Changes in the Nitrate and Nitrite Contents of Fresh and Processed Spinach during Storage

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The influence of storage on the nitrate- and nitrite-N contents of fresh and processed spinach was studied. Under conditions applicable to the consumer, nitrite-N accumulated in some samples of fresh spinach, but not in canned, baby food, or frozen spinach. Nitrite-N accumulated if frozen spinach was allowed to thaw at room temperatures for excessively long periods. Cooking reduced the total nitrate- and nitrite-N contents of the edible portions of fresh spinach by extraction into the cooking water. The nutritional significance is discussed.

The possible production of methemoglobinemia in infants due to ingestion of foods containing large amounts of nitrates has been discussed from time to time. Comly (1945) demonstrated that some cases of methemoglobinemia observed in babies were caused by a high nitrate content of well water. It has been well documented (Knoteck and Schmidt, 1964; Steyn, 1960; Werner, 1965; World Health Organization, 1949; Simon *et al.*, 1964) that a high nitrate content in the water supply has resulted in illness and infant deaths and constitutes the major health hazard in regards to nitrate toxicity. In few cases was the contribution of the nitrate content of the food determined. Age of the child is one of the most important considerations in evaluating nitrate toxicity. Infants under 3 months of age are more susceptible than older infants, probably owing to the presence of fetal hemoglobin (Betke and Rau, 1952) and the reduction of nitrates to nitrites by intestinal bacteria which ascend to the duodenum in infants with gastroenteritis. Burden (1961) considers that the high fluid intake in proportion to body weight is a significant contributing factor in infants. There is a paucity of data from direct clinical studies of effects from feeding nitrate-containing foods to humans. One study, however, was conducted by Kubler (1958). Seven infants ($3^1/_2$ to 8 months of age) were fed spinach containing nitrate at an intake of up to 21 mg. of nitrate per kg. of body weight. Of the seven infants, six showed no increase in methemoglobin, but one showed a very slight increase which was not sufficient to cause any symptoms of methemoglobinemia. The latter infant exhibited elevated levels of methemoglobin prior to the feeding of the nitrate-rich spinach. Previously Wilson (1949) claimed from theoretical considerations that a toxic quantity of nitrate for adults would be ingested from 3.9 oz. of rhubarb or about 2.3 oz. of spinach, turnips, or certain other vegetables. Assuming the average adult to weigh 70 kg., the dose becomes 14.3 mg. of nitrate per kg. of body weight. The studies by Kubler (1958) were conducted on infants, and they are more susceptible to nitrate poisoning than adults. Even so, after feeding nearly twice the level considered dangerous by Wilson (1949), no evidence of toxicity was apparent. More recently the *British Medical Journal* (1966) discussed the findings of Sinios and Wodsak (1965) with the heading "Spinach—A Risk to Babies." The authors describe 14 children, age 2 to 10 months, that developed methemoglobinemia (including one death) after eating spinach. In nearly all cases, the spinach was purchased as a fresh vegetable. Although clinical symptoms were not apparent in 12 cases when the food was ingested soon after preparation, it became toxic after storage for 24 to 48 hours, even when under refrigeration in some cases. It was concluded that nitrate was not the toxic principle but, rather, that nitrite had been formed during storage. Our laboratory and others (Steyn, 1960; *Nut. Rev.*, 1950) found evidence lacking for the toxicity of nitrates per se in foods. Nitrites are the toxic principle which may be formed prior to ingestion or during digestion and absorption of food. Nitrate may be considered as the index or precursor to the amount of nitrite which may be formed. If a food contains large amounts of nitrate, it then is a potential hazard if conditions during storage or processing are conducive to conversion to nitrite.

Studies were conducted on fresh and processed spinach as purchased on the Canadian retail market to determine the nitrite-N content under conditions of storage and cooking which would be used by the consumer. The work described deals only with the formation of nitrite-N prior to ingestion and does not deal with the possible conversion of nitrate in foods to nitrite by intestinal microflora.

MATERIALS AND METHODS

During 1966 and 1967 unprocessed fresh spinach and three types of processed spinach (baby food, canned, and frozen) were purchased in local retail outlets and analyzed for nitrate- and nitrite-nitrogen by the method of Kamm *et al.* (1965). Samples for series 1, 2, and 3 were purchased in August and November 1966 and January 1967, respectively. For each study, three different samples were divided into two portions and stored either at room temperature in the dark or under refrigeration. Spinach leaves and stalks were left intact during storage to simulate ordinary conditions of use by the consumer. At specified periods, 10-gram samples of spinach were removed, mechanically homogenized in water, and the nitrate- and nitrite-N contents were determined as cited above. This yielded variable nitrate- and nitrite-N levels. Canned and baby food spinaches were opened, sampled, exposed briefly to the air, resealed, stored under refrigeration, and

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the sampling procedure repeated after various time intervals. Frozen spinach was sampled while frozen and stored for periods up to 5 months. In one series, frozen spinach was allowed to thaw at room temperature and the nitrate- and nitrite-N contents were determined. The pH of samples was not determined.

The distribution and changes in the nitrate- and nitrite-N contents of the vegetable were determined after cooking. The spinach was cooked under practical conditions by adding 30 grams of fresh spinach to 200 ml. of boiling water. Samples were cooked for approximately 10 minutes and the spinach was separated from the cooking water. Analyses were conducted on each fraction.

RESULTS

Fresh Spinach. Spinach used in the first series was purchased during the summer and stored at room temperature. This series was sampled after 4, 6, and 8 days' storage. Nitrate-N decreased extremely rapidly (Figure 1). During the first 4 days of storage, nitrate-N was reduced to approximately 30% of its initial level and was practically absent by the eighth day. An accumulation of nitrite-N was not apparent during this sampling. To study the changes occurring during the first 4 days of storage, a second series of samples was studied during the winter months. Room temperature was similar to the previous study (70° F.). The concentration of nitrite-N began increasing immediately on storage at room temperature while nitrate-N decreased. This indicated conversion of nitrate to nitrite occurred during the first 4 days followed by a decomposition of nitrite during the next 3 days.

Under refrigeration (Table I), the reduction of nitrate was delayed. The nitrate-N content of the spinach was extremely variable between sampling days. In series 2 and 3, no significant conversion of nitrate to nitrite took place within the first 14 or 22 days, respectively. In only one case (series 1) did nitrite-N accumulate rapidly within 8 days of storage. The nitrite-N formed did not decrease as observed when spinach was stored at room temperature.

Cooking of fresh spinach demonstrated that the total nitrate- and nitrite-N content was reduced to approximately 20 to 25% of the initial value. The decrease was accounted for by extraction into the cooking water. For example, when a quantity of spinach was cooked, the total nitrate and nitrite-N contents decreased from 4.5 to 0.97 mg. and 3.4 mg. was found in the cooking water.

Baby Foods. Three jars of baby food (one pureed spinach and two jars of mixed vegetables containing spinach) were opened and sampled for periods up to 35 days. The nitrate-N content of the mixed vegetables (Table II) was lower than fresh spinach. In both cases, the nitrite-N content was less than 1 p.p.m. For 15 days after opening the jars and with subsequent storage under refrigeration, there was no increase in the nitrite-N content. By day 35 the nitrite-N content of spinach had increased to 3 p.p.m., but no increase was observed in the mixed vegetables. The nitrate-N content did not decrease further over the storage period, indicating no major conversion of nitrate.

Frozen Spinach. The nitrate-N content of frozen spinach varied from 97 to 556 p.p.m., depending on the source. However, the nitrite-N content (Table III) was approximately 1 p.p.m. or less. There was no significant increase in the nitrite-N content with periods of storage

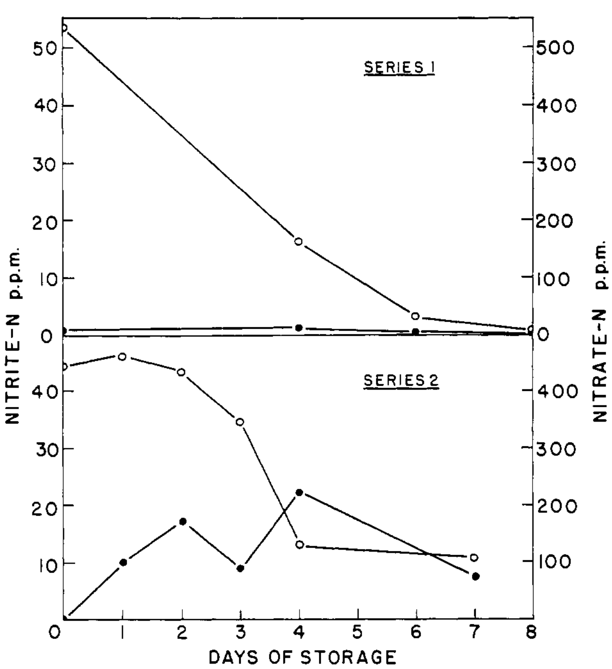

Figure 1. Effect of storage at room temperature on the nitrate- and nitrite-N contents of fresh spinach

●——● Nitrite-N content
O——O Nitrate-N content

Table I. Summary of Changes of Nitrate-N and Nitrite-N Contents of Fresh Spinach Stored under Refrigeration

		Duration of Storage, (Days)					
		0	8–11	14	21–22	28	39
Series 1	Nitrite-N[a]	0.4	32	210	543	439	...
	Nitrate-N	526	685	430	33	12	...
Series 2	Nitrite-N	0.4	0.2	0.3	26	27	...
	Nitrate-N	445	479	337	412	326	...
Series 3	Nitrite-N	0	0.3	...	0.4	17	18
	Nitrate-N	339	374	...	304	553	453

[a] Values in p.p.m.

Table II. Effect of Storage on Nitrate- and Nitrite-N Contents of Baby Food Samples

Sample		Time, Days 0	2	6	9	15	35
Spinach	Nitrite-N[a]	0.4	0.1	0.2	0.3	0.1	3.1
	Nitrate-N	180	192	214	197	208	214
Mixed vegetables	Nitrite-N	0.5	0.2	0.4	0.3	0.2	0.04
	Nitrate-N	42	37	43	42	44	46

[a] Values in p.p.m.

Table III. Effect of Storage and Thawing on Nitrite-N Content of Frozen Spinach

Storage time, months	**0**	**1**	**3**	**4**	**5**	**5**	**5**
Thawing time, hours	**0**	**0**	**0**	**0**	**0**	**15**	**39**
	Nitrite-N, P.P.M.						
Sample 7	0.4	0.4	0	0.1	1.0	0	30
Sample 8	0.5	0.3	0	0.3	0.6	0	19
Sample 9	0.3	0.5	0	0.4	1.2	0	74

in the frozen state of up to 5 months. The length of time samples were allowed to thaw did influence the nitrite-N content. Thawing up to 15 hours at room temperature did not increase the nitrite-N content, but a longer period of 39 hours did.

Canned Spinach. Three different brands of canned spinach were studied for a period of up to 7 days after opening (Table IV). The nitrate-N content was lower than the fresh vegetables studied and varied from 114 to 324 p.p.m. with an average of 200 p.p.m. The nitrite-N content was less than 1 p.p.m. when opened and did not increase significantly over the 7-day period.

DISCUSSION

The data presented above demonstrate that the selected samples of fresh spinach chosen from the Canadian market contained little or no nitrite when purchased. The processing procedures used in the production of the canned, pureed baby food, and frozen spinach did not give rise to appreciable quantities of nitrite. The nitrate present in the fresh leafy vegetable was reduced to nitrite under conditions of storage. The reduction was accelerated when the vegetable was stored at room temperature as compared with refrigeration. The nitrite, however, was in a dynamic state and decreased at room temperature so that high levels did not accumulate. High levels of nitrite did accumulate, in one case, when the fresh vegetable was stored under refrigeration. This may have been the result of the length of time the spinach had been stored prior to purchase and will require further study. Nitrite did not accumulate when frozen spinach was allowed to thaw for periods of up to 15 hours but did if an excessive period of 39 hours was permitted. Nitrite, therefore, would not be expected to increase in frozen spinach following recommended practices for the use of this vegetable. Cooking of fresh spinach decreased the total nitrate- and nitrite-N contents of the edible material to approximately 20% of the initial value. The findings reported above are similar to and in agreement with those observed in Germany by Sinios and Wodsak (1965).

The establishment of tolerance levels for nitrate and nitrite is extremely difficult. The World Health Organization (1949) has reviewed the toxicity of nitrate and nitrites and has stated that the sensitivity of normal babies and apparently dyspeptic babies makes it impossible to establish an acceptable dose level for babies of 6 months of age or less on the basis of animal experimentation or clinical experience. For nitrite, however, the problem can be simplified in regard to the feeding of vegetables. In the newborn, fetal hemoglobin accounts for 60 to 80% of the total hemoglobin. This is reduced to approximately 30% by 3 months of age. Fetal hemoglobin (Betke and Rau, 1952) is more readily oxidized. Thus, children under 3 months of age are more susceptible to methemoglobinemia. It is not until after this age that recommended feeding practices ("Canadian Mother and Child," 1953) suggest the introduction of vegetables to the diet. In considering the toxicity of nitrite in older children and adults, the amounts of nitrite required to produce methemoglobinemia can be estimated. About 1% of the total hemoglobin normally is present as methemoglobin (Lane, 1952). With amounts of methemoglobin under 5%, no clinical symptoms are observed in children, while light cyanosis is obvious (Knoteck, 1964) with greater than 5%. Death from asphyxia due to methemoglobinemia occurs when the concentration in the blood exceeds 70% of the total blood pigment present, provided there is no anemia initially (Locket, 1957). In the presence of anemia, lower concentrations are fatal, since the deciding factor is the amount of oxygen-carrying pigment available in the body. Although both methemoglobin-forming and methemoglobin-reducing reactions take place concurrently (Bodansky, 1951), only the former reactions will be considered for the purpose of calculation. The data were calculated for a 1-year old child having a body weight of 22.5 lb. (Falkner, 1962) with a blood volume of 9% of body weight (Long, 1961). Assuming a hemoglobin content of 17 grams per 100 ml. of blood, this is equivalent to 1.118 mmoles of hemoglobin. Greenberg *et al.* (1943) have shown that one molecule of nitrite reacts with two molecules of hemoglobin to form two molecules of methemoglobin. Therefore, 1 μMole or 14 μg. of nitrite-N could convert 2 μmoles or 30.4 mg. of hemoglobin. The amounts of nitrite-N and the equivalent amounts of spinach which would need to be ingested to produce methemoglobinemia of varying degrees are shown in Table V. These figures cannot be exact and provide a safety factor because the reconversion of methemoglobin to hemoglobin has been neglected. A further four fold increase in the amount of spinach which could be safely ingested would be introduced during cooking by extraction of the nitrate and nitrite into the cooking water. The nitrite concentrations found in the present investigation in samples of pureed baby foods, canned spinach, or frozen spinach, even after long periods of storage, suggest they could be eaten with impunity with little or no hazard from nitrite toxicity. In most cases, fresh spinach presents no hazard. However,

Table IV. Effect of Storage under Refrigeration on the Nitrate-N and Nitrite-N Contents of Canned Spinach[a]

	Storage Time, Days			
	0	1	4	7
Nitrite-N	0.2	0.5	0.4	0.7
Nitrate-N	200.0	210.1	190.4	176.3

[a] Each value represents the average of three brands. Values in p.p.m.

Table V. Postulated Relationship of Ingested Nitrite-N to Methemoglobinemia

Age	Weight, Lb.	Total Blood Vol., Ml.	% Methemoglobin	NO_2-N Required, Mg.	Spinach Required Containing 10 P.P.M. of NO_2-N,[a] Lb.
1 year	22.5	900	5	3.52	0.78
1 year	22.5	900	30	21.13	4.65
1 year	22.5	900	70	49.30	10.86
Adult	148	6000	5	23.45	5.17
Adult	148	6000	30	140.84	31.02
Adult	148	6000	70	328.72	72.41

[a] = 4.54 mg. NO_2-N/lb.

storage under refrigeration can produce conditions such that nitrite may accumulate to toxic amounts. From the data observed in series 1 (Table II), storage of spinach for 14 days resulted in 210 p.p.m. nitrite-N which would require ingestion (if eaten uncooked) of only 0.037 lbs. to yield sufficient nitrite for a detectable cyanosis in a 1-year old child. The cause of the variability of nitrite accumulation may be related to the length of storage prior to purchase or bacterial contamination. Further research is needed to establish the incidence of spinach samples which will accumulate nitrite on storage.

From a standpoint of nitrite toxicity due to the presence of nitrite prior to ingestion, frozen, canned, or baby food spinaches present little or no hazard. Fresh spinach may accumulate nitrite. As stated previously, these studies have not been extended to measure the conversion of nitrate to nitrite by the intestinal microflora in humans. In agreement with Simon (1966), it is recommended that unprocessed spinach be stored a minimum length of time and that recommended infant feeding practices be adhered to in regard to the age of the child at the time of introduction of vegetables to the diet. Spinach should not be introduced prior to 3 months of age.

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