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ACCUMULATION OF NITRATES IN LETTUCE *(Lactuca sativa)* DURING STORAGE FROM DIFFERENT GROWING MEDIA

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ABSTRACT

The values of nitrate accumulation in lettuce in all the media (bagasse, rice, coffee, soil, wood shaving, horse manure and sawdust) over the 20 day storage period was above the recommended WHO limit of 500 ppm. Lettuce grown in coffee exhibited the highest NO_3–N (2700–2880 ppm) after 10 days. Lettuce from soil (control), rice, and wood shavings had the highest NO_3–N after 5 days whereas in horse manure, coffee, sawdust and bagasse the highest NO_3–N was after 10 days. Lettuce from horse-manure, coffee and bagasse had a general decline in NO_3–N after 10 days unlike soil, rice, wood shavings and sawdust which had the opposite effect. Despite the varying trend in NO_3–N during storage, for lettuce from all the media except rice, visual quality was good after 20 days. Bagassee and sawdust appeared to produce the lowest NO_3–N and highest quality ratings during storage.

RESUMEN

En todos los medios (fibra de caña de azúcar, arroz, cafe, tierra, virutas de madera, abono de caballo y serrín), la acumulación de nitratos en lechugas durante un período de almacenamiento de 20 días fue superior al límite de 500 ppm recomendado por la OMS. Las lechugas cultivadas en el medio con base de cafe presentaron el nivel más alto de NO_3 – N (2700 - 2880 ppm) al cabo de 10 días. Las lechugas cultivadas en tierra (el control), arroz y virutas de madera presentaron el nivel más alto de NO_3 – N al cabo de 5 días, mientras que las que fueron culitvadas en abono de caballo, cafe, serrín y fibra de caña de azúcar sufrieron un descenso global al cabo de 10 días; se produjo lo contrario en la tierra, el arroz, las virutas de madera y el serrín. A pesar de las variaciones en los cambios del contenido de NO_3 – N durante el almacenamiento en todos los medios – salvo el arroz – las lechugas presentaron una buena apariencia al cabo de 20 días. Según las observaciones, el cultivo de lechugas en un medio de fibra de caña de azúcar o de serrín produce el nivel más bajo de NO_3 – N y la mejor calidad durante el almacenamiento.

Nitrate accumulation in plants is a natural phenomenon resulting from uptake of the nitrate ion in excess of its reduction and subsequent assimilation. The accumulation of nitrate is dependent on and related to the genetic makeup of the plant, the nitrate-supplying power of the soil and the environmental conditions under which the plant is grown (14). Previously, the main interest in connection with nitrates in vegetables and drinking water was geared towards cyanosis (methaemoglobinaemia), caused by the reduced form of nitrates namely nitrites. More recently, the main concern is the in vivo synthesis of nitrosamines, because of their potential carcinogenicity (10, 20). Taylor and Lijinsky (26) and Sander *et al.* (21) postulated that repeated intake of minute amounts of nitrosamines is more dangerous than a larger single dose. Although the nitrate content of vegetables can be high, nitrite content of vegetables is relatively low (7).

Nitrate may be converted to nitrite during storage of plant products as a result of bacterial action or plant reductase activity (13). Nitrite enters the stomach, where amides or amines are present to form nitrosamines (9, 11, 16, 18). There is no obvious direct link between total nitrate intake and nitrate concentration in the saliva. However, an intake above 40g of nitrate per day will lead to a pronounced increase of nitrate in the saliva (24). It is therefore necessary to aim for the lowest possible intake of nitrate and nitrite for humans and animals (23).

Most vegetables respond to a high nitrogen supply with increased yields, but they also show high nitrate accumulations in the tissue. This is enhanced in so-called nitrophilic plants like head lettuce, spinach, radish and various crucifers (27). Although various local conditions such as soil, climate, temperature, water relations and light intensity may play important roles in nitrate accumulation, fertilization deserves special attention since the availability of nitrate in the root zone and the nitrate concentrations of plant tissues can be related to the amount and kind of nitrogen fertilizer used.

The availability of a wide range of nitrogen fertilizers, indicates a potential risk to growers attempting to utilise the cheapest soil medium supplemented with artificial fertilizers to boost growth and subsequent yields in leafy vegetables such as lettuce. The attainment of higher yields would necessitate some form of storage for the increased supplies. In view of the conversion of nitrates of nitrites and the subsequent build up of nitrites that can occur under refrigeration, this study was initiated to examine the accumulation of nitrates in lettuce grown in different media during storage at 8–9 °C.

Materials and methods

Lettuce heads *(Lactuca sativa,* cv. 'Mignonette Bronze') were obtained from the Vegetable Section, Central Experiment Station, Ministry of Agriculture, Lands and Food Production, Trinidad and Tobago, where they were grown in separate plots consisting of seven different media: bagasse, coffee, horse manure, rice husks, sawdust, wood shavings and soil (control). The fertilizers used were 13:13:21 and sulphate of ammonia at 13 days after transplanting, followed by another application of 12:12:17 (2), 23 days after transplanting. The application rate was 14g per plant.

Lettuce heads were transported in plastic crates to the Biochemistry Laboratory 10 minutes after harvest, washed thoroughly and submerged in water for 20–25 minutes to simulate pre-storage hydro-cooling conditions. Initial water temperature was 2–3°C; the temperature rose no more than 5°C during the treatments. Lettuce heads were air-dried for 15 minutes at ambient temperature and individually seal-packaged in high density polyethylene (HDPE 0.025mm thick) and stored at 8–9 °C for 1, 5, 10, 15 and 20 days.

After each storage interval, each head of lettuce was examined for visual quality which was rated as 1 = unusable, 3 = unsalable, 5 = fair, 7 = good and 9 = excellent (6); fresh weight; percentage fresh weight loss; dry matter which was taken after drying in a hot air oven at 70°C for 48 hours; and nitrate content. The nitrate content was determined by the method of Harper (8). An extracting solution was made up of cupric sulphate pentahydrate, silver sulphate and distilled water and added to the sample (250mg) and shaken with activated charcoal. Calcium hydroxide and magnesium carbonate were added after shaking and the mixture was left to stand for not more than 20 minutes. The solution was filtered and an alliquot of the filtrate was evaporated to dryness on a sand bath. After removal from the sand bath, phenol disulphonic acid, water and sodium hydroxide solution with E.D.T.A. di-sodium salt were added. After cooling the colour developed was measured at 440mu on colorimeter. The range of the nitrate standard solution used for calibration was from 0 to 20 mg NO_3.

There were two replicates, with each replicate being an individual head of lettuce. Data were analysed as a completely randomized design, with a factorial arrangement of variables, and significance tested by the F-test and Duncan's multiple range test where applicable, after trans-formation for ranking (25).

Results

One day refrigerated storage

Samples of head lettuce from all the media had NO_3–N values above the recommended World Health Organisation (WHO) limit of 500 ppm (22) on a dry weight basis on the first day of harvest (Table 1). Heads from horse manure had the highest fresh weight (Fig. 1) and dry weights (Table 2). This corresponded with the highest NO_3–N level (Table 1). Similarly, lettuce heads from coffee and soil (control) had high fresh weights (Fig. 1), high dry weights and high NO_3–N values (Tables 1, 2). An opposite trend was noted for heads from the rice husk medium, i.e. low dry weight and fresh weights but a corresponding high NO_3–N content (Fig. 1, Tables 1, 2). Sawdust grown heads had low fresh and dry weights as well as low NO_3–N content while bagasse and wood-shavings showed minor differences in dry weight, 3.1 and 3.3g and NO_3–N content, 1718 and 1604 ppm (Tables 1, 2).

Five days' refrigerated storage

Despite a mean fresh weight loss of 4.1 per cent from all heads, there was no indication of wilting or discolouration (Table 4). Ratings for visual quality (Table 3) was not affected after 5 days at 8–9 °C. The relationship between dry weight and NO_3–N content of lettuce heads grown in horse manure, soil (control), coffee, rice and sawdust appeared to be consistent with the results obtained when heads were examined previously and after a post-harvest period of 5 days at 8–9 °C. Although heads taken from sawdust had the lowest dry weight, they also had the lowest NO_3–N content together with high quality ratings (Tables 1, 2, 3). The difference in dry weight between bagasse and wood shavings was 0.3 per cent while that of NO_3–N content was 830 ppm. The NO_3–N value of lettuce heads from sawdust was significantly lower ($P = 0.01$) than all the other media except bagasse (Table 1).

Ten day's refrigerated storage

During this storage period both NO_3–N content and dry weight of lettuce heads from soil (control) were lower than from coffee and horse manure, even though visual quality ratings were still high for all three media (Tables 1, 2, 3). Among all the media, samples from horse manure had the highest NO_3–N

Table 1. Effect of different soil media on the accumulation of NO_3 -N in lettuce after 1, 5, 10, 15 and 20 days at 8 - 9°C.

	Storage period (days)					
Growing medium	1	5	10	15	20	Mean
Bagasse	1718	959	1133	931	866	1121
Rice husks	2251	2463	1625	2289	2330	2192
Coffee	2047	2339	2747	2430	2238	2360
Wood shavings	1604	1789	1643	1446	1777	1652
Horse manure	2658	2777	2872	2460	2339	2621
Sawdust	811	743	909	717	1058	848
Soil (Control)	2184	2528	1972	1984	2421	2218
Mean	1896	1943	1843	1951	1861	1859

Error Variance = 32,426 Error d.f. = 28

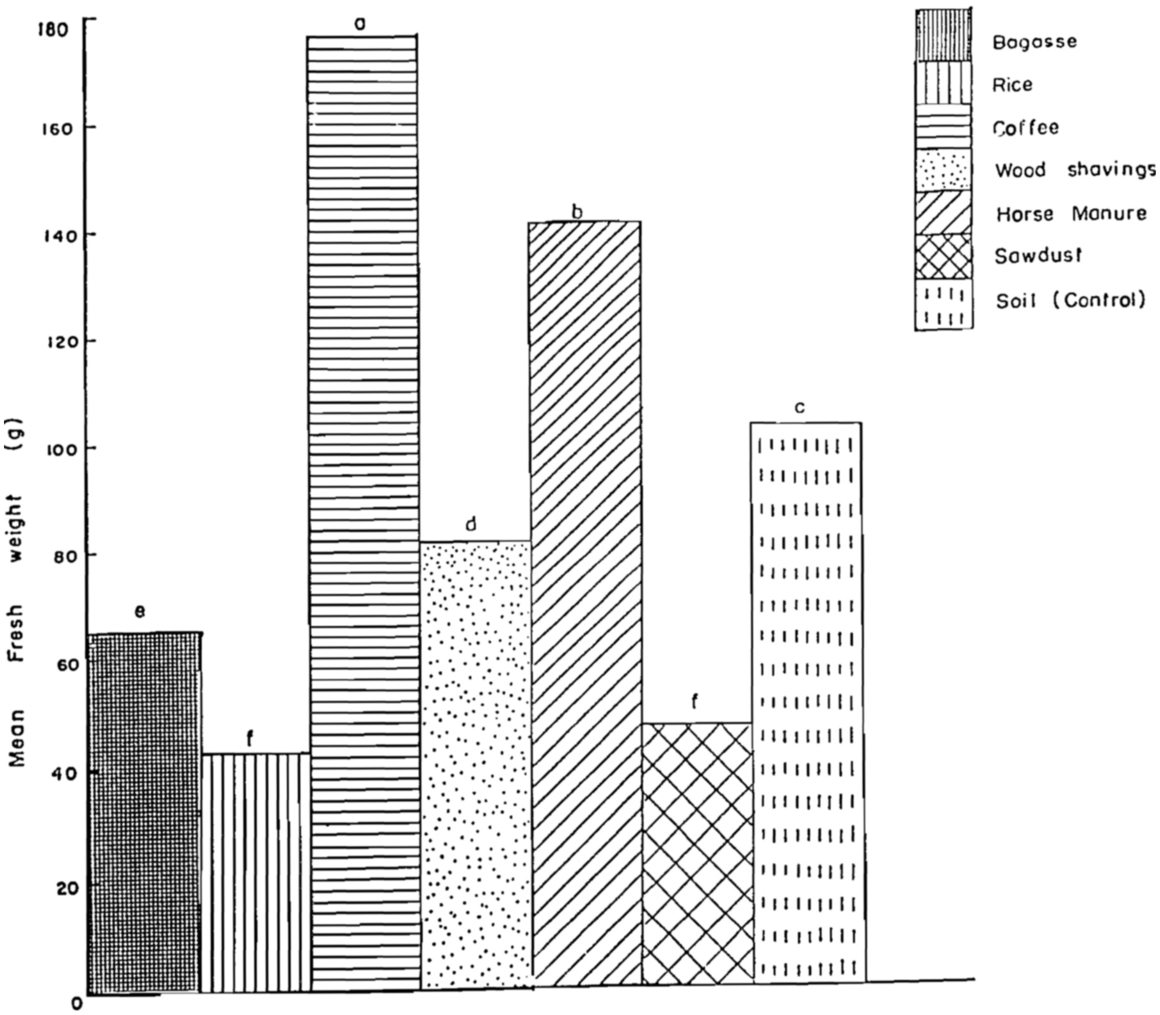

Figure 1: Mean pre-storage fresh weight (g) of lettuce heads from different media[z].

z Mean separation in each coloum for each treatment by Duncan's multiple range test; means not suffixed by the same letter are statistically different (P = 0.05).

Table 2. Effect of different growing media on the dry weight of lettuce after 1, 5, 10, 15 and 20 days at 8 - 9°C.

	Storage period (days)					
Growing medium	1	5	10	15	20	Mean
Bagasse	3.1	2.9	3.0	3.4	3.2	3.1
Rice husks	2.2	2.1	3.0	1.9	2.4	2.3
Coffee	6.0	5.1	7.1	7.3	6.3	6.4
Wood shavings	3.3	4.0	3.7	3.9	4.1	3.8
Horse manure	6.6	6.4	6.8	5.9	3.8	5.3
Sawdust	2.0	2.6	3.4	2.3	1.7	2.4
Soil (Control)	4.2	5.0	5.0	4.7	3.6	4.5
Mean	3.9	4.0	4.6	4.1	3.6	4.0

Error Variance = 2.4 Error d.f. =2.8

value, the lowest percentage fresh weight loss and no effect on quality (Tables 1, 3, 4). Samples from the rice husk medium had a significant ($P = 0.05$) reduction in NO_3–N after 10 days at 8–9°C with excellent keeping quality. From the sawdust medium, lettuce heads had high quality ratings but the lowest NO_3–N when compared to the other soil media (Tables 1, 3). Although the dry weight and quality of samples from bagasse and wood shavings were similar (Tables 2, 3), the percentage fresh weight loss in the former medium was significantly higher ($P = 0.01$) while NO_3–N content was significantly lower ($P = 0.05$) than the latter medium (Tables 1, 4). Among all the media, only samples from sawdust and bagasse had significantly ($P = 0.05$) lower NO_3–N values than soil (Table 1).

Fifteen days' refrigerated storage

The quality ratings, as indicated by distinct brown discolouration at the butt end and slight wilting of the outer leaves of lettuce heads from the rice and soil media, were significantly ($P = 0.05$) lower than the other media (Table 3). Even after 15 days at 8–9°C, the lettuce heads from horse manure and coffee had high quality ratings but elevated NO_3–N values (Tables 1, 3). Lettuce heads from the rice medium had the lowest dry weight and showed a significant decline in quality and an increase in NO_3–N content when compared to the previous storage periods (Tables 1, 2, 3). Although there were only minor changes in storage quality of lettuce heads from bagasse and sawdust, the values of NO_3–N were approximately three times lower than that of rice, coffee or horse manure (Tables 1, 3). Lettuce heads from wood shavings had lower NO_3–N content, dry weight and percentage fresh weight loss but similar quality ratings when compared with coffee and horse manure (Tables 1, 2, 3, 4).

Twenty days' refrigerated storage

Lettuce heads from bagasse and sawdust had lower NO_3–N values ($P = 0.01$) and maintained high quality ratings in contrast to the other media (Tables 1, 3). Although samples from soil (control) appeared to have good keeping quality after 20 days at 8–9°C, they had the highest NO_3–N and fresh weight loss (Tables 2, 3, 4). Samples from coffee and horse manure continued to have good quality ratings but high NO_3–N values (Tables 1, 3). The NO_3–N values from wood shavings were lower than from coffee, horse manure, soil and rice husks but higher than those from bagasse and sawdust (Table 1).

During this storage period, lettuce heads from rice husks had the lowest quality ratings as indicated. Leaves had oval to irregular lesions that ranged from yellow to brown. In some cases the lesions coalesced to form larger, uniformly coloured, depressed patches. The margins of the outer leaves had a scorched soggy appearance making these heads unmarketable. It must be noted that, along with the poor quality, these heads also showed a high NO_3–N content comparable to that of samples grown in coffee, horse manure and soil.

Discussion

Despite a constant fertilizer regime in terms of type and application rate, there were differences in NO_3–N values among the media at the end of every storage period. This is in agreement with the views of Maynard *et al* (14) that different media can result in variations with regards to uptake, assimilation, translocation and accumulation of nitrates in plants. Previous work by Barker (3) and Peet *et al.* (19) indicated that, due to mineralization and nitrification, nitrate is the primary soil-derived N-form

Table 3. Effect of different growing media on the visual quality of lettuce after 1, 5, 10, 15 and 20 days at 8 - 9°C.

	Storage period (days)				
Growing medium	5	10	15	20	Mean
Bagasse	9.0	9.0	8.5	8.0	8.6
Rice husks	9.0	9.0	7.0	4.0	7.3
Coffee	9.0	8.5	8.5	7.5	8.4
Wood shavings	9.0	9.0	8.3	7.5	8.5
Horse manure	9.0	9.0	8.8	8.0	8.7
Sawdust	9.0	9.0	8.5	8.3	8.7
Soil (Control)	9.0	9.0	7.0	7.5	8.1
Mean	9.0	8.9	8.1	7.3	8.3

Error Variance = 17.2 Error d.f. =28

Ratings : 1= unusuable 9= excellent

Table 4. Effect of different growing media on percentage fresh weight loss of lettuce after 1, 5, 10, 15 and 20 days at 8 - 9°C.

	Storage period (days)				
Growing Medium	5	10	15	20	Mean
Bagasse	5.4	6.2	7.3	7.6	6.6
Rice Husks	3.9	5.1	6.2	6.1	5.4
Coffee	3.6	4.6	5.4	6.1	4.9
Wood shavings	3.6	3.9	4.4	5.4	4.3
Horse manure	3.2	3.6	4.9	6.3	4.5
Sawdust	4.2	4.5	5.1	6.5	5.1
Soil (Control)	4.9	5.0	5.1	8.0	5.8
Mean	4.1	4.7	5.5	6.6	5.2

Error Variance = 7.3 Error d.f. =28

regardless of the source of N applied. Therefore, within limits as much nitrate can be accumulated from organic fertilizers as from nitrate carriers if sufficient time is allowed for mineralization to occur.

It is possible that due to greater microbial activity application of the fertilizers had an additive effect in causing a faster uptake, assimilation, translocation and accumulation of NO_3–N in the coffee, horse manure and soil media. The elevated levels of NO_3–N in these media can be related to a more rapid rate for mineralization and nitrification. Barker (3) pointed out that media composed of dried materials would mineralize slowly resulting in lesser nitrate accumulation in the plant tissue. Among the dried materials making up the media in this study, rice husks appeared to mineralize fastest, and consequently had heads with the highest NO_3–N values while sawdust was the slowest resulting in heads with the lowest NO_3–N value.

The fact that NO3–N values from lettuce heads grown in the rice husk medium were comparable to those of horse manure, coffee and soil may be due to (a) the rapid uptake mechanism and (b) the low capacity for the root system to reduce nitrate, thus a greater proportion of N may have been transported to the shoots as nitrate or a combination of (a) and (b) as suggested by Maynard *et al.,* (14).

Minotti (17) claimed that environmental factors such as light, temperature, carbon dioxide concentration, fertilizer, soil type, etc., may modify tissue nitrate by affecting any or all of the processes of absorption, assimilation and translocation. It may be deduced that, despite the constant fertilizer regime applied to all the media, absorption of NO_3–N in the wood shavings, bagasse and sawdust media was limited due to low availability to nitrates in the root zones thereby resulting in minimal assimilation and translocation rates. By contrast, lettuce heads from the other media produced the opposite effect. The need for further research to investigate the factors that may affect assimilation or translocation and the role involved in regulating nitrates under production systems where a range of media are utilised is therefore warranted.

In contrast to previous studies by Asseo-Bickert (2) which indicated an increase in NO_3–N values for greenhouse lettuce during storage at 5–12°C, this investigation did not reflect a similar relationship. However, in the present study the use of different media, temperature and package may have influenced the variations obtained as the number of days in storage increased. Results from both studies, however, indicate the threat of nitrate toxicity to man from ingestion of lettuce since the values were above the recommended WHO limit of 500 ppm (22).

It was obvious from this study that the technique of individual seal-packaging of lettuce heads from all the media with the exception of rice husks in high density polyethylene (HDPE) extended shelf life up to 20 days. Similar results were obtained by Aharoni *et al.* (1) for lettuce, fruits and other vegetables by Ben-Yehoshua (5) and Mohammed (15) using the same type of packaging material.

The relationship between quality and NO_3–N values of lettuce heads during storage suggested a need for educating those involved in growing and marketing about the risks involved in high NO_3–N levels. Consumer demand for lettuce heads based on quality factors such as size of heads, compactness as well as greeness and lack of leaf wilting without considering the type of growing medium or fertilizer may lead to elevated NO_3–N intake as well. Although size, fresh weight and compactness of heads were not used to evaluate quality in this study, growers should possibly be encouraged to utilise sawdust in preference to other media because of low NO_3–N values and high quality rating. Despite this, consumers may still opt for heads grown in horse manure, coffee or soil since their evaluation of quality usually does not include a knowledge of NO_3–N levels as an important factor. This lack of consumer knowledge emphasizes the need to establish baseline data for other nitrophilic vegetables. Lettuce heads from the rice husk medium may receive unfavourable recommendation because of their low fresh weight (Fig. 1), low dry weight (Table 2) and high NO_3–N values (Table 1), with a tendency to senesce most rapidly. It is possible that the drastic increase in NO_3–N after 15 and 20 days of storage which also corresponded with a similar decline in quality (Table 3) could have been related to microbial contamination as suggested by Vogtmann *et al.*

Visible wilting was noted in lettuce heads that had 6 percent loss in fresh weight. (This is in agreement with Barger (4) and Lipton and Barger (12)). Samples from bagasse and rice husks reflected this after the 10 and 15 day storage intervals (Table 4). Although the sealed high density polyethylene bags (HDPE) would have insured an effective barrier to water vapour transmission and attained a relative humidity of 95–100 per cent, the type of heads from bagasse and rice husks, i.e. not as compact and having a greater surface area, may explain the fresh weight loss differences as indicated in this study.

The results of this study indicate that the demand from toxicologists for a reduction in the daily nitrate intake from lettuce ought to be given serious consideration. Important inputs may come from proper refrigeration, timely consumption patterns, the incorporation of low nitrate-accumulating cultivars, fertility and management practices to result in restricted nitrate concentration, and the manipulation of the most suitable medium as well as environmental variables into the crop production scheme.

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