

support other data that the lowest Cr concentration will be found in the fruit, with increases in the stem and the highest concentration in the leaf (Desmet et al., 1975; Lahouti and Peterson, 1979; Ramachandran et al., 1980; Cary et al., 1977a,b). Possibly, contamination of leaves by soil is a function of morphology. Leaves that are hairy, as tomato leaves, might be more efficient in collecting and retaining soil particles than a smooth-leaf species. Leaf age may be an important factor because needles appeared to contain higher concentrations of Ti than leaves of deciduous trees. However, more research is required to prove this hypothesis. There is a need to identify the source of Cr and Ti occurring on plants growing on high-Cr soils.

Registry No. Cr, 7440-47-3; Ti, 7440-32-6.

LITERATURE CITED

- Anderson, R. A. Nutritional role of chromium. *Sci. Total Environ.* 1981, 17, 13-29.
- Bartlett, R. J.; Kimble, J. M. Behavior of chromium in soils: I. Trivalent forms. *J. Environ. Qual.* 1976, 5, 379-386.
- Bartlett, R.; James, B. Behavior of Chromium in Soils: III. Oxidation. *J. Environ. Qual.* 1979, 8, 31-35.
- Bartlett, R.; James, B. J. Studying dried, stored soil samples - some pitfalls. *Soil Sci. Soc. Am. J.* 1980, 44, 721-724.
- Cary, E. E. Chromium in air, soil and natural waters. In *Biological and Environmental Aspects of Chromium*; Langard, S., Ed.; Elsevier Biomedical Press: New York, 1982.
- Cary, E. E. Electrothermal atomic absorption spectroscopic determination of chromium in plant tissues: Interlaboratory study. *J. Assoc. Off. Anal. Chem.* 1985, 495-498.
- Cary, E. E.; Olson, O. E. Atomic absorption spectrophotometric determination of chromium in plants. *J. Assoc. Off. Anal. Chem.* 1975, 58, 433-435.
- Cary, E. E.; Rutzke, M. Electrothermal atomic absorption spectroscopic determination of chromium in plant tissues. *J. Assoc. Off. Anal. Chem.* 1983, 850-852.
- Cary, E. E.; Allaway, W. H.; Olson, O. E. Control of chromium concentrations in food plants. 1. Absorption and translocation of chromium by plants. *J. Agric. Food Chem.* 1977a, 25, 300-304.
- Cary, E. E.; Allaway, W. H.; Olson, O. E. Control of chromium concentrations in food plants. 2. Chemistry of chromium in soils and its availability to plants. *J. Agric. Food Chem.* 1977b, 25, 305-309.
- Cary, E. E.; Grunes, D. L.; Bohman, V. R.; Sanchirico, C. A. Titanium determination for correction of plant sample contamination by soil. *Agron. J.* 1986, 78, 933-936.
- Cherney, J. H.; Robinson, D. L.; Kappel, L. C.; Hembrey, F. G.; Ingraham, R. H. Soil contamination and elemental concentrations of forages in relation to grass tetany. *Agron. J.* 1983, 75, 447-451.
- Desmet, E.; Levi, C.; Myttenaer, R. A.; Verfaillie, G. The Behaviour of Chromium in Aquatic and Terrestrial Food Chains. EUR 5475e Boite postale 1003, Luxemburg, 1975; pp 43-81.
- Jones, B.; Buckley, R. A. Levels of chromium in wheats and some other animal feedstuffs in Australia. *J. Sci. Food Agric.* 1977, 28, 265-268.
- Lahouti, M.; Peterson, P. J. Chromium accumulation and distribution in crop plants. *J. Sci. Food Agric.* 1979, 30, 136-142.
- Lyon, G. L.; Brooks, R. R.; Peterson, P. J.; Butler, G. W. Some trace elements in plants from serpentine soils. *N.Z. J. Sci.* 1970, 13, 133-139.
- Mertz, W. Chromium occurrence and function in biological systems. *Physiol. Rev.* 1969, 49 (2), 163-239.
- Mitchell, R. L. Contamination problems in soil and plant analysis. *J. Sci. Food Agric.* 1960, 11, 553-560.
- Ramachandran, V.; D'Souza, T. J.; Mistry, K. B. Uptake and transport of chromium in plants. *J. Nucl. Agric. Biol.* 1980, 9, 126-128.
- Welch, R. M.; Cary, E. E. Concentration of chromium, nickel, and vanadium in plant materials. *J. Agric. Food Chem.* 1975, 23, 479-482.

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Nonprotein Nitrogen Contents of Animal and Plant Foods

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Nonprotein nitrogen (NPN) was extracted from 20 primary food products and purified by constant-volume ultrafiltration (10 000 MW cutoff) before separation and quantification of free and acid-hydrolyzable amino acids (AA) and their amides. Animal, fish, and poultry products contained 0-35 mg, cereal and pulse grains had 12-44 mg, and roots, vegetables, and fruits contained 65-240 mg of NPN/g of N. Free AA constituted one-third and half of total hydrolyzable AA in the animal and plant foods, respectively, and 70-90% of NPN was composed of non amino acid nitrogen. Glu/Gln and Asp/Asn were prominent in most free AA and peptide fractions, and Lys was a major AA in the free AA pool.

Nonprotein nitrogen (NPN) of food products is of interest to food processors, nutritionists, and dieticians for quite different reasons. NPN is defined as peptides too small to be precipitated and filtered, free amino acids

(AA), amides, and other nonpolymeric nitrogen (N) constituents of the plant or animal product. The interactions of free AA with simple sugars in Maillard reactions are important contributors to food color and flavor (Buck-

Table I. NPN Contents of Food Products, As Determined by Ethanol Extraction and Ultrafiltration, and Their Composition of Amino Acids, Dry Basis

| class of food product | total N, % dry wt | filtrate NPN | | | AAN of NPN, % | filtrate NAAN, mg/g sample N |
|------------------------|-------------------|--------------|---------------|-----------|---------------|------------------------------|
| | | mg/g sample | mg/g sample N | % total N | | |
| Dairy Products and Egg | | | | | | |
| casein | 13.7 | 0.1 ± 0.01 | 0.4 | 0.0 | 30.4 | 0.3 |
| egg | 7.7 | 0.4 ± 0.03 | 5.7 | 0.6 | 24.4 | 4.3 |
| cheese | 6.4 | 2.3 ± 0.36 | 35.5 | 3.5 | 25.5 | 26.4 |
| milk | 4.1 | 1.2 ± 0.02 | 29.5 | 2.9 | 10.0 | 26.5 |
| Meat and Fish Products | | | | | | |
| chicken | 10.8 | 1.6 ± 0.36 | 14.8 | 1.5 | 30.2 | 10.3 |
| fish | 12.8 | 2.1 ± 0.33 | 16.6 | 1.7 | 14.3 | 14.2 |
| beef | 12.2 | 2.4 ± 0.03 | 17.5 | 1.7 | 15.8 | 14.7 |
| Cereals and Pulse | | | | | | |
| sorghum | 2.0 | 0.2 ± 0.00 | 12.5 | 1.2 | 32.8 | 8.4 |
| rice | 0.9 | 0.1 ± 0.00 | 13.1 | 1.3 | 20.0 | 10.5 |
| wheat | 1.9 | 0.3 ± 0.03 | 14.9 | 1.5 | 18.3 | 12.2 |
| corn | 1.6 | 0.3 ± 0.02 | 21.6 | 2.2 | 21.7 | 16.9 |
| field pea | 3.5 | 1.5 ± 0.29 | 44.1 | 4.4 | 20.3 | 35.1 |
| Tuber and Roots | | | | | | |
| potato | 2.0 | 4.8 ± 0.12 | 238.5 | 23.8 | 18.6 | 194.1 |
| beet | 1.7 | 4.0 ± 0.07 | 235.3 | 23.5 | 18.6 | 191.5 |
| carrot | 0.6 | 0.8 ± 0.07 | 131.9 | 13.2 | 11.1 | 117.2 |
| Leafy Vegetables | | | | | | |
| lettuce | 2.6 | 5.3 ± 0.07 | 205.3 | 20.5 | 19.5 | 165.3 |
| cabbage | 2.4 | 5.5 ± 0.14 | 229.2 | 22.9 | 18.9 | 185.9 |
| Fruits | | | | | | |
| apple | 0.2 | 0.1 ± 0.01 | 64.8 | 6.5 | 12.7 | 56.6 |
| banana | 0.7 | 1.1 ± 0.01 | 153.1 | 15.3 | 18.6 | 124.6 |
| tomato | 2.0 | 4.0 ± 0.01 | 199.6 | 20.0 | 13.0 | 173.7 |

holz, 1988). Irwin and Hegsted (1971) and Kies (1972) indicate that NPN can spare specific essential AA and protein. If NPN contains, predominantly, non amino acid nitrogen (NAAN), the protein nutritive value would be reduced proportionately. Also, the NPN proportion of total protein does not contribute to the functional properties of vegetable protein flours, and yields of protein concentrates and isolates would be reduced (Fan and Sosulski, 1974).

Accurate quantitation of the protein contents of foods and feeds is essential in food processing and marketing. The widespread use of Kjeldahl N values, multiplied by an appropriate N to protein conversion factor, for determination of protein content assumes the absence of significant or variable quantities of NPN. It would be of great advantage for the food industry and nutritionists to know the quantity and nature of NPN in primary foods and their processed products.

A variety of approaches have been taken to extract and quantitate the NPN in unprocessed grains (Bhatty et al., 1973; Holt and Sosulski, 1981), giving quite variable results. Bell (1963) concluded that ultrafiltration measured NPN as theoretically defined, but there is no agreement on a uniform procedure for routine analysis.

The purpose of this investigation was to use both ethanol extraction and ultrafiltration to quantitate NPN levels in 20 food products in relation to their protein contents. Amino acid and amide analyses of NPN before and after protein hydrolysis were conducted to assess the characteristics and nutritive value of the NPN fractions.

MATERIALS AND METHODS

Materials. The food products were purchased from commercial outlets in Saskatoon, SK, except casein (Sheffields Co., Norwich, NY), field pea (obtained from Department of Crop Science and Plant Ecology, University of Saskatchewan, SK), and sorghum (from Senegal, West Africa).

Table II. Free AA Composition of the Nonhydrolyzed NPN of Animal Food Products ($\mu\text{M}/\text{mg}$ of NPN), Dry Basis

| | milk products and egg | | | | meat and fish products | | |
|----------------|-----------------------|------|--------|------|------------------------|------|------|
| | casein | egg | cheese | milk | chicken | fish | beef |
| NPN, mg/g of N | 0.4 | 5.7 | 35.5 | 29.9 | 14.8 | 16.6 | 17.5 |
| Arg | | 5.5 | 5.7 | 0.6 | 1.7 | 0.4 | 0.7 |
| His | 4.4 | 1.7 | 0.9 | 0.2 | 2.6 | 0.5 | 1.2 |
| Ile | | 3.5 | 1.0 | | 1.3 | | 1.1 |
| Leu | | 6.7 | 11.4 | 0.5 | 2.5 | 0.3 | 0.1 |
| Lys | 7.2 | 6.5 | 5.9 | 0.5 | 5.1 | 0.7 | |
| Met | | 1.5 | 1.3 | | 0.9 | 0.2 | 0.3 |
| Phe | 9.4 | 5.2 | 5.7 | 0.9 | 1.8 | 0.4 | 0.4 |
| Thr | | 6.5 | | | 2.4 | 0.5 | 2.9 |
| Val | | 6.2 | 4.6 | 0.6 | 3.3 | 0.4 | 0.7 |
| Tyr | 6.4 | 5.2 | 2.5 | 0.9 | 1.8 | 0.5 | 0.6 |
| Ala | | 3.5 | 1.8 | | 5.1 | 2.0 | 0.3 |
| Asp | | 4.2 | 1.5 | | 2.9 | | 0.7 |
| Glu | 4.2 | 8.7 | 11.2 | 1.0 | 6.9 | 0.4 | 0.7 |
| Gly | | 2.7 | 1.3 | 0.5 | 5.0 | 3.2 | 2.1 |
| Pro | | 5.2 | 0.9 | | 3.2 | | 0.4 |
| Ser | | 7.7 | 5.9 | 0.4 | 5.3 | 0.6 | |
| total AA | 31.6 | 81.0 | 61.5 | 6.1 | 51.7 | 10.0 | 12.1 |
| amide | 2.4 | 8.3 | 11.7 | 0.6 | 3.0 | 0.1 | 1.1 |

Analyses. The animal products (except casein), leafy vegetables, fruits, potato, carrot, tomato, and beet were freeze-dried and defatted where necessary. All samples were finely ground in a Krups coffee grinder to 0.25-mm mean particle size.

Moisture and total N were determined by standard procedures (AOAC, 1984) with the exception that, in the micro-Kjeldahl method for total N, a 100:3 mixture of K_2SO_4 and CuSO_4 was substituted for K_2SO_4 and mercuric oxide (AOAC Method 7.033) to achieve a boiling temperature of 345 °C for the 1-h digestion period.

Extraction. Duplicate 1-g samples of finely ground materials were stirred for 30 min with 35 mL of 25% (v/v) ethanol. The extract was centrifuged for 20 min at 27000g, after which the supernatant was flash-evaporated to remove the solvent. The residue was resuspended in 2.5 mL of distilled deionized

Table III. Average Free AA Composition of the Nonhydrolyzed NPN of Plant Food Products ($\mu\text{M}/\text{mg}$ of NPN), Dry Basis

| | sorghum | rice | wheat | corn | pea | potato | beet | carrot | lettuce | cabbage | apple | banana | tomato |
|-------------|---------|------|-------|------|------|--------|-------|--------|---------|---------|-------|--------|--------|
| NPN, mg/g N | 12.5 | 13.1 | 14.9 | 21.6 | 44.1 | 238.5 | 235.3 | 131.9 | 205.3 | 229.2 | 64.8 | 153.1 | 199.6 |
| Arg | 2.8 | | 4.0 | 3.8 | 8.1 | 3.6 | 1.5 | | 1.3 | 6.7 | 8.6 | 3.2 | 0.5 |
| His | 2.1 | 1.0 | 1.7 | 1.0 | 0.5 | 1.1 | 0.2 | | 0.6 | 0.7 | | 10.5 | 0.4 |
| Ile | | | | | 1.1 | 0.8 | 1.9 | 0.4 | 1.6 | 0.7 | | | 0.4 |
| Leu | | 5.6 | | 2.7 | 0.3 | 2.6 | 2.3 | 1.4 | 0.2 | | | 5.8 | 5.4 |
| Lys | 1.7 | | 1.2 | 2.6 | 0.8 | 0.9 | 0.5 | | 0.6 | 0.3 | | 1.4 | 0.2 |
| Met | | | | | | | 1.0 | 0.5 | 0.8 | 0.5 | | | 0.2 |
| Phe | 3.4 | | 3.3 | 1.5 | 0.9 | 37.5 | | | 0.3 | 0.4 | | 0.9 | 0.1 |
| Thr | | 6.6 | | | | | 37.2 | 2.7 | 53.0 | 21.0 | | | 13.0 |
| Val | | | 1.7 | | 0.8 | 2.1 | | | 1.8 | | | 3.2 | |
| Tyr | 3.6 | | 3.0 | 1.4 | 0.7 | 0.8 | 0.5 | 0.8 | 1.0 | 0.5 | | 2.1 | 1.0 |
| Ala | 3.3 | 4.6 | | | 0.8 | 4.3 | 1.3 | 1.6 | 0.2 | 1.7 | | 0.9 | 0.3 |
| Asp | 13.9 | 10.6 | 4.1 | | 6.2 | 3.9 | 2.4 | 6.7 | 2.4 | 5.1 | 14.4 | 1.8 | 7.0 |
| Glu | 3.5 | 8.6 | 2.9 | | 17.1 | 3.5 | 1.9 | 10.2 | | 5.1 | 4.2 | 0.8 | 30.4 |
| Gly | 1.9 | 4.0 | 1.0 | 0.8 | 0.8 | 1.4 | 1.7 | 1.4 | 2.8 | 1.6 | | 1.2 | 0.2 |
| Pro | 4.1 | | | | 1.3 | 1.9 | | | 0.7 | 2.1 | | 1.9 | |
| Ser | 4.1 | | 1.6 | | 2.8 | | | | 3.9 | | 9.2 | 20.0 | |
| total AA | 44.4 | 41.0 | 24.5 | 13.8 | 41.5 | 64.4 | 52.4 | 25.7 | 71.2 | 46.4 | 36.4 | 53.7 | 59.1 |
| amide | 14.6 | 14.4 | 5.1 | 0.0 | 12.8 | 5.8 | 3.7 | 9.3 | 2.2 | 6.5 | 11.3 | 1.5 | 12.6 |

Table IV. Average AA Composition of the Acid-Hydrolyzed NPN of Animal Food Products ($\mu\text{M}/\text{mg}$ of NPN), Dry Basis

| | milk products and egg | | | | meat and fish products | | |
|----------------|-----------------------|-------|--------|------|------------------------|------|------|
| | casein | egg | cheese | milk | chicken | fish | beef |
| NPN, mg/g of N | 0.4 | 5.7 | 35.5 | 29.5 | 14.7 | 16.6 | 17.5 |
| Arg | 7.2 | 6.1 | 7.3 | 0.9 | 2.0 | 0.8 | 1.0 |
| His | 28.8 | 1.8 | 4.2 | 1.7 | 11.0 | 0.5 | 20.3 |
| Ile | 11.8 | 4.0 | 6.9 | 1.8 | 2.1 | 0.3 | 0.8 |
| Leu | 9.8 | 7.9 | 17.4 | 4.2 | 3.1 | 0.9 | 1.4 |
| Lys | 14.7 | 8.1 | 1.1 | 3.5 | 41.5 | 4.7 | 7.2 |
| Phe | 15.2 | 4.8 | 6.1 | 4.2 | 1.9 | 0.7 | 1.3 |
| Thr | | 7.2 | 1.9 | 1.9 | 4.5 | 1.8 | 1.8 |
| Val | 7.4 | 6.9 | 11.3 | 4.9 | 3.5 | 8.7 | 1.5 |
| Tyr | 11.8 | 7.1 | 0.3 | 4.4 | 1.9 | 0.3 | 1.0 |
| Ala | 5.2 | 5.8 | 4.7 | 2.4 | 28.6 | 28.3 | 7.2 |
| Asp | | 11.2 | | 4.2 | 5.0 | 11.8 | 2.5 |
| Glu | 5.8 | 22.2 | 35.5 | 7.5 | 3.7 | 1.2 | 1.4 |
| Gly | 8.0 | 5.4 | 5.0 | 3.9 | 11.4 | 1.6 | 5.8 |
| Pro | | 7.7 | 16.7 | 1.1 | 1.8 | 6.0 | 3.4 |
| Ser | | 7.6 | 4.5 | 2.9 | 3.2 | 1.0 | 1.1 |
| total AA | 125.7 | 113.8 | 122.9 | 49.5 | 125.2 | 68.6 | 57.7 |
| amide | 3.3 | 21.3 | 21.3 | 6.8 | 4.4 | 4.7 | 1.1 |
| total AAN | 21.7 | 17.4 | 18.2 | 7.1 | 21.6 | 10.2 | 11.3 |

water, stirred, and centrifuged to extract any additional NPN. The combined supernatants were ultrafiltered as described below.

Ultrafiltration. Constant-volume ultrafiltration was used for the determination of low-MW NPN in the samples as

described by Holt (1976). An Amicon Model 402 stirred cell with a PM10 noncellulosic membrane (Amicon Canada Ltd., Oakville, ON) with a nominal MW cutoff of 10 000 was used for the ultrafiltration. The supernatants were applied to the cell and dialyzed with distilled water at room temperature and 210-kPa pressure until 250 mL of ultrafiltrate was collected for determination of total N, free and total AA, and amides.

Free AA in Ultrafiltrate. Duplicate aliquots of 50 mL of the 250-mL ultrafiltrate were evaporated to dryness at 50–55 °C. The residue was taken up in 10 mL of citrate buffer (pH 2.2) and stored at 5 °C before being applied to a Beckman 119 BL AA analyzer. The AA were identified by their relative retention times compared with those of known AA. The procedure for HCl hydrolysis of the amide N and NH_3 titration is described in Bailey (1967).

Total Amino Acids in Ultrafiltrate. The total AA content of the ultrafiltrate was determined by hydrolysis with 6 N HCl. Duplicates of 50-mL samples of the original 250 mL of the ultrafiltrate were evaporated to dryness (50–55 °C). The residue was taken up in 8 mL (3 + 3 + 2) of 6 N HCl and placed in a 10-mL ampule that was evacuated, sealed, and heated at 110 °C for 24 h. After hydrolysis, the HCl was removed by flash evaporation and the residue dissolved in 10 mL of citrate buffer, as above, for AA and amide analysis.

RESULTS AND DISCUSSION

Nonprotein Nitrogen. The filtrate NPN values obtained by dialysis are reported in milligrams of NPN/gram of sample, milligrams of NPN/gram of N, and as percent of total N (Table I). Despite the comparatively

Table V. Average AA Composition of the Acid-Hydrolyzed NPN of Plant Food Products ($\mu\text{M}/\text{mg}$ of NPN), Dry Basis

| | sorghum | rice | wheat | corn | pea | potato | beet | carrot | lettuce | cabbage | apple | banana | tomato |
|----------------|---------|------|-------|------|------|--------|-------|--------|---------|---------|-------|--------|--------|
| NPN, mg/g of N | 12.5 | 13.1 | 14.9 | 21.6 | 44.0 | 238.5 | 235.5 | 131.9 | 205.3 | 229.2 | 64.8 | 153.1 | 199.6 |
| Arg | 3.4 | | 2.2 | 2.4 | 11.4 | 0.4 | 1.3 | 2.6 | 0.8 | 6.7 | 5.4 | 4.7 | 0.6 |
| His | 7.2 | 2.0 | 3.0 | 1.9 | 4.7 | 0.6 | 0.6 | 0.6 | 0.7 | 1.5 | | 14.1 | 0.5 |
| Ile | | | 2.6 | | 1.2 | 0.9 | 1.3 | 1.4 | 1.2 | 1.3 | | 1.0 | 0.4 |
| Leu | 7.1 | | 3.3 | 2.7 | 1.6 | 2.0 | 2.8 | 0.6 | 1.0 | 0.8 | | 8.4 | 0.5 |
| Lys | 8.9 | 5.0 | 4.9 | 3.9 | 3.9 | 0.6 | 1.1 | 0.4 | 0.7 | 1.0 | 1.5 | 3.3 | 0.8 |
| Phe | 9.4 | 5.0 | 5.1 | 5.7 | 2.0 | 0.8 | | 1.7 | 1.1 | 0.9 | 6.0 | 0.6 | 1.2 |
| Thr | 3.5 | | 2.3 | 2.4 | 3.4 | 2.5 | 1.2 | 1.8 | 2.3 | 1.5 | 3.2 | 1.8 | 1.1 |
| Val | 23.5 | 9.0 | 3.9 | 9.5 | 2.8 | 1.8 | 2.1 | 4.6 | 2.0 | 2.1 | | 5.4 | 0.5 |
| Tyr | 5.3 | 2.0 | 5.0 | 1.5 | 1.8 | 0.4 | | 0.9 | 0.7 | 0.6 | 3.0 | 0.6 | 4.6 |
| Ala | 9.8 | 10.0 | 5.3 | 8.5 | 4.0 | 3.8 | 2.5 | 4.4 | 5.2 | 3.9 | | 1.8 | 0.8 |
| Asp | 20.8 | 17.0 | 9.7 | 16.1 | 16.1 | 29.2 | 7.6 | 11.2 | 16.2 | 12.4 | 26.5 | | 20.5 |
| Glu | 15.9 | 16.0 | 12.0 | 13.3 | 15.1 | 29.3 | 49.3 | 14.6 | 48.4 | 37.2 | 7.5 | 22.8 | 38.2 |
| Gly | 10.3 | 9.0 | 7.7 | 7.5 | 7.6 | 1.5 | 1.6 | 3.1 | 1.2 | 1.8 | 1.1 | 3.2 | 1.7 |
| Pro | 18.3 | | 15.1 | 20.7 | 2.1 | 3.0 | | | | | | 2.6 | |
| Ser | 5.7 | 15.0 | 3.7 | 4.1 | 2.6 | 2.3 | 4.3 | 2.9 | 4.3 | 3.4 | | 4.3 | 1.1 |
| total AA | 149.1 | 90.0 | 82.1 | 97.4 | 79.8 | 79.1 | 75.7 | 50.8 | 85.9 | 75.1 | 54.2 | 55.8 | 72.5 |
| amide | 31.8 | 24.8 | 15.8 | 23.2 | 17.2 | 44.0 | 48.4 | 14.2 | 44.2 | 37.0 | 22.0 | 13.5 | 21.3 |
| total AAN | 23.4 | 14.3 | 13.1 | 15.5 | 14.5 | 13.3 | 13.3 | 7.9 | 13.9 | 13.5 | 9.1 | 13.3 | 9.3 |

high N contents of the animal products including poultry and fish, the NPN values were less than 2.5 mg/g of sample or 3.5% of total N. The four cereals were characterized by low N and NPN levels, averaging 0.2 mg of NPN/g of sample, which was 1.5% of total N. The field pea sample has 1.5 mg of NPN/g of sample or 44.1 mg/g of N. The tuber and root crops, leafy vegetables, banana, and tomato were also low in N content, but 13.2–23.8% of total N was filtrate NPN.

The values obtained in this study for egg, milk, fish, and beef round muscle were lower than certain published values (Alexander and Elvehjem, 1956; Bell, 1963; Walstra and Jenness, 1984). Degradation of beef (Gardner and Stewart, 1966), poultry (Khan and van den Berg, 1964), and fish (Hodgekiss and Jones, 1955) muscle during storage is common. NPN values similar to those of the present investigation were reported for cereals (Tkachuk, 1977, 1979), field pea (Holt, 1976), and potato (Chandra and Mondy, 1981).

Free Amino Acids. The ultrafiltrates from the ethanol (25% v/v) extracts of the finely ground food products were analyzed for free AA (Tables II and III). The levels of free AA in the food products were not proportional to the total NPN. Egg, cheese, and chicken muscle contained comparatively high levels of free AA while milk, beef, and fish had only trace quantities of several free AA. Due to processing, casein contained only His, Lys, Phe, Tyr, and Glu, the latter being partially in the amide (Gln) form. Glu and Gln were the principal free AA in most animal food products, followed by Lys, Phe, Leu, and Tyr.

The plant foods contained 13.8–71.3 μ M free AA/mg NPN (Table III), which was similar to the range among animal foods (Table II), with lettuce, potato, tomato, banana, and beet having the highest concentrations. Glu, Asp, and Arg were the major free AA in most plant food products, including Gln and Asn, while Thr and Ser were important in several products but absent in others. Phe constituted 58% of the total free AA in potato, and apple had only four free AA. Tkachuk (1979) has reported that stage of maturity and germination have a marked effect on free AA in wheat. The present wheat sample, in fully mature and dormant condition, contained only 10 identifiable free AA of which Asp/Asn, Arg, Phe, and Glu/Gln predominated. Tkachuk (1979) found that Asn/Asp, Glu, Try, and Arg predominated in a sound wheat sample.

It should be noted that many compounds were separated by column chromatography of the nonhydrolyzed NPN, and no attempt was made to identify substances other than the standard 16 protein AA. Interfering peaks of unknown compounds made quantitation of Cys and Try impossible, and there was poor separation of Thr, Tyr, and Glu in certain samples.

Amino Acids in Acid Hydrolysates. Acid hydrolysis of the NPN in the ultrafiltrate improved separation of the AA, but sulfur-containing AA and Tyr were destroyed. Total AA in animal food products were nearly 3 times greater than the free AA values, ranging from 49.5 to 125.7 μ M/mg of NPN (Table IV). Amide N values also increased in proportion to the higher Asp and Glu values. The major AA in the animal products were Ala, Lys, Glu/Gln, and His, which differed in part from the most prominent in nonhydrolyzed NPN (Table II).

Total AA in the acid hydrolysates of the plant ultrafiltrates (Table V) were only twice the levels of free AA (Table III), indicating that peptides provided about half of the AA. Aspartic acid and Glu, partially in the amide

form, were the principal AA in most plant ultrafiltrates, with Pro and Val also being important in the cereal hydrolysates. Note that the high concentrations of Thr in free AA of beet, lettuce, cabbage, and tomato (Table III) were largely destroyed by the hydrolysis procedure (Table V). Similar AA distributions have been reported for hydrolyzed NPN in corn (Christianson et al., 1965), field pea (Holt and Sosulski, 1981), potato (Kapoor et al., 1975), and carrot (Alabran and Mabrouk, 1973).

The amino acid N (AAN) contents of the acid-hydrolyzed NPN were calculated for each food product (Tables IV and V). The AAN as percent of NPN constituted 10.0–30.4% of animal foods (Table I). In plant food products the AA portion of NPN also represented 11.1% (carrot) to 32.8% (sorghum) of total NPN. Thus, the major portion of the high levels of NPN in this wide range of food products was NAAN (Table I).

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LITERATURE CITED

- Alabran, D. M.; Mabrouk, A. F. Carrot flavor. Sugars and free nitrogenous compounds in fresh carrots. *J. Agric. Food Chem.* **1973**, *21*, 205–208.
- Alexander, J. C.; Elvehjem, C. A. Isolation and identification of nitrogenous components in meat. *J. Agric. Food Chem.* **1956**, *4*, 708–711.
- AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.
- Bailey, J. L. *Techniques in Protein Chemistry*; Elsevier: Amsterdam, 1967.
- Bell, P. A. A critical study of methods for the determination of nonprotein nitrogen. *Anal. Biochem.* **1963**, 443–451.
- Bhatty, R. S.; Sosulski, F. W.; Wu, K. K. Protein and nonprotein nitrogen contents of some oilseeds and peas. *Can. J. Plant Sci.* **1973**, *53*, 651–657.
- Buckholz, L. L., Jr. The role of Maillard technology in flavoring food products. *Cereal Foods World* **1988**, *33*, 547–551.
- Chandra, S.; Mondy, N. I. Effect of foliar application of auxin on the quality of potatoes. *J. Food Sci.* **1981**, *46*, 1870–1873.
- Christianson, D. D.; Wall, J. S.; Cavins, J. F. Nutrient distribution in grain. Location of nonprotein nitrogenous substances in corn grain. *J. Agric. Food Chem.* **1965**, *13*, 272–276.
- Fan, T. Y.; Sosulski, F. W. Dispersibility and isolation of proteins from legume flours. *Can. Inst. Food Sci. Technol. J.* **1974**, 256–259.
- Gardner, G. A.; Stewart, D. J. Changes in the free amino and other nitrogen compounds in stored beef muscle. *J. Sci. Food Agric.* **1966**, *17*, 491–496.
- Hodgekiss, W.; Jones, N. R. The free amino acids of fish. *Biochem. J.* **1955**, *61*, 4–5.
- Holt, N. W. Amino acid and nonprotein nitrogen contents of grain legumes. Ph.D. Dissertation, University of Saskatchewan, SK, 1976.
- Holt, N. W.; Sosulski, F. W. Nonprotein nitrogen contents of some grain legumes. *Can. J. Plant Sci.* **1981**, *61*, 515–523.
- Irwin, M. I.; Hegsted, D. M. A conspectus of research on protein requirements of man. *J. Nutr.* **1971**, *101*, 385–429.
- Kapoor, A. C.; Desborough, S. L.; Li, P. H. Extraction of nonprotein nitrogen from potato tuber and its amino acid composition. *Potato Res.* **1975**, *18*, 582–587.
- Khan, A. W.; van den Berg, L. Some protein changes during post-mortem tenderization in poultry meat. *J. Food Sci.* **1964**, *29*, 597–601.
- Kies, C. Nonspecific nitrogen in the nutrition of human beings. *Fed. Proc.* **1972**, *31*, 1172–1177.
- Tkachuk, R. Calculation of the nitrogen-to-protein conversion factor. In *Nutritional Standards and Methods of Evaluation for Food Legume Breeders*; Hulse, J. H., Rachie, K. O.,

Billingsley, L. W., Co-chairmen; International Development Research Centre: Ottawa, ON, 1977; pp 78-82.
 Tkachuk, R. Free amino acids in germinated wheat. *J. Sci. Food Agric.* 1979, 30, 53-58.
 Walstra, P.; Jenness, R. Outline of milk composition and struc-

ture. *Dairy Chemistry and Physics*; Wiley: New York, 1984; Chapter 1.

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Nucleic Acid Nitrogen of Animal and Plant Foods

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Meat products contained greater concentrations of nucleic acid nitrogen (NAN) (0.35-0.56 mg/g) than dairy and egg products (0.05-0.15 mg/g) but, relative to product nitrogen, the compositions were less than 0.5%. The intermediate levels of NAN in cereals and field pea (0.22-0.51 mg/g) represented 1.4-2.5% of total nitrogen. Leafy vegetables, roots, tuber, and fruits contained 0.20-0.73 mg/g of NAN, which constituted 1.3-9.6% of total nitrogen. A highly significant correlation ($r = -0.63^{**}$) between NAN content (mg/g of N) and product nitrogen concentration should aid in prescription of dietary limits on intake of purines and in calculation of nitrogen to protein conversion factors.

Purines and pyrimidines of nucleic acids are not required in the animal diet and can be synthesized in vivo (Lehninger, 1982). But there is ample evidence that dietary nucleic acids are hydrolyzed to nucleosides and free bases in the intestinal mucosa and fluids for absorption and transformation into nucleoproteins and other metabolites. DNA and RNA contain approximately 14% nitrogen and constitute an important factor in the determination of nitrogen to protein conversion factors (Benedict, 1987; Imafidon, 1982).

In man, the purine portion of nucleic acids has low solubility at physiological pH and, at high concentrations, is poorly excreted by the urinary system. High levels in serum can result in urate crystal formation in the tissues and joints (Clifford et al., 1976; Pachla et al., 1987). Several investigations (Bowering et al., 1969; Clifford and Story, 1976; Waslien et al., 1968) suggest that the maximum safe limit of RNA in the diet is 2 g/day. Therefore, accurate quantification of nucleic acids levels in food products would enhance our understanding of nucleic acid intakes and provide better guidelines for recommending safe maximum levels of nucleic acids (purines) in human diets (Young, 1980).

The purpose of this investigation was to quantitate the total nucleic acid content in a wide range of food products commonly consumed in significant amounts.

MATERIALS AND METHODS

The animal and plant products except casein (ANRC reference protein, obtained from the Sheffields Co., Norwich, NY) were purchased from each of two local commercial outlets in Saskatoon, SK. The standard RNA and DNA were purchased from Sigma Chemical Co., St. Louis, MO.

Moisture and total nitrogen were determined by the standard procedures (AOAC, 1984) with the exception that, in the micro-Kjeldahl method for total nitrogen, a 100:3 mixture of K_2SO_4 and $CuSO_4$ was substituted for K_2SO_4 and mercuric oxide, as applied in AOAC Method 7.033, to achieve a boiling temperature of 345 °C for the 1-h digestion period. Crude fat was determined by AACC Official Method 30-25 on freeze-dried samples to overcome, in part, the problems associated with petro-

leum ether extraction of lipids from animal products (AOAC, 1984). The crude fiber content of defatted samples was determined by AACC Method 32-10 (AACC, 1983), and AACC Method 08-01 was employed for ash analysis.

Total nucleic acid nitrogen (NAN) of the food products was determined as follows. One hundred milligrams of the respective samples and 10 mL of cold 10% TCA were stirred in 50-mL centrifuge tubes in an ice water bath for 10 min. After 15 min of centrifugation at 20000g at 0 °C, the supernatant was decanted. The precipitate was washed twice with hot ethanol to remove impurities that may absorb in the UV range 200-300 nm. Each wash was followed by centrifugation at 20000g for 10 min. The residual nucleic acids were hydrolyzed with 15.0 mL of 5% TCA for 25 min at 90 °C in centrifuge tubes, capped with perforated plastic stoppers designed to minimize evaporation. The samples were cooled to 5 °C and centrifuged at 20000g and 0 °C to precipitate colloidal starch. Finally, the samples were scanned from 220 to 300 nm on a Perkin-Elmer double-beam spectrophotometer (Coleman Model No. 128). Regression analysis of the UV absorbance of the standard RNA/DNA solutions at a range of concentrations yielded the following equation (Holt, 1976):

$$\text{NAN (mg/mL)} = \text{absorbance} \times \frac{1}{0.02866} \times 0.148 \times 10^{-3}$$

RESULTS AND DISCUSSION

The food products selected for the present investigation represented a wide range in proximate composition (Table I). On a dry basis, protein contents ranged from 1.3% (apple) to 85.9% (casein) and crude fat from 0.2% (banana) to 47.5% (cheese). Crude fiber and ash levels varied from 0.8 to 0.4%, respectively, in polished rice and 8.6 to 10.2%, respectively, in tomato.

The concentrations of NAN in food products and in proportion to their nitrogen concentrations are presented in Table II. The meat and fish products were rich in protein (11.0-12.8% N) and NAN (0.347-0.558 mg/g) although the NAN concentrations, relative to total nitrogen, were only 0.3-0.5%. Arasu et al. (1981) reported that beef muscle contained 1.77 mg of total nucleic acid/g of fresh sample, which would correspond to 0.62 mg of NAN/g of sample, assuming 60% moisture and 14% nitro-