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Nutritionally Important Chemical Constituents and Yield of Carrot (Daucus carota L.) Roots Grown Organically Using Ten Levels of Green Manure


In a field experiment carried out over two years (1995, 1996) carrots were grown organically on a sandy loam soil. A broad range of mineral nitrogen (N-min) levels at carrot emergence was obtained by depleting or amending the soil by removing or supplying different amounts of green manure. With N-min values ranging from 22 to 162 kg N ha$^{-1}$ at carrot emergence, the N uptake of the carrot crop increased linearly from 70 to 200 kg N ha$^{-1}$. The yield increased with N-min at lower levels of N, but levelled off above 90–100 kg N ha$^{-1}$. In fresh carrot roots the concentrations of total N, ten individual amino acids, total sum of amino acids, two amides, asparagine and glutamine, and β-carotene increased linearly with the soil concentration of N-min at carrot emergence. Nitrate N increased exponentially with N-min, reaching levels of 340 mg NO$_3$ kg$^{-1}$ fresh weight under some conditions. The concentrations of potassium, calcium, glucose, fructose, sucrose and six individual free amino acids were unaffected by the N-min level. Magnesium was decreased at the lowest deliberately depleted N-min levels, whereas the concentration of dry matter and vitamin C decreased linearly with increasing N-min levels. Significantly lower concentrations of dry matter, total N, nitrate N, vitamin C and total sum of free amino acids were found in the warm and sunny year 1995 with the highest yield of carrot roots, whereas the contrary was found for β-carotene.

Introduction

Carrot ranks as the fifth most consumed vegetable in the European Union. It is a rich source of the provitamin β-carotene, which is absorbed in the human body and converted to vitamin A, an essential vita-

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consumption of these nutrients. Sufficient intake of minerals such as calcium (Ca) is important in preventing osteoporosis (Anderson & Garner, 1996). Sugars in carrots are closely related to sweetness (Martens et al., 1985; Hogstad et al., 1997) and the amino acids affect their bitterness and cosweetness (Solms, 1969). Nitrate in vegetables is considered unwanted because a high concentration increases the risk for methaemoglobinaemia among babies and generally is considered to be involved in the formation of carcinogenic nitroso compounds in the processing and consumption of foods (Phillips, 1971; Hofmann, 1995; Derache & Derache, 1997). The concentration of nitrate is relatively low in carrots (Chessin & Hicks, 1987; Wiebe, 1987). Superoptimal nitrogen (N) supply has been shown to cause undesirable high nitrate levels in carrot, whether supplied as mineral fertilizer (Schaller & Schnitzler, 2000) or by mineralization of residues of previous crops (Sorensen, 1993). The concentration of essential amino acids may have importance for vegetarians.

When carrots are grown organically on farms with animal husbandry, the normal practice is to grow carrots after a clover grass ley has been ploughed in. Here, the N supply is typically well above the N demand of carrots. In Danish organic farming, N as a plant nutrient is supplied by crop rotation and using manure, which may be composted or not. Green manure is an important alternative source of N in organic farming without animal husbandry, and it can be used to manipulate the links between amount of available N and the temporal progression of N uptake in the carrot crop (Thorup-Kristensen & Bertelsen, 1996; Thorup-Kristensen & van den Boogaard, 1999). However, organic farmers are not used to considering the risk that too high nutrient availability may have adverse effects on crop quality. So, it is not unusual that crops such as carrot, with low nutrient requirements, are inadvertently subjected to a superoptimal N supply (K. Thorup-Kristensen, 2001, unpublished).

For carrot cultivation a target amount of 100 kg N ha⁻¹ of mineralized N (N-min) at the beginning of the growing season is considered optimal (Scharpf & Weier, 1994; Rühlmann et al., 1996). Continued mineralization of the organic matter in the soil during the season can then supply the remaining N to reach the 160 kg N ha⁻¹ required in total by the carrot crop (Scharpf & Weier, 1994).

Earlier investigations on organic fertilization strategies have lacked consideration of mineralization of the N pool of the soil, or covered only a narrow range of soil fertilities, or both. By monitoring both N-min and measures of yield and quality across a wide range of N availability, the data can be used both to optimize the organic system and to make objective comparisons possible with the results from conventional systems. Even without any treatment, the mineralization of soil organic matter can provide substantial amounts of N. So, to extend the range to mimic a less fertile soil, it is necessary actively to deplete the soil of N by growing and removing a non-legume crop shortly before sowing. Some organic farmers grow winter-hardy cereals or other grasses on the fields during the winter, to ensure continuous soil cover. They may thus inadvertently deplete the plant-available N-min content of the soil, if the cover crop is not incorporated in time to decompose before the next crop is sown.

Earlier studies have shown N-min at the time of emergence to be the best measure of overall N availability (Scharpf & Weier, 1994; Rühlmann et al., 1996).

The aim of this research was to study the effects of a wide range of N-min levels in the soil, established by different green manure treatments, on the concentrations of nutritionally important compounds in carrot roots.

The hypothesis was that the yield of carrot roots on a fertile soil depleted for N would increase significantly with the supply of N-min from green manure and that the concentration of amino acids, amidases, and other N constituents would increase proportionally with the N supply, with the risk of an unhealthily high concentration of nitrate.

Material and methods

Design of the field experiment

The field experiment took place at the Research Centre Aarslev (10°27'E, 55°18'N), on a fertile sandy loam soil, using a randomized complete block design. A time schedule for the experiment is shown in Table 1. The crop for production of green manure was a mixture of winter rye (Secale cereale L.) and hairy vetch (Vicia villosa Roth.) sown at the beginning of August after harvest of a barley crop. Ten levels of N availability expressed in values of N-min at carrot emergence were established in three replicates: after fallow field, a crop of winter rye, and four levels of green manure that were rotted onto the soil in autumn or spring (Table 1). The green manure treatments were: 0, growing of green manure, but removing the plant material before soil tillage; 1, normal incorporation of the green manure grown on the plot; 2, incorporating double amounts of green manure; and 3, incorporating triple amounts of green manure. The additional amounts of green manure were obtained from an area adjacent to the experimental green manure plots. The amount of rye/vetch incorporated as roots using these amounts encom-
Table 1. Crops, activities and dates for sowing of carrot seeds, incorporation of green manure, removal of the depletion crop (rye), sowing, soil sampling at carrot emergence and the first harvest date for harvest in 1994/95 and 1995/96

<table>
<thead>
<tr>
<th>Crop and green manure</th>
<th>Activity</th>
<th>1994/95</th>
<th>1995/96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye and rye/vetch</td>
<td>Sowing</td>
<td>1 August</td>
<td>1 August</td>
</tr>
<tr>
<td></td>
<td>Autumn incorporation</td>
<td>31 October</td>
<td>30 October</td>
</tr>
<tr>
<td></td>
<td>Spring incorporation</td>
<td>24 March</td>
<td>16 April</td>
</tr>
<tr>
<td>Rye</td>
<td>Removal</td>
<td>8 May</td>
<td>6 May</td>
</tr>
<tr>
<td>Carrot</td>
<td>Sowing</td>
<td>23 May</td>
<td>23 May</td>
</tr>
<tr>
<td></td>
<td>Soil sampling</td>
<td>11 June</td>
<td>11 June</td>
</tr>
<tr>
<td></td>
<td>First harvest date</td>
<td>30 August</td>
<td>2 September</td>
</tr>
</tbody>
</table>

passed roots from one level of green manure only. The fallow field was considered as a non-treatment level of N-min and a depletion treatment was accomplished by removal of a rye crop 15 and 17 days before soil rotovation in 1995 and 1996, respectively.

Carrot seeds of the cultivar “Fancy” were sown on 23 May in both years (Table 1), at a row distance of 50 cm and 100 seed m\(^{-1}\). The field management was as described in detail by Thorup-Kristensen (1993) and Thorup-Kristensen & Bertelsen (1996).

The carrots were harvested on four dates with intervals of 2 weeks, with the first harvest on 30 August and 2 September in 1994 and 1995, respectively. The leaves were removed from the roots before sorting, the weights of the carrot roots and leaves were determined separately and samples were taken at random for chemical analyses. The precipitation from sowing of the rye and rye/vetch crop on 1 August until carrot emergence on 11 June (Table 1) was 798 and 296 mm and the average temperature during this period was 8.1 and 7.1°C in 1994/95 and 1995/96, respectively. The sum of the average daily temperature (\(\Sigma (°C_{\text{min}} + °C_{\text{max}})/2\)) in the period from the beginning of May to the end of October was 2624°C and 2348°C in 1995 and 1996, respectively. The accumulated global radiation in the same period was 2877 MJ m\(^{-2}\) in 1995 and 2605 MJ m\(^{-2}\) in 1996.

**Chemicals**

Methanol (MeOH), tetrahydrofuran (THF) and acetone of high-performance liquid chromatography (HPLC) grade were obtained from either Merck (Darmstadt, Germany) or Sigma (Diesenhofen, Germany), and the water was either distilled or ultrapure, generated by an Elgastat Maxima Analytica (Elga, UK) water purification system. All HPLC eluents were filtered using a 0.45 μm nylon filter (Cameo, Sigma) before use. Magnesium carbonate (MgCO\(_3\)), sodium sulfate (Na\(_2\)SO\(_4\)), sodium acetate (CH\(_3\)CO\(_2\)Na), trichloroacetic acid (TCA) and HCl were of analytical grade from Sigma. Amino acids, ethanolamine, 2-mercaptopetanol (2-Me), O-phthalaldehyde reagent solution (OPA) and 2,4-dichloro-indophenol were all purchased from Sigma.

**Mineral nitrogen**

At the time of carrot emergence soil samples were taken from four layers from 0.25 m down to 1 m, with nine subsamples for each plot in each depth as described by Thorup-Kristensen & Bertelsen (1996) and Thorup-Kristensen & van den Boogaard (1999). Ammonium N and nitrate N was extracted with 1 M KCl for 2 h, measured spectrophotometrically (Best, 1976) and the sum (N-min) was corrected for soil density (Thorup-Kristensen, 1993; Thorup-Kristensen & Bertelsen, 1996).

**Plant material and sample preparation**

Immediately before incorporation of the green manure samples were taken at random from the thoroughly mixed harvested above-ground material and total N content was measured.

At harvest 3 kg of thoroughly mixed carrot leaves was sampled from each plot and treated in a meat mincer (model 2964; Ingvald Christensen, Odense, Denmark) to provide samples for analyses. All saleable carrot roots from the middle of the 3.5 m\(^2\) of each plot were mixed thoroughly and transferred one by one into a number of successive wood boxes until each box contained 5 kg carrots. For each plot one box of carrot roots was selected using randomly generated numbers. The carrots were washed in cold water at 12°C, then cut into cylindrical slices 2.5 cm thick that were mixed and divided into seven subsamples each containing 500 g, and used for determination of amino acids, vitamin C, sugars, β-carotene and dry matter (DM). The samples for determination of amino acids were packed in flexible aluminium bags (Lamofoil M 12/9/75; Danisco, Højbjerg, Denmark), then frozen and stored at \(-24°C\).
Dry matter, nitrogen, nitrate and minerals

DM was determined by drying at 80°C for 20 h in a heating cabinet (CBM; Lytzen A/S, Herlev, Denmark). The dried plant material was milled in a Retsch mill (Haani, Germany) and stored at 20°C. Total N, nitrate N and minerals [potassium (K), magnesium (Mg) and Ca] in the milled DM of carrot roots were measured as described by Hansen (1989), Best (1976) and King (1984), respectively. Chemical analysis of the carrot leaves encompassed the determination of DM and total N.

Sugars and vitamin C

Fresh carrot root slices were homogenized in distilled water (1:1) for 2 min in a Waring blender (B012-34-BL 99; Waring Products, New Hartford, USA) and centrifuged at 15,000 × g in a Sorwall Products centrifuge (RC-SB, UK). Supernatants were stored at −24°C until analysis. Thawed samples were filtered through Schleicher and Schuell filter paper (no. 589, white ribbon, reference no. 300111, diameter 125 mm), diluted 40–100 times, and fructose, glucose and sucrose were measured using HPLC as described by Kaack et al. (1993).

The concentrations of ascorbic and dehydroascorbic acid were determined using a method adopted from Pongracz (1971). Fresh carrot slices (200 g) were homogenized in 1 g kg⁻¹ oxalic acid solution (1:1) and, to a 15 ml subsample, 1 ml 100 g kg⁻¹ CH₃CO₂Na solution was added. The mixture was flushed with N₂ for 3 min and the solutions were titrated using a reagent of 0.3 g 2,4-dichloroindophenol in 1 l distilled water, until a colour change occurred.

β-Carotene

Sample preparation for HPLC. Fresh carrot slices were homogenized in distilled water (1:1) for 2 min in a Waring blender, frozen and stored for less than 1 month at −24°C. Samples for HPLC were prepared according to the method of Bushway & Wilson (1982). Under red light, homogenized carrot samples (3 g), MgCO₃ and anhydrous Na₂SO₄ in the proportion 1 : 0.1 : 2 w/w, respectively, were mixed with 20 ml acetone and treated for 2 min in an ultrasonic cell disrupter (Sonifier 250; Branson Sonic, Danbury, USA). The mixture was filtered through a funnel with porosity 3 (Schott, Duran, Germany), and the remaining solids were dissolved in 20 ml acetone, disrupted and filtered. The total filtrate was dissolved in 100 ml acetone and filtered (0.45 μm nylon filter).

Instrumentation and chromatographic conditions. Samples were analysed on a Shimadzu HPLC system (Kyoto, Japan) with ultraviolet detection at 450 nm. Separations were performed on a Supelcosil LC-18-DB column (5 μm, 150 × 4.6 mm; Supelco, Bellefonte, USA), at 35°C, by isocratic elution using water-THF (25 : 75 v/v) as the mobile phase. The flow rate was 1 ml min⁻¹ and the injection volume 150 μl. Standard solutions of β-carotene in acetone were used for quantification.

Amino acids

Sample preparation for HPLC. Five samples of 50 g carrot slices from each plot were frozen in liquid N₂, freeze-dried at 10⁻⁶ mmHg at 25°C and ground in a Retsch mill (particle sizes < 0.5 mm). Then, 50 mg of the milled material was placed in 40 ml centrifuge tubes and 10 ml 3% TCA was added. The centrifuge tubes were placed in an ultrasound cell disrupter for 90 s before adding a further 10 ml 3% TCA. After 30 min the samples were centrifuged at 23,000 × g for 20 min and the supernatant (0.2 ml) was mixed with 0.4 ml internal standard solution and 2.4 ml 0.05 M CH₃CO₂Na buffer.

Instrumentation and chromatographic conditions. The free amino acid concentration in milled plant material was determined using procedures adapted from White & Hart (1992). A Shimadzu HPLC system (Kyoto, Japan) was used, consisting of a quaternary pump system with an on-line degassing device (LC-10AT, FCV-10AL), a column oven (CTO-6A), an autoinjector (SIL-6A) and a fluorescence detector (RF-551) operated at an excitation wavelength of 340 nm and emission wavelength of 440 nm. Separations were performed on a Superspher 100 RP-18 column (4 μm, 250 × 4.0 mm i.d.; Merck), at 30°C, using a ternary solvent system consisting of solvent A (MeOH–THF−0.05 M CH₃CO₂Na buffer; 19 : 1 : 180, v/v/v, pH 5.9), solvent B (MeOH–THF−0.05 M CH₃CO₂Na buffer; 32 : 1 : 7, v/v/v, pH 5.9) and solvent C (80% MeOH). The elution profile was: 0–2 min 0% B and C; 17 min 10% B and 0% C; 27 min 15% B and 0% C; 32 min 20% B and 0% C; 37 min 25% B and 0% C; 42 min 40% B and 0% C; 50–60 min 50% B and 0% C; 85 min 90% B and 0% C; 87 min 100% B; 89–96 min 100% C. The flow rate was 1 ml min⁻¹ and the injection volume 20 μl. A standard mixture of amino acids was used for the identification and quantification of the free amino acids in HPLC samples.

Derivatization procedure. The derivatization reagent (OPA, 2-Me) was prepared by adding 0.5 μl of 2-Me to 1 ml OPA. For automated derivatization, 100 μl OPA, 2-Me reagent and 100 μl aliquots of samples to be analysed were dispensed into an empty vial and
Table 2. Amount of depletion crop (rye) removed, green manure removed or supplied and concentration of total nitrogen (N) in the relevant herbage in both years

<table>
<thead>
<tr>
<th>Crop</th>
<th>Green manure (kg ha^{-1})</th>
<th>Total N (g ha^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>5360</td>
<td>3710</td>
</tr>
<tr>
<td>Rye/vetch, autumn</td>
<td>2330</td>
<td>3340</td>
</tr>
<tr>
<td>Rye/vetch, spring</td>
<td>1860</td>
<td>3050</td>
</tr>
</tbody>
</table>

mixed by dispensing twice. After waiting for 187 s, 20 µl of this mixture was injected. For calibration a standard mixture containing 33.2 µM of each of the 18 amino acids in 0.1 M HCl was used. The internal standard solution contained 0.33 nM of ethanolamine in 0.1 M HCl. The standards were stored at 4°C and prepared freshly every 3 weeks.

Statistical analysis

The basic sources of variation were green manure (df 9), harvest time (df 3), year (df 1) and block (df 2). A variance analysis revealed no significant differences in the concentration of chemical compounds between harvest times, and no significant interactions were found between year and harvest time. Therefore, the average values of the results from the four harvest times were calculated for each of the three blocks, and these results were used as replicates. If the difference in concentration between years for a constituent was non-significant, as found for K, Mg, Ca and sugars, the average for both years is presented. Variance homogeneity was tested using Bartlett’s test and the data were subject to analysis of variance using GLM, paired and multiple comparison t-tests and mean separation multiple-stage tests (REGWQ), respectively (SAS Version 8.00; Cary, NC, USA).

Data processing by linear or exponential regression included calculation of regression coefficients and intercepts. Linearity of the statistical models and significant differences between the calculated variables were found using F- and t-tests, respectively. A 5% level of probability was used for statistical tests unless stated otherwise.

Results

Green manure and mineral nitrogen

The amount of rye crop and green manure and the concentration of total N in this above-ground herbage are shown in Table 2, and the amount of N supplied and N-min values obtained at carrot emergence are shown in Table 3. During the two years a range from 22 to 162 kg N ha^{-1} was obtained using green manure. The depleted (rye) treatment resulted in significantly lower N-min values at carrot emergence than the fallow treatment, as did level zero (0) green manure treatments in 1996. The N-min values for level 1 of green manure treatment differed less between years than the fallow treatment, whether the

Table 3. Treatment and nitrogen (N) supplied by green manure (GM) and mineral N (N-min) (kg N ha^{-1}) in the soil at carrot emergence

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1994/95</th>
<th>1995/96</th>
<th>1995/96</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N supplied by rye crop and GM (kg N ha^{-1})</td>
<td>N-min at carrot emergence (kg N ha^{-1})</td>
<td>N supplied by rye crop and GM (kg N ha^{-1})</td>
</tr>
<tr>
<td>Rye</td>
<td>70</td>
<td>48</td>
<td>70</td>
</tr>
<tr>
<td>Fallow field</td>
<td>–</td>
<td>80</td>
<td>–</td>
</tr>
<tr>
<td>Autumn incorporation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM 0</td>
<td>–</td>
<td>92</td>
<td>–</td>
</tr>
<tr>
<td>GM 1</td>
<td>98</td>
<td>98</td>
<td>137</td>
</tr>
<tr>
<td>GM 2</td>
<td>196</td>
<td>124</td>
<td>274</td>
</tr>
<tr>
<td>GM 3</td>
<td>294</td>
<td>151</td>
<td>411</td>
</tr>
<tr>
<td>Spring incorporation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM 0</td>
<td>–</td>
<td>104</td>
<td>–</td>
</tr>
<tr>
<td>GM 1</td>
<td>67</td>
<td>114</td>
<td>125</td>
</tr>
<tr>
<td>GM 2</td>
<td>134</td>
<td>162</td>
<td>250</td>
</tr>
<tr>
<td>GM 3</td>
<td>201</td>
<td>133</td>
<td>375</td>
</tr>
</tbody>
</table>

LSD_{(0.05)} for N-min was 3 kg N ha^{-1}.
roots were incorporated in autumn or in spring; however, on average fallow and level 1 of green manure were not different. The atypically low N-min value found for treatment 3 by incorporation in spring 1995 is probably an underestimate due to non-efficient mixing and sampling of soil, since this treatment had the highest N uptake of the study (Fig. 1D) and the highest nitrate concentration in 1995 (Fig. 2C).

**Nitrogen uptake and yield**

The yield of saleable carrot roots increased to between 90 and 100 kg N-min ha\(^{-1}\) in the soil at carrot emergence and levelled off at higher values (Fig. 1A). The average yield of carrot roots was significantly different between years and the highest yield was found in the sunnier year, 1995. The total uptake of N by carrot roots and leaves increased linearly and to the same extent with N-min level in the soil at carrot emergence (Fig. 1D). The same trend was found when calculated only for saleable roots, by excluding the contributions from leaves and discarded roots (data not shown).

N-min values measured at later growth stages, 26 September and 3 October in 1995 and 1996, respectively, were not as well correlated with N uptake as those measured at emergence (data not shown), so in all calculations only the N-min values measured at carrot emergence were used.

**Fig. 1.** (A) Total yield of saleable carrot roots, (B) yield of dry matter (DM) in leaves, (C) yield of DM in roots, and (D) total nitrogen (N) uptake by total yield of carrot roots and leaves versus mineral N (N-min) at carrot emergence in two years.

**Fig. 2.** Concentration of (A) dry matter (DM), (B) total nitrogen (N), and (C) nitrate N in carrot roots versus mineral N (N-min) at carrot emergence in two years. FW: fresh weight.
In both years the total sum of free amino acids and amides (TFA) increased significantly with N-min at carrot emergence (Fig. 3C). The concentration of DM in saleable roots decreased significantly with increasing level of N-min at emergence (Fig. 2A) and differed significantly between years.

The concentration of total N and nitrate N in saleable roots increased linearly and exponentially, respectively, with the level of N-min in both years (Fig. 2B, C).

No significant differences were found for the content of minerals between years (data not shown) and the concentrations of K and Ca were not significantly affected by N-min level (Table 4). For Mg only the depleted (rye) treatments resulted in a significantly lowered concentration of Mg in the carrots.

The K uptake in both years was linearly correlated with uptake of N (data not shown), with the highest levels of K in 1994/95. The calculated regression functions with K and N uptake as y and x were y = 110.1 + 0.54x; P for slope < 0.01, R² = 0.652; and y = 105.7 + 0.37x; P for slope < 0.01, R² = 0.719 for 1994/95 and 1995/96, respectively.

Sugars, vitamin C and β-carotene

The large differences in the established N-min did not affect the concentrations of glucose, fructose or sucrose significantly (Table 4), nor were they different between years.

Vitamin C decreased significantly with N-min (Fig. 3A) and it was significantly affected by the growing conditions in the two years, with the lowest level in the warmer and sunnier year, 1995.

Fig. 3B shows that the concentrations of β-carotene increased significantly with the level of N-min at carrot emergence and were significantly affected by the growing conditions in the two years, being highest in 1995.

Amino acids

In both years the total sum of free amino acids and amides (TFA) increased significantly with N-min at carrot emergence (Fig. 3C). The concentration of amino acids was lowest in 1995. Slopes calculated using a linear regression analysis of the dependency of the individual amino acid concentration (y) on the level of N-min (x) at carrot emergence are shown in Table 5. The concentrations of arginine, aspartic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and the amides asparagine and glutamine increased significantly with
Fig. 3. Concentration of (A) vitamin C, (B) β-carotene, and (C) total free amino acids (TFA) versus mineral N (N-min) at carrot emergence in two years. FW: fresh weight; DM: dry matter.

the level of N-min. However, the concentrations of glutamic acid, glycine, serine, tryptophan, alanine and tyrosine were not significantly affected by N-min. The concentration of amino acids at the target level of N-min (100 kg N ha⁻¹) varied from 0.12 mg kg⁻¹ DM for glycine to 2.7 mg kg⁻¹ DM for glutamic acid. The concentration of glutamine was very high compared with the other amino acids and asparagine.

Discussion

Mineral nitrogen, nitrogen uptake and yield

The difference in N-min between the two seasons may be explained by the difference in precipitation, 798 and 296 mm in 1994/95 and 1995/96, respectively. Therefore, leaching of N-min was probably much higher in the first period than in the second period, and this resulted in the difference of 41 N-min units between the fallow treatments (Table 3). The higher temperature sum of 2624°C in the growing period of 1995 compared with 2348°C in the growing period of 1996 may have contributed to increased mineralization of the green manure material and therefore higher level of N-min at carrot emergence relative to the amount of plant material supplied, as shown in Table 3. These differences in climate resulted in a mineralization that corresponded to more than 100% of the N supplied by green manure in 1994/95. However, the maximum capacity for mineralization was not exploited at the time of carrot emergence in 1996 and the percentage of exploitation decreased with increasing amounts of green manure (Table 3).

The increase in yield of saleable carrot roots up to a level of 85-95 t ha⁻¹ at an N-min level from 90 to 100 kg N ha⁻¹ at carrot emergence, corresponding to a total N uptake of 150-160 kg ha⁻¹, is typical on sandy loam in this area. It is also typical that further increases in N availability did not result in significantly increased yields, even though N uptake continued to increase, so a level of N-min of about 100 kg ha⁻¹ at emergence must be considered optimal. This also corresponds to what was found previously (Scharpf & Weier, 1994). Therefore, the objective of covering the entire range of N availability likely to be experienced in organic agriculture was fulfilled. By growing of carrots at a wide range of N-min, obtained by depletion with a non-leguminous crop and oversupply with mineralized nitrate, there was a significant increase in carrot yield by increasing N supply, as assumed in the hypothesis. On a less fertile sandy soil a similar situation must occur in most years.

The range of N-min covered both the levels used by experienced carrot growers (supposedly close to the optimum) and those used by less experienced growers, who may encounter substantially higher or lower values. Only the depleted treatments showed any definitive decrease in yield, so in most years, mineralization from a soil with a good content of organic matter can provide all the nutrients needed to support a good yield of carrots. No manure is needed to obtain the necessary nutrients, and even though the type of green manure used here does not always provide more N to the crop than a fallow treatment, a green manure crop still has all the advantages of a catch crop (Thorup-Kristensen, 1993; Thorup-Kristensen & Bertelsen, 1996). It preserves the level of organic matter in the soil, reduces leaching during wet periods in the winter, and provides a more predictable N supply to the crop than a fallow treatment. The last two effects are clearly reflected in the N-min data presented in Table 3. However, on a less fertile soil, such as the sandy soils often used for carrots, a fallow field is more prone to leaching of the N mineralized during the winter, so here the green
Table 5. Amino acid or amide, slope, probability and correlation coefficient found by regression analysis using a linear model \( y = a + bx \), where \( y \) is the amino acid concentration, and \( x \) the level of mineral nitrogen (N-min) (kg N ha\(^{-1}\)) in the soil at carrot emergence.

<table>
<thead>
<tr>
<th>Amino acid or amide</th>
<th>Slope (mg ha(^{-1}))</th>
<th>Correlation coefficient</th>
<th>Concentration at N-min = 100 kg ha(^{-1}) (mg kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>-0.0015</td>
<td>0.143</td>
<td>2.44</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.0121**</td>
<td>0.842</td>
<td>1.32</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.0054*</td>
<td>0.640</td>
<td>2.23</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.0044</td>
<td>0.539</td>
<td>2.79</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.0003</td>
<td>0.481</td>
<td>0.12</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.0022**</td>
<td>0.835</td>
<td>0.14</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.0018**</td>
<td>0.774</td>
<td>0.43</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.0016**</td>
<td>0.844</td>
<td>0.27</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.0009*</td>
<td>0.701</td>
<td>0.14</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.0007***</td>
<td>0.913</td>
<td>0.04</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.0013*</td>
<td>0.732</td>
<td>0.37</td>
</tr>
<tr>
<td>Serine</td>
<td>0.0047</td>
<td>0.583</td>
<td>1.01</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.0019*</td>
<td>0.626</td>
<td>0.57</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.0035</td>
<td>0.480</td>
<td>0.20</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.0060</td>
<td>0.549</td>
<td>1.75</td>
</tr>
<tr>
<td>Valine</td>
<td>0.0032**</td>
<td>0.815</td>
<td>0.75</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.0201***</td>
<td>0.929</td>
<td>3.20</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.1470***</td>
<td>0.869</td>
<td>15.57</td>
</tr>
</tbody>
</table>

DM: dry matter.

Slope is the increase in concentration of the compounds by each unit of N-min. N-min = 100 is the target level. Significance: *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).

manure will be important and sufficient to ensure an adequate N supply.

Dry matter

The yield and concentration of DM in the carrot roots was significantly different between years, and the highest production of leaves and carrot roots occurred in the sunny year, 1995 (Fig. 1B, C). The concentration of DM was lowest in 1995. One explanation for this could be a higher rate of photosynthesis and incorporation of carbon in polymer carbohydrates and lignins in the cell walls and storage organs in the sunny year. The increased growth was followed by an increase in the uptake of water and a higher yield of saleable carrots (Fig. 1A). The effect of the increased amount of plant material per hectare was a decrease in the concentrations of DM, total N, nitrate N, vitamin C and total sum of amino acids (Figs. 2, 3).

The DM concentrations in the present investigation were between 107 and 116 g kg\(^{-1}\) fresh weight. This is in accordance with the values for conventional carrots found in the literature (Bajaj et al., 1980; Kidmose & Martens, 1999). As also shown in Fig. 2A, the concentration of DM decreased significantly with increasing levels of N-min, as was found previously by Evers (1988) and Lieblein (1993).

Total nitrogen and nitrate nitrogen

The concentration of total N and nitrate N varied between 6.5 and 12.9 g kg\(^{-1}\) DM and between 0.01 and 2.9 g kg\(^{-1}\) DM, respectively, with total N as a linear and nitrate as an exponential function of N-min in both years (Fig. 2B, C). These levels and interrelations are in accordance with previously published results (Stolley et al., 1978; Hofsommer & Gheradi, 1985; Matthies, 1991; Lieblein, 1993; Schaller & Schnitzler, 2000). Since yield appears to be limited by climate and N uptake depended directly on the amount of N-min available, the differences found in concentration of total N and nitrate were expected. Nitrate accumulation at high N-min levels only occurred when the crop received a surplus of N, as in the colder and less sunny year, 1996. In years such as 1995, with favourable growth conditions, the carrot plant utilizes the surplus of N for synthesis of organic compounds including protein, carotenes and amino acids. Because the synthesis of amino acids requires energy and is promoted by higher temperature (Sebanek, 1992; Mohr & Schopfer, 1995), this hypothesis is supported by very large and significant differences in the concentration of total N and amino acids between the two years.

Carrots intended for processing to produce baby food should contain less than 50 mg nitrate kg\(^{-1}\).
fresh weight. In a colder and darker year like 1996, excessive levels of nitrate can be obtained even at low N-min values, and certainly very high concentrations of nitrate were obtained at high levels of N-min. A concentration of 2–3 g nitrate N kg⁻¹ DM corresponds to 170–260 mg nitrate kg⁻¹ fresh weight of carrots with 115 g DM kg⁻¹ fresh weight. This is very high compared with the concentrations normally found in carrots grown with the application of mineral fertilizer (Chessin & Hicks, 1987; Cserni & Prohászka, 1987; Sørensen, 1993). In contrast, the concentrations of nitrate in carrots grown in 1995 with maximum N-min at 160 kg N ha⁻¹ were less than 25 mg nitrate kg⁻¹ fresh weight, which corresponds to very good quality and an excellent raw material for industrial processing of baby foods.

It is against the principles of organic farming to use more fertilizer than necessary, even if not explicitly banned by the formal rules. Obtaining higher N availability than necessary in most respects reduces the quality, as reflected in a strongly increased risk of accumulation of undesirable high levels of nitrate. For most consumers low nitrate is generally considered more important than a high level of free amino acids. However, there are recent indications that nitrate may be beneficial for some consumer groups (Knight, 1999). If this is confirmed, the combination of high nitrate with high amino acid concentrations must be reconsidered as better quality, and the target for optimal N supply would then have to be raised.

Minerals

The concentrations of K, Mg and Ca found in this experiment were 188–230, 8.7–9.4, and 29–30 mg kg⁻¹ DM, respectively. These concentrations are within the range found earlier for these compounds in carrots (Bajaj et al., 1980).

The close relationship between uptake of K and N was expected because K is absorbed as the positively charged K ion and N as the negatively charged nitrate ion.

Sugars and vitamin C

A lack of significant differences with respect to the three sugars between the years, as well as non-significant regressions between the sugar concentrations and N-min, showed that neither the year nor the level of N-min had any clear, significant effect on the concentrations of sugars in carrots. The range of sugar concentrations in carrot roots normally varies considerably, depending largely on the cultivar, climate and soil conditions (Bajaj et al., 1980). In previous experiments with carrots the average total concentrations of sugars were found to be between 50 and 70 g kg⁻¹ (Kidmose & Martens, 1999). This is the same level as in the present investigation.

The decreasing concentration of vitamin C (Fig. 3A) owing to an increasing availability of N occurs in several plant species, in the roots, leaves and fruit (Mozafar, 1993), and is probably due to a general effect on plant tissues.

β-Carotene

The concentration of β-carotene in the cultivar “Fancy” grown in the two previous years on the same soil type with conventional production was on average 124 mg kg⁻¹ fresh weight (Kjeldsen et al., 1994), which is between the values found in 1995 and 1996 (Fig. 3B). These results are in accordance with the literature, where concentrations between 50 and 200 mg kg⁻¹ fresh weight have been reported (Bradley et al., 1967; Bajaj et al., 1980; Kjeldsen et al., 1994; Kidmose & Martens, 1999).

In 1995, with a higher temperature sum and a larger accumulated radiation than in 1996, the more intense photosynthesis and the higher energy status appear to have promoted an accumulation of β-carotene, as has been observed in other experiments (Sebanek, 1992; Mohr & Schopfer, 1995).

Amino acids

The concentration of all amino compounds (Table 5) except for tyrosine was within previously reported ranges of amino acids and their amides (Solms, 1969; Matthies, 1991; Lieblein, 1993). An explanation for the higher concentration of tyrosine in the present study may be a superior extraction and improved derivatization procedure. The relatively high concentrations of asparagine and glutamine compared with the concentrations of the other amino acids corresponds with the function of these two amides, which are stored as N sources, and are involved in the biosynthesis of other amino acids (Sebanek, 1992; Mohr & Schopfer, 1995).

Both the total N and TFA were high in 1996 and low in 1995 as expected, because more of the absorbed N is incorporated in proteins in years with increased plant growth.

A study of biocrystallization as a term for the description of raw material quality showed that the major significant differences detected in carrots were related to differences in the concentration of N metabolites (Andersen et al., 2000).

Conclusions

This study found that the concentrations of nutritionally important chemical constituents in carrots grown by organic farming using green manure showed
values and dependency on N availability that were comparable with those found earlier in carrots grown using mineral fertilizers.

The concentrations of total N, ten individual free amino acids, asparagine and glutamine increased linearly and nitrate N exponentially with the levels of N-min established at carrot emergence. Overfertilization using green manure resulted in an extremely high and unwanted concentration of nitrate.

A high yield of carrot roots obtained in the warmer and more sunny season may be associated with a lower content of DM, total N, nitrate N, vitamin C and total free amino acids in 1996, whereas the content of β-carotene may have increased.

So, to produce a good crop of high-quality carrots under organic conditions, the advice to growers with similar soil and climate as in the present investigation is to establish a green manure crop in the year before, e.g. by undersowing in a cereal crop, and incorporate the material during the winter, no later than 1–2 months before sowing the carrots. Other procedures easily cause too low, too variable or too high concentrations. However, the strong effect of climatic variation between years shows that the procedure must be carefully adjusted to local conditions, to ensure the best quality together with a good yield.

References


