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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 petroleum 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 20000*g* 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 20000*g* 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 20000*g* 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-

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Table I. Composition of Proximate Constituents in Several Classes of Food Products (% Dry Basis)[a]

food product by class	moisture	crude protein, N × 6.25	crude fat	crude fiber	ash
		Meat Products			
chicken muscle	1.4[b]	68.5	28.2	0.4	3.7
beef steak	4.2[b]	76.5	10.9	1.5	4.0
fish fillet (perch)	3.7[b]	80.0	12.0	0.5	7.6
		Dairy Products and Egg			
whole milk	4.1[b]	25.9	18.6	1.1	5.8
cheese	2.0[b]	40.3	47.5	0.3	6.4
whole egg	1.2[b]	48.4	36.8	0.2	3.9
casein	9.2	85.9	0.1	0.8	1.7
		Cereals and Pulse			
polished rice	12.1	5.9	0.3	0.8	0.4
whole corn	10.1	9.8	3.6	1.5	1.4
whole wheat	11.9	12.0	1.7	2.7	1.5
whole sorghum	9.2	12.4	2.7	2.0	1.5
field pea	10.0	21.6	1.0	6.4	2.4
		Leafy Vegetable			
lettuce	4.4[c]	16.0	1.0	8.6	6.3
cabbage	4.1[b]	15.0	0.7	7.6	6.4
		Roots and Tuber			
carrot	7.0	3.9	2.7	5.4	5.8
beet	6.6[b]	10.6	0.4	4.8	7.4
potato	4.6[b]	13.0	0.4	2.9	4.6
		Fruits			
apple	5.8[b]	1.3	1.2	6.0	1.8
banana	5.7[b]	4.6	0.2	1.7	3.3
tomato	10.0[b]	12.4	2.1	8.6	10.2
CV, %	1.5	0.8	0.2	1.4	0.2

[a] Values are means of duplicate analyses. [b] Freeze-dried before analysis.

Table II. Concentrations of NAN in Food Products and in Proportion to Their Nitrogen Contents, Dry Matter Basis

food product by class	N, %	nucleic acid nitrogen (NAN) mg/g sample	mg/g N[a]	% total N
		Meat Products		
chicken	11.0	0.558	5.2 ± 0.00	0.5
beef	12.2	0.439	3.6 ± 0.04	0.4
fish	12.8	0.347	2.7 ± 0.01	0.3
		Dairy Products and Egg		
milk	4.1	0.151	3.7 ± 0.15	0.4
cheese	6.4	0.108	1.7 ± 0.00	0.2
egg	7.7	0.050	0.6 ± 0.05	0.1
casein	13.7	0.130	0.9 ± 0.01	0.1
		Cereals and Pulse		
rice	0.9	0.221	24.6 ± 0.20	2.5
corn	1.6	0.332	21.1 ± 0.00	2.1
sorghum	2.0	0.423	21.1 ± 0.10	2.1
wheat	1.9	0.403	21.2 ± 0.10	2.1
field pea	3.5	0.507	14.6 ± 0.10	1.4
		Leafy Vegetables		
lettuce	2.6	0.728	28.0 ± 0.01	2.8
cabbage	2.4	0.654	27.3 ± 0.02	2.7
		Roots and Tubers		
carrot	0.6	0.426	67.1 ± 0.00	6.7
beet	1.7	0.551	32.4 ± 0.00	3.2
potato	2.1	0.252	12.6 ± 0.19	1.3
		Fruits		
apple	0.2	0.201	95.9 ± 0.00	9.6
banana	0.7	0.506	70.4 ± 0.15	7.0
tomato	2.0	0.278	13.9 ± 0.36	1.4

[a] Values are means ± SE.

gen. Clifford and Story (1976) obtained RNA concentrations of 2.03–3.43 mg/g for several fish species, which was in the range of the present samples.

Although lowest in protein content among the dairy products, the NAN concentration of 0.151 mg/g resulted in 3.7 mg/g of N in milk, similar to that of beef (Table II). Eggs and casein were particularly low in NAN concentration. The NAN value for milk obtained in this study was within the range of values reported by Wastra and Jenness (1984).

Compared to the animal products, the grains contained high concentrations of NAN (0.221–0.423 mg/g) relative to their lower range in protein contents (Table II). Thus, the range in NAN content was 21.1–24.6 mg/g of N for the four cereal grains, with field pea having a value 14.6 mg/g of N. Holt and Sosulski (1981) previously reported that 17 lines of field peas contained an average of 27.4 mg of NAN/g of sample.

The leafy vegetables contained the highest concentrations of NAN in the present study: 0.728 mg/g for lettuce and 0.654 mg/g for cabbage (Table II). Because of their intermediate protein contents, these values represented 27–28 mg/g of N or 2.7–2.8% of total leaf nitrogen. The carrot, beet, and banana also contained high concentrations of NAN, between 0.426 and 0.551 mg/g of sample, which constituted 6.7, 3.2, and 7.0%, respectively, of total plant nitrogen. The 9.6% NAN in total nitrogen in apple was likely the result of a low tissue nitrogen content of 0.2%.

For the 20 samples, the correlation of NAN (mg/g of N) contents with product nitrogen concentrations (N, %) was negative and highly significant: $r = -0.63^{**}$. Particularly within classes of food product, there was a marked decrease in NAN content of the total nitrogen as nitrogen concentration in the product increased (Table II). Since the ranges in total nitrogen and NAN content differed greatly among classes of food product, the correlation value was of lower magnitude than would occur if only one class of food product were investigated. Knowledge of the specific relationship of NAN content to total protein for each class of food product would aid in the prescription of dietary intakes of purines and in the calculation of nitrogen to protein conversion factors.

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Essential Oil Constituents of *Ocimum micranthum* Willd.

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The essential oil from the leaves, flowers, and stems of *Ocimum micranthum* Willd., a strongly aromatic annual herb used as a local beverage and medicinal plant and native to the lowlands of Central and South America and the West Indies, has for the first time been examined. The essential oil content between plant parts varied significantly with 1.54, 0.63, and 0.08 (percent volume/fresh weight) yield from the leaves, flowers, and stems, respectively. Twenty compounds in the essential oil were identified: 1,8-cineole, eugenol, β-caryophyllene, β-selinene, and elemene isomers were found to be the major constituents. Essential oil composition also varied by plant part. Eugenol, the major constituent in leaves, was present only in trace amounts in flowers and stems. β-Selinene, a minor component in the leaves, was a major constituent in the flowers and stems. Total sesquiterpenes accounted for 48.4, 85.8, and 78.5% of the oil in the leaves, flowers, and stems, respectively. This is the first report of an *Ocimum spp.* to be high in elemenes, 1,8-cineole, β-caryophyllene, and β-selinene.

Ocimum micranthum Willd., a strongly aromatic annual herb native to the lowlands of Central and South America and the West Indies, is used locally to flavor beverages and soups (Morton, 1981). It is also used in domestic medicine for treating colds, fever, stomach disturbances, and dysentery and as a remedy for screwworms parasitizing nasal passages of people in the tropics (Morton, 1981; Standley and Williams, 1978). A decoction of the plant is also used to kill the larvae. The plant is locally used in the treatment of epilepsy, nervous trouble, and earaches, as a remedy for influenza, colic, and convulsion in children, and for painful menstruation (Morton, 1981).

A large part of the aroma and flavor of this plant is due to the presence of essential oils, some constituents of which have also been shown to have biological activity and could be responsible for the plant's use in traditional medicine. While the constituents in the essential oils of *Ocimum basilicum* have been reviewed (Guenther, 1949; Lawrence et al., 1971; Simon et al., 1984), the essential oils of other *Ocimum* species have not been as extensively studied. Many *Ocimum spp.* have been selected and bred for specific essential oil constituents for use in flavoring and perfume products (Sobti et al., 1982a–c). Lesser known yet highly aromatic plants such as *O. micranthum* could serve directly as natural plant sources for specific natural products or used in interspecific hybridizations. Thus, the objective of this study was to determine the essential oil content and composition extracted from fresh leaves, flowers, and stems from *O. micranthum* Willd. in order to evaluate its potential use as a source of aroma chemicals.

MATERIALS AND METHODS

Plant Materials. Seeds of *O. micranthum* Willd. were obtained from Companion Plants (Athens, OH) where it was listed commercially as Peruvian basil. Seeds were sown in the greenhouse and transplanted into the field at the Purdue University Vegetable Research Farm (Oakley silt loam soil) during the summer of 1987. The entire plants were harvested in full-bloom (in October), weighed, and leaves, flowers, and stems immediately separated. Essential oils were then extracted from each of three 50-g samples of fresh leaves, flowers, and stems. Another set of three 50-g samples of leaves, flowers, and stems was dried in an oven at 35 °C and essential oil extracted from the dried plant material. A dried plant specimen was deposited in the Herbarium of the Field Museum of Natural History, Chicago, IL.

Essential Oil Extraction and Isolation. Essential oil was extracted by hydrodistillation for 1 h (fresh samples) and 1 h 15 min (dry samples) with a modified clevenger trap (ASTA, 1968). The essential oil content was determined on a volume to fresh weight or dry weight basis. The values for essential oil content of the three replications were averaged and standard deviations calculated. The essential oil samples were stored in silica vials with Teflon-sealed caps at 2 °C in the absence of light.

Gas Chromatography. Essential oil samples from each of the distillations were analyzed separately and the relative peak areas for individual constituents averaged for each plant part.

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