Nitrate and Nitrite Content of Some Fresh and Processed Market Vegetables

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The nitrate and nitrite contents of 34 vegetables, fresh and processed, as available at retail markets are reported. Samples included 17 fresh, 23 frozen, and 14 canned vegetables. Nitrate values in general were in the ranges expected from literature values. The nitrite values for vegetables available in the market, many reported here for the first time, are reassuringly low. Ranges found were 0.2–6.0 ppm of nitrite for fresh, 0.4–6.1 for frozen, and 0.2–0.7 for canned vegetables. The tenfold higher nitrite values reported by Richardson in 1907, and still cited, are not confirmed for vegetables produced and processed under today's conditions.

Until recently, concern over the amounts of nitrate and nitrite in our diet has been due to the relationship between nitrate and nitrite, and infant methemoglobinemia. Currently, the role of nitrites in formation of the carcinogenic nitrosamines has led to some public apprehension regarding the nitrite content of our food. These interrelationships have recently been reviewed by Phillips (1971), Wolff and Wasserman (1972), Fassett (1973), Aune (1972), and the National Research Council (1972). Intensive research attention is being devoted to the nitrosamine problem.

Increased use of chemical fertilizers, widespread adoption of feedlot systems for meat production, and continuing problems in disposal of wastes combine to bring increasing amounts of nitrate into the environment (National Research Council, 1972).

To evaluate amounts of nitrate and nitrite presently entering our diet, recent data on their levels in U.S. dietary components are needed. Richardson (1907) calculated that vegetables provided the major portion of nitrate in the U.S. diet at that time. Jackson et al. (1967) found no difference in nitrate content between 1964 crops and those analyzed by Richardson. Processed meat has generally been considered to be the major source of nitrite (Fassett, 1973; Wolff and Wasserman, 1972), but there is very little information available on the nitrite content of vegetable foods.

Some attention has been given to the influences of variety, fertilization, and other agronomic factors on nitrate content of such U.S. vegetable crops as beets, spinach (Lee et al., 1971; Barker et al., 1971; Peck et al., 1971), potatoes (Heisler et al., 1973), tomatoes (Luh et al., 1973), and lettuce, mustard, collards, cabbage, snap beans, beets, corn, tomatoes, and peppers (Splittstoesser et al., 1974). A relatively great amount of data was generated in a large-scale cooperative study of detinning of cans by tomatoes, carrots, green beans, and spinach (Farrow et al., 1969). In that work, the influence of agronomic and environmental effects on nitrate accumulation and resulting can detinning was studied. The distribution of nitrate content of 373 samples of processed tomatoes from four cooperators over two seasons is given as ranging from 0 to 109 ppm of NO_3^-, with 78% below 30 ppm; similar information is presented for the other vegetables.

While such data are needed in studying the factors controlling nitrate accumulation in vegetables, they are not greatly useful for estimating the amounts of nitrate and nitrite in the United States diet. For this purpose it is more appropriate to examine analytical data on vegetables and vegetable products actually available in the consumer market. Data of this type are available only for nitrate, having been reported by Richardson (1907) for 25 vegetables, mostly fresh, by Wilson (1949) for 9 vegetables, fresh and frozen, and by Jackson et al. (1967) for 34 fresh and processed vegetables. Viets and Hageman (1971) pointed out the "paucity of data on the nitrate content of human foods"; since then, Maynard and Barker (1972) published data for 12 fresh vegetables. All reported for their local markets. Heisler et al. (1973) reported nitrate and nitrite values for fresh potatoes from five representative markets in the United States.

The situation with respect to data on nitrite content of vegetables available at the market is far less reassuring. Except for the report on potatoes, only two papers contain data on nitrite content of vegetables. Richardson (1907) gave rather high values (30–76 ppm) for four of five vegetables; Lee et al. (1971) have reported the following results for locally purchased canned vegetables: four samples of beets ranged from a trace to 3.9, and two samples of spinach averaged 24.1 ppm of NO_2^-.

Nitrite ion is the form implicated in toxicity to humans, and since infants are at far greater risk of methemoglobinemia than adults, virtually all of the nitrite data that are available are for prepared baby foods. Since these appear to have been adequately studied (Phillips, 1968a, 1969, 1971; Kamm et al., 1965; Fogden and Fogden, 1969; Achtzehn and Hawat, 1969; Lee et al., 1971; National Research Council, 1972), they have not been included in this study.

Table I. Nitrate and Nitrite Content of Fresh Vegetables

	ppm, fresh wt		
Vegetable	Nitrate	Nitrite	pH
Cabbage	784	0.5	6.48
Celery	1600	0.5	6.08
	2390	0.4	6.20
	2670	0.5	6.19
Cucumber	24	0.5	6.20
Eggplant	302	0.5	5.17
Endive	663	0.5	5.88
Lettuce, iceberg	1100	0.4	6.15
	1130	0.3	5.88
Lettuce, romaine	1400	0.4	6.16
Mushrooms	63	0.8	6.48
Peppers, sweet green	62	0.4	5.52
Radishes, red	2400	0.2	6.11
	3000		6.09
Squash, acorn	34	0.4	6.60
butternut	678	0.4	6.08
zucchini	665	0.6	6.30
Salad, mixed	819	0.7	6.20

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Table II. Nitrate and Nitrite Content of Selected Fresh Vegetables

		ppm, fresh wt				
		Nitrate		Nitrite		
Vegetable	No. samples	Mean	Std dev	Mean	Std dev	pH range
Beets	12	3010	688	6.0	4.6	5.72–6.46
Carrots	8	72	56	0.6	0.5	5.85–6.41
Spinach	7	2220	375	0.7	0.3	6.13–6.42

Table III. Nitrate and Nitrite Content of Processed Vegetables

			ppm, fresh wt				
			Nitrate		Nitrite		
Vegetable	No. samples	No. brands	Av	Std dev	Av	Std dev	pH range
			Frozen				
Artichoke	2	1	12	1.4	0.4	0.1	5.12–5.19
Asparagus spears	6	6	16	9.0	0.9	0.5	6.32–6.52
Beans, green	4	3	270	41.2	0.9	0.7	6.08–6.22
Beans, lima	4	4	27	9.8	1.1	0.6	6.60–6.82
Broccoli, spears	6	6	464	171	1.0	0.5	6.45–6.55
Broccoli, chopped	4	4	573	164	1.0	0.4	6.35–6.55
Brussels sprouts	7	4	84	65.9	1.0	0.5	6.13–6.53
Carrots	5	4	97	39.1	1.0	0.5	6.13–6.43
Cauliflower	5	5	254	56.2	1.1	0.7	6.58–6.92
Collard greens	4	2	2450	1300	1.7	0.5	6.17–7.00
Corn	3	3	45	14.8	2.0	0.3	7.06–7.28
Kale	3	2	2770	750	1.8	1.6	6.63–6.74
Mustard greens	4	3	2390	868	1.6	1.2	6.45–7.10
Okra (whole)	3	3	74	34.0	0.7	0.1	6.32–6.43
Onions, chopped	1	1	33		1.0		5.30
Onions, whole	1	1	128		0.4		5.63
Pea pods, chinese	2	2	13	1.4	0.6	0.2	6.02–6.03
Peas, blackeye	2	1	9	0.7	2.6	0.3	6.75–6.85
Peas, green	4	4	20	6.5	0.7	0.2	6.50–6.92
Peppers, sweet	3	2	50	40.4	0.7	0.3	4.90–5.38
Potatoes, hash brown	2	1	37	1.4	0.7	0.1	6.15–6.28
Potatoes, small whole	1	1	150		0.8		6.18
Spinach	4	3	2140	283	6.1	7.7	6.77–6.90
Squash	2	1	160	20.5	0.9	0.1	6.45–6.55
Turnip greens	3	2	3460	358	4.4	3.0	6.70–7.06
Zucchini	4	3	533	146	1.0	0.4	6.28–6.53
			Canned				
Asparagus	1	1	3		0.2		6.1
Beans, green	5	5	100	68.4	0.2	0.1	6.0–6.3
Beets	4		1450	249	1.8	0.6	5.20–5.34
Carrots	18	9	205	129	1.1	0.4	4.70–5.48
Collard greens	2	1	2640	856	0.2	0	5.7–6.2
Kale	3	2	1600	871	0.2	0	5.8–7.0
Mushrooms, sliced	1	1	6		0.2		7.0
Mushrooms, whole	1	1	17		0.2		6.7
Mustard greens	2	1	1360	334	0.3	0.1	5.8–6.2
Okra	1	1	2		0.7		4.5
Peas	3	3	6	1.9	0.4	0.3	7.0–7.1
Potatoes, whole	2	2	63	14.1	0.5	0.4	6.6–6.7
Potatoes, sliced	1	1	69		0.4		6.4
Sauerkraut	1	1	68		0.4		4.2
Spinach	1	1	573		0.7		
Turnip greens	2	2	2230	541	0.2	0.1	6.2

A related matter is the possible accumulation of nitrite during marketing or storage of fresh high-nitrate vegetables. Sixteen cases of infant methemoglobinemia in Germany were traced to feeding of improperly stored home-prepared spinach puree which had developed over 1000 ppm of nitrite (Phillips, 1968b). Fresh spinach and beets are known to accumulate appreciable amounts of nitrite during extended (abusive) storage (Phillips, 1968a; Keybets et al., 1970; Heisler et al., 1974).

The primary objective of the work reported herein was to obtain information on the nitrite content of fresh and processed vegetables purchased at retail markets. Nitrate values were also obtained. We report nitrate and nitrite contents for 34 vegetables obtained at retail markets. These included 17 fresh, 23 frozen, and 14 canned. In addition, special attention was given to fresh spinach, beets, and carrots, and the effect of refrigerated storage on the nitrate and nitrite contents of lettuce, a commonly stored high-nitrate vegetable, was examined.

MATERIALS AND METHODS

Samples and Preparation. All samples were purchased from local supermarkets, with as wide a representation of brand names as practicable. Fresh vegetables were obtained in December 1973 and January 1974 and were representative in quality of those available at that season. Fresh vegetables were analyzed either on the day of purchase or refrigerated 1 day until analysis. Only the solids of canned products were analyzed. Frozen products were kept frozen until analysis.

Samples were disintegrated with an equal weight of water in a Waring Blendor for about 5 min until homogeneous. A 50-g sample of the blend was stirred for 15 min in a beaker with 75 ml of water, its pH was determined with a Beckman Expandomatic SS-2 meter, it was then centrifuged 15 min at 900*g*, and the supernatant was filtered and analyzed for nitrate and nitrite content.

Nitrate Analysis. The method of Lipp and Dölberg (1964) as modified by Heisler et al. (1973) was used; details may be found in the latter paper. A 5-ml sample of the filtrate was used in most cases, with appropriately smaller samples for high-nitrate vegetables.

Nitrite Analysis. The method using sulfanilamide and *N*-(1-naphthyl)ethylenediamine hydrochloride exactly as described by Fudge and Truman (1973) was applied to extracts clarified as follows.

For nitrite analysis, 25 ml of the filtrate was pipetted into a 200-ml volumetric flask and 50 ml of $Al(OH)_3$ suspension (American Public Health Association, 1965) was added to decolorize; the flask was made to volume with water, shaken, let stand 30 min, and filtered through Whatman No. 1 paper.

To analyze the largest possible sample because of low nitrite levels, 20 ml of the filtrate was placed in a 50-ml volumetric flask, 7 ml of reagents was pipetted in, and the mixture was made to volume with the filtrate, providing a sample aliquot of 43 ml.

All analyses were carried out in duplicate and triplicate and are expressed as parts per million of nitrate or nitrite, fresh weight. The minimum detectable concentration of nitrate was 5 ppm; of nitrite, 0.2 ppm.

RESULTS AND DISCUSSION

Results of analyses of 14 fresh vegetables available in local supermarkets during December 1973–January 1974 are given in Table I. Several samples of high-nitrate vegetables were analyzed. In Table II are given more extensive results for the high-nitrate vegetables beets and spinach. A recent report of methemoglobinemia ascribed to ingestion of juice of fresh carrots (Keating, 1974) prompted a more extensive examination of carrots.

Nitrate data for vegetables previously studied are similar to values in the literature. In no case was the nitrite content of fresh vegetables as purchased over 100 ppm, or high enough for concern; only one, beets, consistently contained between 1 and 10 ppm of nitrite.

Since lettuce is commonly stored under refrigeration for several days, a sample was so stored and analyzed at 0, 3, 5, and 12 days, at which time the sample was slightly discolored but considered edible. At that point the nitrate content had declined from 1130 to 940 ppm, while nitrite never exceeded 0.9 ppm.

As noted above, very few data are available for the nitrite content of fresh United States vegetables. Lee et al. (1971) in fertilizer response studies report a range for beets of 3–6.6 ppm and for spinach of 1.6–2.0 ppm. Richardson's early values are considerably higher: celery, 33; lettuce, 76; turnips, 4; radish, 76; and beets, 59 ppm of NO_2^- fresh weight. Russian data (Rooma, 1971) for ten vegetables (including beets, not spinach) are all less than 1.4 ppm. The data in Table II for beets agree with the more recent values of Lee, rather than those of Richardson; those for carrot (Table II), lettuce, celery, and radish (Table I) are similar to Rooma's data, rather than the considerably higher values of Richardson.

Table III lists nitrate and nitrite contents for 26 frozen vegetables and 16 types of canned vegetables. Included are 89 samples of the former (71 brand name–vegetable combinations) and 48 samples of the latter (32 brand name–vegetable combinations). The pH range of the samples is also shown. Nitrate values are those expected from previous work: leafy vegetables (collard, kale, mustard, spinach, turnip) are highest; legumes are generally low; roots, fruits, stems, and inflorescences are moderate.

Results for canned products are generally lower than for corresponding frozen products, probably reflecting the distribution of nitrate between the solid and the unanalyzed liquid portion (Jackson et al., 1967). None of the nitrite values for processed vegetables exceeded 2.6 ppm, except for frozen spinach (6.1) and frozen turnip greens (4.4); most were 1.0 or lower.

Values found for carrots in fresh, frozen, or canned form, a total of 31 samples, were in general agreement with previously recorded data (Maynard and Barker, 1972; Jackson et al., 1967; Herrmann, 1972). The nitrate levels found for carrots are, at most, a tenth of those commonly reported for spinach, which has been implicated in infantile methemoglobinemia. Thus, no obvious explanation is apparent for the case reported by Keating (1974).

On the basis of the data presented herein, there need be no cause for concern regarding the nitrite content of commercial fresh, frozen, or canned vegetables as available to the consumer, but it must be kept in mind that prolonged storage of opened, thawed, cooked, or uncooked vegetables or their storage under improper conditions may lead to conversion of part of their nitrate to nitrite. No unexpectedly high values for nitrate were encountered for the fresh and processed market vegetables as analyzed in this study, without additional storage after opening or thawing for analysis. Under certain abusive storage conditions, nitrite may accumulate but nitrosamines were not detected (Heisler et al., 1974).

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Amino Acid Composition of the Morel Mushroom

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Freeze-dried samples of the morel mushroom (*Morchella spp.*) as well as other genera were hydrolyzed with hydrochloric acid and the free amino acids isolated by ion exchange chromatography. The amino acids were derivatized with *n*-propyl alcohol and heptafluorobutyric anhydride. The volatile *N*-heptafluorobutyl-*n*-propyl derivatives were separated and quantitatively determined by gas chromatography. Seventeen amino acids were identified as major components in most species. The method of analysis did not permit the identification of tryptophan, although a few unidentified minor components were detected. Variation of composition as a function of species, genus, and sample source is discussed as well as the efficacy of the gas chromatographic technique.

The morel mushroom (*Morchella spp.*) is avidly sought by connoisseurs throughout the world. Because of its delicate flavor and nutritional potential as a protein source (Litchfield et al., 1963a; Litchfield, 1967a) morel mycelia have been grown in submerged culture on a commercial scale while fruiting bodies of the morel have so far resisted efforts toward cultivation.

Previous investigators have reported marked variances in chemical composition among samples of cultured mushroom mycelium as a function of growth conditions and nutrients (Litchfield et al., 1963a; Litchfield, 1967b).

In the case of the cultivated mushroom, *Agaricus bisporus*, alanine, ammonia, and arginine contents of the tissue decreased, and the aspartic acid content increased when gelatin was added to the compost (Kissmeyer-Nielsen et al., 1966). However, when hydrolyzed casein was added to the compost, the arginine content decreased. There is a lack of information in the literature on the chemical composition of mushrooms as affected by environmental conditions in natural habitats.

While morel mushrooms are easy to recognize in the field (Boudier, 1897), distinguishing between various species and varieties is difficult and is a matter of some uncertainty (Gilbert, 1960; Imbach, 1968). The amino acid composition of morels might provide an improved means for identifying these organisms as well as provide an index of protein quality and flavor characteristics.

Previous studies have determined qualitatively (Litchfield et al., 1963b; Litchfield, 1967b; Janardhanan et al., 1970) and quantitatively (Litchfield et al., 1963b) the amino acid pattern in cultured morel mycelium, while Hatanaka and Terakawa (1968) have reported the qualitative examination of morel fruiting bodies for nonprotein amino acids and recently Hatanaka (1969) has isolated a new amino acid, *cis*-3-aminoproline, from three morel species. The literature contains no quantitative data that compare the amino acid composition of naturally occurring morels with that of the cultured mycelium.

Interest in gas chromatography as a reasonably inexpensive and rapid means of determining amino acid composition has increased with particular emphasis upon single column analysis. Gehrke and others have developed the derivatizing techniques enabling efficient separation (Gehrke et al., 1971). A recent study suggests that complete single column separation of 20 amino acids may be effected via the *N*-heptafluorobutyl-*n*-propyl derivatives (Moss et al., 1971).

The rapidity with which gas chromatographic techniques may be applied suggested the applicability of the single column technique to amino acid analysis of fungal tissue. This paper reports the results of such a study.

EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard Model 5750 gas chromatograph, equipped with a flame ionization detector, was used in all of the analyses. The recorder has an input of 0–5 mV and a chart speed of 0.25 in./min with a working sensitivity of 16×10^{-11} A. The column was a 3.65 m × 3 mm i.d. coiled glass column packed with 3% OV-1 on Chromosorb W (HP), 80–100 mesh. A detector temperature of 290° and an injector temperature of 270° were maintained throughout the investigation. The column

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