# Amino Acid Compositions of Cereals and Oilseed Meals<sup>1</sup>

R. TKACHUK and G. N. IRVINE, Grain Research Laboratory, Winnipeg 2, Manitoba

#### ABSTRACT

Quantitative amino acid compositions were determined for samples of wheat, barley, oats, rye, soybean, buckwheat, millet, sunflower, yellow mustard, rapeseed, and flax. The results were obtained by ion-exchange chromatographic analyses of 6N HCl and barium hydroxide hydrolysates. Hydrolyses were carried out for various intervals to correct for the decomposition of threonine and serine and for the increasing yield of valine and isoleucine during hydrolysis.

The present work is concerned with quantitative amino acid compositions of various cereals and oilseeds grown in Canada. Data of this type are necessary for accurate nutritional research and for calculating the correct protein contents of food and feed materials. Use of quantitative amino acid data for the latter purpose is the subject of an accompanying publication (1).

## MATERIALS

# Samples

Representative samples of cereals and oilseeds were chosen for analysis. All extraneous and foreign material was removed by hand from all samples previous to any analyses; for example, in the case of wheat germ, 22% of the original sample consisted of wheat bran. All samples were ground to a fine flour before analysis, except oilseeds which were previously defatted in a Soxhlet extractor with petroleum ether, b.p. 35° to 60°C. Analyses for protein, moisture, and oil content were carried out according to standard AACC methods (2). A description of the samples is given in Table I.

## Reagents

All chemicals used in the preparation of buffer and analytical solutions were "Baker-Analyzed," reagent-grade, obtained from J. T. Baker Chemical Co., except for ninhydrin, Brij-35, octanoic acid, and thiodiglycol which were "specially purified for amino acid analysis" as supplied by Pierce Chemical Co. Concentrated HCl was diluted to 6N, and the 6N constant-boiling azeotrope was distilled twice. Methyl Cellosolve used for dissolving ninhydrin was distilled before use, and checked to see that it contained no peroxides.

## **METHODS**

# Hydrolysis with HCI

Direct hydrolysis of samples was carried out with 6N HCl to obtain hydrolysates suitable for analysis of all amino acids except for cystine+cysteine, and tryptophan.

Hydrolysis was carried out by adding 4.0 ml. of twice-distilled 6N HCl to 20 to 40 mg. of sample in 18-mm. Pyrex test tubes. Just previous to use, the test tubes were washed with dilute NaOH solution, rinsed with distilled water, and oven-dried. The mixture was frozen in a bath at -80°C, and the test tubes were evacuated to less than 50 microns; the contents were then allowed to melt so that any entrapped air bubbles could escape, and the test tube was sealed. Hydrolysis of samples was carried out at 110° ± 2°C. for 24, 48, and 72 hr. in a forced-draft oven.

<sup>&</sup>lt;sup>1</sup>Paper No. 276 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba, Canada. Presented in part at the 51st annual meeting, New York, April 1966.

TABLE I. DESCRIPTION OF CEREALS AND OILSEEDS ANALYZED<sup>a</sup>

| Sample                 | Source                           | Protein <sup>b</sup><br>content | Moisture |
|------------------------|----------------------------------|---------------------------------|----------|
| Manitou wheat          | Whole seed, Western Canada;      | · ·                             |          |
|                        | 1966 composite                   | 15.40                           | 08.10    |
| Selkirk wheat          | Whole seed, Western Canada;      | 10.40                           | 00.10    |
|                        | 1966 composite                   | 15.00                           | 08.10    |
| Triticale              | Whole seed, grown in 1966        |                                 | 00.10    |
|                        | in Manitoba                      | 15.60                           | 13.10    |
| Wheat endosperm        | Commercial "wheatlets" milled    | 10.00                           | 15.10    |
| rinoat ona osporm      | from No. 2 Northern wheats, ap-  |                                 |          |
|                        | proximately 2% extraction        | 12.20                           | 13.90    |
| Wheat germ             | Commercial sample from Maple     | 12.20                           | 13.90    |
| willedt geriff         | Leaf Mills Ltd. All bran         |                                 |          |
|                        | originally present removed by    |                                 |          |
|                        | hand                             | 32.70                           | 00.40    |
| Wheat bran             | Commercial sample from Ogilvie   | 32.70                           | 08.40    |
| wheat bran             | Flour Mills Co. Ltd.             | 14.40                           | 1410     |
| Barley                 |                                  | 14.40                           | 14.10    |
| bariey                 | Whole, six-row barley, Western   | 44.40                           |          |
| D- + tt                | Canada 1964 composite sample     | 11.10                           | 08.05    |
| Pot barley             | Western Canada 1962 average;     |                                 |          |
|                        | (six-row) (29% of outer kernel   |                                 | 223323   |
| •                      | removed by pearling)             | 09.75                           | 07.50    |
| Oats                   | Western Canada 1963 average;     | 1999                            |          |
| _                      | hulled                           | 16.60                           | 07.00    |
| Rye                    | Dark rye flour from Maple Leaf   |                                 |          |
|                        | Mills Ltd.; ash content 1.1%     |                                 |          |
|                        | (dry basis)                      | 13.20                           | 08.80    |
| Buckwheat              | Hulled seed, from 1966 Manitoba  |                                 |          |
|                        | crop                             | 10.70                           | 09.34    |
| Millet                 | Whole seed, individual plot      |                                 |          |
|                        | sample                           | 12.10                           | 10.50    |
| Soybean                | Whole seed from 1963 Manitoba    |                                 |          |
|                        | crop; defatted                   | 41.50                           | 07.44    |
| Flaxb                  | Whole seed from 1963 Manitoba    |                                 |          |
|                        | crop; defatted                   | 35.40                           | 07.90    |
| Rapeseed <sup>b</sup>  | Whole seed from 1963 Manitoba    |                                 |          |
|                        | crop; defatted                   | 35.10                           | 07.40    |
| Sunflower <sup>b</sup> | Hulled seed, defatted meal,      |                                 |          |
|                        | variety Peredovik; breeder       |                                 |          |
|                        | seed grown 1964 Morden, Manitoba | 54.10                           | 03.50    |
| Mustard <sup>b</sup>   | Yellow mustard, Western Canada,  | ••                              | 55.56    |
|                        | 1963 average; defatted           | 36.50                           | 07.00    |

<sup>&</sup>lt;sup>a</sup>Oil content of original sample: flax 40.7; rapeseed 42.2; sunflower 53.1; and mustard 27.2.

After hydrolysis, HCl was removed from the hydrolysate mixture by placing the frozen mixture in a desiccator containing NaOH pellets and evacuating to approximately 0.1 mm. This vacuum is necessary to ensure that the HCl is removed within 10 to 12 hr. (3). A specified volume (usually 25 ml.) of citrate buffer 0.20N Na<sup>+</sup>, pH 2.2, containing Brij-35 detergent and octanoic acid, was added to the residue. The insoluble humin in the resulting solution was removed by vacuum filtration through Whatman No. 52 filter paper. Aliquots of the supernatant or filtrate were then used for amino acid analysis.

Samples previously oxidized with performic acid were also hydrolyzed with 6N HCl as described above. This was done to obtain samples suitable for the analysis of cystine+cysteine content in the form of the more stable derivative, cysteic acid, as described by Schram et al. (4).

# Hydrolysis with Barium Hydroxide

To 30 to 50 mg. of sample in an 18-mm. Pyrex test tube, 1.0 g. barium hy-

bAs-is basis, N X 5.7.

TABLE II. AMINO ACID RECOVERIES FROM SELKIRK WHEAT (micromoles per g. sample protein (N  $\times$  5.7) )

|               | Hydrolysis Time <sup>a</sup> |   |           |           | HCOOOH<br>sample | Amino<br>acid, g.<br>per 100 g.<br>sample N |                    |        |
|---------------|------------------------------|---|-----------|-----------|------------------|---|--------------------|--------|
|               | 4<br>hr.                     | 15<br>hr.                               | 24<br>hr. | 48<br>hr. | 72<br>hr.        |   |                    |        |
| Tryptophan    |                              |   |           |           |                  | 0,082 <sup>b</sup>                          | 0,082.0            | 009.55 |
| Lysine        | 0.141                        | 0,170                                   | 0,175     | 0,178     | 0,170            | -,  | 0.174.0            | 014.50 |
| Histidine     | 0,125                        | 0,151                                   | 0,157     | 0,163     | 0,149            |   | 0.156.0            | 013.80 |
| Ammonia       | 2,300                        | 2,234                                   | 2,328     | 2,345     | 2,378            |   | 0,156.0<br>2,297.0 | 022.30 |
| Arginine      | 0,199                        | 0,251                                   | 0,260     | 0,252     | 0,240            |   | 0.251.0            | 024.90 |
| Cysteic acid  | 150000000                    | 000000000000000000000000000000000000000 |           |           |                  | 0,206                                       |                    |        |
| Aspartic acid | 0,352                        | 0,383                                   | 0,379     | 0,385     | 0,390            |   | 0,385.0            | 029.20 |
| Meth, sulfone | 8                            | 6366                                    | 100       | 20.0      | i.               | 0,123                                       | 10.                |        |
| Threonine     | 0,219                        | 0,234                                   | 0,244     | 0,233     | 0,228            |   | 0,254.0            | 017.30 |
| Serine        | 0,479                        | 0,464                                   | 0,467     | 0,413     | 0,355            |   | 0,523.0            | 031.30 |
| Glutamic acid | 2,436                        | 2,457                                   | 2,495     | 2,479     | 2,439            | 2,326                                       | 2,471.0            | 207.00 |
| Proline       | 1,026                        | 1,015                                   | 1,014     | 1,010     | 1,061            | 0,909                                       | 1,061.0            | 069.60 |
| Glycine       | 0,514                        | 0,532                                   | 0,543     | 0,552     | 0,548            | 0,527                                       | 0,548.0            | 023.50 |
| Alanine       | 0,370                        | 0,391                                   | 0,404     | 0,396     | 0,402            | 0,370                                       | 0,401.0            | 020.40 |
| Cystine       | 0,054                        | 0,048                                   | 0,042     | 0,036     | 0,000            | 0,000                                       | 0,118.0            | 016.20 |
| Valine        | 0,236                        | 0,360                                   | 0,393     | 0,394     | 0,418            | 0,336                                       | 0,418.0            | 027.90 |
| Methionine    | 0,093                        | 0,087                                   | 0,108     | 0,103     | 0,098            | 0,000                                       | 0,123.0            | 010.50 |
| Isoleucine    | 0,161                        | 0,266                                   | 0,284     | 0,319     | 0,319            | 0,262                                       | 0,319.0            | 023.90 |
| Leucine       | 0,469                        | 0,547                                   | 0,548     | 0,592     | 0,544            | 0,509                                       | 0,561.0            | 042.00 |
| Tyrosine      | 0,137                        | 0,159                                   | 0,162     | 0,150     | 0,126            | 0,000                                       | 0,162.0            | 016.70 |
| Phenylalanine | 0,285                        | 0,294                                   | 0,304     | 0,324     | 0,317            |   | 0,315.0            | 029.70 |
| N recovery, % |                              |   |           |           |                  |   | 096.4              |        |

AMINO ACID COMPOSITIONS

droxide monohydrate was added, followed by 1 ml. water. After the mixture was frozen, the test tube was evacuated to approximately 50 microns, and the line connecting the test tube to the vacuum was closed. While still under vacuum the contents of the test tube were allowed to thaw. Further pumping then was carried out, combined with judicious cooling of the test tube with a "dry ice"-ethanol mixture to prevent excessive foaming; after 15 to 30 sec. the test tube was sealed. Hydrolysis of samples was carried out at  $110^{\circ} \pm 2^{\circ}$ C. in a forced-draft oven for 16 to 18 hr. After hydrolysis, the mixture was acidified to pH 2 with 6N HCl, and the Ba<sup>++</sup> was precipitated by adding 2% sodium sulfate (5). After removal of the barium sulfate by centrifugation, aliquots of the supernatant were analyzed for tryptophan content.

#### **Amino Acid Analysis**

Analyses for amino acid content by the method of Spackman et al. (6) were carried out with a Beckman-Spinco model 120 amino acid analyzer modified to carry out 4.25-hr. accelerated amino acid analysis; spherical resins (7) and a 2-mm. cuvet were used. Type PA-27 resin was used for the separation of all basic amino acids; a 5-cm. column was used to separate lysine, histidine, and ammonia; a 12-cm. column was used for tryptophan analysis. The longer column was used to obtain good resolution of tryptophan from lysine. Type AA-15 resin was used in the 57-cm. column for separation of acidic and neutral amino acids. To retard decom-

<sup>&</sup>lt;sup>a</sup>Analysis of 6N HCl hydrolysates.

<sup>&</sup>lt;sup>b</sup>From analysis of barium hydroxide hydrolysate.

<sup>&</sup>lt;sup>C</sup>Extrapolated value. The 4-hr. valid value was 2,300 micromoles.

TABLE III. AMINO ACID COMPOSITION OF WHEAT AND WHEAT PRODUCTS (g. amino acid per 100-g. sample N)

|   | Selkirk  | Manitou  | Triticale  | Wheat  | Wheat   | Wheat  |
|---|--|--|--|--|---|--|
|   | Wheat  | Wheat  | 1966   | Endosperm  | Germ  | Bran   |
| Tryptophan  | 009.55   | 009.20   | 009.90   | 009.43   | 08.85   | 010.60   |
| Lysine  | 014.50   | 014.80   | 019.00   | 012.30   | 39.00   | 021.20   |
| Histidine   | 013.80   | 014.30   | 015.50   | 013.30   | 14.30   | 015.60   |
| Ammonia   | 022.30   | 022.10   | 018.60   | 025.40   | 13.90   | 013.20   |
| Arginine  | 024.90   | 024.40   | 030.50   | 020.80   | 46.60   | 037.10   |
| Aspartic acid Threonine Serine Glutamic acid Proline Glycine Alanine Cystine + cysteine Valine Methionine Isoleucine Leucine Tyrosine Phenylalanine | 029.20<br>017.30<br>031.30<br>207.00<br>069.60<br>023.50<br>020.40<br>016.20<br>027.90<br>010.50<br>023.90<br>042.00<br>016.70 | 030.90<br>017.20<br>029.70<br>207.00<br>067.50<br>021.20<br>015.10<br>027.80<br>010.60<br>021.80<br>041.30<br>041.30 | 036.90<br>019.60<br>028.50<br>193.00<br>066.90<br>024.70<br>017.40<br>031.30<br>012.00<br>025.90<br>044.00<br>014.50<br>029.70 | 024.10<br>019.00<br>030.90<br>229.00<br>074.20<br>020.30<br>015.10<br>026.90<br>010.90<br>024.50<br>042.50<br>046.70 | 50.90<br>24.00<br>26.70<br>83.40<br>23.40<br>33.80<br>35.50<br>09.04<br>34.20<br>09.95<br>19.50<br>34.70<br>19.40 | 038.10<br>017.90<br>027.10<br>105.00<br>035.60<br>028.90<br>011.80<br>022.50<br>006.29<br>016.90<br>031.70<br>015.10 |
| N recovery, %   | 096.40   | 095.80   | 095.30   | 098.10   | 83.10   | 079.00   |

position, the ninhydrin reagent was kept in the dark at 8° to 12°C., and also was protected with a quadruple 4-liter water-bottle system interconnected with thick rubber vacuum tubing to reduce oxidation of the reagent by diffusion of atmospheric oxygen. As a result of these precautions, the ninhydrin yielded virtually identical recoveries on analysis of standard amino acid mixtures over a period of 30 days when the same lot of ninhydrin solution was used.

The response of the amino acid analyzer was checked by analyzing a standard mixture of the 17 commonly occurring amino acids in a protein hydrolysate and ammonia. A linear response was obtained over the range 0.05 to 2 micromoles. A standard amino acid mixture containing 0.5 micromole of the aforementioned amino acids was analyzed in duplicate for each lot of ninhydrin used during analysis of the various samples. Recoveries obtained on analysis of the standard mixture of amino acids were used to calculate the amounts of amino acids obtained on the various samples.

#### Amino Acid Compositions

#### RESULTS

Selkirk wheat was analyzed extensively to reveal details of amino acid recoveries as a function of hydrolysis time, and to determine how best to proceed to analyze the other samples. All of the data obtained for Selkirk wheat are given in Table II.

The amino acid analysis of each sample reported on is the net result of analysis of five hydrolysates, four acid and one basic. These included a 24-, a 48-, and a 72-hr. acid hydrolysate for analysis of all amino acids except cystine and tryptophan; 16-hr. acid hydrolysis of a sample previously performic acid-oxidized to recover cystine+cysteine quantitatively in the form of cysteic acid; and one barium hydroxide hydrolysate for quantitative recovery of tryptophan.

All of the acidic and neutral amino acids present in the HCOOOH hydrolysate are listed in Table II, as the amounts of these amino acids are a useful check as to whether or not any of the HCOOOH hydrolysate has been lost during drying in a vacuum. Occasionally HCOOOH samples will "bump" during vacuum-drying, resulting in loss of some of the sample.

Threonine and serine content was estimated by linear extrapolation of the acid

TABLE IV. AMINO ACID COMPOSITION OF BARLEY, OATS, RYE, BUCKWHEAT, AND MILLET (g. amino acid per 100-g. sample N)

|                  | Barley<br>(6-row) | Barley<br>(pot,<br>6-row) | Oats<br>(hulled) | Oats<br>(flour) | Rye<br>(dark<br>flour) | Buckwheat | Millet |
|------------------|-------------------|---------------------------|------------------|-----------------|------------------------|-----------|--------|
| Tryptophan       | 008.85            | 009.43                    | 010.0            | 010.1           | 007.57                 | 014.2     | 008.62 |
| Lysine           | 021.70            | 020.40                    | 023.2            | 023.7           | 018.10                 | 034.2     | 010.70 |
| Histidine        | 013.30            | 014.10                    | 013.5            | 014.5           | 013.10                 | 013.4     | 014.30 |
| Ammonia          | 017.70            | 019.50                    | 016.2            | 017.6           | 018.20                 | 017.1     | 020.30 |
| Arginine         | 027.30            | 028.70                    | 041.3            | 042.3           | 026.20                 | 050.9     | 020.70 |
| Aspartic acid    | 038.20            | 037.60                    | 048.6            | 050.3           | 040.40                 | 058.0     | 040.00 |
| Threonine        | 022.00            | 023.80                    | 020.9            | 020.4           | 020.90                 | 023.0     | 019.10 |
| Serine           | 026.50            | 029.80                    | 030.1            | 029.7           | 026.80                 | 029.8     | 043.00 |
| Glutamic acid    | 153.00            | 175.00                    | 131.0            | 130.0           | 172.00                 | 105.0     | 139.00 |
| Proline          | 068.40            | 079.30                    | 029.3            | 032.2           | 065.00                 | 022.1     | 042.90 |
| Glycine          | 026.20            | 025.60                    | 030.0            | 028.4           | 022.70                 | 036.8     | 014.50 |
| Alanine          | 025.40            | 024.40                    | 028.6            | 028.2           | 023.20                 | 025.8     | 070.80 |
| Cysteine+cystine | 015.50            | 010.10                    | 020.6            | 020.7           | 014.30                 | 018.1     | 011.20 |
| Valine           | 034.00            | 035.90                    | 034.9            | 034.1           | 030.60                 | 030.9     | 034.00 |
| Methionine       | 010.00            | 009.95                    | 011.0            | 010.8           | 007.31                 | 010.8     | 014.80 |
| Isoleucine       | 023.90            | 027.30                    | 026.0            | 026.0           | 022.80                 | 022.6     | 027.10 |
| Leucine          | 043,40            | 045.70                    | 044.8            | 045.8           | 037.40                 | 038.7     | 082.00 |
| Tyrosine         | 015.80            | 020.30                    | 019.0            | 019.2           | 011.80                 | 013.1     | 023.10 |
| Phenylalanine    | 031.70            | 036.10                    | 030.9            | 032.2           | 028.00                 | 025.9     | 034.90 |
| N recovery, %    | 090.50            | 098.80                    | 092.7            | 093.4           | 089.60                 | 089.5     | 098.50 |

TABLE V. AMINO ACID COMPOSITION OF OILSEED MEALS

(g. amino acid per 100-g. sample N)

|                  | Soybean | Flax   | Mustard (yellow) | Rape-<br>seed | Sunflower |
|------------------|---------|--------|------------------|---------------|-----------|
| Tryptophan       | 011.10  | 012.2  | 010.90           | 010.4         | 010.0     |
| Lysine           | 038.30  | 023.1  | 036.30           | 036.6         | 019.5     |
| Histidine        | 017.00  | 013.6  | 018.20           | 016.7         | 014.4     |
| Ammonia          | 010.50  | 013.5  | 014.20           | 013.1         | 016.0     |
| Arginine         | 042.20  | 055.6  | 035.80           | 038.5         | 056.2     |
| Aspartic acid    | 070.80  | 058.3  | 045.80           | 045.9         | 054.9     |
| Threonine        | 022.10  | 023.9  | 025.80           | 027.2         | 022.3     |
| Serine           | 029.00  | 027.4  | 025.00           | 027.6         | 026.3     |
| Glutamic acid    | 113.00  | 131.00 | 101.00           | 113.0         | 143.0     |
| Proline          | 032.10  | 022.6  | 037.90           | 038.3         | 031.1     |
| Glycine          | 026.30  | 037.7  | 033.60           | 031.4         | 033.9     |
| Alanine          | 026.10  | 027.6  | 025.50           | 027.1         | 024.5     |
| Cysteine+cystine | 012.90  | 011.0  | 010.80           | 017.1         | 013.4     |
| Valine           | 033.30  | 036.5  | 033.30           | 033.9         | 034.9     |
| Methionine       | 008.08  | 010.9  | 008.34           | 013.2         | 011.4     |
| Isoleucine       | 029.60  | 028.0  | 025.70           | 027.1         | 029.2     |
| Leucine          | 044.00  | 036.9  | 041.20           | 042.3         | 038.4     |
| Tyrosine         | 019.00  | 013.8  | 018.60           | 016.2         | 014.2     |
| Phenylalanine    | 028.80  | 028.0  | 024.50           | 024.4         | 029.4     |
| N recovery, %    | 091.30  | 095.0  | 089.60           | 092.1         | 097.3     |

hydrolysis values to zero time hydrolysis (Fig. 1). Tyrosine was determined by analysis of 24-hr., and isoleucine and valine, 72-hr., hydrolysates (Fig. 2). Isoleucine and valine are virtually completely released after 72 hr. of hydrolysis (3,7,8,9). Methionine was estimated as methionine sulfone by analysis of the performic acid sample. In many instances analysis of the 24-hr. 6N HCl hydrolysates indicated identical recovery of methionine as compared with recoveries of methionine

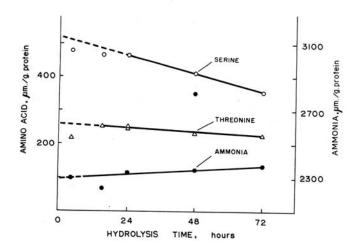


Fig. 1. Recoveries of threonine, serine, and ammonia as a function of hydrolysis time for Selkirk wheat. The extrapolated recoveries at zero time for ammonia, threonine, and serine were found to be 2,297, 254, and 523 micromoles per g. protein (N  $\times$  5.7). A very high recovery value of 2,800 micromoles is shown for ammonia for a 48-hr. hydrolysate; it was obtained by filtering a 48-hr. hydrolysate through filter paper.

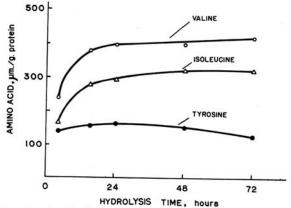


Fig. 2. Recoveries of valine, isoleucine, and tyrosine as a function of hydrolysis time of Selkirk wheat.

sulfone. Values for the rest of the amino acids were obtained by averaging results of the 24-, 48-, and 72-hr. acid hydrolyses.

It seems unnecessary to illustrate all of the data obtained from the above analyses. The data given are the final computed amino acid compositions for the various materials analyzed, as illustrated in the last column of Table II for Selkirk wheat. The amino acid compositions for wheat and its products, for barley, oats, rye, buckwheat, and millet, and for oilseeds are given in Tables III, IV, and V respectively.

# **Extraneous Ammonia in Protein Hydrolysates**

Two unexpected sources of extraneous ammonia have been discovered while

TABLE VI. CORRECTION FACTORS FOR AMINO ACIDS AND AMMONIA

| Cereal or Oilseed | Correction Factors   |             |                     |                     |                         |  |  |  |
|-------------------|----------------------|-------------|---------------------|---------------------|-------------------------|--|--|--|
|                   | Ammonia <sup>a</sup> | Threonine a | Serine <sup>a</sup> | Valine <sup>b</sup> | Isoleucine <sup>b</sup> |  |  |  |
| Wheat: Manitou    | 0.98                 | 1.03        | 1.10                | 1.07                | 1.02                    |  |  |  |
|                   | 0.99                 | 1.04        | 1.12                | 1.06                | 1.10                    |  |  |  |
| Selkirk           | 0.99                 | 1.03        | 1.07                | 1.05                | 1.07                    |  |  |  |
| Triticale         |                      | 1.07        | 1.06                | 1.01                | 1.05                    |  |  |  |
| Wheat endosperm   | ••••                 | 1.02        | 1.09                | 1.04                | 1.06                    |  |  |  |
| Wheat germ        | ••••                 | 1.07        | 1.06                | 1.02                | 1.05                    |  |  |  |
| Wheat bran        | ••••                 | 1.06        | 1.07                | 1,11                | 1.08                    |  |  |  |
| Barley            | ••••                 |             | 1.09                | 1.14                | 1.15                    |  |  |  |
| Barley (pot)      | ••••                 | 1.05        | 1.08                | 1.16                | 1.12                    |  |  |  |
| Oats (hulled)     | ••••                 | 1.03        | 1.09                | 1.01                | 1.02                    |  |  |  |
| Oats (flour)      | ••••                 | 1.02        |                     | 1.15                | 1.11                    |  |  |  |
| Rye (dark flour)  | ****                 | 1.05        | 1.06                | 1.10                | 1.09                    |  |  |  |
| Soybean           |                      | 1.03        | 1.07                | 1.19                | 1.07                    |  |  |  |
| Flax              | ••••                 | 1.08        | 1.11                |                     | 1.07                    |  |  |  |
| Buckwheat         |                      | 1.04        | 1.07                | 1.06                | 1.06                    |  |  |  |
| Millet            |                      | 1.03        | 1.12                | 1.02                |                         |  |  |  |
| Mustard (yellow)  |                      | 1.07        | 1.11                | 1.07                | 1.06                    |  |  |  |
| Rapeseed          | ••••                 | 1.05        | 1.10                | 1.08                | 1.09                    |  |  |  |
| Sunflower         |                      | 1.08        | 1.14                | 1.12                | 1.07                    |  |  |  |
| Averages          | 0.99                 | 1.05        | 1.09                | 1.08                | 1.07                    |  |  |  |

a(Extrapolated value at "zero" time hydrolysis) ÷ (24-hr. hydrolysate value).

amino acid analysis was being carried out. The first source was that present in floor wax (10); the second was found in filtering protein hydrolysates through filter paper to remove humin. Analysis revealed significant contents of ammonia in various filter papers, especially in the acid-washed samples. For example, 11.0-cm. Whatman No. 42 and No. 52 paper was found to contain 1.1 to 1.6, and 2.1 to 6.8 micromoles of ammonia per disc. This is a significant amount of extraneous ammonia, especially if smaller amounts of samples are being hydrolyzed. Figure 1 illustrates a value for extraneous ammonia obtained by filtering through unwashed filter paper.

As most of the analyses were completed before discovery of the high ammonia content of the filter paper used to remove humin, 4-hr., 110 °C., 6N HCl hydrolysates were obtained and analyzed for all the samples to obtain valid values for ammonia content. Hydrolysis for this duration is sufficient to release all amide ammonia (11,12,13). Humin was removed by high-speed centrifugation, which results in a firm pellet of humin in the centrifuge tube from which most of the clear supernatant can be drawn off.

The redistilled 6N HCl and pH 2.2 buffer used to dissolve the hydrolyzed sample contained 0.012 and 0.021 micromoles of ammonia per ml. The analytical

results were corrected for these amounts of ammonia.

#### DISCUSSION

# **Expression of Results**

There are many ways in which amino acid content may be expressed. This often results in data which are difficult if not impossible to compare. The author has previously reported amino acid compositions as percent amino acid N of total sample N (10), as this is a simple and unambiguous way of expressing results and

b(72-hr. hydrolysate value) ÷ (24-hr. hydrolysate value).

also enables one to calculate very easily the recovery of amino acid N. However, workers in nutritional research desire amino acid compositions to be expressed as g. amino acid per unit of food or feed, the commonest unit of this type being "g. amino acid per 16 g. sample N" which implies that the protein content of food and feeds is equivalent to the weight of N multiplied by the factor 6.25. As is demonstrated in an accompanying article (1), this implication is not valid, and accordingly, we have chosen to express our results as "g. amino acid per 100 g. sample N."

# Applicability of Results

It must be emphasized that composite samples were analyzed in the present work, with the exception of millet, which was a sample from a single location. Accordingly, the present results cannot be used to indicate differences in amino acid compositions as influenced by variety or protein content.

# Amino Acid Compositions

Generally, the amino acid compositions given in the present work agree quite well with the few available reports of recent origin. In most of the latter, results were obtained by ion-exchange chromatographic procedures. However, comparisons between present results and the majority of compositions listed in the literature reveals numerous and wide discrepancies. These should not be interpreted too strictly, since compositions are being compared of different samples grown in different environments and locations.

The amounts of tryptophan reported in the present work are significantly larger than most reports in the literature. This is due to removal of Ba++ from the barium hydroxide hydrolysates at an acidic pH, as described recently by Miller (5). The latter author discovered that when Ba++ is precipitated from a basic solution, losses of tryptophan due to absorption occur. It is somewhat surprising that our results show larger recoveries of tryptophan than even those reported by Miller; for example, our average recovery for wheat was 9.55, as compared to Miller's 6.88 g. tryptophan per 100 g. of sample N (5). Experiments were also run in an attempt to increase the recovery of tryptophan by addition of histidine to the samples previous to hydrolysis of barium hydroxide, as described by Spies (14). The findings reported by Spies do not seem to apply to wheat or wheat bran, since similar recoveries of tryptophan were obtained when samples of wheat or wheat bran were hydrolyzed in the presence or absence of added histidine.

Recoveries of cystine, methionine, threonine, and serine are higher than those in most literature reports. Higher recoveries of cystine probably result from analyzing for it in the more stable form of cysteic acid. The higher yields of threonine and serine are due to correcting for decomposition which occurs during hydrolysis. The higher recovery of methionine is possibly due to using thiodiglycol as an antioxidant, as recommended by Moore and Stein (15).

The amino acid compositions are compared with some compositions listed in the literature. Variations in these compositions were all calculated as follows: [(value, this work, minus literature value)/value, this work] × 100. For example, for glycine content in wheat, our result was 23.7, whereas one literature value is 44 g. per 100 g. of sample; accordingly, the variation is  $[(23.7 - 44)/23.7] \times 100 =$ -86%. Only variations exceeding 19% are mentioned.

#### Wheat

The present results are in good agreement with the compositions published by

Sosulski et al. (16) and Waggle et al. (17). Good agreement is to be found also between the results reported by Kohler and Palter (18) and Hepburn and Bradley (19), except for the differences of +30% for tryptophan (18), and +23 and +27% for tryptophan and cystine (19). Comparison of the results with those reported by McDermott and Pace (20) shows differences of +34, -22, and +30% for histidine, ammonia, and alanine (20). Comparison of the present results with two average compositions reveals variations as high as -50 (21) and -86% (16) for glycine and -42% for arginine (22).

### Wheat Flour

The present results are in good agreement with results published earlier from this laboratory (10) except for the variation of +42% for tryptophan content. Reasonably good agreement is to be found between the present results and those given by Waggle et al. (17), McDermott and Pace (23), Hepburn et al. (24), and by Orr and Watt (21), and by Ewart (25), except for the following differences: +22% for valine (17); +24 and -25% for tryptophan and tyrosine (23); +35, +28, and -25% for tryptophan, cystine, and tyrosine (24); +26, -22, +25, and +31% for tryptophan, arginine, cystine, and methionine (21); and +32 and +28% for tryptophan and methionine (25).

# Wheat Germ

The results given in this work seem to be the first report for quantitative amino acid analyses for pure wheat germ. Recovery of only 82% of the germ N in the form of amino acid N indicates that 18% of the germ N may be due to the presence of nucleotides and other nonamino acid sources.

There are a number of reports which give the amino acid composition of commercial samples of germ (17,18,21,24). Commercial samples of germ usually have a significantly lower protein content because of the large amount of bran contamination. Accordingly, the amino acid compositions given in the literature are not compared with the compositions given in the present work for pure wheat grain.

#### Wheat Bran

The data given herein seem to be the first complete amino acid composition given for bran from HRS wheat. In general, the present values are in reasonable agreement with partial analysis given by Lyman et al. for HRW bran (26), and by Hepburn et al. (24) and Kohler and Palter (18) for various blends of HRS and HRW wheats. The present results vary significantly from recent amino acid data (complete except for tryptophan and ammonia content) given by Waggle et al. for bran obtained from Pembina wheat (17).

# Barley

The present results for six-row barley are in poor agreement with the two average compositions listed in the literature, the values for lysine, proline, glycine, cystine, isoleucine, and tyrosine varying by -35(21), +23(21), +27(22), +25(21) and +38(22), -21(21), -34(21) and -20%(22).

The amino acid compositions given for pot barley seem to be the first reported. The composition for pot barley is quite similar to that of whole barley. Comparison of our results for pot barley with Ewart's results for barley flour (25) shows that differences are present of +25, +22, +28, +25, -68, +22, +23, and +21% for

histidine, ammonia, aspartic acid, glycine, cystine, valine, isoleucine, and tyrosine.

#### Oats

The results reported in this work are in excellent agreement with those given by Slump and DeGroot (27), except for tyrosine, where a recovery lower by -32% was reported. Good agreement is found between the present results for hulled oats and those given by Ewart for oat flour except for the differences of +28 and +24% for ammonia and tyrosine content (25).

The present results are in very poor agreement with three other published average amino acid compositions of oats (21,22,28). The discrepancies vary as much as +75 and +64% for glycine and cystine (22).

#### Rye

Good agreement was found between the present results and those given by Slump and DeGroot (27), except for tyrosine content which differed by -43%. Comparison of the present results with the results given by Ewart shows differences of -29, +20, -21, and -33% for tryptophan, aspartic acid, cystine, and methionine (25). Poor agreement is observed in comparing our results with two other reports of averaged compositions (21,22).

# Soybean

The results agree with a partial composition report in the literature (22), except for the variation of +27% for tryptophan content, and with the composition given by Orr and Watt (21) except for a +23, -41, and -32% variation in tryptophan, serine, and proline content. Numerous discrepancies are to be found between the results in the present work and results reported by Rackis et al. (29) and by Block and Weiss (30).

#### Flax

The composition in this work is in good agreement with that reported by Block and Weiss (30), except for the variations of +28, -28, and -38% for tryptophan, serine, and tyrosine content. The present results do not agree with those presented by Holmes (31); for example, the recoveries of lysine, tyrosine, and histidine vary by -165, -97, and -93%.

#### Buckwheat

The present results seem to be the first complete amino acid composition for buckwheat. The results are in agreement with the partial compositions given in the literature, except for a +38 and +33% variation in tryptophan content (21), and a +30, +22, and -48% variation in tryptophan, leucine, and tyrosine content (22).

## Millet

The present results seem to be the first report of a complete amino acid composition for millet. Very poor agreement is to be found between these results and the partial compositions reported in the literature; for example, variations of -63, -62, and -50% are found for tryptophan, threonine, and lysine (21); and -48 and -83% for tryptophan and lysine (22). It is seen that millet is a rich source of leucine, as it contains approximately twice the amount present in any cereal or oilseed except for sorghum and corn.

#### Mustard

The composition listed in the present work is in good agreement with that reported by Miller et al. (32), except for the variation of +34% for threonine content.

# Rapeseed

The present results are in good agreement with the partial results published by Bell (33), except for tyrosine content which was found to differ by -155%. The present results are not in agreement with those published by Ägren (34); for example, lysine and serine contents vary by +41 and -95%.

## Sunflower

The present results are in reasonable agreement with the partial composition given by Block and Weiss (30), except for the variations by +31, +24, +21, and +25% for tryptophan, histidine, glutamic acid and cystine content.

# **Correction Factors for Hydrolysis Time**

The amino acid compositions reported in this work were obtained from analyses of 24-, 48-, and 72-hr. 6N HCl hydrolysates. This procedure allows one to correct for the amino acids which decompose during hydrolysis, and for other amino acids which are hydrolyzed very slowly. The recoveries of amino acids as a function of time have been indicated for Selkirk wheat in Figs. 1 and 2.

Analyses of multiple hydrolysates of numerous samples are, however, very time-consuming, and it would be advantageous to use one hydrolysis time combined with appropriate correction factors. Accordingly, values obtained by extrapolating to "zero hydrolysis time" divided by 24-hr. hydrolysis values for ammonia, threonine, and serine, and 72-hr. hydrolysis values divided by 24-hr. hydrolysis values for valine and isoleucine, are given in Table VI.

It is seen in Table VI that although the factors vary somewhat in value, 0.99, 1.05, 1.09, 1.08, and 1.07 should be reasonably accurate correction factors for the 24-hr. 110°C. hydrolysate recovery values of ammonia (provided extraneous ammonia is not present), threonine, serine, valine, and isoleucine. These figures are in excellent agreement with the values of 1.04, 1.08, 1.08, and 1.08 published recently for HRS wheat and soft wheat bran (18). The use of these factors applied to the results of an analysis of a 24-hr. acid hydrolysate, combined with the results of an analysis of two other samples — one previously oxidized with performic acid and then hydrolyzed with acid in order to recover cystine + cysteine quantitatively in the form of cysteic acid (4) and another hydrolyzed with barium hydroxide to recover tryptophan (5) — would then give a complete and quantitative amino acid composition of a sample.

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