# Automatic Simultaneous Determination of Nitrogen and Moisture in Grain with or without Weighing

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Investigation showed that dry flours of different cultivars of the same species of grain have, within very narrow limits, the same contents of carbon and hydrogen and that even those of many different species have nearly the same contents of these elements. It is therefore possible to carry out a simultaneous C, H, N, and S analysis of an unweighed sample and to use the obtained carbon figure as an internal standard for the determination of the nitrogen, sulfur, and moisture contents of the grain. It is also possible to weigh the sample and to calculate its moisture content from the difference between the known carbon content of the dry grain and that obtained in the analysis. The methods can be automatized and computerized. Procedures for the simultaneous determination of nitrogen and moisture are described.

Fast, cheap, and accurate analyses of grain are most important for food and forage industry, and their importance is increasing rapidly as a consequence of the population explosion, which calls for a most economic use of all available food resources.

A rapid, automatic, high-capacity Dumas method for the determination of nitrogen in agricultural and industrial products and raw materials, based upon the Carlo Erba automatic nitrogen analyzer, ANA 1400 (Colombo and Giazzi, 1982), was recently described by Kirsten and Hesselius (1983). When used in connection with an efficient mill like the Retsch ZM1, which rapidly grinds the grain to a very fine powder, it replaces very favorably the Kjeldahl method in many instances of industrial, agricultural, and food analysis.

The method described below is a first step to further automatization and rationalization by elimination also of the weighing step and by simultaneous determination of nitrogen, sulfur, and moisture.

## THEORY OF THE METHOD

We found that dry flours of different strains of the same species of grain within very narrow limits have the same contents of carbon and hydrogen. It should, therefore, be possible to carry out a C, H, N, and S analysis without weighing, to use the obtained carbon figure as an internal standard, and to calculate the nitrogen, sulfur, and moisture contents from the ratios N/C, S/C, and H/C, as had been suggested by the Working Group for the Analysis of Nutrition Protein (1972).

If the sample is weighed, the moisture content can also be calculated from the difference between the carbon content of the dry grain and that obtained in the analysis.

## EXPERIMENTAL SECTION

**Apparatus.** The following were used: automatic elemental analyzer, Carlo Erba, Milan, Italy, Model 1104 or 1106; centrifugal mill, ZM1, with 24 wings and an 0.08-mm sieve from Retsch KG, 5657 Haan b, Düsseldorf, FRG; spot welding equipment for the encapsulation of samples under inert gas as described by Kirsten and Kirsten (1979), Figures 1 and 2; solid samples injector as shown in Figure 3.

**Materials and Reagents.** Tin capsules No. 84 0180 41, weight 34 mg, were from Lüdi and Cie, CH-9230 Flawil, Switzerland. Granulated nickel oxide and granulated cobalt oxide–silver were from Carlo Erba or prepared as described by Kirsten and Hesselius (1983) and by Kirsten (1983). The combustion tube of nickel was as shown in Figure 3.

Spot welding and encapsulation equipment, combustion tube, and tube fillings are available from Mikro Kemi AB, 750 19 Uppsala, Sweden.

**Adjustment of Apparatus.** The elemental analyzer is used as described in the manual of the instrument, except that a 10-mL oxygen injection loop and the combustion tube shown in Figure 3 are used. The weight of the sample should be around 5 mg.

It is important that the time, during which the carrier gas passes through the oxygen loop, is sufficient to sweep out all oxygen into the carrier gas line. When a gas flow rate of 30 mL/min and a gas pressure of 1 kg/cm$^2$ are used, the loop contains 20 mL of oxygen, and it takes 40 s to sweep it out. In the Model 1104 instrument the closing of the loop is controlled with a separate timer, which can be adjusted to the right time. In the Model 1106 instrument the loop is closed when the sample falls into the combustion tube. With the ordinary arrangement this time is too short. This can be remedied by introducing a 10-mL delay loop into the carrier gas line after the oxygen injection valve. The optimal inlet delay time of the sample is then determined as described below. It will be long enough to allow all oxygen to be swept out from the loop.

To determine the inlet delay time a few empty capsules were placed in the sampler and analyzed by using different inlet delay times, say, 30, 40, 50, and 60 s, and observing the flashes in the combustion tube. The inlet delay time was adjusted to about 5 s before the maximum flash.

It is important that the connections between the combustion tube and the reduction tube and between the reduction tube and the separation column are well heated to avoid a broadening of the water peak.

After several weeks of use some hygroscopic nickel halide can sublime into the connection between the combustion tube and the reduction tube and cause a tailing of the water peak: if this occurs, the connection should be replaced with a new one. The ordinary 2-mm steel tubing is satisfactory for this purpose.

Some of the newest Carlo Erba analyzers are provided with gas flow regulators instead of only pressure regulators and safety capillaries. This has a disadvantage: The absorption of the oxygen in the reduction tube causes a pressure drop. The pressure must be quickly restored through a rapid helium flow from the membrane valve. The flow regulator prevents this and causes a peak in the

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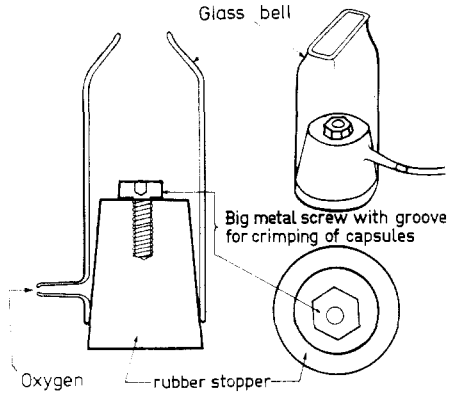


**Figure 1.** Glass bell for transfer of sample into capsule under a noninterfering gas. Height of vessel is about 13 cm; outer diameter is about 4.5 cm [reprinted with permission from Kirsten and Kirsten (1979); copyright 1979 Academic Press].

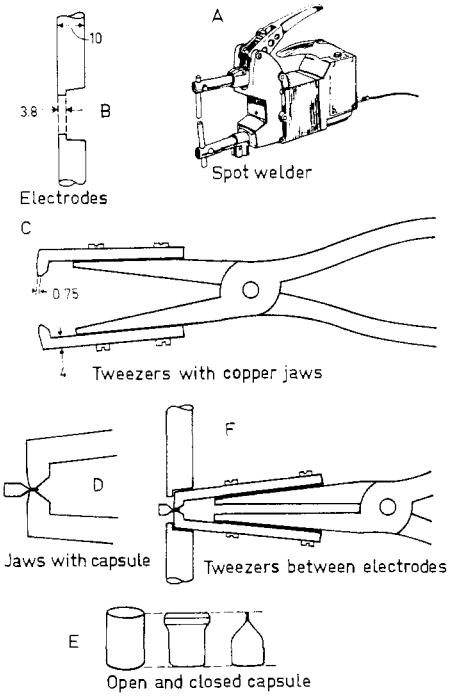


**Figure 2.** Spot welder with tweezers for the sealing of the capsules [reprinted with permission from Kirsten and Kirsten (1979); copyright 1979 Academic Press]. When the sample has been injected, the capsule is immediately grasped with the tweezers. The tweezers with the capsule are inserted between the electrodes of the welder, and the welder is activated.

chromatogram before the nitrogen peak, which can interfere with the correct integration. If this happens, the flow regulating valve should be opened and the flow rate regulated with the membrane valve.

**Procedure.** The grain should be ground in the ZM1 mill with the 0.08-mm sieve and placed into a tight container. The tin capsule is placed into the screw head in the glass bell of the encapsulation equipment under oxygen. A sample with the solid sample injector is taken under the surface of the flour and transferred quickly to the capsule. The capsule is grasped quickly with the tweezers and sealed in the spot welder. It is placed in the drum of the analyzer.

The elemental analyzer is calibrated with unweighed samples of phenacetin run at intervals of about 15 analyses. An analysis of a nitrogen-free substance, e.g., sucrose, is run at intervals of about 30 analyses. When the sucrose gives unacceptably high nitrogen blanks, the nickel oxide filling in the combustion tube should be replaced. About

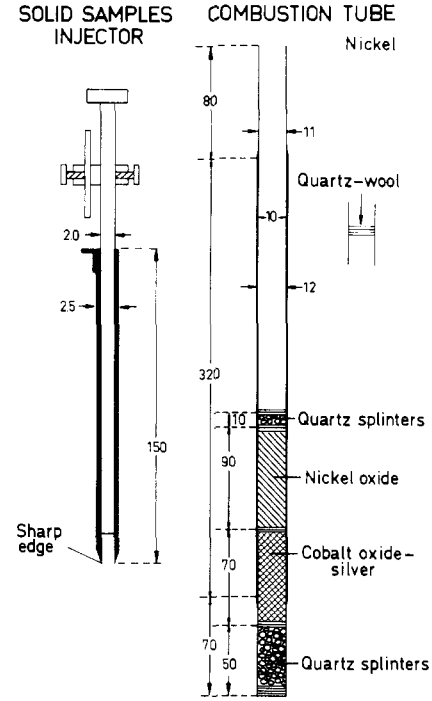


**Figure 3.** Solid samples injector and combustion tube. The fillings of the nickel combustion tube should not strongly restrict the gas flow through the tube. Use only coarse filling material, discard dust, and do not compress the quartz wool too tightly. Use quartz splinters 0.5–1.5 mm. The wall surfact of the nickel tube contains some carbon, which must be burned away. Let the analytical cycle go on for about 2 h with a very slow flow of carrier gas, before connecting the combustion tube to the reduction tube.

400 analyses can be carried out with one nickel oxide filling.

If the water is to be determined from the difference between the carbon content of the dry flour and the carbon content obtained in the analyses, the samples and also the calibration substances should be weighed.

*Calculations without Weighing.* The nitrogen content of the sample is calculated according to eq 1:

$$\% \text{ N} = N \times A \times D/C \tag{1}$$

The moisture content is calculated according to eq 2:

$$\% \text{ moisture} = \frac{100 \times 9(H \times B \times D/C - E)}{100 + 9(H \times B \times D/C - E)} \tag{2}$$

in which $A$ and $B$ are calibration ratios, calculated from the averages of the calibration analyses: $A$ = % N × peak area of C/(% C × peak area of N); $B$ = % H × peak area of C/(% C × peak area of H); $C$ = carbon peak area; $N$ = nitrogen peak area; $H$ = hydrogen peak area; $D$ = % carbon in dry grain; $E$ = % hydrogen in dry grain. $D$ and $E$ are read from Table I.

*Calculation with Weighing.* The moisture content of the sample is calculated according to eq 3:

$$\% \text{ moisture} = \frac{(D - \% \text{ C obtained}) \times 100}{D} \tag{3}$$

in which $D$ again is % carbon in dry grain, read from Table I.

The nitrogen content of the sample, obtained in the analysis for the moisture content, is corrected by using eq 4:

$$\% \text{ N} = \frac{\% \text{ N obtained} \times 100}{100 - \% \text{ moisture}} \tag{4}$$

Table I. Carbon and Hydrogen Contents of Some Dried Species of Grains

| species | no. of cultivars analyzed | % C | SD, % | % H | SD, % |
|---|---|---|---|---|---|
| wheat | 10 | 45.81 | 0.140 | 6.43 | 0.029 |
| maize | 7 | 45.89 | 0.177 | 6.45 | 0.041 |
| rye | 10 | 45.52 | 0.135 | 6.34 | 0.064 |
| barley | 10 | 45.67 | 0.249 | 6.32 | 0.054 |
| peas | 10 | 45.58 | 0.183 | 6.32 | 0.052 |
| soy, defatted | 10 | 46.44 | 0.185 | 6.23 | 0.058 |
| oats with hulls | 9 | 46.72 | 0.391 | 6.32 | 0.086 |
| oats, hull-less | 1 | 47.42 | | 7.08 | |
| oat flakes | 1 | 47.10 | | 7.09 | |

Table II. Theoretical Calculated Standard Deviations of Nitrogen and Moisture Results Caused by Differences of the Samples' Individual Carbon and Hydrogen Contents

| | | moisture | |
|---|---|---|---|
| species | nitrogen, % | calcd from H/C | calcd from % C |
| wheat | 0.0059 | 0.26 | 0.31 |
| maize | 0.0062 | 0.37 | 0.39 |
| rye | 0.0040 | 0.58 | 0.30 |
| barley | 0.0101 | 0.49 | 0.55 |
| peas | 0.0131 | 0.47 | 0.40 |
| soy, defatted | 0.0302 | 0.52 | 0.40 |
| oats with hulls | 0.0141 | 0.77 | 0.84 |

In routine work the integration of the peaks and the calculation of the results are carried out automatically with a minicomputer directly connected to the elemental analyzer (Haraldsson, 1980).

## ANALYSES

The basic requirement for the correctness of the method is that different cultivars of the same species have the same carbon and hydrogen contents. We have, therefore, analyzed different cultivars of grains. The samples were finely ground, dried at 1-mm pressure at 100 °C, weighed with a Cahn 27 electronic microbalance, and analyzed with a Carlo Erba elemental analyzer. At least three analyses were carried out for every cultivar to make certain that accurate results were obtained. The results are reported in Table I.

Surprisingly not only many cultivars but also many species of grain, like wheat, maize, rye, barley, and peas, have very nearly the same content of carbon and hydrogen.

From the figures of Table I we can calculate the errors that would result in the nitrogen and moisture determinations from the differences of the compositions of different cultivars alone, if an error-free CHN determination method were used. The results are reported in Table II.

We can also calculate the following: When the moisture content of the samples is calculated from the relationship H/C, an error of 0.1% in the hydrogen determination causes an error of 0.9% in the determination of the moisture content. When it is calculated from the difference between the carbon content obtained in the analysis and that of the dry grain, reported in Table I, an error of 0.1% in the carbon determination causes an error of 0.22% in the moisture determination. This appeared quite favorable, and we decided to test the methods with the analytical equipment available in our laboratory.

We ground a number of grains with the Retsch mill as described above and moistened the flour with different amounts of water. Then we analyzed the specimens with weighing according to the procedures described above, and we determined their moisture contents also by conventional drying for 5 h at 105 °C in air and weighing. The analytical results, calculated in the conventional manner, are reported in Table III.

Table III. Elemental Analyses and Moisture Determinations through Drying and Weighing in Moist Samples of Grain Flour

| specimen | wt of sample, mg | carbon, % | hydrogen, % | nitrogen, % | moisture, % |
|---|---|---|---|---|---|
| rye A1 | 5.368 | 42.10 | 6.72 | 1.26 | 6.94 |
| | 4.696 | 41.85 | 6.71 | 1.26 | |
| | 4.315 | 41.80 | 6.62 | 1.25 | |
| rye A2 | 2.729 | 41.68 | 6.53 | 1.23 | 6.89 |
| | 3.469 | 41.83 | 6.60 | 1.30 | |
| | 2.277 | 41.76 | 6.49 | 1.18 | |
| rye B1 | 5.826 | 39.82 | 6.97 | 1.17 | 11.66 |
| | 4.414 | 39.83 | 6.85 | 1.27[a] | |
| | 3.867 | 41.87[a] | 7.30 | 1.20 | |
| rye B2 | 2.647 | 39.72 | 6.78 | 1.20 | 15.65 |
| | 2.825 | 39.70 | 6.80 | 1.16 | |
| | 3.195 | 39.59 | 6.80 | 1.18 | |
| rye C1 | 5.096 | 39.23 | 7.14 | 1.12 | 15.65 |
| | 4.432 | 38.02 | 6.94 | 1.29[a] | |
| | 6.023 | 38.22 | 7.04 | 1.12 | |
| rye C2 | 2.516 | 37.88 | 6.55 | 1.06 | 15.42 |
| | 2.627 | 37.90 | 7.25 | 1.13 | |
| | 2.835 | 38.04 | 6.90 | 1.07 | |
| wheat A1 | 4.903 | 42.03 | 6.75 | 1.43 | 7.57 |
| | 5.792 | 42.08 | 6.65 | 1.57[a] | |
| | 3.969 | 42.07 | 6.57 | 1.40 | |
| wheat A2 | 3.033 | 41.75 | 6.60 | 1.47 | 7.46 |
| | 2.873 | 41.73 | 6.60 | 1.46 | |
| | 2.439 | 41.72 | 6.57 | 1.41 | |
| wheat B1 | 5.672 | 40.22 | 6.85 | 1.37 | 11.76 |
| | 5.521 | 40.08 | 6.84 | 1.56[a] | |
| | 6.708 | 40.22 | 6.87 | 1.37 | |
| wheat B2 | 3.057 | 39.73 | 6.83 | 1.32 | 11.71 |
| | 3.126 | 39.96 | 6.87 | 1.33 | |
| | 2.885 | 39.56 | 6.80 | 1.31 | |
| wheat C1 | 3.106 | 37.60 | 5.65[a] | 1.24 | 15.80 |
| | 3.448 | 37.62 | 7.03 | 1.28 | |
| | 2.956 | 37.52 | 6.95 | 1.25 | |
| wheat C2 | 2.280 | 37.73 | 6.90 | 1.23 | 15.97 |
| | 2.533 | 37.98 | 6.96 | 1.25 | |
| | 3.020 | 38.06 | 7.01 | 1.26 | |
| oats A1 | 2.962 | 43.58 | 6.58 | 2.08[a] | 5.46 |
| | 3.145 | 43.88 | 6.72 | 2.21 | |
| | 2.794 | 43.94 | 6.71 | 2.21 | |
| oats A2 | 2.805 | 43.95 | 6.67 | 2.09 | 5.36 |
| | 2.495 | 43.94 | 6.65 | 2.11 | |
| | 2.480 | 44.71[a] | 6.80 | 2.14 | |
| oats B1 | 2.761 | 41.02 | 6.96 | 1.99 | 11.40 |
| | 2.866 | 41.04 | 6.91 | 1.98 | |
| | 2.957 | 40.91 | 6.98 | 1.97 | |
| oats B2 | 2.401 | 41.17 | 6.17[a] | 1.94 | 11.47 |
| | 2.644 | 41.03 | 6.79 | 1.93 | |
| | 2.469 | 40.99 | 6.86 | 1.94 | |
| oats C1 | 2.681 | 39.27 | 7.11 | 1.92 | 15.12 |
| | 2.492 | 39.45 | 6.99 | 1.90 | |
| | 3.392 | 39.23 | 7.21 | 1.92 | |
| oats B1 | 2.090 | 38.94[a] | 6.32 | 1.83 | 15.17 |
| | 2.476 | 39.31 | 7.07 | 1.88 | |
| | 1.990 | 39.16 | 5.91[a] | 1.83 | |
| barley A1 | 1.924 | 41.17 | 6.02[a] | 1.55 | 8.20 |
| | 2.829 | 41.32 | 6.58 | 1.67 | |
| | 2.029 | 41.17 | 6.52 | 1.60 | |
| barley A2 | 2.785 | 41.22 | 6.55 | 1.60 | 8.30 |
| | 1.736 | 41.21 | 6.40 | 1.54 | |
| | 2.255 | 41.29 | 6.52 | 1.59 | |
| barley B1 | 2.879 | 39.87 | 6.76 | 1.60 | 11.47 |
| | 3.991 | 40.06 | 6.86 | 1.72 | |
| | 2.841 | 39.98 | 6.77 | 1.66 | |
| barley B2 | 2.758 | 39.69 | 5.97[a] | 1.53 | 11.62 |
| | 2.314 | 39.82 | 6.73 | 1.56 | |
| | 3.040 | 40.21 | 6.57 | 1.56 | |
| barley C1 | 2.908 | 38.09 | 6.96 | 1.49 | 15.66 |
| | 1.939 | 38.29 | 6.87 | 1.61[a] | |
| | 2.646 | 38.07 | 6.94 | 1.51 | |
| barley C2 | 2.772 | 38.48 | 5.78[a] | 1.48 | 15.54 |
| | 3.247 | 38.18 | 5.74[a] | 1.49 | |
| | 2.826 | 37.92 | 5.68[a] | 1.47 | |

[a] Gross errors: in the hydrogen determination mainly caused by untightness of capsules, in the nitrogen determination caused by incorrect integration.

Table IV. Nitrogen and Moisture Contents of Moist Grain Flours Calculated from Analyses of Table III[a]

| | nitrogen, %, calculated | | | moisture, %, calculated | | |
|---|---|---|---|---|---|---|
| | according to | | | according to | | |
| specimen | eq 1 | eq 4 | from weight | eq 2 | eq 3 | from drying and weighing |
| rye A1 | 1.36 | 1.38 | 1.35 | 7.66 | 8.76 | 6.94 |
| | 1.37 | 1.37 | 1.35 | 7.91 | 8.19 | |
| | 1.36 | 1.37 | 1.34 | 7.26 | 8.77 | |
| rye A2 | 1.34 | 1.34 | 1.32 | 6.70 | 8.43 | 6.89 |
| | 1.42[b] | 1.41[b] | 1.41[b] | 7.10 | 8.10 | |
| | 1.28 | 1.29 | 1.27 | 6.28 | 8.26 | |
| av | 1.34 | 1.35 | 1.33 | 7.15 | 8.42 | 6.92 |
| SD | 0.036 | 0.038 | 0.034 | 0.60 | 0.29 | 0.04 |
| rye B1 | 1.34 | 1.34 | 1.32 | 12.75 | 12.52 | 11.66 |
| | 1.45 | 1.44 | 1.44 | 11.81 | 12.50 | |
| | 1.31 | 1.37 | 1.36 | 12.57 | 12.41 | |
| rye B2 | 1.37 | 1.37 | 1.36 | 11.46 | 12.74 | 11.64 |
| | 1.33 | 1.33 | 1.31 | 11.68 | 12.78 | |
| | 1.36 | 1.36 | 1.34 | 11.77 | 13.03 | |
| av | 1.36 | 1.37 | 1.36 | 12.01 | 12.66 | 11.65 |
| SD | 0.049 | 0.042 | 0.046 | 0.52 | 0.23 | |
| rye C1 | 1.34 | 1.33 | 1.33 | 16.25 | 16.01 | 15.65 |
| | 1.54[b] | 1.54[b] | 1.53[b] | 15.03 | 16.48 | |
| | 1.33 | 1.33 | 1.33 | 15.56 | 16.04 | |
| rye C2 | 1.27 | 1.27 | 1.25 | 12.19[b] | 16.78 | 15.42 |
| | 1.35 | 1.36 | 1.34 | 17.61 | 16.74 | |
| | 1.29 | 1.28 | 1.27 | 14.81 | 16.43 | |
| av | 1.32 | 1.31 | 1.30 | 15.85 | 16.41 | 15.54 |
| SD | 0.034 | 0.038 | 0.041 | 1.13 | 0.33 | 0.16 |
| wheat A1 | 1.55 | 1.56 | 1.55 | 7.73 | 8.25 | 7.57 |
| | 1.71[b] | 1.71[b] | 1.70[b] | 6.81 | 8.14 | |
| | 1.53 | 1.53 | 1.51 | 6.15 | 8.16 | |
| wheat A2 | 1.63 | 1.62 | 1.59 | 7.18 | 9.08 | 7.46 |
| | 1.62 | 1.60 | 1.58 | 7.20 | 8.91 | |
| | 1.56 | 1.55 | 1.52 | 7.02 | 8.93 | |
| av | 1.58 | 1.57 | 1.55 | 7.19 | 8.58 | 7.52 |
| SD | 0.044 | 0.037 | 0.035 | 0.44 | 0.34 | 0.08 |
| wheat B1 | 1.56 | 1.56 | 1.55 | 10.98 | 12.20 | 11.76 |
| | 1.79[b] | 1.78[b] | 1.77[b] | 11.11 | 12.51 | |
| | 1.56 | 1.56 | 1.55 | 11.13 | 12.20 | |
| wheat B2 | 1.54 | 1.52 | 1.50 | 11.90 | 13.27 | 11.71 |
| | 1.54 | 1.53 | 1.51 | 11.93 | 12.77 | |
| | 1.54 | 1.52 | 1.48 | 11.90 | 13.64 | |
| av | 1.55 | 1.54 | 1.52 | 11.49 | 12.77 | 11.74 |
| SD | 0.011 | 0.021 | 0.031 | 0.46 | 0.59 | 0.03 |
| wheat C1 | 1.51 | 1.51 | 1.47 | 3.89[b] | 17.92 | 15.80 |
| | 1.56 | 1.56 | 1.52 | 16.09 | 17.88 | |
| | 1.53 | 1.53 | 1.48 | 15.60 | 18.10 | |
| wheat C2 | 1.52 | 1.49 | 1.46 | 15.30 | 17.64 | 15.97 |
| | 1.52 | 1.51 | 1.49 | 15.41 | 17.09 | |
| | 1.54 | 1.52 | 1.50 | 15.72 | 16.92 | |
| av | 1.53 | 1.52 | 1.49 | 15.62 | 17.59 | 15.89 |
| SD | 0.018 | 0.024 | 0.021 | 0.31 | 0.41 | 0.12 |
| oats A1 | 2.21 | 2.23 | 2.20 | 6.06 | 6.72 | 5.46 |
| | 2.35 | 2.35 | 2.34 | 6.90 | 6.08 | |
| | 2.35 | 2.35 | 2.34 | 6.68 | 5.95 | |
| oats A2 | 2.25 | 2.22 | 2.21 | 6.73 | 5.93 | 5.36 |
| | 2.27 | 2.24 | 2.23 | 6.86 | 5.95 | |
| | 2.26 | 2.24 | 2.26 | 6.86 | 4.30[b] | |
| av | 2.28 | 2.27 | 2.26 | 6.68 | 6.13 | 5.41 |
| SD | 0.057 | 0.061 | 0.063 | 0.32 | 0.34 | 0.07 |
| oats B1 | 2.26 | 2.27 | 2.25 | 12.50 | 12.20 | 11.40 |
| | 2.25 | 2.26 | 2.23 | 12.10 | 12.16 | |
| | 2.25 | 2.25 | 2.22 | 12.79 | 12.44 | |
| oats B2 | 2.22 | 2.20 | 2.19 | 6.02 | 11.88 | 11.47 |
| | 2.23 | 2.20 | 2.18 | 11.57 | 12.18 | |
| | 2.24 | 2.21 | 2.19 | 12.15 | 12.20 | |
| av | 2.24 | 2.23 | 2.21 | 12.22 | 12.18 | 11.43 |
| SD | 0.015 | 0.032 | 0.028 | 0.46 | 0.18 | 0.05 |
| oats C1 | 2.28 | 2.28 | 2.26 | 16.05 | 15.95 | 15.12 |
| | 2.25 | 2.25 | 2.24 | 14.89 | 15.56 | |
| | 2.29 | 2.29 | 2.26 | 16.83 | 16.25 | |
| oats C2 | 2.20 | 2.19 | 2.16 | 9.89[b] | 16.45 | 15.17 |
| | 2.24 | 2.23 | 2.22 | 15.55 | 15.85 | |
| | 2.19 | 2.18 | 2.16 | 5.93[b] | 16.18 | |
| av | 2.24 | 2.24 | 2.22 | 15.83 | 16.07 | 15.15 |
| SD | 0.041 | 0.046 | 0.046 | 0.82 | 0.38 | 0.04 |

Table IV (*Continued*)

| specimen | nitrogen, %, calculated according to eq 1 | according to eq 4 | from weight | moisture, %, calculated according to eq 2 | according to eq 3 | from drying and weighing |
|---|---|---|---|---|---|---|
| barley A1 | 1.72 | 1.85 | 1.69 | 3.12[b] | 9.85 | 8.20 |
| | 1.85 | 1.77 | 1.82 | 7.85 | 9.52 | |
| | 1.78 | 1.77 | 1.74 | 7.57 | 9.85 | |
| barley A2 | 1.78 | 1.77 | 1.74 | 7.64 | 9.74 | 8.30 |
| | 1.71 | 1.71 | 1.68 | 6.41 | 9.77 | |
| | 1.77 | 1.76 | 1.73 | 7.29 | 9.59 | |
| av | 1.72 | 1.77 | 1.73 | 7.35 | 9.72 | 8.25 |
| SD | 0.050 | 0.045 | 0.050 | 0.56 | 0.14 | 0.07 |
| barley B1 | 1.83 | 1.83 | 1.81 | 11.43 | 12.70 | 11.47 |
| | 1.96 | 1.96 | 1.94 | 11.95 | 12.38 | |
| | 1.90 | 1.89 | 1.88 | 11.37 | 12.46 | |
| barley B2 | 1.76 | 1.76 | 1.73 | 4.64[b] | 13.09 | 11.62 |
| | 1.96[b] | 1.78 | 1.77 | 11.95 | 12.38 | |
| | 1.78 | 1.77 | 1.77 | 9.25[b] | 11.96 | |
| av | 1.87 | 1.83 | 1.82 | 11.68 | 12.49 | 11.55 |
| SD | 0.088 | 0.079 | 0.079 | 0.32 | 0.38 | 0.11 |
| barley C1 | 1.79 | 1.79 | 1.77 | 15.43 | 16.60 | 15.66 |
| | 1.92[b] | 1.92[b] | 1.91[b] | 14.47 | 16.38 | |
| | 1.81 | 1.81 | 1.79 | 15.37 | 16.64 | |
| barley C2 | 1.76 | 1.76 | 1.75 | 4.52[b] | 15.74 | 15.54 |
| | 1.79 | 1.78 | 1.76 | 4.57[b] | 16.40 | |
| | 1.77 | 1.77 | 1.74 | 4.41[b] | 16.97 | |
| av | 1.78 | 1.78 | 1.76 | 15.09 | 16.46 | 15.60 |
| SD | 0.020 | 0.019 | 0.019 | 0.54 | 0.54 | 0.08 |

[a] Nitrogen values are percent of nitrogen in dry substance. Moisture values are percent of moisture in moist substance. Column nitrogen calculated from weight reports nitrogen values from Table III corrected for moisture content obtained through drying and weighing. [b] Gross errors.

There are six gross nitrogen errors in the table, that is, results in which one nitrogen result in a group differs more than 0.1% from the average of the two others in the group. Four of these gross errors occur in the five groups of analyses with samples larger than 4 mg. The errors were caused by the fact that the analyses of Table III—only these—were carried out with an obsolete, time-controlled integrator, which calculated a part of the carbon peak as nitrogen when large samples were analyzed, though the chromatographic separation was quite complete.

There are also a number of gross errors in the hydrogen determination, all low, obviously caused by looseness of the spot welded capsules. This would not have happened if a somewhat stronger current had been used in the spot welder.

From the results of Table III the nitrogen and the moisture contents of the samples were calculated according to the different formulas given above. The nitrogen results were also calculated by correcting the nitrogen results obtained in the analyses for the moisture contents of the samples, found by drying and weighing. The results are reported in Table IV.

Of course, the gross errors from Table III show up also in Table IV. Unfortunately, we have no more means to continue the work with this project and to redo these analyses. We hope, however, that the tables also in their present state give an idea about the possibilities lying in the described methods.

## DISCUSSION

**Grinding of the Samples.** We had hoped that it would be possible to find a mill that could grind a representative sample of grain—200–300 g—without affecting its moisture content. In this case no other moisture determination than that obtained in the reported procedure would have been necessary. Unfortunately, all mills with a well-closed grinding chamber and with a sufficient efficiency can only manage much smaller samples.

The centrifuge mill, ZM1, from Retsch grinds 200–300 g of grain to a sufficiently fine powder for the analysis in about 1 min, but a strong air flow passes through the material and affects its humidity. The humidity of the original grain must, therefore, be determined in a separate analysis. Since the moisture content of the ground material must be determined anyway, the method provides still a good advantage.

**Encapsulation of the Samples.** Grain flour is easy to encapsulate. It does not form preventing films on the walls of the capsules. Cold welding of the capsules with other equipment than that described should, therefore, be possible. The described method has, however, the advantage that no direct gas flow passes over the sample and dries it. It is also cheaper than most other available equipment.

**Analysis.** It would be desirable to determine also sulfur together with nitrogen and moisture. Methods for the simultaneous determination of carbon, hydrogen, nitrogen, and sulfur have been described by Dugan (1977), Pella and Colombo (1978), and Kirsten (1979). The determination of the sulfur in these methods is, however, not sensitive enough for the determination of sulfur in grain. A method for the determination of traces of sulfur simultaneously with the other elements is being studied in our laboratory. This method (Kirsten, 1981, 1983) is new, and we have not yet applied it to grain.

The Carlo Erba elemental analyzer is originally designed for samples between 0.5 and 1.2 mg. The homogenization with the Retsch mill was not fine enough for the use of so small samples. It was, therefore, necessary to modify its combustion tube and to increase the amount of oxygen used in the combustion, so that it can handle samples up to 10 mg. With the rather low nitrogen contents of the agricultural materials, no further modification of the analyzer is necessary. With samples of very high nitrogen contents, a longer separation column might be necessary to provide for a good separation between the nitrogen and

the carbon peaks. The separation needs, however, not to be complete, because any modern chromatographic integrator computes a complete separation also when the base line is not reached between the peaks.

**Calculation of the Results.** The dry weight of the sample is $C \times F_c \times 100/D$, where $C$ is the area of the carbon peak, $F_c$ is the calibration factor defined by $C \times F_c$ = mg of carbon, and $D$ is the percentage of carbon in dry grain. $D$ is read from Table I.

The sample's content of nitrogen can, therefore, be calculated with the equation

$$\% \text{ N} = N \times F_n \times D/(C \times F_c) \quad (5)$$

in which $N$ is the area of the nitrogen peak and $F_n$ the calibration factor for nitrogen defined by $N \times F_n$ = mg of N.

The sample's content of sulfur and hydrogen is calculated in the same manner with $S$ and $F_s$ and $H$ and $F_h$ instead of $N$ and $F_n$.

For the calculation of the moisture content we have to substract the hydrogen content of the dry grain—E, read from Table I—from the total hydrogen content of the sample to obtain the water hydrogen, which we convert to water with the factor 9. Hence, we obtain

$$\% \text{ moisture in the } \textit{dry} \text{ grain} = 9[H \times F_h \times D/(C \times F_c) - E] \quad (6)$$

The percentage of moisture in the fresh grain is then given by

$$\% \text{ moisture} = \frac{100 \times 9[H \times F_h \times D/(C \times F_c) - E]}{100 + 9[H \times F_h \times D/(C \times F_c) - E]} \quad (7)$$

In eq 5, 6, and 7 we can set $F_n/F_c = A$, $F_h/F_c = B$, and $F_s/F_c = C$.

Quotients $A$, $B$, and $C$ are obtained from the calibration analyses:

$$A = \% \text{ N} \times \text{peak area of C}/(\% \text{ C} \times \text{peak area of N})$$

$$B = \% \text{ H} \times \text{peak area of C}/(\% \text{ C} \times \text{peak area of H})$$

$$C = \% \text{ S} \times \text{peak area of C}/(\% \text{ C} \times \text{peak area of S})$$

When we introduce $A$ and $B$ into eq 5 and 7, we obtain the calculation formulas 1 and 2, reported above in the description of the analytical method.

Equation 3 is based upon the assumption that the presence of moisture in the sample causes a corresponding deviation of its carbon content from that of Table I.

Equation 4 is a mere correction of the sample's nitrogen content for its moisture content.

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**Registry No.** Carbon, 7440-44-0; nitrogen, 7727-37-9; hydrogen, 1333-74-0; water, 7732-18-5.

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# Determination of Protein Hydrophobicity Using a Sodium Dodecyl Sulfate Binding Method

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An attempt to determine the protein hydrophobicity was made by using the sodium dodecyl sulfate (SDS) binding method. The SDS-binding capacity of proteins in the presence of a low concentration of SDS was proportional to the surface hydrophobicity determined by the fluorescence probe method. The electrostatic effect on the SDS-binding capacity was not observed. As protein denaturation proceeded, the SDS-binding capacity of proteins changed correspondingly to changes in the protein hydrophobicity. A good correlation was observed between the surface hydrophobicity and SDS-binding capacity of 42 native and denatured proteins. The SDS-binding method was applied to determine the surface hydrophobicity of insoluble denatured ovalbumins. It was suggested that this method was suitable for the determination of the surface hydrophobicity of insoluble proteins.

Attempts to determine the surface hydrophobicity of proteins have been made by some investigators (Shanbhag and Axelsson, 1975; Keshavarz and Nakai, 1979; Kato and Naki, 1980). Shanbhag and Axelsson (1975) established the hydrophobic partition method to determine the surface hydrophobicity of proteins. Keshavarz and Nakai (1979) applied hydrophobic chromatography to assess the surface hydrophobicity. Kato and Nakai (1980) reported the

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