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Effects of Nitrogen Fertilization, Harvest Time, and Species on the Concentration of Polyphenols in Aerial Parts and Seeds of Normal and Tartary Buckwheat (*Fagopyrum* sp.)

K. B. Christensen[1)], M. Kaemper[2)], R. Loges[2)], X. C. Fretté[3)], L. P. Christensen[3)] and K. Grevsen[4)]
([1)]Department of Food Science, [4)]Department of Horticulture, University of Aarhus, Denmark, [2)]Institute of Crop Science and Plant Breeding, University of Kiel, Germany and [3)]Institute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Denmark)

Summary

Buckwheat (*Fagopyrum* sp.) is a well-known source of many health beneficial components such as starch, dietary fibres, proteins, and polyphenols such as flavonoid glycosides, especially rutin, and phenolic acids. Use of buckwheat for medicinal and health purposes demands that the content of these components are as high as possible. In this study, we have investigated the influence of species, variety, nitrogen (N) fertilization, and harvest time on the content and yield of polyphenols in buckwheat aerial parts and seeds. Experiments were performed as a joint project in both Denmark and Germany; however, all treatments were not repeated in both locations. Three varieties of normal buckwheat (*F. esculentum*) and one of tartary buckwheat (*F. tataricum*) were included in the experiments on the two locations. Experiments with high and low N fertilization were performed on both locations and in Germany combined with three harvest times of aerial parts. Seeds at maturity were only harvested and analysed in the Danish experiments.

For aerial parts of both normal and tartary buckwheat from both locations there was no significant effect on the concentration of flavonoids with either increasing at high and low N-application. N-fertilization did not show a conclusive affect on the concentration of phenolic acids in the aerial parts as well. Concentration of rutin in aerial parts harvested at flowering stage in the Danish experiments varied between 1.7 and 3.9 % dry matter content (DM) in tartary buckwheat and between 2.2 and 2.4 % DM in normal buckwheat. Harvest time or development stage had a highly significant influence on the concentration of polyphenols in the aerial parts, as concentrations at harvest before flowering were up to four times higher than for harvest after flowering. In aerial parts the species/varieties did not differ significantly in content of polyphenols, except for kaempferol-3-*O*-rutinoside, which was higher in the tartary buckwheat 'Lifago' than in the other normal buckwheat varieties. Seeds of tartary buckwheat had a significantly higher concentration of flavonoids than normal buckwheat. In tartary buckwheat seeds, the kaempferol-3-*O*-rutinoside content decreased with increasing N-fertilization, but no significant effect on flavonoids in normal buckwheat seeds was found. Concentration of rutin in normal and tartary buckwheat seeds differed significantly and was between 0.03 % and 0.11 % DM and 1.4 % and 1.5 % DM, respectively.

Key words. cultivation techniques – *Fagopyrum esculentum* – *Fagopyrum tataricum* – flavonoid glycosides – phenolic acids – rutin

Introduction

Buckwheat (*Fagopyrum* sp.) originates from Southwest China, but is nowadays cultivated all over the world and in particular in Asia, Europe, and North as well as South America. Buckwheat belongs to the family *Polygonaceae* and is a pseudo-cereal as it is not a grass like for example wheat. Common buckwheat (*F. esculentum* Moench) accounts for up to 90 % of the world's buckwheat production, and China and Russia are some of the biggest producers (Campbell 1997). Tartary buckwheat (*F. tataricum* Gaertn.) is less common and often referred to as bitter buckwheat. The bitterness of tartary buckwheat makes this species less suitable for food consumption (Campbell 1997). Products such as flour, groats, tea, noodles, beer, and vinegar are produced from the seeds of normal buckwheat. The aerial parts of buckwheat are also used for foods, and buckwheat sprouts are for example very popular in Japan in various dishes (Campbell 1997).

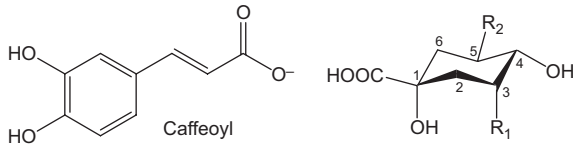
In traditional Chinese medicine normal buckwheat (seeds, stems, and leaves) has been used for the treatment of gastrointestinal problems, zoster, inflammation, and hypertension as it has been found to, e.g., loosen the intestine, resolve toxins, close sores, stanch bleeding, and lower blood pressure (Zhou 2003). In addition, extracts of buckwheat inhibit progression of renal failure in rats (Yokosawa et al. 2002). The health-promoting and nutritional effects of buckwheat are generally ascribed to the

high content of dietary fibres, proteins, minerals, vitamins, and antioxidants such as flavonols, catechins, phenolic acids, and tocopherols (Quettier-Deleu et al. 2000; Holasova et al. 2002; Yokosawa et al. 2002; Zhou 2003; Krkosova and Mrazova 2005; Kalinova et al. 2006).

The very high content of the flavonol glycoside quercetin-3-*O*-rutinoside (rutin) (Fig. 1) in buckwheat has made this plant a primary food source of this flavonoid, which has several health beneficial activities besides being an antioxidant (Kreft et al. 2006). Rutin can reduce high blood pressure, decrease the permeability of blood vessels, reduce the risk of arteriosclerosis, and has an anti-oedema effect (Kreft et al. 2006).

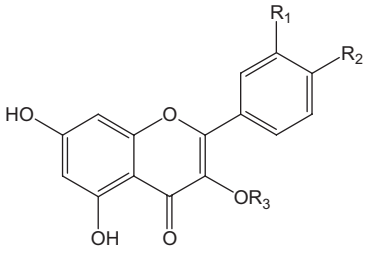
Polyphenols such as flavonoids and phenolic acids constitute the major part of the secondary metabolites produced by buckwheat (Hagels et al. 1998; Quettier-Deleu et al. 2000; Fabjan et al. 2003; Kalinova et al. 2006; Jiang et al. 2007). Hence, it is important to know the composition and content of these polyphenols in buckwheat and how various factors such as species, variety, harvest time, fertilization etc. affect the content of these compounds in cultivated buckwheat to be used for medicinal preparations and/or functional foods. The concentration of polyphenols in plants may increase in response to biotic and abiotic stress (Stewart et al. 2001). Low fertilizer levels in different cultivated plant species has for example resulted in higher concentrations of polyphenols in the leaves of barley (Noerbaek et al. 2003), in leaves of tomato (Bongue-Bartelsman and Phillips 1995), in apple skin (Awad and De Jager 2002), in fruits of strawberry (Anttonen et al. 2006), and in the aerial parts of stinging nettle (Grevsen et al. 2008). Only one study has investigated the effects of nitrogen (N) fertilization on buckwheat and it was found that increasing N-fertilization increased yield of aerial parts of normal buckwheat but the concentration of rutin decreased around 20 % (Honermeier and Wagenbreth 2000). With developmental stage or harvest time Honermeier and Wagenbreth (2000) showed that the aerial parts reached a maximum in rutin content after 50 to 60 d after sowing, that is around flowering for buckwheat, and decreased hereafter. In other plants like for instance oregano the concentration of polyphenols in the aerial parts were highest in the early stages of development, before flowering, and decreased after the flowering stage until near senescence (Grevsen et al. 2009).

In this study, we hypothesized that species, variety, N-fertilization, and harvest times would affect both the yield of plant material as well as the composition and concentration of flavonoids and phenolic acids in buckwheat aerial parts and seeds, and hence the quality of the harvested plant material. To test this hypothesis, we cultivated different varieties of the two species using different nitrogen levels. The experiments were performed over two seasons at two locations, Aarslev (Denmark) and Lindhof (Germany), although not all treatments or combinations were investigated at both locations. The two locations were used to strengthen the outcome of the experiments and the results from plants grown under different circumstances. The German location Lindhof cultivated buckwheat as organic production however, it is not possible and not the intention to compare conventional and organic growing within this work. Samples from both locations were collected at different harvest times and analysed for yield, dry matter content (DM), concentration, and composition of flavonoids and phenolic acids.



3-*O*-caffeoylquinic acid: R_1 = caffeoyl, R_2 = OH

5-*O*-caffeoylquinic acid: R_1 = OH, R_2 = caffeoyl



Quercetin-3-*O*-rutinoside: R_1 = R_2 = OH, R_3 = rutinose

Quercetin-3-*O*-glucoside: R_1 = R_2 = OH, R_3 = glucose

Kaempferol-3-*O*-rutinoside: R_1 = H, R_2 = OH, R_3 = rutinose

Fig. 1. Chemical structures of the major polyphenolic compounds identified in MeOH extracts of aerial parts and seeds of *Fagopyrum tataricum* and *F. esculentum*.

Materials and Methods

Plant material

For the field experiments in 2006 and 2007 seeds of tartary buckwheat (*F. tataricum*) variety 'Lifago' were purchased from the German seed company Deutsche Saatveredelung AG (Lippstadt, Germany) (hereafter 'Lifago' (D)) and the Danish seed company Hunsballe Froe A/S (Soerbymagle, Denmark) (hereafter 'Lifago' (DK)). For the 2007 experiments, additionally seeds of normal buckwheat (*F. esculentum*) varieties 'Lileja', 'Bamby', and 'Hruszowska' were purchased from the seed companies Feldsaaten Freudenberger (Krefeld, Germany), Saatzucht Gleisdorf Ges.mbH (Gleisdorf, Austria), and Camena Samen (Lauenau, Germany), respectively.

Field experiments in Denmark

The Danish buckwheat field experiments were sown on May 10, 2006 and May 22, 2007, respectively. Seeds were sown on a sandy loam soil (ca. 11 % clay and ca. 2.4 % organic material) at Research Centre Aarslev (55° 18' N; 10° 27' E). The individual plots were 10 m long and 1.2 m wide. The plants in the plots were established in 10 rows with a plot drill machine (Hege 80) and with a plant density of about 250 plants m^{-2}, which is normal for these plants. The spacing between individual plots was 0.65 m. The relative wide spacing between plots was to avoid contamination with applied N between neighbouring plots. The space between plots was kept weed free and as bare soil. The experiments were established on new field area

each year. The crop grown in the rotation before buckwheat in 2006 and in 2007 was spring barley for grain harvest.

Experiment 1: N-application, Denmark. In the 2006 N fertilizer experiment two tartary buckwheat 'varieties' (seed origins) 'Lifago' (DK) and Ligfago (D) were tested with four different N-applications: 0, 30, 60, or 120 kg N ha^{-1}. In the 2007 experiment the two species of buckwheat, namely the tartary buckwheat variety 'Lifago' (DK) and the normal buckwheat variety 'Lileja' were tested with four different N-applications: 0, 40, 80, or 160 kg N ha^{-1}. The N was applied as granulated urea fertilizer (Kemira A/S, 46 % N) on the soil surface and all the N was given at the beginning of the season in June. The N_{-min} (mineral N) content of the topsoil was very low May 2006 and May 2007 as it was 28 and 25 kg N ha^{-1}, respectively. The statistical design of the N fertilizer field experiment in both years was in randomized complete blocks with two replicates (total of 2 × 4 × 2 = 16 plots).

Experiment 2: Variety, Denmark. In 2007, a variety/species experiment with two normal buckwheat 'varieties' 'Hruszowska', 'Bamby', and one tartary buckwheat 'variety' 'Lifago' (D) was performed. The plot size and plant density was as stated above in the N-application experiment and the site was next to the fertilizer experiment area in the same field. The plots in the variety experiment were given 80 kg N ha^{-1} at the start of cultivation. The statistical design of the 'variety' trial was in randomised blocks with four replicates (total of 3 × 4 = 12 plots). Only seeds for yield and polyphenol analysis were harvested at maturity in this Danish 'variety' trial.

Samples. In all Danish experiments, samples of aerial parts were harvested and the yield parameters determined. The samples for aerial parts were harvested on 2.25 m^2 of the plot area. The dry matter contents of all harvested samples of aerial parts were determined at harvest by drying at 80 °C until constant weight was obtained. The rest of the plot area (9 m^2) was used for buckwheat grain harvest. The seeds were harvested by means of a plot combine harvester (NJF, Denmark). The seeds were air dried at 25 °C to near 12 % water content after harvest then cleaned in a stationary seed cleaner by sieving and a system of hoppers (Askov, Denmark). The seed yield is given as mass of dry cleaned seeds per area. The concentration of polyphenols were determined on deep-frozen (−24 °C) samples of aerial parts and on dry and cleaned whole seeds.

Field experiments in Germany

The German buckwheat field experiments were sown on May 4, 2007. Seeds were sown on a sandy loam soil (12–14 % clay and 1.9–2.1 % organic material) at Research Farm Lindhof (53° 40' N; 10° 35' O) 20 km northwest of Kiel. The soil at the research farm is managed as certified organic arable crop production. The main plots were 12.0 m long and 3.1 m wide (main plot area 37.2 m^2). The plants were established with 12 cm distance between rows with a plot drill machine (Wintersteiger) and with a plant density of about 250 plants m^{-2}. The spacing between individual plots was 1 m and was kept weed free as bare soil.

Experiment 3, Variety × N-application, Germany. The N-fertilizer treatments in the German organic field experiment were 0 or 120 kg N ha^{-1} applied. The N was applied as 3.4 t ha^{-1} dry coarse pea-meal (*Pisum sativum*) (3.51 % N). Half of the N was applied before sowing in May and incorporated in the topsoil and the rest was applied as topdressing four weeks after without incorporation. The N_{-min} (mineral N) content of the topsoil was low; 19 kg N ha^{-1} in 2007.

In the 2007 fertilizer experiment four varieties ('Lifago' (D), 'Bamby', 'Hruszowska', and 'Lileja') were grown with two N treatments (0 or 120 kg N ha^{-1} applied). The statistical design of the 2007 field experiment was a split-plot design with varieties as main-plots and fertilizer treatment as sub-plots and with three replicates (a total of 4 × 2 × 3 = 24 plots). The fertilizer treatments (120 kg N ha^{-1} applied) were given to half of the main-plot area (sub-plot) i.e. on 18.6 m^2 (6.0 m × 3.1 m) for each variety (main-plot) in the experiment. The crop grown in the rotation before buckwheat in 2007 was maize (*Zea mays* L.) for silage.

Experiment 4, Harvest time, Germany. Next to the German fertilizer experiment in 2007, a harvest time experiment was performed with two buckwheat varieties 'Lifago' (D) and 'Lileja' and two N-applications (0 and 120 kg N ha^{-1}). The harvest time experiment in 2007 was designed as split-split-plot with varieties as main-plot, and N-applications as sub-plots and three harvest times as sub-sub-plots and the experiment was performed with three replicates (a total of 2 × 2 × 3 × 3 = 36 plots). The plot sizes in the harvest time experiment was 37.2 m^2 (main plot), 18.6 m^2 (1st sub-plot), and 6.2 m^2 (2nd sub-plot), respectively, and the buckwheat crop was established as in the fertilizer experiment described previously. The aerial parts of the two buckwheat varieties were harvested at three different dates: June 26, July 18, and August 20, respectively. In buckwheat plant development, these harvest dates corresponded to before flowering, full flowering, and late end of flowering, respectively.

Samples. In all German experiments only samples of aerial parts and not seeds were harvested in the experiments. The samples of aerial parts were harvested on 0.5 m^2 of the plot area in all experiments.

Chemicals

Methanol (MeOH), acetonitrile (MeCN), and formic acid were all 99.9 % high performance liquid chromatography (HPLC) grade (Sigma-Aldrich Chemie, Germany). Trifluoroacetic acid (TFA) was 99 % ReagentPlus® grade (Sigma-Aldrich Chemie, Germany). The water used was ultra pure generated by an Elgastat water purification system (Elga Ltd., United Kingdom). All eluents were degassed with ultrasound for 30 min before use. Authentic samples of 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, epicatechin, quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside were purchased (Sigma-Aldrich Chemie and PhytoLab, Germany; Extrasynthese, Genay, France).

Extraction of flavonoids and phenolic acids

300 g frozen aerial parts (leaves and stalks) of buckwheat were homogenized (Robot Coupe® R5 Plus) at −20 °C and

two samples (2 × 5 g) were taken for extraction. 30 g of buckwheat seeds were ground (Retsch mill ZM1, Jawo Handling Aabenraa, Denmark; 1.0 mm) and two samples (2 × 2 g) were taken for extraction. All samples were extracted with MeOH (40 mL) and left overnight with stirring in the dark at 5 °C. The extracts were then filtered (S & S 5892 ashless filter discs) and the material re-extracted with MeOH (40 mL) overnight and filtered. The combined extracts of the aerial parts and seeds, respectively, were each transferred into a 100 mL volumetric flask, adjusted to a final volume of 100 mL with MeOH. The extracts were filtered (Cronus syringe filter nylon 13 mm 0.45 µm, Frisenette Aps, Denmark) into 2 mL brown vials and analysed by RP-HPLC for flavonoids and phenolic acids. The efficiency and the reproducibility of the extraction procedure were determined by multiple extractions (3 × 25 and 3 × 40 mL, MeOH) of frozen aerial parts (5 g) or seeds (2 g) in duplicates. For determination of the efficiency of the extraction method, the extract obtained after each extraction-step was analysed for flavonoids and phenolic acids by RP-HPLC. The above extraction procedure ensured extraction of >95 % of total flavonoids and phenolic acids. Furthermore, the extraction method was found to be reproducible (CV<5 %). It should be stressed that it is very important to extract the frozen aerial parts immediately in the organic solvent to avoid enzymatic degradation of flavonoids and phenolic acids in the plant material (data not shown).

Identification of flavonoids and phenolic acids by LC-MS

Identification of flavonoids and phenolic acids in MeOH extracts of aerial parts and seeds of buckwheat 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 RP-C18 end-capped column (5 µm particle size; 250 × 4 mm i.d., Merck, Darmstadt, Germany) protected with a RP-18 guard cartridge (5 µm; 15 × 4 mm i.d., Merck, Darmstadt, Germany) at 35 °C. The mobile phase consisted of solvent A (aqueous 1 % formic acid) and solvent B (100 % MeCN). The following solvent gradient was used: 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 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 and monitored at 280, 320, 360, and 500 nm. MS spectra were recorded consecutively in negative and positive 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: N_2 used as drying gas at a flow of 7 L min^{-1} and as nebulising gas at 300 °C. A potential of 3 kV was used on the capillary and the corona current was set to 15 µA (negative mode) and 4 µA (positive mode). Flavonoids and phenolic acids were identified in the buckwheat 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 flavonoids and phenolic acids by RP-HPLC

Analytical RP-HPLC was performed on a Shimadzu HPLC LC-10 Series System equipped with a DAD (Hitachi l-7450). Flavonoids and phenolic acids were monitored at 280 and 360 nm and UV-vis spectra were recorded

Table 1. Characteristic ions of phenolic acids and flavonoids in aerial parts and seeds of *Fagopyrum esculentum* and *F. tataricum* determined by LC-MS (APCI, Negative and Positive mode) and their UV spectra determined by HPLC-DAD.

Compound no.*	RT HPLC (min)	Compound	HPLC-DAD UV λ_{max} (nm)	LC-MS APCI *m/z* (% base peak)
1	6.9	3-*O*-caffeoylquinic acid (neochlorogenic acid)	300sh, 325	353 $[M-H]^-$ (85), 191 (75)
2	9.4	flavonol triglycoside	255, 264sh, 353	773 $[M+H]^+$ (100), 630 (10), 465 (10), 303 (10)
3	9.5	coumaroylquinic acid derivative	310	337 $[M-H]^-$ (100), 191 (20), 163 (45)
4	10.3	5-*O*-caffeoylquinic acid (chlorogenic acid)	300sh, 325	353 $[M-H]^-$ (33), 191 (100)
5	11.0	feruloylquinic acid derivative	292sh, 325	577 $[M-H]^-$ (100), 367 (20)
6	12.9	epicatechin	278	289 $[M-H]^-$ (100)
7	16.2	orientin or isoorientin	255sh, 265, 350	447 $[M-H]^-$ (100)
8	19.2	quercetin-3-*O*-rutinoside (rutin)	255, 264, 301, 354	611 $[M+H]^+$ (100), 465 (70), 303 (60)
9	20.2	quercetin-3-*O*-glucoside (isoquercitrin)	255, 265sh, 300sh, 355	463 $[M-H]^-$ (100), 301 (10)
10	20.7	quercetin derivative	255, 265sh, 300sh, 354	453 $[M+H]^+$ (30), 303 (20)
11	22.2	kaempferol-3-*O*-rutinoside	265, 298sh, 345	595 $[M+H]^+$ (100), 449 (45), 287 (25)
12	23.8	quercetin derivative	256, 263, 303, 348	447 $[M-H]^-$ (100), 301 (10)

* Numbering corresponds to peak numbering in HPLC chromatograms (Fig. 2).

between 200 and 600 nm. Separations were carried out on a LiCrospher® 100 RP-18 column (5 μm particle size; 250.0 × 4.6 mm i.d., Merck, Darmstadt, Germany) at 35 °C. The mobile phase consisted of solvent A (aqueous 0.5 % TFA) and solvent B (100 % MeCN). The following solvent gradient was used: 10 to 20 % B (15 min), 20 to 30 % B (10 min), 30 to 40 % B (15 min), 40 to 50 % B (15 min), 50 to 90 % B (10 min), 90 % B isocratic (15 min), 90 to 10 % B (5 min), 10 % B isocratic (10 min). All changes of solvent B were linear programmed. The flow rate was 1.0 mL min^{-1}, the injection volume 20 μL, and acquisition was off at 75 min. Contents of phenolic acids and flavonoids were determined by external calibration curves of chlorogenic acid and rutin, respectively. The precision of the HPLC method was determined by four injections of a buckwheat 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 was 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 and interactions (F-tests) were calculated and tested using the ANOVA procedure except one analysis with a few missing values where the GLM procedure was used. In the split-plot and split-split-plot designed experiments the proc MIXED procedure was used and the appropriate error terms of interactions of main-plots and replicate as well as main-1stsub-plot and replicate were used to calculate significance of treatments. Regression analyses on the effects of N-application were performed with the Proc REG procedure in the Danish nitrogen fertiliser experiments with four N-treatments. Significance tables of Wald-type F-statistics are shown for the results of individual experiments in the detailed tables of results. If the F-tests showed significant treatment effects LSD values (P = 0.05) are used to separate means in tables of treatments effects.

Results and Discussion

Flavonoids and phenolic acids in normal and tartary buckwheat

Polyphenols (Fig. 1; Table 1) in extracts of aerial parts and seeds of normal and tartary buckwheat were monitored by RP-HPLC-DAD and LC-MS (APCI, in negative and positive mode).

Compounds 1, 4, and 5 all had UV absorptions clearly indicating that they were caffeic acid derivatives, which was also confirmed by LC-MS data (Table 1). Compounds 1 and 4 both had a pseudomolecular ion $[M-H]^-$ at *m/z* 353, compatible with caffeoylquinic acids and were identified as 3-*O*-caffeoylquinic acid (neochlorogenic acid) and 5-*O*-caffeoylquinic acid (chlorogenic acid), respectively, by comparison with authentic standards. Chlorogenic acids have previously been reported in buckwheat seeds, seedlings, and aerial parts (Tsuzuki and Yamamoto 1987; Hagels et al. 1998; Golisz et al. 2007; Kim et al. 2008). Other phenolic acids previously reported in aerial parts of buckwheat are ferulic acid, caffeic acid, and gallic acid but none of these were identified in this study. Syringic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and dihydroxybenzoic acid have previously been reported in buckwheat seeds but again none of these phenolic acids was detected in this study (Durkee 1977; Watanabe et al. 1997; Hung and Morita 2008). These differences are probably due to differences in extraction methods, cultivars, and varieties used as well as sampling techniques.

Compound 6 had a UV absorption similar to that of flavanones/catechins which was also confirmed by LC-MS. Compound 6 showed a pseudomolecular ion $[M-H]^-$ at *m/z* 289 indicating that this compound was epicatechin, which was confirmed by comparison with an authentic standard. Epicatechin has previously been reported as a constituent of both seeds and aerial parts of buckwheat (Quettier-Deleu et al. 2000; Kalinova et al. 2006; Golisz et al. 2007).

Compounds 2 and 7–12 showed typical UV spectra of flavonol glycosides in accordance with their LC-MS data (Table 1). Compounds 2, 8–10, and 12 all gave an ion at *m/z* 301 or *m/z* 303 in negative and positive mode, respectively, corresponding to the aglycone quercetin. Compound 2 showed a pseudomolecular ion $[M+H]^+$ at *m/z* 773 and losses of 308 and 162 Da corresponding to the loss of a rutinose moiety and a glucose moiety, respectively (Table 1) giving that this compound was a flavonol triglycoside. Compound 2 corresponds to a substance previously mentioned by Tian et al. 2002 however, no comprehensive identification was performed. Compound 7 showed a pseudomolecular ion $[M-H]^-$ at *m/z* 447 corresponding to orientin or isoorientin (Kim et al. 2008). Compounds 8 and 9 showed pseudomolecular ions $[M+H]^+$ at *m/z* 611 and $[M-H]^-$ at *m/z* 463, respectively, and were identified as quercetin-3-*O*-rutinoside and quercetin-3-*O*-glucoside, respectively. Compound 11 showed a pseudomolecular ion $[M+H]^+$ at *m/z* 595 and a loss of 308 Da corresponding to the loss of a rutinose moiety (Table 1). Consequently, compound 11 was identified as kaempferol-3-*O*-rutinoside, which was also confirmed by comparison with an authentic standard. The flavonoids identified in the present investigation have all previously been identified in aerial parts and seeds of normal buckwheat (Sato and Sakamura 1975; Golisz et al. 2007) as well as the seeds of tartary buckwheat (Tian et al. 2002; Kim et al. 2008). However, the flavone *C*-glycosides isovitexin, vitexin, and the flavonol quercetin, which have all previously been identified in buckwheat seed extracts (Margna and Margna 1982; Watanabe et al. 1997; Krkosova and Mrazova 2005) were not detected in this study. As mentioned before, these differences are most likely due to the different extraction methods, cultivars, and varieties used as well as sampling techniques.

A clear distinction between the two species; *F. esculentum* and *F. tataricum* can be seen in the metabolite profiles from the HPLC analyses at 280 nm (Fig. 2). It is evident that the compound profile of the aerial parts of normal buckwheat is more complex than for tartary buckwheat, and this was consecutively found throughout our analyses. Compounds 2–4, 6, 8, 10, and 11 were found in aerial parts of both species whereas compounds 7, 9, and 12 were only found in normal buckwheat. For the seeds a much more limited amount of polyphenolic compounds were detected (Fig. 2).

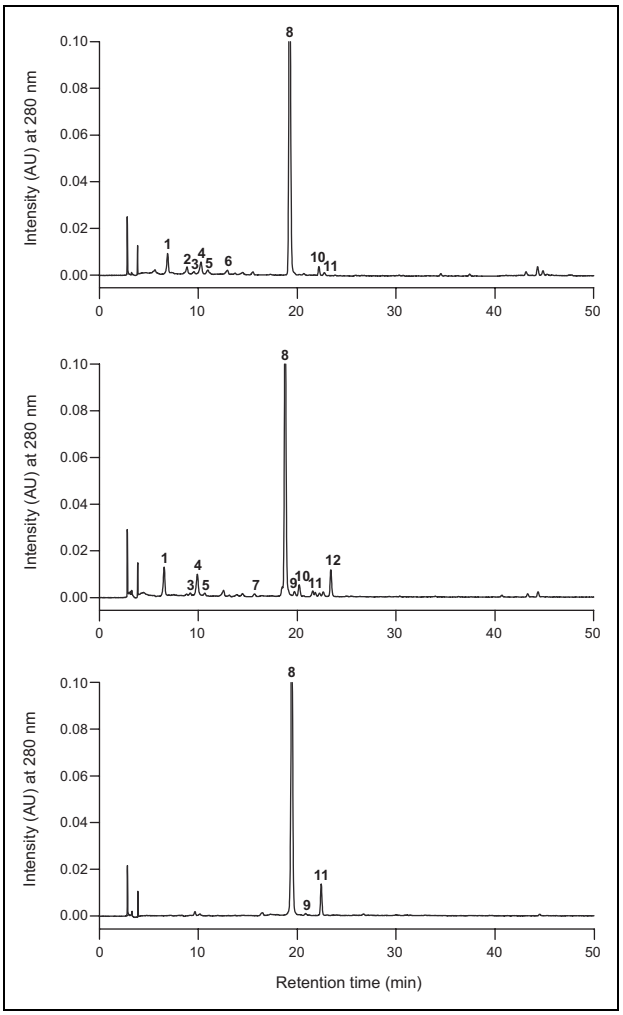


Fig. 2. Representative HPLC chromatograms at 280 nm for aerial parts of *Fagopyrum tataricum* (variety 'Lifago') (top), aerial parts of *F. esculentum* (variety 'Lileja') (middle), and seeds of *F. tataricum* (variety 'Lifago') (bottom). Numbers refer to Table 1 where identified polyphenols in the two buckwheat species are specified. (1) 3-*O*-caffeoylquinic acid, (2) flavonol triglycoside, (3) coumaroylquinic acid derivative, (4) 5-*O*-caffeoylquinic acid, (5) feruloylquinic acid derivative, (6) epicatechin, (7) orientin/isoorientin, (8) quercetin-3-*O*-rutinoside, (9) quercetin-3-*O*-glucoside, (10) quercetin derivative, (11) kaempferol-3-*O*-rutinoside, (12) quercetin derivative.

Effects of nitrogen fertilization, species/variety, and harvest time on yield parameters

Aerial parts. High N-levels increased the fresh weight (FW) yield of aerial parts for both normal and tartary buckwheat. FW yields increased from about 22 to 34 t ha^{-1} for tartary buckwheat and from 22 to 29 t ha^{-1} for normal buckwheat by going from 0 kg N to 120–160 kg N ha^{-1} added. The DM yield only showed a minor increase for both tartary and normal buckwheat, and this was not significant. DM yield was near constant around 4 t ha^{-1} DM for the different N-applications (Table 2). For buckwheat cultivated in 2007 in Germany similar effects of N-application was seen although there was a slightly significant increase in DM yield by high N-application (Table 3). Regression analyses on N-level treatments (as average over year and variety) showed only significant negative correlated effects on dry matter content (DM %, $R^2 = 0.9969$; $P = 0.0016$). DM yields of buckwheat varieties cultivated in Germany in 2007 (Table 3) all increased just slightly significant with increased N-fertilization; whereas DM % decreased highly significantly (Table 3). The magnitude and effects of N-fertilization on FW and DM yields seen in this study correspond to the findings by Honermeier and Wagenbreth (2000), who found an increase of FW yield of about 30–40 % by the application of 30 kg N ha^{-1} compared to no N-application. The DM yield and the DM % of common buckwheat (variety 'Lileja') and tartary buckwheat (variety 'Lifago') cultivated in Germany and harvested at three different dates in 2007 showed very significant increases with later harvest (Table 4) at both 0 and at 120 kg N ha^{-1}.

Seeds. The yield of normal buckwheat seeds decreased with increasing N-application and for variety 'Lileja' the yield changed from 2.6 to 2.0 t ha^{-1} for 0 and 160 kg N ha^{-1}, respectively (Table 5). The seed yield of the tartary buckwheat variety 'Lifago' (DK) in the same experiment was not clearly decreasing (Table 5). The seed yield in relation to N-application is not commented in the buckwheat experiments by Honermeier and Wagenbreth (2000), but stagnation in seed yield in relation to high N-applications are not uncommon in grain crop cultivation (Novoa and Loomis 1981). The high N fertilization favours the vegetative growth over the generative and the buckwheat plant can not utilise the high N for seed production.

The yield of seeds was also highly species dependant (Table 6), where the tartary buckwheat yielded (3.2 t ha^{-1}) about 1 t ha^{-1} of grain more than normal buckwheat (2.2 t ha^{-1}).

Effects of nitrogen fertilization on polyphenol content

Aerial parts. For both tartary buckwheat (variety 'Lifago') and normal buckwheat (variety 'Lileja', only in 2007) cultivated at four different N-levels no significant effect on the concentration of flavonoids between low and high levels of N could be detected in the aerial parts (Table 2). The average concentration of flavonoids for, e.g., Danish 'Lifago' (DK) 2007 was 25.4 mg g^{-1} DM and for normal buckwheat (variety 'Lileja') it was 26.5 mg g^{-1} DM. For aerial parts of both normal and tartary buckwheat, the concentration of phenolic acids was also not significantly affected by N-levels. For aerial parts of tartary buckwheat the average concentration of total phenolic acids was 2.3 mg g^{-1} DM and for normal buckwheat 3.1 mg g^{-1} DM (Table 2). Regression analyses on effects of N-application on the concentrations of flavonoids and phenolic acids showed no significant correlation in the Danish N-experiment (Table 2). In the German N-application experiment (Table 3), there was also no significant effect of high or low N on the content of phenolic acids and flavonoids in the aerial parts. In both experiments (Table 2 and 3), there was a tendency to decreasing flavonoid concentration (average 16 % decrease in rutin concentration) with higher N-application. The reason for this weak reaction of the buckwheat plants to N-fertilization in contrast to the findings in other plant species, e.g., barley (Noerbaek et al.

Table 2. Effects of nitrogen (N)-application in two buckwheat varieties on dry matter (DM) yield of aerial parts, dry matter content (DM %), content of major phenolic acids (3-*O*-caffeoylquinic acid (1), 5-*O*-caffeoylquinic acid (4)), and flavonoids (epicatechin (6), quercetin-3-*O*-rutinoside (8), quercetin-3-*O*-glucoside (9), kaempferol-3-*O*-rutinoside (11)) (Experiment 1: Denmark).

Year	Variety	N-applied (kg N ha^{-1})	Harvest time	DM yield (t ha^{-1})	DM (%)	Phenolic acids		Flavonoids				Total	
						1	4	6	8	9	11	phenolic acids	flavonoids
						(mg g^{-1} DM)							
2006	'Lifago'[1)]	0	14/7	4.5 b[2)]	14.6 a	1.2 a	0.9 a	0.15 a	35.4 a	nq	0.7 a	2.5 ab	36.2 a
	(D)	30		5.3 a	13.8 a	1.4 a	0.9 a	0.17 a	39.2 a	nq	0.7 a	2.7 a	40.1 a
		60		5.3 a	14.4 a	1.2 a	0.8 a	0.15 a	32.2 a	nq	0.6 a	2.3 b	32.9 a
		120		5.7 a	13.5 a	1.3 a	0.8 a	0.14 a	32.9 a	nq	0.6 a	2.5 ab	33.7 a
	'Lifago'	0	14/7	3.0 a	15.4 a	1.1 a	0.9 a	0.18 a	35.6 a	nq	0.7 a	2.3 a	36.4 a
	(DK)	30		4.6 a	15.1 a	1.1 a	0.9 a	0.16 a	32.3 a	nq	0.6 a	2.4 a	33.1 a
		60		4.1 a	15.1 a	0.9 a	0.7 a	0.15 a	26.4 a	nq	0.5 a	1.9 a	27.0 a
		120		4.1 a	14.0 b	1.2 a	0.8 a	0.13 a	25.9 a	nq	0.5 a	2.3 a	26.5 a
2007	'Lifago'	0	10/7	3.1 a	15.3 a	1.0 a	0.8 a	0.20 a	29.1 a	nq	0.6 a	2.2 a	29.9 a
	(DK)	40		3.3 a	15.2 a	1.0 a	0.8 a	0.20 a	26.2 a	nq	0.6 a	2.3 a	26.9 a
		80		4.2 a	13.5 ab	1.0 a	0.8 a	0.14 a	17.6 a	nq	0.4 a	2.2 a	18.1 a
		160		3.9 a	11.4 b	1.3 a	1.0 a	0.17 a	20.9 a	nq	0.5 a	2.7 a	21.5 a
	'Lileja'	0	10/7	3.5 a	15.7 a	1.5 a	1.3 a	0.28 a	24.3 a	0.3 a	0.3 a	3.0 a	27.3 a
	(D)	40		3.7 a	14.3 ab	1.6 a	1.2 a	0.30 a	23.6 a	0.3 a	0.3 a	3.1 a	26.7 a
		80		3.6 a	13.5 ab	1.5 a	1.2 a	0.28 a	22.3 a	0.2 b	0.3 a	2.9 a	25.1 a
		160		3.5 a	12.2 b	1.8 a	1.2 a	0.32 a	24.3 a	nq	0.3 a	3.3 a	27.1 a
Statistical analysis													
2006	N-application			ns[3)]	ns	ns	ns	ns	ns	–	ns	ns	ns
	Variety			ns	*	ns	ns	ns	ns	–	ns	*	ns
	N-application*variety			ns	ns	ns	ns	ns	ns	–	ns	ns	ns
2007	N-application			ns	***	ns	ns	ns	ns	–	ns	ns	ns
	Species/variety			ns	ns	*	**	*	ns	–	*	*	ns
	N-application*variety			ns	ns	ns	ns	ns	ns	–	ns	ns	ns

[1)] 'Lifago' (D) and 'Lifago' (DK) are tartary buckwheat and 'Lileja' (D) is normal buckwheat.
[2)] Means followed by the same letter are not significantly different ($LSD_{0.05}$). Mean comparison by letters are for N-application in each year and for each variety.
[3)] ns, *, **, ***: not significant, or significant at P≤0.05, P≤0.01, and P≤0.001, respectively.
nq = not quantified. Amounts under the quantification level of 0.05 mg g^{-1} dry matter.

2003), strawberries (Anttonen et al. 2006), and stinging nettle (Grevsen et al. 2008) could be a relatively low N-uptake of buckwheat, which is known to be a pioneer crop that can grow well on low fertile soil (Marquard and Kroth 2001). In contrast with our study, a more clear indication of the fact that the rutin content decreases (18–29 % decrease) in aerial parts of normal buckwheat with increasing N-fertilization (0 to 90 kg N ha^{-1}) was reported by Honermeier and Wagenbreth (2000), although they also write about a 'tendency' that was confirmed in studies of other varieties.

Fabjan et al. 2003 found rutin contents of up to 3 % dry weight (DW) in aerial parts of tartary buckwheat corresponding with our findings of a range from 1.7 to 3.9 % DW rutin depending on N-fertilization (Table 2). Other studies have found rutin contents of 3.0–9.6 % DW, but here only the leaves were analysed and they are known to have a much higher concentration of flavonol glycosides than the stalks (Honermeier and Wagenbreth 2000; Kalinova et al. 2006).

In sprouts of both normal and tartary buckwheat concentration of chlorogenic acids (5-*O*-caffeoylquinic acid, 4) was found to be 0.8–1.7 mg g^{-1} DW (Kim et al. 2008) and the results from our study are of the same magnitude 0.7–1.4 mg g^{-1} DW for aerial parts (Table 2 and 3). Golisz et al. 2007 found significantly higher concentrations of rutin, chlorogenic acid, and epicatechin in aerial parts of normal buckwheat 50.5, 1.79, and 0.98 mg g^{-1}

Table 3. Effects of variety and nitrogen (N) fertilization on dry matter (DM) yield of aerial parts, dry matter content (DM %), content of major phenolic acids (3-*O*-caffeoylquinic acid (1), 5-*O*-caffeoylquinic acid (4)), and flavonoids (epicatechin (6), quercetin-3-*O*-rutinoside (8), quercetin-3-*O*-glucoside (9), kaempferol-3-*O*-rutinoside (11)). The buckwheat was harvested on June 26, 2007. (Experiment 3: Germany). The experiment was in a split-plot design and the table of significance show effects of variety as main-plot and N-application as sub-plot (3 replicates).

Year	Variety	N-applied (kg N ha^{-1})	DM yield (t ha^{-1})	DM (%)	Phenolic acids		Flavonoids				Total	
					1	4	6	8	9	11	phenolic acids	flavonoids
					mg g^{-1} DM							
2007	'Lifago' (D)	0	3.2 a[2)]	11.1 a	1.6 a	1.3 a	0.15 a	41.5 a	nq	0.9 a	3.3 a	42.6 a
	'Lileja'		3.3 a	12.4 a	1.8 a	1.4 a	0.22 a	30.3 a	nq	0.3 b	3.2 a	33.4 a
	'Hruszowska'		3.1 a	11.8 a	1.7 a	1.2 a	0.21 a	28.8 a	nq	0.4 b	2.9 a	31.2 a
	'Bamby'		2.3 b	12.1 a	1.8 a	1.5 a	0.26 a	30.9 a	nq	0.4 b	3.5 a	34.3 a
	'Lifago' (D)	120	4.0 a	9.8 ab	1.5 a	1.1 a	0.11 a	29.5 a	nq	0.7 a	3.0 a	30.2 a
	'Lileja'		3.5 ab	9.4 b	1.7 a	1.3 ab	0.18ab	26.0 a	nq	0.4 b	2.9 a	28.2 a
	'Hruszowska'		3.6 a	9.4 b	1.7 a	1.2 ab	0.23 b	29.9 a	nq	0.4 ab	2.9 a	32.4 a
	'Bamby'		2.5 b	10.5 a	1.7 a	1.3 b	0.23 b	25.8 a	nq	0.3 b	3.3 a	28.2 a
Statistical analysis												
Variety			*[2)]	ns	ns	ns	ns	ns	–	*	ns	ns
Replicate			ns	*	ns	ns	ns	ns	–	ns	ns	ns
Variety*replicate			ns	ns	ns	ns	ns	ns	–	ns	ns	ns
N–application			*	***	ns	ns	ns	ns	–	ns	ns	ns
variety*N–application			ns	ns	ns	ns	ns	ns	–	ns	ns	ns

[1)] Means followed by the same letter are not significantly different ($LSD_{0.05}$). Mean comparison by letters are for variety and for each N level.
[2)] ns, *, **, ***: not significant, or significant at P≤0.05, P≤0.01, and P≤0.001, respectively.
nq = not quantified. Amounts under the quantification level of 0.05 mg g^{-1} DM.

DW, respectively, than in the present study, but this could be due to the fact that they analysed leaves, stems, and inflorescences individually, and did not present a weighted average.

Seeds. For tartary buckwheat (Table 5, variety 'Lifago' (DK)) the content of total flavonoids decreased from 16.1 mg g^{-1} DW at low N to 15.2 mg g^{-1} DW at high N, but only the decrease in the content of kaemferol-3-*O*-rutinoside was significant (Table 5). For seeds of normal buckwheat (variety 'Lileja') a decrease in total flavonoids was also seen from 1.3 mg g^{-1} DW at low N to 0.5 mg g^{-1} DW at high N, but again this was not significant (Table 5). Quercetin-3-*O*-glucoside and kaempferol-3-*O*-rutinoside were quantified in the tartary buckwheat seeds but in the normal seeds, only trace amounts were observed. Furthermore, a large difference in rutin content, and thereby total flavonoid content, was seen between the two species (Table 5 and 6). For the normal buckwheat seeds, the rutin concentrations were in the range 0.3–1.1 mg g^{-1} DW and for tartary buckwheat seeds, it was 14.0–15.0 mg g^{-1} DW. Tartary and normal buckwheat seeds have previously been reported to contain 8–16 mg g^{-1} DW and 0.1–0.6 mg g^{-1} DW rutin, respectively, (Bonafaccia et al. 2003: Fabjan et al. 2003; Jiang et al. 2007; Kim et al. 2008) and this corresponds well with the results obtained in this study.

Effects of harvest time on polyphenol content in aerial parts

As illustrated in Fig. 3, harvest time or development stage has a great impact on the content of flavonoids in aerial parts of both normal and tartary buckwheat. For tartary buckwheat (variety 'Lifago' (D)) at low N-level, concentration of flavonoids decreased from 42.6 to 21.2 to 8.9 mg g^{-1} DM at harvest in June, July, and August, respectively (Table 4). The same was true for normal buckwheat although the total content of flavonoids was lower (Table 4). Concentration of phenolic acids was also highly significantly affected by harvest time as the total content of these decreased from 3.21 mg g^{-1} DM at first harvest to 0.46 mg g^{-1} DM at last harvest for normal buckwheat at the low N-level (variety 'Lileja'). The same effect could also be observed for tartary buckwheat (variety 'Lifago' (D)) (Table 4). In the significance table of Table 4 it is seen that harvest time had a very significant effect on all the measured variables from yield and DM % to content of all polyphenols, whereas again the N-application had only minor effects on DM % and rutin concentration (Table 4). The data analyses show some significant interactions of Harvest × Variety and Harvest × N-application in the polyphenol content (Table 4). The variety "Lileja" shows a stronger reaction to harvest time than "Lifago" and there is a stronger reaction to harvest

Table 4. Effects of three harvest times for two varieties and two nitrogen (N) fertilization levels on dry matter (DM) yield of aerial parts, dry matter content (DM %), content of major phenolic acids (3-*O*-caffeoylquinic acid (1), 5-*O*-caffeoylquinic acid (4)), and flavonoids (epicatechin (6), quercetin-3-*O*-rutinoside (8), quercetin-3-*O*-glucoside (9), kaempferol-3-*O*-rutinoside (11)). (Experiment 4: Germany). The experiment was a split-split-plot design. In the significance table, the effects of varieties and replicates were tested with the Variety*replicate error term. Variety*N-application interaction was tested with Variety* N-application*replicate error term and the remaining effects and interactions with the experiment-wise error term.

Year	Variety	N-applied (kg N ha^{-1})	Harvest time	DM yield (t ha^{-1})	DM (%)	Phenolic acids		Flavonoids				Total	
						1	4	6	8	9	11	phenolic acids	flavonoids
						mg g^{-1} DM							
2007	'Lifago'	0	26/6	3.2 b[1)]	11.1 a	1.6 a	1.3 a	0.15 a	41.5 a	nq	0.9 a	3.3 a	42.6 a
	(D)		18/7	7.3 a	18.8 b	0.5 b	0.5 b	0.12 a	20.6 b	nq	0.5 b	1.3 b	21.2 b
			20/8	7.1 a	26.0 c	0.2 c	0.2 c	0.10 a	8.5 c	nq	0.3 c	0.6 c	8.9 c
		120	26/6	4.0 b	9.8 a	1.5 a	1.1 a	0.11 a	29.5 a	nq	0.7 a	3.0 a	30.2 a
			18/7	6.9 a	15.5 b	0.7 b	0.7 b	0.14 a	21.9 ab	nq	0.6 ab	1.7 b	22.6 ab
			20/8	7.7 a	24.2 c	0.2 c	0.2 c	0.07 a	8.9 b	nq	0.3 b	0.6 c	9.3 b
	'Lileja'	0	26/6	3.3 c	12.4 a	1.8 a	1.4 a	0.22 a	30.3 a	nq	0.3 a	3.2 a	33.4 a
			18/7	5.6 b	20.2 b	0.7 b	0.5 b	0.14 b	12.2 b	0.2	0.2 b	1.5 b	13.9 b
			20/8	7.1 a	30.1 c	0.3 c	0.2 c	0.05 c	6.7 c	0.2	0.1 b	0.5 c	7.4 c
		120	26/6	3.5 b	9.4 a	1.7 a	1.3 a	0.18 a	26.0 a	nq	0.4 a	2.9 a	28.2 a
			18/7	6.3 a	17.4 b	0.7 b	0.5 b	0.13 a	12.7 b	0.4	0.2 b	1.4 b	14.8 b
			20/8	7.2 a	25.6 c	0.3 b	0.2 b	0.06 b	6.0 b	0.3	nq	0.5 b	6.9 b
Statistical analysis													
Variety				ns[2)]	ns	ns	ns	ns	ns	–	*	ns	ns
Replicate				ns	ns	ns	ns	ns	ns	–	ns	ns	ns
Variety*replicate				ns	ns	ns	ns	ns	*	–	*	ns	ns
N-application				ns	*	ns	ns	ns	*	–	ns	ns	*
Variety*N-appl.*replicate				ns	ns	ns	ns	ns	ns	–	ns	ns	ns
Harvest time				***	***	***	***	***	***	–	***	***	***
Harvest*variety				ns	ns	ns	ns	*	ns	–	*	ns	ns
Harvest*N-appl.				ns	ns	ns	*	ns	*	–	ns	ns	*
Harvest*variety*N-appl.				ns	ns	ns	ns	ns	ns	–	ns	ns	ns

[1)] Means followed by the same letter are not significantly different ($LSD_{0.05}$). Mean comparison by letters are for variety and for each N-application.
[2)] ns, *, **, ***: not significant, or significant at P≤0.05, P≤0.01, and P≤0.001, respectively.
nq = not quantified. Amounts under the quantification level of 0.05 mg g^{-1} DM.

time at low N-application. Honermeier and Wagenbreth (2000) also investigated the impact of development stage of the plant on rutin content (normal buckwheat, variety 'Prego') and reported a decrease in rutin content from about 63 d after sowing (5.8 % rutin) to 92 d after sowing (3.7 % rutin). This would correspond to the first harvest time (53 d after sowing, 3.03 % rutin) in our German harvest time experiment (Table 4, 'Lileja', low N) and the last harvest time (98 d after sowing, 0.67 % rutin). Again the difference in magnitude of rutin content may be explained by the fact that Honermeier and Wagenbreth (2000) only analysed the leaves whereas we analysed total aerial parts including stalks.

Effects of species/variety on polyphenols in seeds

It is only in the seeds that a very significant difference in flavonoid concentration between normal and tartary buckwheat can be seen. The rutin content of tartary buckwheat seeds (variety 'Lifago') is near 14 mg g^{-1} DM, whereas the normal buckwheat varieties 'Hruszowska' and 'Bamby' only have 0.7 and 0.3 mg g^{-1} DM, respectively (Table 6). In the aerial parts, on the other hand, there is no big difference in the polyphenol content of the two species (Table 2, 3 and 4).

In conclusion, we identified most of the major polyphenols previously reported in *Fagopyrum* sp. in the

Table 5. Effects of nitrogen (N) fertilization on seed yield of two buckwheat species/varieties and content of major flavonoids (quercetin-3-*O*-rutinoside (8), quercetin-3-*O*-glucoside (9), and kaempferol-3-*O*-rutinoside (11)). (Experiment 1: Denmark).

Year	Variety	N-applied (kg N ha^{-1})	Seed yield (t ha^{-1})	Flavonoids			
				8	9	11	Total
				mg g^{-1} DM			
2007	'Lileja'[1)]	0	2.6 a[2)]	1.1 a	nq	0.05 a	1.3 a
	(D)	40	2.6 a	0.4 a	nq	0.05 a	0.6 a
		80	1.9 b	0.3 a	nq	0.05 a	0.4 a
		160	2.0 b	0.4 a	nq	0.05 a	0.5 a
	'Lifago'	0	3.1 ab	14.7 a	0.07 a	1.11 a	16.1 a
	(DK)	40	3.6 a	15.0 a	0.07 a	1.07 ab	16.3 a
		80	2.8 b	14.6 ab	0.07 a	0.99 bc	15.8 ab
		160	3.0 ab	14.0 b	0.07 a	0.92 c	15.2 b
Statistical analysis							
Variety			***[3)]	***	–	***	***
N-application			**	ns	–	**	ns
Variety*N-application			ns	ns	–	ns	ns

[1)] 'Lileja' (D) is a normal and 'Lifago' (DK) a tartary buckwheat variety.
[2)] Means followed by the same letter are not significantly different ($LSD_{0.05}$). Mean comparison by letters are for N-application for each species/variety.
[3)] ns, *, **, ***: not significant, or significant at P≤0.05, P≤0.01, and P≤0.001, respectively.
nq = not quantified.

Table 6. Effects of species/varieties on seed yield and content of major flavonoids (quercetin-3-*O*-rutinoside (8), quercetin-3-*O*-glucoside (9), and kaempferol-3-*O*-rutinoside (11)). (Experiment 2: Denmark).

Year	Variety	N-applied (kg N ha^{-1})	Seed yield (t ha^{-1})	Flavonoids			
				8	9	11	Total
				mg g^{-1} DM			
2007	'Hruszowska'	80	2.2 a[2)]	0.7 a	nq	0.02 a	0.9 a
	'Bamby'		2.2 a	0.3 a	nq	nq	0.4 a
	'Lifago'		3.2 b	13.7 b	nq	0.95 b	14.8 b
Statistical analysis							
Species/variety			***[3)]	***	–	***	***

[1)] 'Hruszowska' and 'Bamby' are normal buckwheat varieties and 'Lifago' is a tartary buckwheat variety.
[2)] Means followed by the same letter are not significantly different ($LSD_{0.05}$).
[3)] ns: *, **, ***: not significant, or significant at P≤0.05, P≤0.01, and P≤0.001, respectively.
nq = not quantified.

five varieties examined in this study. We generally found lower levels of flavonoids than other studies; however, this is most likely due to the fact that we have used total aerial parts with stalks and others have primarily used only the leaves. We can also conclude that cultivation of buckwheat for medicinal purposes with a high yield of bioactive polyphenols can be manipulated markedly by the choice of species for seed harvest and in aerial parts especially by harvest time or development stage, whereas N-fertilization seemed to have only minor effects on the polyphenol content in dry matter.

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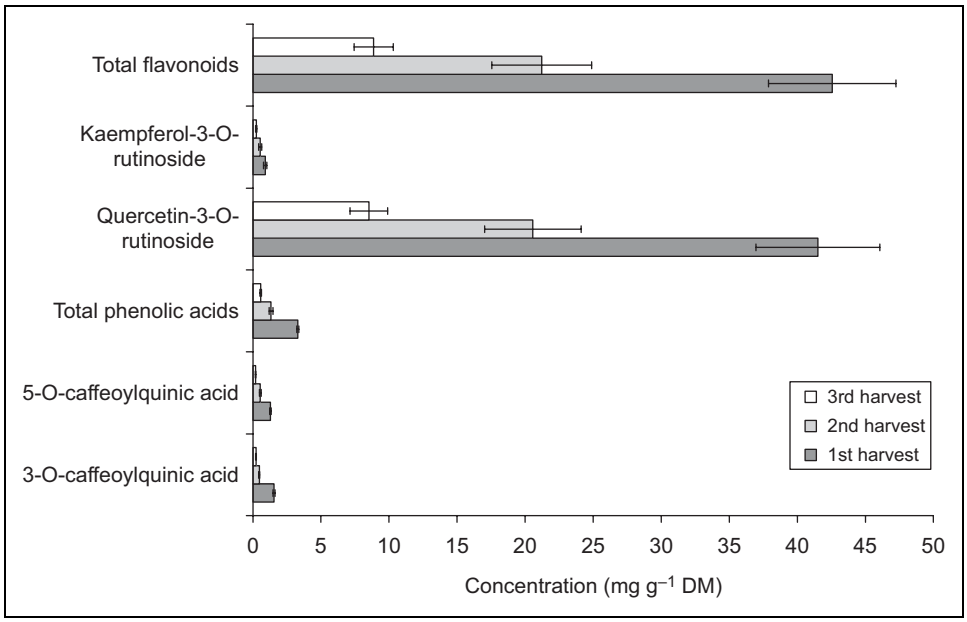


Fig. 3. Effect of harvest time on the concentration of flavonoids and phenolic acids in aerial parts of *Fagopyrum tataricum* (variety 'Lifago') cultivated with 0 kg N ha^{-1} in Germany in 2007. Bars are ± SE.

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Addresses of authors: Kathrine B. Christensen (corresponding author), Department of Food Science, and Kai Grevsen, Department of Horticulture, Faculty of Agricultural Sciences, University of Aarhus, Kirstinebjergvej 10, 5792 Aarslev, Denmark. Mirja Kaemper and Ralf Loges, Institute of Crop Science and Plant Breeding – Grass and Forage Science/Organic Agriculture, University of Kiel, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany. Xavier C. Fretté and Lars P. Christensen, Institute of Chemical Engineering, Biotechnology and Environmental Technology, Faculty of Engineering, University of Southern Denmark, Niels Bohrs Allé 1, 5230 Odense M, Denmark, e-mail: kbch@kbm.sdu.dk.