene sample boxes or 405 samples perdoor or approximately 4000 samples per cabinet. Individual trays being used are place alone in a door section to permit easy access to samples.

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2. No moisture conversion required for data obtained on "pre-adjusted" weights.
3. Samples can be held and rechecks make if necessary with a minimum of effort.

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ANALYTICAL PROCEDURES AND EQUIPMENT REQUIREMENTS

by

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Analytical Procedures

Sampling

For large samples some method must be used to obtain representative portions for analysis. Various commercial sample splitters would be useful. Ten-gram samples from each replication from International Winter Wheat Performance Nurseries (IWWPN) are returned to Nebraska for analysis. Normally we process five grams for lysine and protein determinations.

Grinding Equipment

Several years ago there was a problem to find a grinder which would process 1-10 g. of wheat and not require clean out and also give good recovery of the original sample.

Several cereal laboratories have reported the use of a hand or power burr mill followed by sub-sampling and grinding on the Wig-L-Bug. However, the system is fairly time consuming and the samples are generally less than 1 g. It does offer potential with extremely small genetic samples in that kernels may be cut transversely, the germ ends saved for planting, and the remainder ground with the Wig-L-Bug.

Our first approach was to use the Udy Cyclone Sample Mill which was a modification of the Weber Bros. Laboratory Pulverizing Mill. This was a small hammer mill which was used with a 0.024" screen and a vacuum operated cyclone for sample pickup. An additional vacuum cleaner was required for a source of vacuum for the cyclone. We cleaned the grinder between samples, when processing 5-10 g. of wheat.

We are now using the newly designed Udy Cyclone Sample Mill. The grinding principle has been completely changed. Impact fins of a propellor force grain to an abrasive surface. The mill grinds wheat quickly without an additional vacuum source. In our experience, clean out between samples is not required for samples of 5 g. or larger.

Cereals containing higher levels of lipid often adhere to the sides of the cyclone. A removal plate over the cyclone permits one to clean the grinder easily. This mill is operated with the 0.020" (0.5 mm) screen when grinding wheat for use with normal dye binding methods (1).

Blending Ground Samples

Following grinding the samples are brushed through two sieves into a 2" x 2" storage box. If the container is approximately 1/2 full it can be further blended by tumbling. In our experience sampling was adequate following the brushing through the sieves.

Moisture Equilibration of Samples

Moisture contents of cereal samples are necessary to calculate analytical data to a constant moisture value. These determinations waste sample and are time consuming for a large laboratory screening effort.

In 1965 we started to equip and test an available baking fermentation cabinet (Fig. 1) from the National Manufacturing Company, 1218 North 22nd, Lincoln, Nebraska 68503, as a moisture equilibrating cabinet.[1] Since we had adequate control of room temperature we did not use the temperature controls provided in the cabinet. Air circulation was provided with a built in squirrel cage fan. Humidity control was our main concern. To provide maximum space for storage the humidifier unit (Walton Montclair Model WF-225, Walton Laboratories, Inc., 1835 Burnett Avenue, Union, New Jersey 07083 which is supplied by the National Manufacturing Co.) was placed below the cabinet. The output of the humidifier was transported with the use of a rubber stopper reducing unit and 1/4" I.D. pressure rubber tubing through the bottom of the cabinet. This small opening was extremely important to prevent drastic over-shooting of humidity in the cabinet during cycling of the humidity controller unit.

Since the fermentation cabinet was already provided with a satisfactory air circulating system the main problem was to provide humidity controls, racks and shelving for sample containers.

The following items for humidity control are available from Hygrodynamics, Inc., 949 Selim Road, Silver Springs, Maryland 20910:

1. Electronic hygrometer controller, Model 15-3216
2. Wall mounting unit no. 15-6100 (for sensor)
3. Humidity sensor no. 4-4819 (color code green)
4. Sensor cable no. 88-301-37 from the wall mounting unit to the hygrometer controller box (6 feet normally would be sufficient)

At a setting on the hygrometer controller to maintain a relative humidity (R.H.) of 46% in the cabinet one can maintain ground wheat at a moisture content of 11.7-11.9 at approximately 21-22°C.

Depending on the moisture content of the cereals when placed in the cabinet, constant moistures are obtained in 3-5 days for ground material and 21 days for whole kernels of wheat.

Various cereals come to their own specific moisture levels at the constant condition in the equilibrating cabinet. Differing protein levels and previous drying treatments may influence final moisture slightly.

The moisture equilibrating cabinet is supplied with 12 small doors. Normally we place 9 or 10 shelves in the unit. Each shelf contains 9 drawers, each drawer holds 45 polystyrene sample boxes or 405 samples per door or approximately 4000 samples per cabinet. Individual trays being used are placed alone in a door section to permit easy access to samples.

[1]Mention of firm names or trade products does not imply that they are recommended by the U. S. Department of Agriculture, U. S. Agency for International Development over other firms or similar products not mentioned.

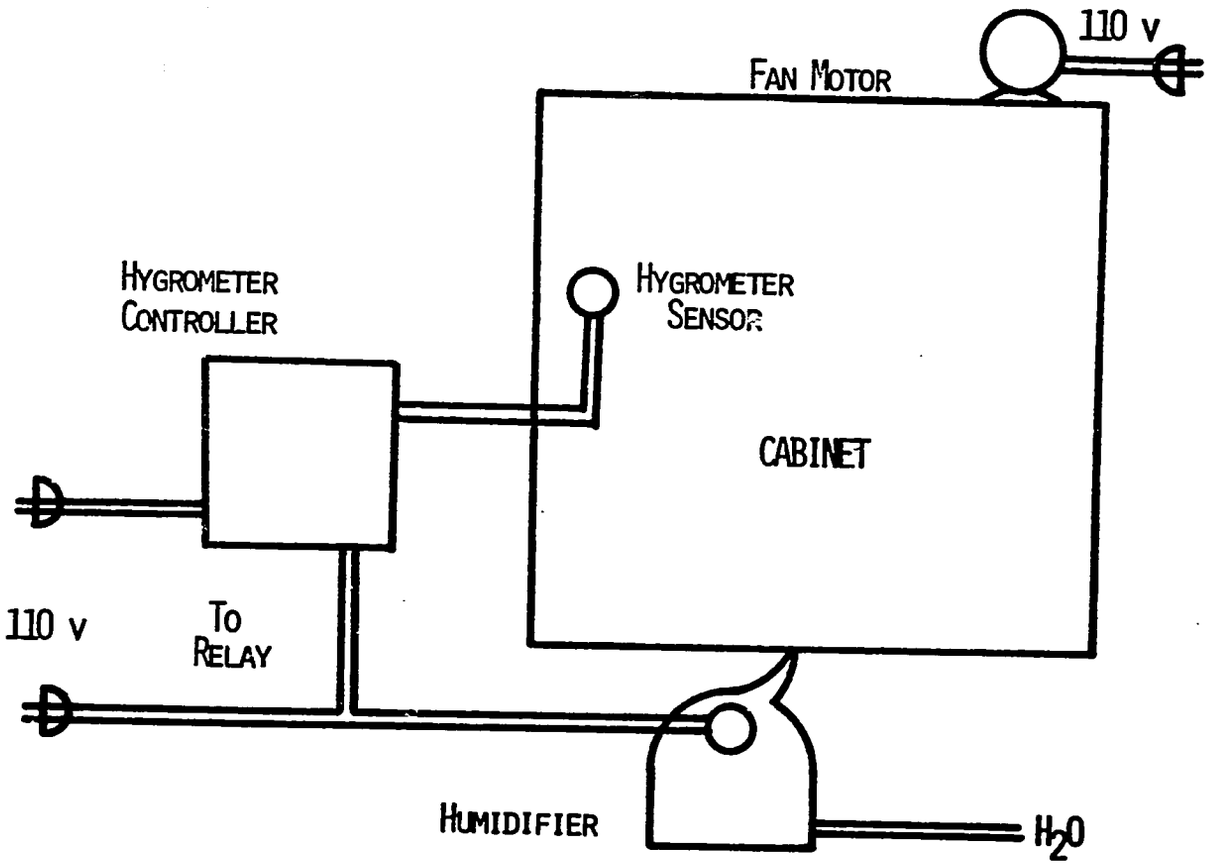

Figure 1.-- MOISTURE EQUILIBRATING CABINET

We routinely place self adhesive teflon on the runner edges of the shelf in order to minimize friction and keep powdered metal from falling into the samples.

Several control samples are weighed each morning for moisture. Since sample moistures are known the technicians can weigh samples to a constant moisture content. Tables for sample weights have been prepared for 0% (dry weight) and 14% moisture levels. Using this technique it is not necessary to make conversions on analytical data after an analysis has been completed.

In summary, the main advantages of using a moisture equilibrating cabinet are:
1. Moisture determinations are limited to two per day per cabinet.
2. No moisture conversion required for data obtained on "pre-adjusted" weights.
3. Samples can be held and rechecks made if necessary with a minimum of effort.

Protein Analyses

In order to select wheat varieties with improved protein potential we have primarily concerned ourselves with screening methods for total protein and lysine. To gain information on varietal differences and environmental effects we have also analyzed selected samples for their complete amino acid content.

Protein by Kjeldahl

The macro Kjeldahl continues to be our method of choice providing sample size is adequate. It requires a special laboratory installation and competent technicians. The Kjeldahl is not necessarily easy just because it has been run routinely for more than 80 years. We obtain Kjeldahl values on samples to be analyzed for complete amino acid content.

If samples are small we often run micro Kjeldahls. Digestions for the micro Kjeldahl have been simplified by the use of a 40 unit digestor produced by Tector ab, Kronborgsgatan 6, Helsingborg, Sweden. Samples are digested in 25 x 250 mm tubes. Sulfuric acid will accumulate in a regular laboratory hood. Therefore, one needs a special exhaust system. The manufacturer does supply such an item but it must be used in conjunction with a water aspirator.

Following digestion any number of automatic detection systems can be used. We employ an alkaline-phenol color development recommended by the Technicon Corporation for use with their auto analyzer.

Protein by Dye-Binding

Over the past 25 years dye-binding methods have been developed to estimate crude protein in a variety of materials (2,3). We use an azo sulfonic dye, acid orange 12 (C.I.15970). The acid buffered dye solution is mixed with a ground cereal sample and the single anionic dye bonds with the basic imidazol, quanidine and amino groups of the protein. These groups originate from the basic amino acids histidine, arginine and lysine, or from the free amino groups at the end of the protein chains (4).

We use a modification of the Udy Method (1). One-half gram ground samples are weighed into 50 ml polycarbonate centrifuge tubes. Twenty-five ml of dye are added with a mechanically driven syringe (Brewer Automatic Pipetting Machine).

Tubes are capped and given a hand shaking to wet contents and placed in special racks each holding 24 samples. Racks are placed on the tray of an Eberbach reciprocating shaker. Tubes are agitated in a horizontal position for one hour at 146 excursions per minute.

Tubes are centrifuged for 15 minutes at 5000 rpm in a Model GLC-1 Sorvall Clinical Centrifuge. The supernatant dye solution is evaluated in a Udy colorimeter. The percent transmission is converted to percent protein with appropriate standardized conversion charts.

If sampling is adequate and sample size small one can obtain a dye-binding value with a 0.2 g. sample and 10 ml of dye solution.

Amino Acid Determination

Ion Exchange Chromatography

Ion exchange chromatography (5,6) is still considered the most reliable method for the determination of amino acids. Completely automatic amino acid analyzers are expensive. A laboratory with minimal financial support and technical personnel can employ other methods for screening lysine.

The original lysine screening of the World Wheat Collection and samples from the IWWPN were analyzed with an automatic amino acid analyzer modified with four short columns (7). Samples must be acid hydrolyzed prior to analysis by ion exchange chromatography. The procedure was programmed so that only the lysine peak was recorded and integrated. One operator can analyze 50 samples per day.

Dye-Binding

With samples of barley, wheat, oats, rye, triticale, and corn Mossberg (8) showed that dye-binding capacity (DBC) was more highly correlated with the basic amino acid content, r = 0.940, than with nitrogen and crude protein content, r = 0.767. Lysine was highly correlated with the DBC. The correlation coefficient was 0.819. Munck, et al. (9) incorporated the concept of obtaining DBC with a constant amount of protein.

At the University of Nebraska we are using modifications of the procedures in (8,9) for screening lysine in wheat. First, a crude protein content is determined by Kjeldahl. A ground wheat sample containing 65 mg of protein is shaken for one hour with 25 ml of a dye solution containing 1.3 mg/ml (commercial Udy Analyzer Company dye) in a 50 ml polycarbonate centrifuge tube. The mixture is centrifuged as noted above for the normal Udy method. Apparent "lysine" is determined from a standard curve prepared from lysine data determined by ion exchange chromatography. Our DBC data correlated well with standard lysine values (r = 0.78).

Complete Amino Acid Determinations

We do not see an immediate need for wheat breeding programs in all countries to equip laboratories with amino acid analyzers to evaluate samples for complete amino acid profiles.

We have completed specialized studies on environmental and varietal differences for four selected samples grown at 15 locations in the First IWWPN.

The four varieties were:

NB67730	-- a Nebraska high protein selection'
Triumph 64	-- CI13679, an early-maturing, fairly widely-adapted productive U.S. variety
Bezostaia	-- PI304092, a universally-adapted, productive USSR wheat which is used as a check in the IWWPN
Sturdy	-- CI13684, a Texas semi-dwarf wheat.

The mean differences in the four varieties were quite small, but a large range in values for protein and lysine indicates a sizeable influence of the environment on the amino acid composition of the protein.

Tryptophan

Since tryptophan is destroyed during the normal 6N HCl acid hydrolysis procedure for the complete amino acid profile,it is evaluated on a separate basic hydrolyzate by an ion exchange method (10) which was modified. An 8 cm column of PA 35 resin is equilibrated with a 3.28 pH sodium citrate buffer and developed with a 5.28 pH sodium citrate buffer.

Nutritional Studies

In Vitro Nutritional Assays

We have used an *in vitro* assay for wheat protein to calculate a Pepsin Pancreatin Digest Index which uses hog pepsin and pancreatin (11). The method as given made use of the amino acid analyzer to determine the essential amino acids released by the proteolytic enzyme system. The calculation makes use of acid hydrolysis data for whole egg and the unknown wheat sample and essential amino acid data from proteolytic enzyme treated whole egg and the wheat sample. From these four sets of data the PPDR index is calculated by the use of a computer program. Whole egg is the reference at 100.

One improvement is the use of DEAE-Sephadex to recover the essential amino acids following the pepsin pancreatin digest (12).

Quite often lysine is difficult to determine on the amino acid analyzer because of formation of ornithine during the assay. To separate lysine from ornithine a 20 cm column of PA 35 resin was used. The resin was first equilibrated with 3.28 pH sodium citrate buffer and then the sample was eluted with 5.28 pH sodium citrate buffer.

Data from the *in vitro* test is being compared with mouse feeding values. At present we have a backlog of *in vitro* data awaiting comparison with feeding experiments. The indices on wheat range from 46 to 57. We will soon know how these data compare with PER's from our mouse feeding laboratory.

This particular *in vitro* test requires a sophisticated laboratory. The calculations are very tedious and the use of a computer would be recommended.

For the best results with a dye-binding procedure one should:

1. Grind all samples on the same mill with as uniform conditions as possible.
2. Evaluate a known standard wheat sample daily in order to check accuracy of weighing, solutions, etc.
3. Check calibration of the colorimeter with known dye solutions if the standard wheat sample is in error.
4. Use a centrifuge to remove sample from the dye solution to avoid problems associated with filtering systems.

At present a genetic source for higher lysine in wheat appears to be a reality. If this proves to be true we recommend the dye-binding procedure for lysine screening of nursery materials. This must be used in conjunction with the Kjeldahl procedure as pointed out under the specific procedure.

There are a number of other satisfactory procedures available for lysine. It is not the intent of this report to summarize them. However, if it becomes necessary to set up methods for lysine we could make recommendations at that time for specific laboratories, taking into consideration the number of samples to be analyzed, funding, available laboratory facilities, and equipment, etc.

As higher protein and lysine samples become available, there will be an additional need for complete amino acid determinations, and small animal feeding trials. These tests should perhaps be conducted at larger laboratory centers in order to keep costs reasonable and utilize equipment and personnel efficiently.

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