NITRATE AND NITRITE CONTENTS IN FOOD OF PLANT ORIGIN IN PAKISTAN

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Nitrate and nitrite contents of commonly consumed cereals, pulses and vegetables in four provinces of Pakistan indicated highest levels (6490 ppm) in vegetables. Cereals from the NWFP contained more nitrites than those collected from other provinces. The lowest amounts of nitrate and nitrite were detected in the pulses.

Key words: Nitrates, Nitrites, Cereals, Pulses, Vegetables.

Introduction

Nitrate accumulation in plants is a natural phenomenon resulting from uptake of nitrate ion in excess of its reduction and subsequent assimilation. Accumulation of nitrate depends on genotypes, soils and environmental conditions [1]. Nitrate accumulation in plants had been observed for many years, but interest has intensified since the mid-1950's with increased use of nitrogen fertilizers, particularly in the recent years.

In addition to animal fatalities due to the ingestion of forages containing nitrates, there are reports of cyanosis in infants (blue babies) which occurred after consuming food to which water contaminated with nitrates was added [2]. The incidence of methaemoglobinaemia in infants, was reported to be due to ingestion of foods containing large amounts of nitrates. Nitrates and nitrites combine with haemoglobin in blood thus impairing the oxygen carrying capacity of haemoglobin [3]. The incidence of methaemoglobinaemia, by ingestion of spinach was reported by Sinios and Wodsoke[4]. One of the 14 affected children, 2-10 months of age, died after eating contaminated spinach [4]. Although the disease is commonly referred to as nitrate poisoning, it is caused by nitrites derived from nitrates before ingestion or within the digestive tract. After ingestion, nitrates are reduced to nitrites by bacterial reduction. Nitrites can then react under certain conditions with secondary amino groups to form N-nitroso compounds. Most of the N-nitroso compounds so far tested using laboratory animals have been reported to be carcinogenic, hepatotoxic, mutagenic and teratogenic [5-7].

In view of the toxic nature of these nitrogenous compounds in foodstuffs, a comprehensive study on the level of nitrates was undertaken. The present study reports the contents of nitrate and nitrite in vegetables, cereals and pulses collected from the four provinces of Pakistan.

Materials and Methods

Vegetables, cereals and pulses were procured from the following Agricultural Research Institutes during 1987-89. *Department of Chemistry, F.C. College, Lahore,

**Institute of Chemistry, Punjab University, Lahore.

(i) Ayub Agricultural Research Institute, Faisalabad-Punjab, (ii) Sariab Agricultural Research Institute, Quetta-Baluchistan, (iii) Turnab Agricultural Research Institute, Peshawar, NWFP, (iv) Tandojam Agricultural Research Institute, Tandojam, Sindh.

The crops were grown on fertilized soils and fertilizer used was diammonium phosphate (DAP), urea and potassium sulphate. Following in the fertilization practice for various crops.

| Name of crop | Type of soil | Fertilizer | Method of application |
|-----------------|-----------------|------------|--|
| Wheat | Fertile soil | NP | To be broadcast and mixed in the soil planked and sowing |
| -237 | | | is complete. |
| Rice | Medium | NPK | To be broadcast |
| | fertile soil | | and followed by |
| | | | irrigation at puddling. |
| Maize | Fertile soil | NP | To be broadcast near |
| | | | the crop furrows |
| | | | following by irriga- |
| | 3-6 16 | | tion at sowing. |
| Gram(Mung | Fertile and | NP | To be broadcast |
| Mash etc.) | medium soil | | and mixed in the soil. |
| Vegetables | Medium | NP | To be broadcast |
| | fertile soil | | and mixed in the |
| | | | soil at sowing. |

Addition of potassium was recommended in soils low in potassium particularly sandy soils and irrigated with tubewell waters. The lelvel of fertilizer depends on the nature of soil and varied from crop to crop in the four provinces of Pakistan.

The samples of these matured crops were collected and rapped in polyethylene bags. The samples of leafy and root vegetable were stored at refrigeration temperature during transportation and prior to analysis. This procedure was carried out to avoid the reduction of nitrate into nitrite during storage.

The samples were analyzed for dry matter, nitrate and nitrite contents. The dry matter was determined by AOAC mathod [8]. Nitrates and nitrites were analyzed by the nitroxylenol distillation and diazotiation methods. [9,10]. All results are expressed as ranges and means on dry weight basis (given in parenthesis). The levels of significance were determined statistically by two sample student 't' test.

Results and Discussion

Cereals obtained from the Turnab Agricultural Research Station, NWFP contained the maximum amount of nitrates (Table 1). Two varieties of rice from Swat Centre of the NWFP were found to contain the maximum amount of nitrates (2780 ppm). Reduction of nitrates into nitrites in cereals, ranged from 5-12 ppm. However, nitrite contents of rice varieties were significantly higher (P< .001) in the province of Sindh (Table 1).

Nitrate contents of the pulses grown in different parts of Pakistan, ranged from 72-209 ppm respectively. Nitrate contents of black gram grown in Baluchistan were significantly (P < .001) different from the other providence of Pakistan. Group comparison of lentil varieities showed maximum of nitrates in the samples from NWFP province (138 ± 6) . Significant differences (P<.02, .01) were also observed in the nitrite levels of the pulses (Table 2).

Nitrate contents of common vegetable ranged from 110 to 6490 ppm. Group comparison of vegetables by two sample 't' test showed significant differences (Table 3). Reduction of nitrates into nitrites, in vegetables, was comparatively more than in cereals and pulses. Spinach, turnip, radish, sugar beet and potatoes had nitrite levels up to 47 ppm. Nitrite content of other vegetables ranged from 10-30 ppm. Significant difference in the nitrite levels were observed between the four provinces of Pakistan (Table 3).

Accumulation of nitrates and nitrites was low in cereals, which is in agreement with the finding of Walker [11]. Nitrate

| Name of | f crop Pi | Punjab(A) | | Baluchistan(B) | | NWFP(C) | | Sind(D) | |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--|
| (n=5) | NO ₃ | NO ₂ | |
| Maize | 92-200 | 5-10 | 93-161 | 5-8 | 164-316 | 5-12 | 92-112 | 7-8 | |
| | (138±45)* | (7±2) | (126±30)* | (7±1) | (235±60) | (8±3) | (100±10)** | (7±1) | |
| Rice | 102-255 | 5-11 | 161-270 | 5-6 | 202-2780 | 5-9 | 95-235 | 9-12 | |
| | (170±57) | (8±2) | (225-70) | (6±0) | (1470±1330) | (7±1) | (170±58) | (10±1)** | |
| Wheat | 100-213 | 5-9 | 90-237 | 5-6 | 200-245 | 6-8 | 210-237 | 8-9 | |
| | (150±50)* | (8±1)* | (160±60) | (6±1) | (232±32) | (6±1) | (220±14) | (7±1) | |

** Highly significant (P≤0.001); * Significant (P≤0.02); n = No. of samples.

TABLE 2. NITRATE AND NITRITE CONTENTS (ppm) OF PULSES.

| Name of crop | ere en Pi | injab(A) | Baluc | histan(B) | NWFP(C) Sin | | Sind | ndh(D) | |
|---------------|-----------------|-------------------------|----------------------------|-----------------|-------------------|------------------------|----------------------------|--------------------|--|
| (n=5) | NO ₃ | NO ₂ | NO ₃ | NO ₂ | NO ₃ | NO ₂ | NO ₃ | NO ₂ | |
| Gram(black) | 92-121 | 3-6 | 160-170 | 5-6 | 97-115 | 5-8 | 72-90 | 5-8 | |
| | (105±10)** | (5±1) | (165±10) | (6±1) | (100±10)** | (7±1) | (80±10)** | (7±1) | |
| Gram(white) | 121-146 | 3-4 | 145-188 | 8-10 | be to tom N-min | am <u>n</u> o grea | 90-98 | 5-8 | |
| taaobao | (130±12)* | (3±1)** | (165±20) | (9±1) | apounds so far te | i-nit <u>ro</u> so con | (95±5)** | (8±1) | |
| Cow pea | and the second | - fior r | 100-180 | 8-11 | ported to be care | have_been rs | ratory mimals | odsi <u>gaia</u> i | |
| | na te lime | - | (150±31) | (9±1) | ogonic [5-7]. | unic part term | and a subsice mulage | jeuic, hep | |
| Lentil | 110-118 | 5-6 | 99-146 | 5-10 | 136-145 | 7-8 | 117-127 | 5-9 | |
| | (115±5) | (5±1) | (120±21) | (6±3) | (138±6) | (7±1) | (121±10) | (7±2) | |
| Lobia | 123-133 | 4-5 | Addition of p | - 2105 | 80-122 | 4-10 | smdern s en. Th | aw Polantin | |
| | (125±6) | (5±1) | hooimo , maiaas | iog - ber | (100±20) | (6±2) | and aithin the vege | e stente in | |
| Mash | 109-124 | 5-6 | 90-100 | 6-8 | 84-141 | 5-9 | 75-90 | 4-10 | |
| | (115±8)* | (5±1)* | (92±10) | (7±1) | 120±30) | (6±1) | (80±10) | (8±3) | |
| Mung | 93-160 | 7-8 | 100-209 | 5-10 | 87-187 | 5-7 | 81-129 | 5-10 | |
| 0.00 00100100 | (140±20) | (7±1) | (168±40) | (7±3) | (140±40) | (6±1) | (100±20)* | (8±3) | |
| Pigen pea | t to solutions | an i agai nap | 79-114 | 4-11 | es during 1987.8 | earch Institu | 104-139 | 7-9 | |
| cature during | adloor_uojita | ored <u>a</u> t reirigo | (92±20) | (7±3) | | anonal za | (128±16)* | (8±1) | |

** Highly significant ($P \le 0.001$), * Significant (P < 0.05, $\le .02$, $\le .01$), n = No of samples.

and nitrite contents of the pulses varied with variety and maturity. Most varieties of the pulses contained modest levels of nitrates (up to 209 ppm). Our results agree with the findings of Richardson [12]. Nitrate contents of Pakistani spinach (Khatikal) ranged from 2940-6490 ppm. Maximum accumulation was recorded in the samples supplied by the NWFP (P<.001). Vegetables are generally rich in nitrates [13, 14] and

it is widely known that spinach contains a particularly high nitrate concentration (1000-5000 ppm).

Maximum accumulation of nitrates was observed in the beet roots of NWFP (6000 ± 400 ppm, P<.001). However, accumulation of nitrate in radish was found to be the highest in samples grown in Punjab (P <.001). Bitter gourd, cucumber, calsbash, cabbage procured from the four provinces also

| Name of crop | pullesW) Pun | jab(A) | Balu | chistan(B) | NWFP(C) Sin | | Sin | ndh(D) | |
|----------------|-----------------|-----------------|-----------------|-----------------|----------------------|------------------|-----------------|-----------------|--|
| (n=5) | NO ₃ | NO ₂ | NO ₃ | NO ₂ | NO ₃ | NO ₂ | NO ₃ | NO ₂ | |
| Bitter gourd | 3920-6480 | 25-37 | 2890-4780 | 12-20 | 3320-6440 | 9-18 | 2620-3860 | 16-22 | |
| and the second | (5050±1190) | (29±5) | (3600±745) | (15±3)** | (4670±1170) | $(14\pm 4)^{**}$ | (2990±568)* | (20±3)* | |
| Brinjal | 1300-1880 | 13-20 | 1930-3130 | 14-20 | | and the second | 747-1416 | 21-37 | |
| (round) | (1640±238) | (16±3) | (2480±480)* | (17±2) | ned sont Aroun | noi io asu an | (968±267)* | (28±6)* | |
| Brinjal | 1870-2770 | 17-27 | 1180-1990 | 13-20 | ioned our Aurorea | iratmens ro | 970-1430 | 18-28 | |
| (long) | (2240±380) | (22 ± 4) | (1670±330)* | (17±3) | a petere using a | nust_ascorta | (1180±180)** | (22±4) | |
| Cabbage | 1670-2680 | 21-29 | 780-1140 | 18-27 | oncy in the soll and | is any defici | 740-1420 | 22-36 | |
| wil, 127-134 | (2060±390) | (24±4) | (873±154)** | (24±4) | | to be raised | (995±263)** | (30±5) | |
| Calsbash | 2120-2940 | 12-21 | 1920-2670 | 20-29 | 1700-2320 | 8-11 | 1470-2540 | 13-19 | |
| (Ghia) | (2530±350) | (18±4) | (2310±340) | (25±4)* | (1980±300)* | (19±2) | (2050±440) | (16±2) | |
| Cucumber | 450-830 | 15-19 | 640-940 | 15-20 | ar provinces in col | ons of the for | 440-980 | 19-29 | |
| (Khira) | (654±160) | (16±2) | (770±120) | (17±2) | - | - | (700±206) | (24±4)* | |
| Cucumber | 1630-2630 | 14-27 | 980-1680 | 15-20 | | - | 2920-4130 | 18-34 | |
| (Tar) | (2200±410) | (21±5) | (1360±265)* | (18±2) | - | sectiones | (3520±500)** | (26±6) | |
| Carrot | 770-1460 | 17-26 | 1430-2040 | 14-21 | 640-1140 | 6-11 | 1820-2930 | 19-36 | |
| | (1010±268) | (20±4) | (1740±250)* | (18±2) | (820±200) | (8±2)** | (2500±390)** | (29±6)* | |
| Garlic | 320-640 | 13-20 | 430-770 | 13-19 | 490-740 | 9-15 | 520-720 | 18-20 | |
| | (455±137) | (16±3) | (608±150) | (16±2) | (585±108) | (10±3)* | (624±87) | (19±1) | |
| Ginger | 440-910 | 19-26 | 1420-1790 | 13-17 | 1640-2350 | 12-19 | - | - | |
| | (740±195) | (21±3) | (1560±189)** | (15±2) | (2030±312)** | (16±3) | - | - | |
| Lady finger | 550-980 | 17-29 | 750-940 | 11-18 | 670-1120 | 12-21 | 540-980 | 19-27 | |
| | (757±164) | (22±5) | (801±115) | (16±3)* | (849±200) | (17±3) | (731±159) | (23±4) | |
| Onion | 440-900 | 28-36 | 1060-1740 | 19-26 | 340-910 | 12-28 | 740-1370 | 19-30 | |
| | (730±188) | (31±3) | (1500±265)** | (23±2) | (634±215) | (20±6)* | (988±265) | (22±5)* | |
| Potatoes | 440-870 | 18-34 | 400-660 | 25-29 | 800-1920 | 8-13 | 370-760 | 26-36 | |
| | (670±173) | (25±6) | (524±99) | (27±2) | (1340±436)* | (10±2)** | (610±162) | (31±4) | |
| Radish | 3820-6060 | 15-22 | 3220-4590 | 16-21 | 1420-2340 | 18-26 | 1620-2920 | 17-20 | |
| | (4960±900) | (18±3) | (3750±594)* | (18±2) | (1680±430)** | (21±3) | (2170±540)** | (18±1) | |
| Spinach | 1200-1820 | 23-29 | 3170-3970 | 21-27 | 1830-2370 | 20-29 | - | - | |
| (Desi) | (1540±225) | (29±6) | (3480±269)** | (24±2) | (2050±218)* | (24±5) | - | - | |
| Spinach | 2940-4800 | 29-41 | 4410-5560 | 28-36 | 5640-6490 | 21-30 | 4170-5830 | 18-30 | |
| (Khatikal) | (3960±763) | (34±4) | (5200±660)* | (31±3) | (6210±310)** | (28±4) | (5129±758) | (25±5)* | |
| Sugar beet | 3750-5620 | 19-39 | 2620-3490 | 30-36 | 5480-6390 | 37-42 | - | - | |
| | (4860±760) | (29±8) | (3010±335)** | (33±2) | (6000±400)** | (40±2) | - | - | |
| Tinda | 900-1460 | 21-31 | 920-1130 | 18-24 | - | - | 720-980 | 28-31 | |
| | (1110±220) | (25±2) | (1060±110) | (21±2) | - | 12.14 | (832±97)* | (29±1) | |
| Tomatoes | 140-340 | - | 110-210 | - | - | - | 220-440 | - | |
| | (224±76) | - | (162±41) | - | - | - | (305±85) | - | |
| Turnip | 470-830 | 30-47 | 650-970 | 17-19 | 1640-3130 | 10-17 | 340-680 | 30-40 | |
| | (670±160) | (35±9) | (791±123) | (18±1)* | (2330±665)** | (13±3)** | (495±130) | (35±4) | |

TABLE 3. NITRATE AND NITRITE CONTENTS (ppm) OF VEGETABLES.

** Highly significant (P \leq .001), * Significant (P \leq .05, \leq .02, \leq .01).

contained significantly high nitrate nitrogen contents (Table 3). Potato tubers contained 370-1920 ppm nitrate nitrogen and carrot had nitrate contents ranging from 640-2930 ppm. Potatoes supplied by Turnab Agricultural Institute contained more nitrate as compared to those received from other provinces. Similar finding were reported earlier [12, 15, 16].

The nitrite contents of the vegetable were generally low. Reduction of endogenous nitrates to nitrites was highly significant (P<.001) in case of leafy root vegetables. These results are supported by other workers [17]. On the basis of these findings it can be concluded that contribution of vegetables as a source of nitrates and nitrites is greater than from cereals and pulses in the human diet.

The population of Pakistan is expanding at the rate of more than 3% per annum necessitating demand for more food and subsequent increase in the use of fertilizers. There is a need for proper education of the framers regarding the judicious use of fertilizers and they must ascertain before using such fertilizers (a) whether there is any deficiency in the soil and (b) the requirement of the crop to be raised.

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