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Nitrate and Nitrite Levels in Fresh Spinach as Influenced by Postharvest Temperatures[1]

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Abstract. **Various postharvest temperature-time combinations on nitrate-nitrite conversion in fresh spinach (*Spinacia oleracea* L.) were investigated. At 0°C spinach could be held as long as 40 days without significant changes in these nitrogen components. Substantial loss of nitrate-N and accumulation of nitrite-N occurred in spinach held for 3 to 7 days at 20°. Simulated transit periods of 2 weeks at 0 and 5° and simulated marketing periods of 3 days at 10° were imposed separately and in sequence to spinach in pretransit storage for 15 hours at 21° or to spinach without a pretransit storage period. Accumulations of nitrite-N exceeding 2 ppm fresh weight were found only if the simulated transit period was 5°. In this instance pre- and posttransit storage further increased nitrite accumulation. Nitrite-N levels exceeding 10 ppm were found only in visibly decayed samples.**

The public health implications of nitrate and nitrite in our diet have been reviewed (9, 11, 13). Maynard et al. (8) have recently discussed the factors affecting nitrate accumulation in vegetables. Few studies have dealt with the influence of postharvest handling on the conversion of nitrate to nitrite in spinach. Schuphan (12) reported that increasing N fertilizer from 80 to 320 kg N/ha increased the nitrate-N in leaves at harvest to approx 790 ppm of fresh wt and the nitrite-N accumulated after 4 days of storage at 22-32°C to 108 ppm. Phillips (10) found no accumulation of nitrite-N in one series of spinach samples stored at 21° although nitrate-N fell to about 30% of its initial level during the first 4 days of storage and was practically depleted by the eighth day. In a second series of samples, the concn of nitrite-N began increasing immediately on storage at 21° exceeding 20 ppm on a fresh wt basis after 4 days while nitrate-N decreased. Refrigeration delayed the conversion of nitrate to nitrite. Lee et al. (7) found no conversion of nitrate to nitrite when fresh beets and spinach were stored at 2° regardless of N fertilizer applied. On the other hand, Heisler et al. (5) reported that the nitrite-N concn in shredded fresh spinach increased to approx 91 ppm after 14 days at 5°.

Some of the studies dealing with nitrate and nitrite changes in spinach after harvest have been limited to that purchased from supermarkets. Cultivar, preharvest growing conditions and postharvest handling conditions prior to the time the commodities were purchased were unknown (5, 10). These factors may explain variable results such as reported by Phillips (10) for the 2 series of spinach samples. The objective of this study was to evaluate the influence of postharvest temp and to some extent storage duration on nitrate and nitrite levels in fresh spinach. Treatments were included to simulate pretransit, transit and posttransit conditions which might be encountered in distribution and marketing.

Materials and Methods

'Early Hybrid 7' and 'Virginia Savoy' spinach were grown in the summer of 1975 at the Homer C. Thompson Vegetable Research Farm in Freeville, New York. Nitrogen was added to the soil at 0, 117 or 250 kg N/ha as ammonium nitrate. Two-thirds of the N fertilizer was broadcast at planting and the rest was sidedressed 2 weeks prior to harvest. Each treatment received 40 kg P/ha and 70 kg K/ha broadcast at planting. The plants were harvested at market maturity 42 days after planting. Initial samples of 120 g of intact spinach leaves with petioles attached were taken immediately after harvest, frozen in liquid N and stored at −18°C for subsequent chemical analyses. For each cultivar and N fertilizer treatment, 750 g of intact leaves were held in a glass bottle under humidified air with a flow rate that resulted in no more than 0.25% CO_2 in the effluent gas stream. In one experiment, spinach was held either at 0° for as long as 40 days or at 20° for up to 7 days. About 100 g of spinach leaves were removed from the bottles at intervals for chemical analyses. In a second experiment, spinach was held at 21° for 0 or 15 hr to simulate a delay between harvest and transit and was then transferred to either 0° or 5° for 2 weeks to simulate transit conditions. After the simulated transit treatment, the spinach was held at 10° for 0 or 3 days to simulate marketing. Spinach leaves were scored for visual quality on a 1 to 9 scale: 1 = extremely poor; yellowish-brown color, severe decomposition, not usable; 5 = fair; slight discoloration, lowest limit of sale appeal; 9 = excellent; completely green, no decay.

Nitrate in oven dried tissue was extracted with .025 M $Al_2(SO_4)_3$ solution and analyzed potentiometrically by the method of Baker and Smith (2) using an Orion Model 93-07 nitrate ion electrode. Nitrite was determined colorimetrically on homogenized frozen tissue by a modified Greiss-Ilosvay method (3). Total N in oven dried tissue was measured by a modified microKjeldhal procedure (1) that ensures reduction of nitrate. All chemical analyses were run in duplicate.

Results

The 2 spinach cultivars were similar in initial nitrate-N and total N concn and in the levels of nitrite-N accumulated in storage at the various temps. The average nitrate-N concn at harvest for the three N levels were 820 ppm for 'Early Hybrid 7' and 770 ppm for 'Virginia Savoy' on fresh wt basis. Therefore, the cultivars were considered 2 replications of the experiments and the data presented are the means of the cultivars. Nitrogen fertilization had no effect on the nitrate-N and total N concn in spinach leaves at harvest or the levels of nitrite-N produced in storage.

Effect of storage at 20° or 0°C. Spinach stored at 20° deteriorated rapidly and was considered unmarketable after 48 hr and unusable after 72 hr. Loss of nitrate-N was 40% of the initial level in the first 3 days of holding at 20°, thereafter, the loss was much slower (Fig. 1). The nitrite-N concn increased as the nitrate-N level decreased, reaching a maximum of 73 ppm of fresh wt by the third day and decreasing thereafter (Fig. 1). Essentially no change occurred in the total N concn in spinach held at 20° during the first 2 days of storage. However, a decrease in total N occurred as the spinach decayed with up to

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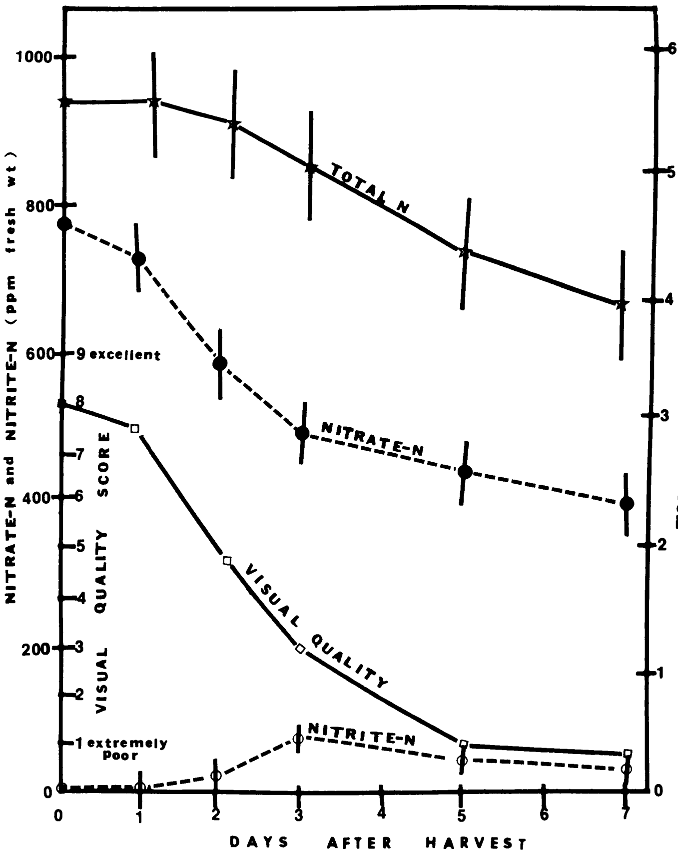

Fig. 1. Effect of holding time at 20°C on visual quality and the concn of total N, nitrate-N and nitrite-N in fresh spinach. Nitrogen fertilization had no significant effect; hence, values from 0, 117 and 250 kg N/ha are combined. Confidence intervals at 5% level are shown for total N, nitrate-N and nitrite-N.

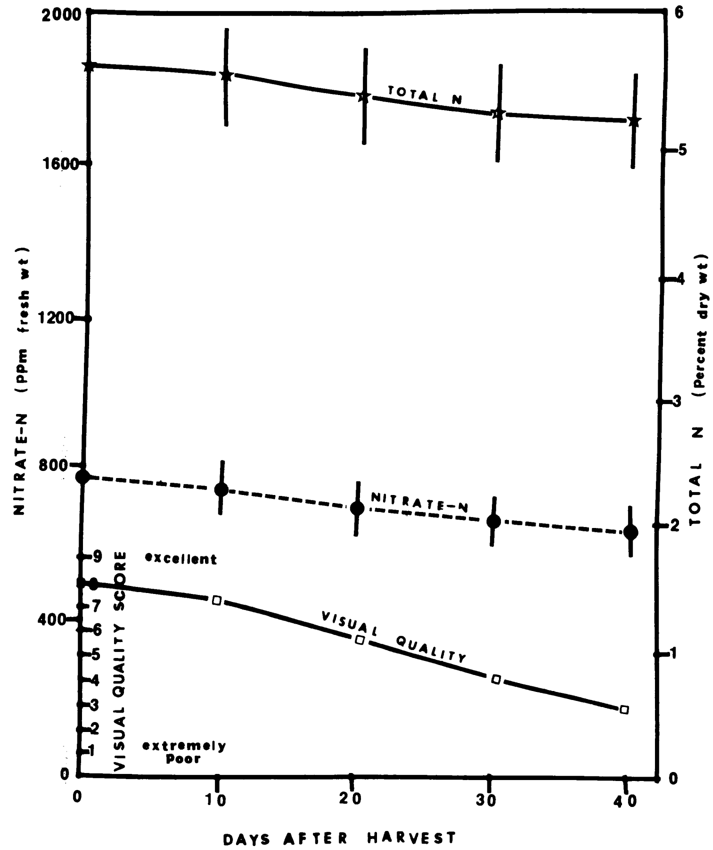

Fig. 2. Effect of holding time at 0°C on visual quality and the concn of total N and nitrate-N in fresh spinach. Nitrogen fertilization had no significant effect; hence, values from 0, 117 and 250 kg N/ha are combined. Confidence intervals at 5% level are shown for total N and nitrate-N.

30% of the initial level being lost by the seventh day (Fig. 1).

In contrast to storage at 20°C, there was no significant change in the nitrate-N or total N concn in spinach held at 0° (Fig. 2). All samples held at 0° for as long as 40 days had less than 5 ppm nitrite-N on fresh wt basis (data not shown) even though the bulk of the spinach had started to decay by the end of the storage period.

Effect of simulated pretransit, transit, and posttransit temp. The simulated transit conditions had a greater effect on the conversion of nitrate to nitrite than did the imposed pretransit and posttransit conditions. Spinach stored at 0°C for 2 weeks had less than 2 ppm nitrite-N, even though preceded by a previous 15-hr period at 21° and followed by a 3-day period at

Table 1. Nitrate-N and nitrite-N in spinach leaves as influenced by simulated pretransit, transit, and posttransit conditions.[z]

Pretransit Time of storage at 21°C (hr)	Transit Temp of storage for 2 wk (°C)	Posttransit Time of storage at 10°C (days)	Nitrate-N (ppm fresh wt)	Nitrite-N (ppm fresh wt)	Visual quality score[y]
–	Initial	–	810a[x]	0.1a	9
15	–	0	778abc	0.4a	9
0	0	0	782ab	0.4a	8
0	0	3	794a	0.5a	7
15	0	0	750abcd	0.7a	7
15	0	3	757abcd	1.6a	5
0	5	0	630cde	4.3ab	3
0	5	3	633bcde	11.3b	2
15	5	0	610de	9.8b	2
15	5	3	563e	24.3c	1

[x]Mean separation in columns by LSD, 5% level.
[y]Rates on a scale of 1 = extremely poor; 9 = excellent.
[z]Nitrogen was applied at 250 kg/ha.

10^{o} (Table 1). On the other hand, spinach held at 5^{o} for 2 weeks accumulated much more nitrite-N, particularly with the pre and posttransit treatments. A delay period of 15 hr at high temp (21^{o}) resulted in a significant accumulation of nitrite-N on subsequent storage for 2 weeks, only when the spinach was stored at 5^{o} instead of 0^{o}. The nitrite-N concn in spinach held at 5^{o} for 2 weeks was increased 3-fold on transfer to 10^{o} for 3 days. The treatments that caused significant loss of nitrate-N and accumulation of nitrite-N also resulted in substantial deterioration in visual quality (Table 1). The bulk of the spinach held at 5^{o} for 2 weeks was decayed beyond acceptability for food use.

Discussion

Nitrogen fertilization had no significant effect on the levels of nitrate-N and total N present in spinach leaves at harvest presumably because of high levels of residual soil N. This might explain why there were no differences in the concn of nitrite-N in fertilized and unfertilized spinach during storage at 0^{o} and 20^{o}C. Schuphan (12) reported that N fertilization increased nitrite accumulation in spinach during storage at 22-32^{o}, but his spinach varied widely in nitrate concn prior to storage as a result of fertilization.

The observation that there was no conversion of nitrate to nitrite in spinach held at 0^{o}C while relatively high levels of nitrite-N were present at 20^{o} is in agreement with previous studies by Phillips (10) and Lee et al. (7). The sudden loss of nitrite at 20^{o} was also observed by Phillips (10) in spinach and by Hicks et al. (6) in carrot juice. The loss of nitrite probably resulted from its conversion to gaseous nitrogenous compounds. The decline in total N at 20^{o} in highly decayed spinach might have been due to the breakdown of protein by microbial enzymes with the release of ammonia.

Exposing spinach to a high temp (21^{o}) for several hr after harvest caused more than a 2-fold increase in nitrite-N levels on subsequent storage at 5^{o} for 2 weeks emphasizing the need for prompt cooling after harvest. By far the most effective means of minimizing nitrite accumulation in spinach would be to hold it as close to 0^{o} as possible from harvest through transit and marketing. This practice would also ensure better produce quality.

From the standpoint of nitrite-induced methemoglobinemia, it is reassuring to note that nitrite-N levels in excess of 10 ppm were present only in decaying spinach considered unmarketable. However, frequent and long term ingestion of low levels of nitrite might be a health risk in light of the recent demonstration of in vivo formation of carcinogenic nitrosamines from dietary nitrite (4). Thus, attempts to minimize its accumulation in fresh vegetables such as spinach appear worthwhile.

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