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Concentration and Composition of Flavonol Glycosides and Phenolic Acids in Aerial Parts of Stinging Nettle (Urtica dioica L.) are Affected by Nitrogen Fertilization and by Harvest Time

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Summary

Plants of Urtica dioica were cultivated in three years with four different nitrogen (N) levels (0, 100, 200 and 400 kg N ha−1) and the aerial parts were harvested three times per year to investigate if the content of flavonoids and phenolic acids were affected. The flavonol glycosides quercetin-3-O-glucoside, quercetin-3-O-rutinoside, isorhamnetin-3-O-rutinoside, and kaempferol-3-O-rutinoside and the phenolic acids 5-O-cafeoylquinic acid, 3-O-cafeoylquinic acid, 5-O-feruloylquinic acid and 2-O-cafeoylmalic acid were identified and quantified in extracts of the aerial parts of U. dioica by LC-DAD-MS and RP-HPLC-DAD, respectively. High N levels reduced the concentration of total flavonoids significantly from an average of 10 mg g−1 dry matter (DM) at 0 kg N ha−1 to 5 mg g−1 at 400 kg N. The effect of N levels on total phenolic acids was only significant in the second harvest each year with a reduction from an average of 30 mg g−1 DM at 0 kg N ha−1 to 23 mg g−1 at 400 kg N ha−1. The composition of flavonoids changed significantly among the major flavonoids with later harvest time resulting in an increase in the content of quercetin-3-O-rutinoside and a decrease of the quercetin-3-O-glucoside content. From the present study it appears that cultivation of U. dioica herba for medicinal purposes with a high yield of bioactive compounds is a compromise between a high yield of plant material and the content of flavonol glycosides and phenolic acids in the harvested product.

Key words. cultivation – medicinal plant – phenolics – caffeic acid derivatives – isoquercitrin – rutin

Introduction

Stinging nettle (Urtica dioica L.) is used as a herbal remedy for the treatment of many diseases. The aerial parts of the plants are used as a blood purifier and diuretic, and infusion of the plant is used for the treatment of nasal and menstrual haemorrhage, diabetes, rheumatism, eczema, and against diarrhoea as well as in the treatment of arterial hypertension (Wagner et al. 1989; Zinyat et al. 1997). Previous studies on water extracts of the aerial parts of U. dioica have in addition to a diuretic and a natriuretic effect also demonstrated a hypertensive action as well as an anti-hyperglycemic action in rats in vivo (Tahir et al. 2000; Legssyer et al. 2002; Bouham et al. 2003) confirming the traditional use of this plant against the development of cardiovascular diseases and diabetes, respectively. Other pharmacological effects, such as stimulation of human lymphocyte proliferation (Wagner et al. 1989), anti-inflammatory activity (Riehmann et al. 1999; AKBAY et al. 2003), anti-ulcer and analgesic activities (Gülcin et al. 2004) have been demonstrated for U. dioica and further it has been suggested that the immunostimulatory activity of leaf extracts could be the reason for the use of this plant in traditional anticancer treatment (Obertreis et al. 1996). The pharmacological effects observed for the aerial parts of U. dioica are believed to be due to the content of flavonol glycosides such as quercetin-3-O-rutinoside (rutin) and quercetin-3-O-glucoside (isoquercitrin), kaempferol-3-O-rutinoside and isorhamnetin-3-O-glucoside and phenolic acids such as 5-O-cafeoylquinic acid (chlorogenic acid) and 2-O-cafeoylmalic acid (Obertreis et al. 1996; AKBAY et al. 2003; Gülcin et al. 2004) (Fig. 1). The content of these phenolics is therefore important for the quality of the plant material sold for the preparation of various medicinal products. This is also in accordance with the quality criteria for U. dioica herba given in the pharmacopoeias such as the European Pharmacopoeia (2007) where minimum concentrations of bioactive components are listed, including also some of the phenolics mentioned above.

The concentrations of phenolics in plants have been shown to increase in response to biotic and abiotic stress. Different types of plant stress have been investigated in relation to the accumulation of phenolics in plants, including UV-light, drought, plant diseases, insect attack, physical scorching and nutrient deficiency such as low nitrogen availability (Bongue-Bartelsman and Phillips 1995; Dixon and Paiva 1995; Stewart et al. 2001; Awad and De Jager 2002; Norbak et al. 2003; Anttonen et al. 2006). Low fertilizer levels in cultivation resulted in higher concentrations of phenolics in leaves of tomato (Bongue-Bartelsman and Phillips 1995), in apple skin (Awad and De Jager 2002), in leaves...
tion of flavonoids and phenolic acids.

ing seasons for analyses of yield, concentration and composi-

and sampled the aerial parts several times during three grow-

this hypothesis, we used four increasing nitrogen supplies

composition of flavonoids and phenolic acids in aerial parts,

more plant material but may alter the concentration and

nitrogen fertilizer in cultivation of 

1993). Therefore, we reasoned that increasing the supply of

therefore produce more plant material to harvest (W EISS

er hand a nitrophile plant and higher amount of nitrogen will

Fig. 1. Flavonoids and phenolic acids identified in aerial

parts of stinging nettle (Urtica dioica) in the present inves-

of barley (NøRBÆK et al. 2003) and in fruits of strawberry

(Anttonen et al. 2006), but no investigations of this fertilizer
effect have been made in U. dioica. Urtica dioica is on the oth-

er hand a nitrophile plant and higher amount of nitrogen will

therefore produce more plant material to harvest (Weiss 1993).

Therefore, we reasoned that increasing the supply of

nitrogen fertilizer in cultivation of U. dioica will produce

more plant material but may alter the concentration and

composition of flavonoids and phenolic acids in aerial parts,

and hence the quality of the harvested plant material. To test

this hypothesis, we used four increasing nitrogen supplies

and sampled the aerial parts several times during three grow-

ning seasons for analyses of yield, concentration and composi-
tion of flavonoids and phenolic acids.

Materials and Methods

Plant material

Transplants of stinging nettles for the field experiments

were made from root cuttings in April 2003. The plant

material for cuttings was obtained from a herb grower

(Søren Kristoffersen, SciCom, Vejen, Denmark), and the

origin of seeds for this planting was again from a collector

of wild herb seeds (Flemming Tommerup, CompleMedic,

Denmark) who harvested the seeds in a natural occurring

nettle population (Seager near Snoldelev, Sealand, Den-

mark). Root cuttings of approx. 3 cm in length were

placed in speedling plugs (VP 77, Vefi A/S, Larvik, Nor-

cay) filled with a soil/sphagnum mixture enriched with

organic fertilizer and lime (Pindstrup 301/024 and

327/024 organic transplant mixture, Pindstrup mose-
brug A/S, Denmark). The transplants (3000 plugs) were

raised in a glasshouse at a minimum temperature of

12 °C and reached a size of 6 to 8 leaves after five weeks.

Chemicals

Methanol (MeOH), acetonitrile (MeCN) and formic acid

were of 99.9 % high-performance liquid chromatography

(HPLC) grade (Aldrich-Chemie, Germany). The water

used was ultra pure generated by an Elgastat water puri-

fication system (Elga Ltd., United Kingdom). All eluents

for HPLC were filtered through nylon 0.45 µm Cameo

25P syringe filters (Bie & Berntsen, Denmark) and de-
gassed with ultrasound for 20 min before use. Authentic

samples of 5-O-cafeoylquinic acid, quercetin-3-O-rutino-

side, quercetin-3-O-glucoside, isorhamnetin-3-O-rutino-

side, kaempferol-3-O-rutinoside were purchased (Extra-

synthese, France) and 3-O-cafeoylquinic acid was kindly
donated by Anne S. Meyer, Department of Chemical En-
gineering, Technical University of Denmark.

Field experiments

The field experiment was planted on May 22, 2003 on a

sandy loam soil at Research Centre Aarslev (10°27’ E,

55°18’ N). The plants were established in a bed system

and through black plastic ground cover to control weeds.

The individual plots were 5 m long and 1.5 m wide beds.

The plant density in beds was about 16 plants per m² in

four rows and with a row distance of 0.2 m and an in-row

spacing of 0.25 m. The plastic cover was 1.2 m in width

and secured with soil along the edges. The spacing be-

 tween individual plots was 0.80 m. The relative wide

spacing between plots was to avoid contamination with

nitrogen (N) between neighbouring plots. In the space be-

tween plots a lawn grass mixture was sown and kept short

by moving all through the three seasons of the experi-

ment. The statistical design of the field experiment was in

randomised blocks with three replicates of the fertilizer


Fertilization

The N treatments in the experiment were as follows:

2003: 0, 75, 150 and 300 kg N ha⁻¹; 2004: 0, 100, 200

and 400 kg N ha⁻¹ and 2005: 0, 100, 200 and 400 kg

N ha⁻¹. The N was applied as urea fertilizer and in 2003

and 2004 all the N was given at the start of the season in

and through black plastic ground cover to control weeds.

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and 400 kg N ha⁻¹ and 2005: 0, 100, 200 and 400 kg

N ha⁻¹. The N was applied as urea fertilizer and in 2003

and 2004 all the N was given at the start of the season in
April. In 2005 the fertilizer applied was also urea but given as a split application with 1/3 at the start of the season and the last 2/3 after the first and second harvest (June and August).

Harvest time (Re-harvest)
The aerial parts of the nettle plots were harvested at two times in 2003 (July and September) three times in 2004 (May, July and October) and also three times in 2005 (June, August and October). The two first cuts in each season were timed to be at onset of flowering, whereas the last cut in each year were timed after the highest possible yield of fresh material before winter deterioration. The plants were cut about 15 cm over ground and the yield of fresh material was recorded by weighing just after cutting. The plots-area for yield analysis was 4.5 m². At each harvest two samples of about 300 g fresh material were taken for dry matter and for chemical analysis and stored in sealed plastic bags. All samples of aerial parts were stored at –24 °C until analysis.

Extraction of flavonol glycosides and phenolic acids
Fresh frozen aerial parts (approximately 2 g leaves and 1 g stalks) of stinging nettle were immediately homogenized in a centrifuge tube using an Ultra-Turrax (model T25; IKA Labortechnik, Staufen, Germany) for 1 min together with 20 mL aqueous 80 % MeOH followed by extraction for 2 h at room temperature. After the extraction the sample was filtered followed by filtration through a nylon 0.45 µm, Cameo 25P syringe filter and then analysed by RP-HPLC for flavonoids and phenolic acids. The efficiency and the reproducibility of the extraction procedure described above were determined by multiple extraction (3×20 mL 80 % MeOH) of fresh frozen leaves in quadruplicates which showed that extraction of 1×20 mL 80 % MeOH as described above ensured extraction of >98 % of the total flavonoids and phenolic acids and further the reproducibility of the extraction method (CV<5 %). For the determination of the efficiency of the extraction method the extract sample was centrifuged for 10 min using a Sorvall SA-600 head (gmax 20.845; Buch & Holm, Herlev, Denmark) between each extraction and the supernatant collected and analysed for flavonoids and phenolic acids. It should be noticed that it is very important that the fresh frozen leaves are extracted immediately in the organic solvent in order to avoid enzymatic degradation of flavonoids and phenolic acids in the plant material (data not shown).

Identification of flavonol glycosides and phenolic acids by LC-MS
Identification of flavonoids and phenolic acids in aqueous 80 % MeOH extracts of aerial parts of stinging nettle were performed on an Agilent HPLC-DAD-MS station (Agilent, Waldbronn, Germany) comprising of a series 1100 HPLC equipped with a diode array detector (DAD) and a LC/MSD SL detector fitted with an atmospheric pressure chemical ionization (APCI) source. Data acquisition and processing were performed using Agilent’s ChemStation software. Separations were carried out on a Purospher® STAR reversed-phase (RP)-C18 end-capped column (5 µm particle size; 250×4.0 mm i.d., Merck, Darmstadt, Germany) protected with a RP-18 guard cartridge (5 µm; 15×4 mm i.d., Merck, Darmstadt, Germany). The column temperature was maintained at 35 °C and the mobile phases consisted of solvent A (aqueous 1 % formic acid) and solvent B (100 % MeCN). Separations were performed by the following solvent gradient: 1 % B isocratic (2 min), 1 to 25 % B (28 min), 25 to 35 % B (10 min), 35 to 95 % B (5 min), 95 % B isocratic (5 min), 95 % B to 1 % B (1 min), 1 % B isocratic (9 min). All changes of solvent B were linear programmed. The flow rate was 0.4 mL min⁻¹ and the injection volume ranged from 1 to 10 µL.

UV-vis spectra of flavonoids and phenolic acids were recorded between 200 and 600 nm at a rate of 1.25 scans/sec and monitored at 280, 320, 360 and 500 nm. MS spectra were recorded consecutively in negative mode with the detector fitted with the APCI chamber. MS spectra were recorded in the range m/z 50–m/z 1,000. The acquisition parameters were as follows: fragmentor 70 V; spray chamber parameters: nitrogen used as the drying gas at a flow of 71 min⁻¹ and as nebulizing gas at a temperature of 300 °C. A potential of 3 kV was used on the capillary and the corona current set to 15 µA. Flavonoids and phenolic acids were identified in U. dioica extracts based on comparison with authentic standards and/or tentatively identified based on their retention time and their LC-MS and UV-data (Table 1).

Quantification of flavonol glycosides and phenolic acids by RP-HPLC
Analytical RP-HPLC was performed on a Shimadzu HPLC LC-10 Series System equipped with a DAD detector (Hitachi L-7450). Flavonoids and phenolic acids were monitored at 280 and 360 nm and UV-spectra were recorded between 200–450 nm. Separations were performed on a LiChrospher® 100 RP-18 column (250×4.6 mm i.d., particle size 5.0 µm, Merck, Darmstadt, Germany) at 35 °C using the following solvent gradient: 0.5 % trifluoroacetic acid (TFA) in H2O–MeCN [0 min (90:10), 15 min (80:20), 25 min (70:30), 35 min (50:50), 45–60 min (10:90), 65–75 min (90:10)]. The flow rate was 1 mL min⁻¹ and the injection volume 20 µL. Acquisition off at 75 min. Flavonoids and phenolic acids were determined by external calibration curves of quercetin-3-O-rutinoside and 5-O-cafeoylquinic acid, respectively. Mean recovery rates (< accuracies) for 5-O-cafeoylquinic acid and quercetin-3-O-rutinoside, respectively, were >98 % with a relative standard deviation (RSD) of <5 %, and were determined by spiking a known amount of authentic standard of 5-O-cafeoylquinic acid and quercetin-3-O-rutinoside to U. dioica extract samples, respectively. The precision of the HPLC method was determined by four injections of a U. dioica extract sample in 1 day (intraday variation) and on four different days (interday variation). The overall intraday and interday variations were found to be less than 5 % for both flavonoids and phenolic acids.

Statistics
Analysis of variance were performed on each variable using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). The variations (standard errors, SE), the significances of treatment effects (F-test) and regression
coefficients were calculated and tested using the General Linear Models procedure and statistical differences were determined with the LSD test (P<0.05).

**Results and Discussion**

**Flavonol glycosides and phenolic acids in stinging nettle**

Phenolic constituents in extracts of aerial parts of *U. dioica* were monitored by RP-HPLC-DAD and LC-MS (APCI, negative mode). Typical HPLC chromatograms at 280 nm and 360 nm, respectively, of an aqueous MeOH extract of aerial parts of *U. dioica* are shown in Fig. 2. Based on the UV spectra the compounds could be grouped into (i) caffeic acid derivatives with an absorption band centred around 325 nm with a shoulder at around 299 nm and (ii) flavonol glycosides with the typical pattern of band I centred around 354 nm with a shoulder at around 299 nm and band II centred around 325 nm (Table 1). The flavonoids identified in the present investigation were derivatives of caffeic acid, which was also confirmed by their LC-MS data. Compounds 1 and 3 had both a pseudomolecular ion [M–H]– at m/z 353, compatible with caffeoylquinic acids and were identified as 3-O-cafeoylquinic acid and 5-O-cafeoylquinic acid, respectively, by comparison with authentic standards. These compounds have previously been identified as constituents in the aerial parts of *Urtica* species including *U. dioica* (BUDZIANOWSKI 1991; BUCAR et al. 2006). Compound 4 showed a pseudomolecular ion [M–H]– at m/z 295 compatible with 2-O-cafeoylmalic acid, which was also confirmed by an ion at m/z 179 corresponding to caffeic acid (FANG et al. 2002) and an ion at m/z 133 corresponding to malic acid. 2-O-Caffeoylmalic acid has previously been identified as a major phenolic acid in the aerial parts of *U. dioica* (BUDZIANOWSKI 1991; BUCAR et al. 2006) in accordance with the results of the present study. Compound 5 gave a pseudomolecular ion [M–H]– at m/z 367 and a major ion at m/z 191 in accordance with this compound being 5-O-feruloylquinic acid which has also previously been identified in the aerial parts of *U. dioica* (BUDZIANOWSKI 1991).

**Table 1.** Characteristic ions of phenolic acids and flavonoids in extracts of aerial parts of *Urtica dioica* as determined by LC-MS (APCI, Negative Mode, Capillary Voltage 70 V) and their UV-Spectra as determined by HPLC-DAD.

<table>
<thead>
<tr>
<th>Peak</th>
<th>R_t (min)</th>
<th>Compound</th>
<th>HPLC-DAD UV spectrum λ_max (nm)</th>
<th>LC-MS APCI m/z (% base peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.4</td>
<td>3-O-cafeoylquinic acid (neochlorogenic acid)</td>
<td>298sh, 327</td>
<td>353 [M–H]– (100), 191 (1), 173 (2)</td>
</tr>
<tr>
<td>2</td>
<td>13.9</td>
<td>quercetin diglucoside</td>
<td>255, 264sh, 301sh, 350</td>
<td>625 [M–H]– (100), 463 (24), 301 (4)</td>
</tr>
<tr>
<td>3</td>
<td>14.6</td>
<td>5-O-cafeoylquinic acid (chlorogenic acid)</td>
<td>300sh, 325</td>
<td>353 [M–H]– (100), 191 (15)</td>
</tr>
<tr>
<td>4</td>
<td>17.9</td>
<td>2-O-cafeoylmalic acid</td>
<td>302sh, 328</td>
<td>295 [M–H]– (100), 179 (8), 133 (16)</td>
</tr>
<tr>
<td>5</td>
<td>20.3</td>
<td>5-O-feruloylquinic acid</td>
<td>300sh, 324</td>
<td>367 [M–H]– (100), 191 (25)</td>
</tr>
<tr>
<td>6</td>
<td>21.8</td>
<td>unknown phenolic acid</td>
<td>300sh, 327</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>22.6</td>
<td>quercetin-3-O-rutinoside (rutin)</td>
<td>256, 263sh, 298sh, 353</td>
<td>609 [M–H]– (100), 463 (2), 301(2)</td>
</tr>
<tr>
<td>8</td>
<td>22.9</td>
<td>quercetin-3-O-glucoside (isorhamnetin)</td>
<td>256, 263sh, 298sh, 353</td>
<td>463 [M–H]– (100), 301 (2)</td>
</tr>
<tr>
<td>9</td>
<td>24.4</td>
<td>kaempferol-3-O-rutinoside</td>
<td>265, 299sh, 347</td>
<td>593 [M–H]– (100), 447 (1), 285(7)</td>
</tr>
<tr>
<td>10</td>
<td>25.3</td>
<td>isorhamnetin-3-O-rutinoside</td>
<td>256, 268sh, 301sh, 352</td>
<td>623 [M–H]– (100), 315 (3)</td>
</tr>
</tbody>
</table>

aPeak numbers correspond to the peak numbers in Fig. 2. bR_t = retention time on HPLC. cR_t, UV and LC-MS data compared with an authentic standard.

**Effects of nitrogen fertilization and re-harvest on yield of aerial parts of stinging nettle**

Higher nitrogen (N) application levels increased the yield of aerial parts substantially. The accumulated yield over...
three years increased from 30 t ha\(^{-1}\) at 0 kg N ha\(^{-1}\) to 50.0, 68.4 and 87.6 t ha\(^{-1}\) at 100, 200 and 400 kg N ha\(^{-1}\), respectively (Fig. 3). The exact harvest dates and yields in every single harvest are listed in Table 2. The 75 percent increase in accumulated yield over three years observed in our experiment going from 100 to 400 kg N ha\(^{-1}\) is, however, considerably lower than the increase found in a similar study by Weiss (1993). Weiss (1993) found a yield increase of about 250 percent in going from 80 to 440 kg N ha\(^{-1}\) also over a three year growing period and yield increase of about 250 percent in going from 80 to 440 kg N ha\(^{-1}\) was the year of establishment with only two harvests. Year 2003 results show, however, also a significant lower difference in amounts of applied N and because this is the case in the study by Weiss (1993) where even the 440 kg N ha\(^{-1}\) treatment did not show any ‘tailing off’ in the yield response curve. In our study the first harvest and second re-harvest (cut) in each year gave the highest yield (Fig. 3) whereas the third re-harvest may under our conditions not be profitable unless a very high N supply is given.

**Effects of nitrogen fertilization and re-harvest on the content of flavonol glycosides and phenolic acids**

Contrasting to yield of bio-mass higher N levels reduced the concentration of flavonol glycosides in the harvested plant material significantly, in almost all harvests (Table 2). If an average over six harvests in two years (2004 and 2005) is calculated there is a significant negative correlation \((R^2 = 0.9973, p = 0.014)\) between N applied and concentration of total flavonol glycosides (Fig. 4). Results from year 2003 are omitted because of the difference in amounts of applied N and because this was the year of establishment with only two harvests. Year 2003 results show, however, also a significant lower concentration of flavonol glycosides in relation to higher N application (Table 2). A decline in content of flavonol glycosides with an increase in N supply (Fig. 4a) is in accordance with the results of similar studies performed on crops like apples (Awwad and DE JAGER 2002), strawberries (ANTTONEN et al. 2006) and tomatoes (STEWART et al. 2001). For example, Anttonen et al. (2006) found that the lowest level of fertilization (not only N) in their experiment with strawberries increased the contents of flavonols in fruits by up to 57 % compared to double the amount of fertilizer. Stewart et al. (2001) found that the ‘flavonol’ content was inversely related to nitrogen and phosphate nutrition of tomato, the effect of nitrogen being more pronounced than phosphate.

The effect of applied N on the concentration of phenolic acids did not show a similar clear correlation. In year
2003 and 2004 there was a tendency to lower concentrations of phenolic acids with higher application of N, although only significant for the highest amount of N compared to the lowest (Table 2). However, in 2005 there was no significant effect of applied N on the content of phenolic acids in the harvested plant material. If again an average over six harvests in two years (2004 and 2005) is calculated, there is a slightly negative correlation between concentration of phenolic acids and applied N, which, however, is not significant (Fig. 4b).

The first harvest time each year tended to be lower in concentration of flavonol glycosides but there was no significant difference in the concentration of phenolic acids over harvest times (Table 2). Looking at the single major flavonol glycosides as percentage of total flavonoids, the percentage of quercetin-3-O-rutinoside (rutin) was significantly increasing with later harvest time (p<0.001) each year, in contrast to quercetin-3-O-glucoside (isoorcitrin) whose relative concentration decreased with later harvest time (Fig. 5a). This result could indicate an

<table>
<thead>
<tr>
<th>Year</th>
<th>Harvest date</th>
<th>Nitrogen applied (kg N ha⁻¹)</th>
<th>Yield (FW) (t ha⁻¹)</th>
<th>Dry matter (DM) (%)</th>
<th>Total flavonoids</th>
<th>Quercetin-3-O-rutinoside</th>
<th>Quercetin-3-O-glucoside</th>
<th>Total phenolic acids</th>
<th>5-O-caffeoylquinic acid</th>
<th>2-O-caffeoyl-malic acid</th>
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</thead>
<tbody>
<tr>
<td>2003</td>
<td>5. July</td>
<td>0</td>
<td>8.0 a</td>
<td>19.1 a</td>
<td>4.8 a</td>
<td>3.2 a</td>
<td>1.4 a</td>
<td>20.7 a</td>
<td>4.1 a</td>
<td>14.9 a</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>8.7 ab</td>
<td>19.0 a</td>
<td>3.7 ab</td>
<td>2.7 ab</td>
<td>0.9 a</td>
<td>11.5 b</td>
<td>3.1 a</td>
<td>7.1 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>12.7 b</td>
<td>17.0 a</td>
<td>2.9 ab</td>
<td>2.1 ab</td>
<td>0.6 a</td>
<td>14.9 ab</td>
<td>3.5 a</td>
<td>10.4 ab</td>
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<td>300</td>
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<td>17.4 a</td>
<td>2.4 b</td>
<td>1.7 b</td>
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<td>12.5 b</td>
<td>2.5 a</td>
<td>9.1 b</td>
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<td>2003</td>
<td>2. September</td>
<td>0</td>
<td>2.9 ab</td>
<td>26.6 a</td>
<td>10.0 a</td>
<td>7.4 a</td>
<td>2.3 a</td>
<td>13.4 b</td>
<td>5.7 b</td>
<td>6.0 a</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>2.6 a</td>
<td>26.6 a</td>
<td>9.6 a</td>
<td>7.2 a</td>
<td>2.2 a</td>
<td>23.1 a</td>
<td>9.2 a</td>
<td>12.3 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5.6 ab</td>
<td>26.0 a</td>
<td>5.4 b</td>
<td>4.0 b</td>
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increasing anabolic activity towards the biosynthesis of quercetin-3-O-rutinoside from quercetin-3-O-glucoside with later harvest, although it cannot be excluded that the changes in the composition of the major flavonoids are due to a catabolic degradation of quercetin-3-O-glucoside and an increased anabolic activity towards the biosynthesis of quercetin-3-O-rutinoside. The percentage of 5-O-caffeoylquinic acid of the total phenolic acid concentration was a little higher in the second harvest each year (p=0.03) and this was contrasted by a tendency to lower percentage of 2-O-caffeoylmalic acid in the second harvest (p=0.10) (Fig. 5b). These results also suggest that the major phenolic acids undergo anabolic and catabolic changes during the growing season. For example, this could be a simple biosynthetic conversion of 2-O-caffeoylmalic acid into 5-O-caffeoylquinic acid by substituting malic acid with quinic acid.

The dry matter (DM) content of plant material (Table 2) is significantly negatively correlated to N application ($R^2=0.9298$, $p=0.036$) and decreases from an average of 23.5 % at 0 kg N ha$^{-1}$ to 21 % at 400 kg N ha$^{-1}$. The theoretical yield of phenolics per area (calculated amounts of harvested phenolics in kg ha$^{-1}$) were highest at an application rate of about 200 kg N ha$^{-1}$ for both flavonol glucosides and phenolic acids (Fig. 6) but the difference between N applications was not significant in either of them.

There was a high variation in the concentrations of phenolics over years, harvests and N applications (Table 2). In the study by WEISS (1993) the phenolics were not determined, but the concentrations found in the aerial parts of stinging nettle in the present investigation are comparable with the values found by SCHOMAKERS et al. (1995). They reported that the variation of quercetin-3-O-rutinoside in leaves of stinging nettle to be between 1 to 6 mg g$^{-1}$ DM and the variation of 2-O-caffeoylmalic acid to be between 0.3 to 16 mg g$^{-1}$ DM. The values in our experiment for quercetin-3-O-rutinoside varied from 1.5 to 9.8 mg g$^{-1}$ DM, and that of 2-O-caffeoylmalic acid between 4.6 to 21.8 mg g$^{-1}$ DM (Table 2) which is in a similar range as found by SCHOMAKERS et al. (1995). According to the EUROPEAN PHARMACOPOEIA (2007) the content of bioactive compounds in nettle leaf (Urticae folium) should be minimum 3 mg g$^{-1}$ DM expressed as the sum of 2-O-caffeoylmalic acid and chlorogenic acid, which is clearly below the amounts found in the present investigation (Table 2).
**Fig. 6.** Average potential yield per area of total flavonoids (a) and total phenolic acids (b) in aerial parts of stinging nettle (*Urtica dioica*) as an effect of applied nitrogen. Data are averages of six harvests over two years (2004 and 2005). Bars are SE (n=6).

The results of the present study may be used to optimise the yield and quality of a stinging nettle product and to lower the cost of cultivation and processing. However, the results of the present investigation clearly shows that cultivation of *U. dioica* herba for a high yield of bioactive compounds is a compromise between a high yield of plant material and the content of flavonol glycosides and phenolic acids in the harvested product.

**Acknowledgement**

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