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◆ Review ◆

Nitrogen Compounds in Vegetable Foods*

(식물성 식품중의 질소화합물)

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The animal body's structure and function is either made of or governed by nitrogen-bearing molecules; such as proteins, nucleic acids, enzymes, and certain vitamins and hormones. While plants form these materials from inorganic sources of nitrogen, man and other higher animals are, for the most part, dependent upon an external source of already formed amino acids to build their body protein. The major source of nitrogen for plants is the nitrate ion present in soils and ground water. It is formed by the decomposition of organic nitrogenous materials of plants and animals that are either in, added to, or returned to the soil according to the nitrogen cycle of the ecosystem. Microorganisms in the soil decompose organic materials and release nitrogen as the ammonium ion. In the presence of air, additional microorganisms oxidize the ammonium ion to nitrate. This form of inorganic nitrogen then becomes the major source of the various nitrogen compounds found in plant foods, such as nitrate itself, free ammonia, alkaloids, amino acids, imines, amides, peptides, and protein.

Under normal conditions of cultivation or under the natural circumstances which occurred before the intrusion of modern agriculture, the relative proportions of these nitrogenous constituents in plants remain fairly constant. However, when unusual conditions exist, such as heavy application of nitrogen fertilizer, certain nutrient deficiencies, damage from insects or herbicides and abnormal climatic conditions, the relative proportions of these nitrogenous compounds may be altered. In recent years, a high rate of nitrogen fertilization has been a common agricultural practice. As a consequence, many plant accumulate or form large quantities of certain nitrogen compounds, which may be found in proportion not considered "normal". During subsequent processing of such crops, these nitrogenous compounds may change form or react with other compounds which can have important effect on the over-all quality and safety of the food product.

Among several factors, conditions for accumulation of some inorganic and organic nitrogen compounds in fresh, stored, and processed vegetables, and their relationship to the food quality, will be presented here, in relation to environmental impact. This discussion will be limited to an examination of the nitrate and nitrite contents of some fresh and processed vegetables and also the accumulation of glutamine, with particular consideration of its relation to the formation of 2-pyrrolidone-5-carboxylic acid (PCA) during processing.

Nitrates are widely distributed in the human environment and they are used often as deliberate food additives. The potential toxicity of nitrate to animals has been recognized for many years. [1] Although the condition is commonly referred to as nitrate poisoning, toxicity is caused by nitrite which is formed from nitrate by microbial action either prior to ingestion or within the gastrointestinal tract. The aspect of the effect of nitrates in the environment has become more important in recent years due to increased use of nitrogen fertilizers in agriculture, use of nitrate accumulating feeds for animals, inadequate sewage systems, water contaminition by animal wastes, and other waste disposal problems. Nitrate poisoning in infants was first reported in 1945 by Comly. [2] Most of the cases of

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illness and death caused by nitrate toxicity have been traced to water supplies.[3] Attention was shifted recently from water to food as a source of nitrate toxicity by several incidences of methemoglobinemia in Europe with infants caused by the consumption of spinach.[4,5] These reports caused public concern in the U. S. over the possible health hazard that might be caused by high levels of nitrates found in some foods.

Nitrate and Nitrite

There have been many reports dealing with the presence of nitrate in vegetables in this country.[6-8] Table 1 lists the nitrate nitrogen content of various

Table 1. Average nitrate nitrogen content of fresh vegetables

Vegetables	Nitrate nitrogen (ppm)	
	Richardson (1907)	Jackson et al. (1967)
Beets	587	275
Cabbage	46	72
Carrots	15	23
Lettuce	387	153
Peas	12	11
Potatoes	18	24
Radish	413	343
Spinach	434	121
Stringbeans	100	53
Tomatoes	12	16

vegetables determined by Richardson[6] and Jackson et al.[8] The vegetables analyzed by Richardson in 1907 were grown primarily with natural manure, while Jackson et al. analyzed samples which were grown mainly using chemical fertilizers. The accumulation of nitrate is apparently independent of the type of fertilizer used but does depend on the vegetable. Such species as beets, spinach, and radishes accumulate relatively large amounts of nitrate. Richardson[6] then compared these values with the nitrate content of cured meats and stated that……

"the average person obtains a larger quantity of nitrates from his vegetables than from his meat foods"……

Table 2 shows the nitrate nitrogen content of various vegetables obtained at Ithaca, N.Y.[7] and at Geneva, N.Y.[9]. Widely differing values for the nitrate content

Table 2. Nitrate nitrogen content of fresh vegetables

Vegetables	Nitrate nitrogen (ppm)	
	Wilson (1949)	Lee (1971)
Beets	306	548
Broccoli	180, 884*	214
Cabbage	511	207
Carrots	101	76
Cauliflower	460	238
Celery	276	226
Lettuce	287	63
Radish	70, 270*	456
Spinach	70, 836*	468

* Nitrate nitrogen content in the juice of vegetables

of individual vegetables occur which are due to the various factors described previously.

It has been known for a long time that amount of nitrogen fertilization affects the content of nitrate in certain crops. Brown and Smith[10] studied the effects of nitrogen fertilizer on various vegetables and reported that the greatest accumulation of nitrate appeared occurs in earlier-maturing vegetable species, such as radish and mustard. They suggested that more than 50 pounds of nitrogen per acre leads to marked nitrate accumulation in garden vegetables. Studies on beets and spinach[11,12] also showed that the rate of nitrogen fertilization is directly related to the accumulation of nitrates (Table 3).

Table 3. Nitrate and nitrite nitrogen contents of fresh beets and spinach at different rates of nitrogen fertilizer[11,12]

Fertilizer lb N/acre	Beets		Spinach	
	NO_3-N (ppm)	NO_3-N* (ppm)	NO_3-N (ppm)	NO_2-N* (ppm)
0	58	0.9	173	0.5
100	150	1.5		
200	415	1.2		
400	455	2.0	552	0.6

* Nitrite nitrogen content as dry basis

Uufertilized fresh spinach contained 173 ppm nitrate nitrogen whereas nitrogen fertilizer (400 lb N/acre) increased the content to 552 ppm. The nitrate nitrogen content of beets was increased from 58 ppm in unfertilized beets to 455 ppm when nitrogen fertilizer was applied at the rate of 400 lb N/acre. However, unlike nitrate there was no direct relationship between the nitrite content of the vegetables and fertilizer rate. The range of nitrite nitrogen content was 0 to 2.5 ppm for beets and 0 to 1 ppm for spinach on dry basis.

Table 4. Nitrate nitrogen content of infant baby foods

Baby Food	Nitrate nitrogen (ppm)	
	Wilson (1949)	Kamm et al. (1965)
Beets	76, 172	222
Carrots	0.7	23
Garden vegetables	—	41
Spinach	141, 191	312
Squash	0	64
Vegetable soup	8.5	—

Table 4 shows the nitrate content of commercially prepared infant foods sold in the U. S.[7] and Canada.[13] The variations in nitrate content of certain vegetables can be seen also from this table. One sample of beets contained 76 ppm nitrate nitrogen, whereas another contained 172 ppm. Likewise, two spinach samples show two different values of 141 ppm and 191 ppm. In general, the values for nitrate nitrogen in baby food analyzed by Kamm et al.[13] were higher than those of Wilson.[7] We found that spinach puree prepared from the unfertilized spinach contained 60 ppm nitrogen, whereas spinach puree prepared from the fertilized spinach contained 204 ppm.[11,12]

Some controversy has been developed over the evidence of the conversion of nitrate to nitrite in foods which presented a problem of toxicity. A report from Germany[14] showed that spinach puree which caused methemoglobinemia in 2 and $3^{-1/2}$ month old infants contained 661 ppm nitrite nitrogen and only a trace of nitrate nitrogen. It was suggested that most of the nitrate was reduced to nitrite during storage. Another report from Germany[15] showed that fertilized spinach, (320 kg N/ha) which contained 30 ppm nitrite in dry matter at harvest time, increased to 3550 ppm after storage for 4 days. Recently, Phillips[16] in Canada reported that the nitrate nitrogen content in spinach stored at room temperature was reduced to approximately 30% of its initial amount during the first 4 days, and after 8 days no nitrate nitrogen remained. However, he did not mention whether the fresh spinach stored at room temperature for 4 or 8 days was still acceptable for consumption. In his storage experiment under refrigeration condition, one series of experiments showed that nitrite accumulated within 8 days, while the other two series did not show any significant nitrite accumulation during storage for 14 or 22 days. We found no significant conversion of nitrate to nitrite in fresh spinach stored at 35°F for a period of up to 3 weeks (Fig. 1). Likewise, fresh beets stored at 35°F for a period of up to 60 and 70 days did not show any significant changes in nitrate content (Fig. 2).

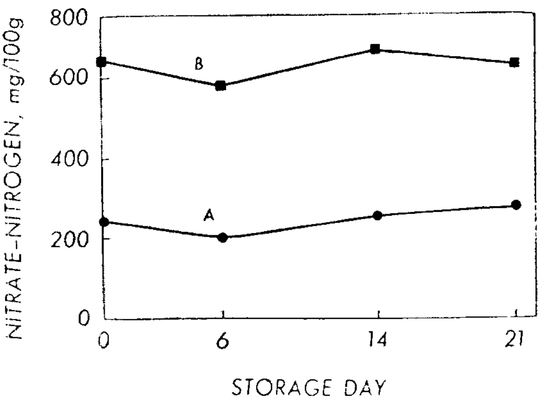

Fig. 1. Changes of nitrate-nitrogen content in fresh spinach during the storage at 35°F. (Nitrogen fertilizer treatment, A:0, and B:400 lb/acre)

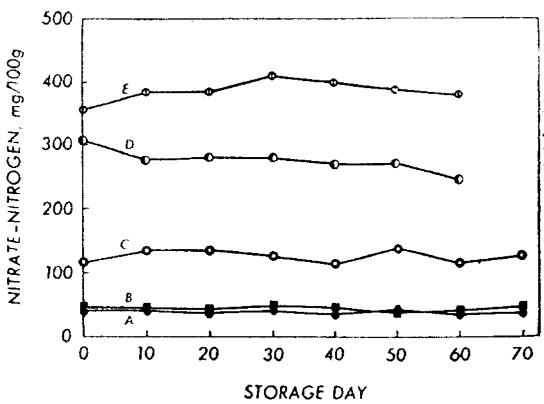

Fig. 2. Changes of nitrate nitrogen content in table beets during the storage at 35°F. (Nitrogen fertilizer treatment, A:0, B:50, C:100, D:200, and E:400 lb/acre)

Nitrite nitrogen appeared in only trace amounts (less than 2 ppm) in both crops. Therefore, we believe that properly handled fresh spinach and beets do not produce nitrite for a certain period of storage time (at least two weeks for spinach and two months for beets at low temperature).

Home prepared spinach baby food has been suspected in Europe as a source of nitrite when partially consumed jars were stored certain periods of time before re-consumption.[17] However, Phillips[16] found no nitrite accumulation in the remainder of partially consumed baby food (spinach and beets) which were held under refrigeration for periods of up to seven days.

It is apparent from these results that nitrate can accumulate to certain toxic levels in fresh beets and spinach, but its conversion to nitrite is variable and unpredictable and depends upon many factors such as handling, processing, and storage conditions, as well as the original nitrate content of vegetables.

Effects of commercial canning process on the nitrate and nitrite content of vegetables have been studied in conjunction with nitrate-nitrite conversion. Beets and spinach grown with varying rates of nitrogen fertilization were processed and stored at 65°F for 6 months and then the nitrate and nitrite nitrogen contents were determined. The canning proecss itself reduced the nitrate content of fresh beets and spinach (Table 5). The average loss of nitrate nitrogen during processing

Table 5. Nitrate nitrogen content of fresh and canned beets and spinach[11,12]

Fertilizer treatment lbs N/acre	Nitrate nitrogen (mg/100g)*			
	Beets		Spinach	
	Fresh	Canned	Fresh	Canned
0	40	25	248	133
100	110	99	—	—
200	310	165	—	—
400	350	250	690	340

* Calculated as dry basis

was 30% for beets and nearly 50% for spinach. This loss can be attributed to leaching during processing. Chemical reduction of nitrate to nitrite during processing was not observed with either spinach or beets.

The nitrate nitrogen content of a few samples of commercially canned beets and spinach obtained from a local market in Geneva, N. Y., ranged from 168 to 290 mg/100 g for beets and 15 to 150 mg/100 g for spinach (dry weight basis). The relatively low nitrate content found in market samples of spinach is also probably due to extensive leaching during processing.

Nitrate and Detinning

Nitrate in certain vegetables is known to be related to detinning of canned products. Hoff and Wilcox[18] reported that nitrate in tomatoes caused detinning upon storage. They found that the correlation coefficient between the nitrate content of tomatoes and the dissolved tin in the can after six months storage was r= .94. Farrow et al.[19] also observed extensive detinning in canned tomatoes due to nitrate. Tomatoes with no nitrate removed only 15% of the tin from their containers during 2 years of storage, while other tomatoes which had about 50~80 ppm nitrate removed about 70 % of the tin from their cans.

Nitrosamines

Associated with the problems of high levels of nitrate and nitrite in foods is the formation of the toxic nitrosamines. The presence of nitrite in combination with secondary or tertiary amines may lead to form carcinogenic nitroso compounds. It was not long ago that the possible occurrence of carcinogenic nitrosamines in food was postulated. In 1960, an outbreak of serious liver disease was observed in sheep fed a diet containing fish meal preserved with nitrite.[20] Sen et al.[21] investigated the formation of nitrosamine from the naturally occurring amines in fish preserved with added nitrite. They found that certain kinds of fish, especially those rich in naturally occurring amines, form dimethylnitrosamine during cooking with nitrite.

Unfortunately, the question as to whether the above mentioned nitrite-secondary amine reactions do occur in foods and/or in the animal stomach after ingestion of such products has not yet been sufficiently proved to permit any definitive conclusions. The major obstacle to progress in such studies is the lack of reliable analytical techniques to provide quantitiative determination as well as unequivocal identification of N-nitrosamines.

A considerable amount of experimental work has been carried out to determine whether any nitrosamines are present in animal foods, such as smoked herring, smoked haddock, smoked sausage, bacon, and smoked ham.[22] However, there have been few reports.

of the presence of nitrosamines in vegetable foods. Hendler and Marquardt[23] demonstrated the presence of nitrosamines as well as nitrite in wheat meal and flour. Keybets et al.[24] studied the possible formation of nitrosamines in nitrite containing spinach but they did not detect any demonstrable nitrosamine under normal processing conditions.

Glutamine and Pyrrolidonecarboxylic Acid

Accumulation of organic nitrogen compounds such as glutamine due to high doses of nitrogen fertilizer and subsequent formation of 2-pyrrolidone-5-carboxylic acid (PCA) during the heat processing is an example of a potential adverse effect on the quality of processed vegetables. PCA causes a detectable off-flavor when its concentration exceeds a certain level which varies with different products.[25,26] According to Archibald[27] nearly complete conversion of glutamine to PCA is accomplished in 2 hrs. at 212°F at pH 2 to 10, whereas complete glutamic acid conversion to PCA required 212°F for 50 hrs. at pH 4 or 10 (Fig. 3). The mild condition required to transform glutamine to PCA would indicate that most of the PCA found in canned food products is derived from glutamine during thermal processing.

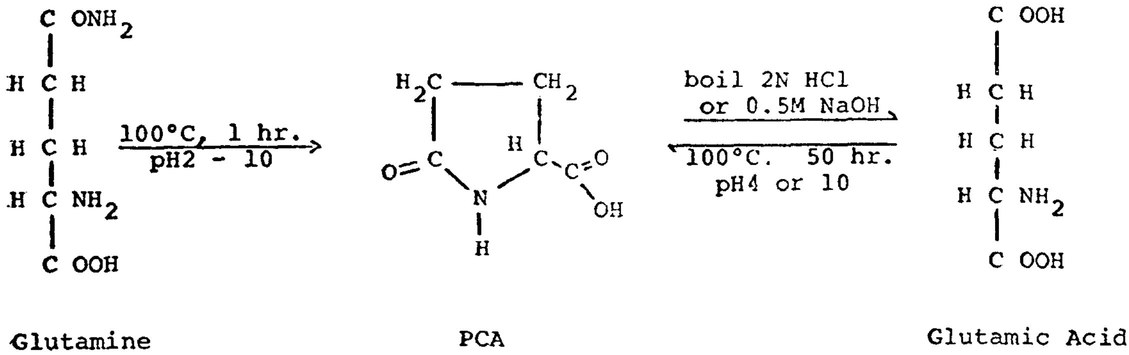

Fig. 3. Chemical relationship of glutamine, PCA and glutamic acid

Vickery et al.[28] first reported the accumulation of glutamine in beets as affected by different nitrogen fertilizers. Shallenberger et al.[29] (1959) studied the formation of PCA during the preparation of beet puree for baby food. Their recommendation for controlling the off-flavor in beets was to use beets with a low glutamine content, and to minimize the time and temperature during processing. Lin et al.[30] reported that among the organic acid, PCA and acetic acid showed striking increases of over 100% during spinach processing at 240°F.

Table 6. Content of glutamine and PCA in fresh and canned beets grown on different rates of nitrogen fertilizer[11,12]

Fertilizer treatment lbs N/acre	Glutamine*(%)		PCA* (%)	
	Fresh	Processed	Fresh	Processed
0	1.89	1.24	0.13	1.40
100	2.25	2.02	0.30	2.20
200	3.12	2.45	0.34	2.51
400	3.46	2.49	0.58	2.78

* Average duplicate determination on duplicate samples on dry weight basis.

In studies on the accumulation of nitrogen compounds in beets, Lee et al.[11,12] observed that the rate of nitrogen fertilization is directly related to the accumu-

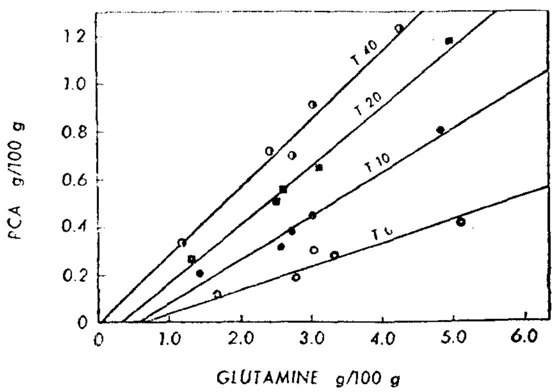

Fig. 4. Changes in glutamine and PCA content of beets after 0, 10, 20, and 40 min. heating in boiling water

lation of glutamine in beets. The PCA content of the canned beets was therefore correspondingly higher. The amount of PCA was also found to be proportional to the glutamine content of fresh beets (Table 6). Changes in the PCA and glutamine contents of beets subjected to various heating periods are shown in Fig. 4. Increasing the length of the heating time increased the PCA content and decreased glutamine content.

To summarize, nitrogen compounds, which accumulate in certain vegetable crops due to heavy use of nitrogen fertilizer, have been discussed in the light of their being potential health hazards and as factors on the over-all quality of processed foods. Much further information is needed on impact of changed agricultural practices to solve and eliminate the problems in public health and the over-all quality of processed foods. In the meantime, studies on what really is proper fertilization, processing, storage, and handling of foods and these should be a continued concern for everyone.

References

1) Mayor, N. S.: *Kan. Agr. Exp. Sta. Bull.*, 49 (1895).
2) Comly, H. H.: *J. Am. Med. Assoc.*, **129**, 112 (1945).
3) Rosenfield, A. B. and Hutson, R.: *Minn. Med.*, **33**, 787 (1950).
4) Simon, C., Manzke, H., Kay, H. and Mrowetz, G.: *Z. Kinderheilk.*, **91**, 124 (1964).
5) Simon, C.: *Lancet*, I (7442). 872 (1966).
6) Richardson, W. D.: *J. Am. Chem. Soc.*, **29**, 1757 (1907).
7) Wilson, J. K.: *Agron. J.*, **41**, 20 (1949).
8) Jackson, W. A., Steel, J. S. and Boswell, V. R.: *Am. Soc. Hort. Sci.*, **90**, 349 (1967).
9) Lee, C. Y.: Unpublished data (1971).
10) Brown, J. R. and Smith, G. D.: *Univ. Missouri Agr. Exp. Sta. Res. Bull.*, 920 (1967).
11) Lee, C. Y., Shallenberger, R. S. and Acree, T. E.: *J. Food Sci.*, **36**, (1971).
12) Lee, C. Y., Shallenberger, R. S., Downing, D. L., Stoewsand, G. S. and Peck, N. H.: *J. Sci. Food Agr.*, **22**, 90 (1971).
13) Kamm, L., McKeown, G. G. and Smith, D. M.: *J. Assoc. Offic. Anal. Chemists*, **48**, 892 (1965).
14) Hölscher, P. M. and Natzschka, J.: *Deut. Med. Wschr.*, **89**, 1751 (1964).
15) Schuphan, W.: *Z. Ernähr. Wiss.*, **6**, 207 (1965).
16) Phillips, W. E. J.: *Can. Inst. Food Technol. J.*, **2**, 160 (169).
17) Sinios, A. and Wodsak, W.: *Med. Wscher.*, **90**, 1856 (1965).
18) Hoff, J. E. Wilcox, G. E.: *J. Am. Soc. Hort. Sci.*, **95**, 2 (1970).
19) Farrow, R. P., Johnson, J. H., Gould, W. A. and Charbonneau, J. E.: *J. Food Sci.*, **36**, 341 (1971).
20) Sakshaug, J., Sögnen, E., Hansen, M. A. and Koppang, N.: *Nature*, **206**, 1261 (1965).
21) Sen, N. P., Smith, D. C. and Schwinghamer, L.: *Food Cosmet. Toxicol.*, **7**, 301 (1969).
22) Ender, F. and Ceh, L.: *Food Cosmet. Toxicol.*, **6**, 569 (1968).
23) Hendler, L. and Marquardt, P.: *Food Cosmet. Toxicol.*; **6**, 341 (1968).
24) Keybets, M. J. H., Groot, E. H., and Keller, G. H. M.: *Food Cosmet. Toxicol.*, **8**, 167 (1970).
25) Mahdi, A. A., Rice, A. C. and Weckel, K. G.: *J. Agr. Food Chem.*, **9**, 143 (1961).
26) Shallenberger, R. S. and Moyer, J. C.: *J. Agr. Food Chem.*, **6**, 604 (1958).
27) Archibald, R. M.: *Chem. Rev.*, **37**, 161 (1945).
28) Vickery, H. B., Pucher, G. W. and Clark, H. E.: *Plant Physiol.*, **11**, 43 (1936).
29) Shallenberger, R. S., Pallesen, H. R. and Moyer, J. C.: *Food Technol.*, **13**, 92 (1959).
30) Lin, Y. D., Clydesdale, F. M. and Francis, F. J.: *J. Food Sci.*, **36**, 240 (1971).