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Effects of Organic and Conventional Growth Systems on the Content of Flavonoids in Onions and Phenolic Acids in Carrots and Potatoes

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The demand for organic food products is steadily increasing partly due to the expected health benefits of organic food consumption. Polyphenols, such as flavonoids and phenolic acids, are a group of secondary plant metabolites with presumably beneficial health effects, and contents in plants are affected by, for example, plant nutrient availability, climate, pathogen infection, and pest attack. In the current study, onions, carrots, and potatoes were cultivated in two-year field trials in three different geographical locations, comprising one conventional and two organic agricultural systems. The contents of flavonoids and phenolic acids in plants were analyzed by pressurized liquid extraction and high-performance liquid chromatography−ultraviolet quantification. In onions and carrots, no statistically significant differences between growth systems were found for any of the analyzed polyphenols. On the basis of the present study carried out under well-controlled conditions, it cannot be concluded that organically grown onions, carrots, and potatoes generally have higher contents of health-promoting secondary metabolites in comparison with the conventionally cultivated ones.

KEYWORDS: Carrots (*Daucus carota* L.); flavonoids; onions (*Allium cepa* L.); organic agriculture; potatoes (*Solanum tuberosum* L.); phenolic acids

INTRODUCTION

Plant production in organic agricultural systems relies on organic manures and biological pest control, excluding the use of inorganic fertilizers and synthetic pesticides. Organic agriculture has developed rapidly in most parts of Europe since the 1990s, especially in the Scandinavian and Mediterranean countries (*1*), and the market shares were around 5% in Denmark at the end of 2007 (*2*). Improved animal welfare, environmental protection, human health, and taste and freshness are the most important reasons for the increasing demand of organic food products (*3*). However, the possible health benefits of organic food consumption are still controversial and not considered scientifically well-documented (*4*).

The fundamental differences in organic and conventional agricultural systems, particularly regarding fertilization strategy and soil fertility management, affect the nutrient composition in plants (*5*). However, previous studies on the nutrient content of organically and conventionally grown plants have generated contradicting results (*6*, *7*). To make valid comparisons of organic and conventional plants, important variation caused by several factors such as geographical location and growth season must be included to ensure that the possible differences found are systematic and reliable, but this has not been the case in most previous studies.

Little consideration has previously been given to bioactive secondary plant metabolites (*8*), which play an important role in the growth, development, and defense system of plants (*9*). Despite being nonessential to humans, various secondary plant metabolites have been proposed to prevent numerous diseases (*10*). Polyphenols have one or more phenol units in their chemical structure (*11*) and are a group of secondary plant metabolites, frequently speculated to have positive health effects including a decreased risk of heart diseases, dementia (*12*), and cancer (*13*, *14*).

Flavonoids are a group of polyphenols that occur widely across the plant kingdom (*15*). Onions are one of the main sources of polyphenols in the daily food intake (*16*, *17*) and a rich source of flavonoids (*18*). Quercetin mono- and diglucosides account for up to 80% of the total content of flavonols in onions, but kaempferol (*19*), isorhamnetin derivates (*20*), and myricetin (*9*) have also been found (**Figure 1**).

Another group of widespread polyphenols are phenolic acids, for example, caffeic, *p*-coumaric, ferulic, and chlorogenic acids (**Figure 1**),

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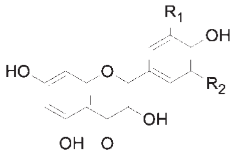
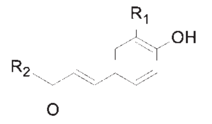




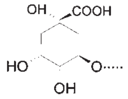
	R_1	R_2		R_1	R_2
Quercetin [a]	OH	H	Caffeic acid [e]	OH	OH
Kaempferol [b]	H	H	*p*-coumaric acid [f]	H	OH
Isorhamnetin [c]	OCH_3	H	Ferulic acid [g]	OCH_3	OH
Myricetin [d]	OH	OH	5-*O*-caffeoyl quinic acid [h]	OH	

Figure 1. Chemical structures of selected flavonoids and phenolic acids. Systematic names: [a]2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one; [b]3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one; [c]3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen-4-one; [d]3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one; [e]2-propenoic acid, 3-(3,4-dihydroxyphenyl)-; [f]2-propenoic acid, 3-(4-hydroxyphenyl)-, (2*E*)-; [g]2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-; and [h]cyclohexanecarboxylic acid, 3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propen-1-yl]oxy]-1,4,5-trihydroxy-, (1*S*,3*R*,4*R*,5*R*)-.

which are found in various beverages, fruits, and vegetables (*21*). 5-*O*-Caffeoylquinic acid (5-CQA) (**Figure 1**) is the predominant phenolic acid in potatoes (*22*) and carrots (*23*), where it constitutes 80−90% of the total content of phenolics.

The content of polyphenols in plants is affected by factors such as cultivar (*24*), pathogen infection and pest attack (*25*), time of harvest, and storage and processing procedures (*26*). The content of nutrients and secondary plant metabolites in food products is also affected by, for example, growth conditions, use of fertilizers, climate (*27*), and plant nutrient availability (*28*).

The objective of our study was to compare the content of selected flavonoids and phenolic acids in organically and conventionally grown onions, potatoes, and carrots and to evaluate if the ability of the crops to synthesize selected secondary metabolites is systematically affected by growth systems across different growth years as well as geographic locations (soil types).

MATERIALS AND METHODS

Reagents and Chemicals. Methanol [high-performance liquid chromatography (HPLC)-grade, Rathburn Chemicals Ltd., Scotland] and Milli-Q water (18 MΩ, Millipore, United States) were used for standards and eluents. Dimethyl sulfoxide (DMSO, > 99%) and formic acid (98−100%) were purchased from Merck (Germany). Purchased were the following standards: quercetin (Q) from Extrasynthése (France), quercetin-3,4′-diglucoside (Q-3,4′-diglu) from Polyphenols (France), quercetin-7,4′-diglucoside (Q-7,4′-diglu) from Apin (United Kingdom), quercetin-4′-glucoside (Q-4′-glu) from Plantech (United Kingdom), and quercetin-3-glucoside (Q-3-glu, purity > 90%), 5-CQA (purity > 95%), and caffeic acid (CA, purity > 99%) from Sigma-Aldrich (Germany).

Individual standard stock solutions were prepared by dissolving the solid compounds in DMSO (100 μg/mL). Working solutions were prepared by 10 times dilutions of the stock solutions with methanol. Stock solutions were stored at −80 °C (< 1 year), while the working solutions were prepared shortly before analyses and kept at −20 °C until analyses.

Samples. Potato, carrot, and onion samples were obtained from field trial studies undertaken in 2007 and 2008 (year 1 and 2). The potatoes (*Solanum tuberosum* cv. *sava*) were grown in the long term CropSys crop rotation experiment (*29*) at three different geographical locations (Flakkebjerg, Foulum, and Jyndevad) (http://www.cropsys.elr.dk/uk/); average temperatures and precipitations at the three locations are shown in Table 4 in the Supporting Information, and planting time, harvest time, and irrigation are shown in Table 5 in the Supporting Information. The carrots (*Daucus carota* cv. *bolero*) and onions (*Allium cepa* cv. *hytech*) were grown at one location (Aarslev) in the VegQure rotation experiment (http://www.vegqure.elr.dk/uk/). The crops were grown in three different agricultural systems at all locations: one conventional system (C) and two organic systems (OA and OB). The systems were all based on stockless cash crop production systems with an identical sequence of main crops (8 year rotation in Aarslev and 4 year rotation at the other locations). In the conventional system, pesticides and inorganic fertilizer were used like generally applied in conventional cultivation of potatoes in Denmark. The OA system relied on the import of animal manure. In the OB system, the nutrient supply was based on the use of cover crops (mainly legumes), but animal manure was also applied to onions to satisfy the high nitrogen demand of this crop. Cover crops were generally grown in the autumn after the main crops and incorporated into the soil in the spring before the main crops were grown. The organic systems were managed in full compliance with the Danish guidelines for organic farming administered by the Danish Plant Directorate (http://pdir.fvm.dk). The potatoes were grown with two replicates of each growth system resulting in six plots per year per location (36 plots in total), while carrots and onions were grown with three replicates of each growth system resulting in nine plots per year (18 plots in total). Table 1 in the Supporting Information presents details on field trial characteristics such as geographical locations, soil types, fertilizer applications, etc.

The crops were harvested at the same day for all systems. A 15 kg sample was collected from each plot using the following inclusion criteria both years (marketable quality): potatoes, 35−60 mm diameter; carrots, 50−250 g fresh weight (fw); and onions, 40−80 mm diameter. Representative sampling was ensured by stepwise mass reduction as described in Petersen et al. (*30*). Only the edible parts of the crops were analyzed, that is, the bulbs of the onions were used for analysis by removing the shoots and outer leaves and the shoot of the carrots was discarded. The samples were washed in Milli-Q water, peeled (only carrots and potatoes), cut into 0.5 cm thick slices, and freeze-dried at 0.08 kPa for 1−2 days at a commercial freeze-drying company (Danish Freeze-Dry A/S, Kirke Hyllinge, Denmark). Afterward, the samples were crushed, homogenized, and stored at −20 °C in an inert nitrogen atmosphere until analysis. In general, the samples were protected from light and oxygen during the entire sample preparation by wrapping them in aluminum foil and storing them in a nitrogen atmosphere.

Chemical Analysis of Flavonoids and Phenolic Acids. The principles of the chemical analysis are summarized below, and further details can be seen in Søltoft et al. (*31*). Extraction of flavonoids and phenolic acids was performed by pressurized liquid extraction using an ASE-200 (Dionex, Sunnyvale, CA). The sample material (0.5 g) was added to 5 mL extraction cells together with 0.5 g of C18-material (Sepra E-C18, 50 μm; Phenomenex, Allerød, Denmark) and extracted with 65% aqueous methanol. The volume of the extracts was adjusted to 20 mL and filtered (0.20 μm, Sartorius Minisart, Aubagne, France) before quantification by HPLC-UV on a Phenomenex Prodigy RP-C18 column (4.6 mm × 250 mm, 5 μm, 30 °C) with 0.1% formic acid in Milli-Q water (v/v) and methanol (90:10, v/v, A eluent) and 100% methanol (B eluent). The flavonoids and phenolic acids were quantified relative to Q according to common practice

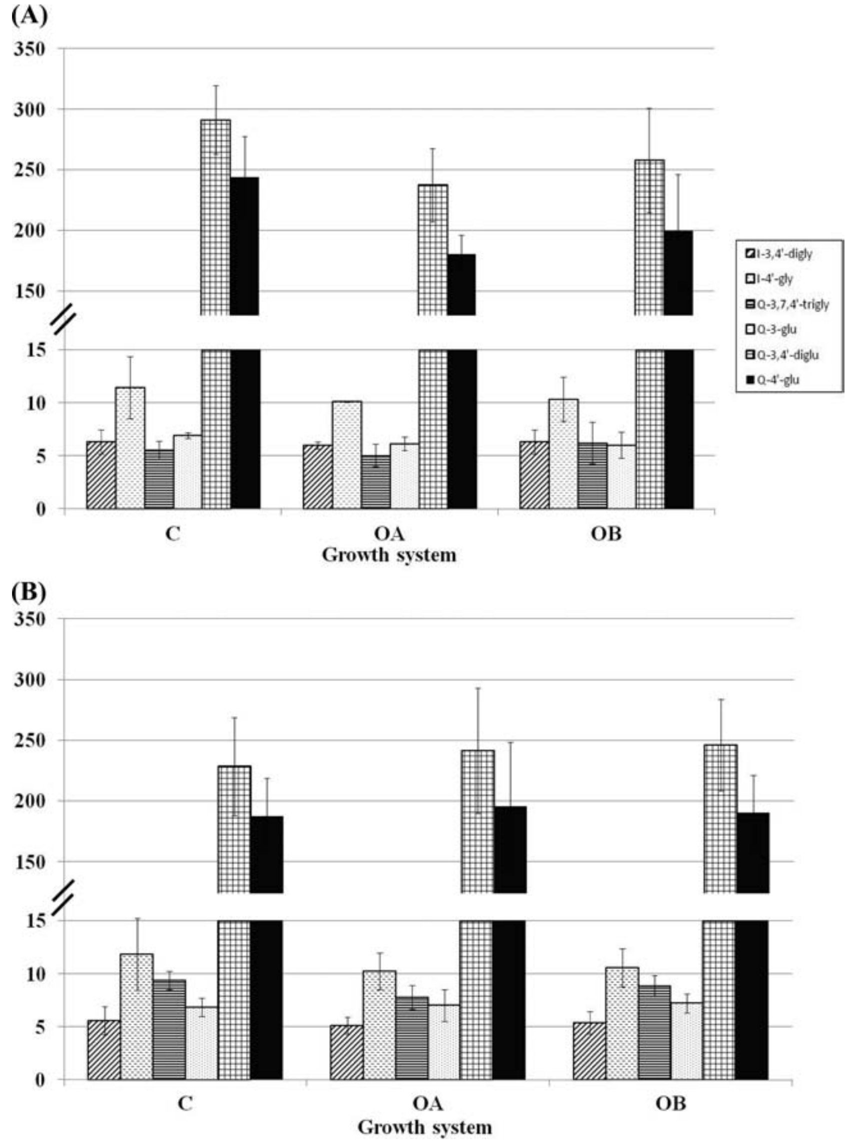


Figure 2. Average concentrations of flavonoids in onions (μg quercetin-equivalents/g fresh weight) in three different growth systems (C: conventional, OA: organic using animal manure, and OB: organic using cover crops) and in two harvest years (A: year 1 and B: year 2). Error bars describe the standard deviation of replicates from the field (n = 3).

for analyses of bioactive compounds (*32*). The chemical structure of the flavonoids and phenolic acids was elucidated by mass spectrometry (MS) and MS/MS analysis and by comparison of retention times, UV, and MS data with available standards (Q, Q-3,4′-diglu, Q-7,4′-diglu, Q-4′-diglu, Q-3-glu, 5-CQA, and CA) according to the principles detailed in Søltoft et al. (*31*), which also presents the validation parameters used.

Quality assurance was performed by including an in-house sample as a reference material (a mix of freeze-dried potato, onion, and carrot) in each series of analyses and yielded an average relative standard deviation (RSD) of 6.2% for all quantified polyphenols (n = 4). Furthermore, duplicate measurements (including weighing and extraction) of randomly selected samples in each series of analyses were included, yielding an average RSD of 6.8, 11, and 2.7% for potatoes, carrots, and onions for all quantified polyphenols in each crop (n = 6 for each compound in potatoes, and n = 2 for carrots and onions).

Elemental Nitrogen Analysis. Nitrogen was measured using isotope ratio mass spectrometry (IR-MS; Europa Scientific, Crewe, United Kingdom). Approximately 4 mg of pulverized material was weighed in tin capsules and introduced to the MS via a combustion interface. Quality assurance was performed using certified reference material, frequent quality control samples, and duplicate measurements of all samples.

Statistical Analysis. The responses y_{ysb} from the Aarslev location were modeled as: $y_{ysb} = \mu + \alpha_b + \beta_y + \delta_s + \varepsilon_{ys} + \varepsilon_{yb} + \varepsilon_{ysb}$, where μ is the generalized intercept; α_b, $b = 1, 2, 3$ is the effect of the blocks; β_y, y = year 1, year 2 is the effect of year; and δ_s, s = C, OA, OB is the effect of growth system. The responses y_{yslb} from the other locations were modeled as: $y_{yslb} = \mu + \beta_y + \delta_s + \gamma_l + \alpha_{lb} + \varepsilon_{ys} + \varepsilon_{yl} + \varepsilon_{sl} + \varepsilon_{ylb} + \varepsilon_{yslb}$, where μ is the generalized intercept; β_y, y = year 1, year 2 is the effect of year; δ_s, s = C, OA, OB is the effect of growth system; γ_l is the effect of location, l = Foulum, Jyndevad, Flakkebjerg is the location; and α_{lb}, b = 1, 2 is the effect of the blocks within each location.

Errors (ε) are considered independently and normally distributed and represent corresponding variance components of interaction. The pair wise comparisons and their confidence intervals between the systems were adjusted to obtain a family wise error rate of 5%. The model was fitted using the proc mixed procedure in the SAS/STAT software packages (Version 9.2, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Flavonoids in Onions. The flavonoid profile of onions was similar to profiles previously identified in southern Italian red onions (*20*) and white onions (*33*) with a high abundance of Q-3,4′-diglu and Q-4′-glu, but traces of quercetin-3,7,4′-triglycoside (Q-3,7,4′-trigly), Q-3-glu, isorhamnetin-3,4′-diglycoside (I-3,4′-digly), and isorhamnetin-4′-glycoside (I-4′-gly) were also detected (**Figure 2**). Q-7,4′-diglu was only present in concentrations below the limit of quantification [6.6 μg quercetin equivalents

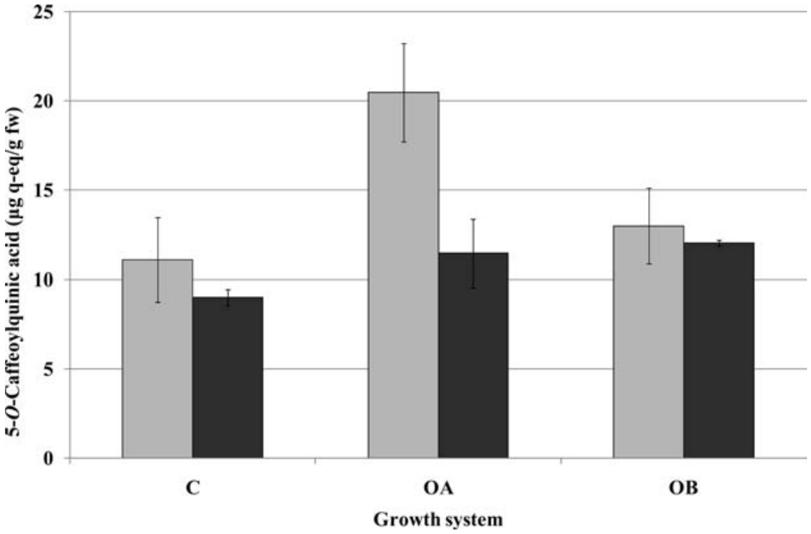


Figure 3. Average concentrations of 5-CQA in carrots (μg q-eq/g fw) in three different growth systems (C, conventional; OA, organic using animal manure; and OB, organic using cover crop) and in two harvest years (gray square, year 1; and black square, year 2). Error bars describe the standard deviation of replicates from the field ($n = 3$).

(q-eq)/g fw (μg q-eq/g fw)]. The average concentrations [±standard deviation (SD), $n = 9$] of Q-3,4′-diglu and Q-4′-glu in year 1 were 262 ± 38 and 208 ± 41 μg q-eq/g fw, respectively, and the concentrations were 9 and 8% lower in year 2, respectively. The concentrations correspond to 530 ± 77 μg Q-3,4′-diglu/g fw and 330 ± 65 μg Q-4′-diglu/g fw, and the concentration levels of flavonoids were thus in accordance with levels previously found in onions (*18*, *34*).

No significant differences ($p > 0.05$) in the content of any of the flavonoids in onions between the three growth systems (C, OA, and OB) were observed. However, a large variation (average RSD for all quantified flavonoids: 16%, $n = 3$) within growth systems was seen in most cases, despite the replicates being located geographically close to one another to minimize the effects of differences in soil fertility and microclimate.

A year-to-year variation was observed for Q-3,7,4′-trigly with a significantly higher content in year 2 ($p < 0.05$). The variation in flavonoid content of onions between growth years has previously been shown by Mogren et al. (*34*, *35*). A significant difference in harvest yield ($p < 0.05$) and onion size ($p < 0.05$) between the two growth years was also found (Table 2 in the Supporting Information). The observed year-to-year variation could, for instance, be related to the weather conditions since year 1 was a growth year with higher temperatures and more precipitation in comparison with year 2.

The use of inorganic nitrogen in conventional agriculture often results in a higher plant nitrogen availability (*5*), harvest yield (*36*), and nitrogen content of the crop (*5*). A higher nitrogen availability has previously caused lower accumulation of flavonoids in tomatoes (*5*). The harvest yield was significantly affected (Table 2 in the Supporting Information) by the different amounts of fertilizer and fertilizer types used for onions in the growth systems in the current study (Table 1 in the Supporting Information). Thus, the application of high amounts of inorganic fertilizer in the growth system C resulted in the highest harvest yield in both years. However, no significant differences between the conventionally and the organically grown onions in the content of nitrogen and flavonoids, size, and dry matter content were found ($p > 0.05$). The nitrogen levels at harvest were within norm values [1.2−1.4% in dry matter (*37*)], but the differences in harvest yield indicated that fertilization of the organic crops during the growth season was suboptimal and below the yield plateau as is often the case in organic farming (*36*).

The results were in accordance with previous onion cultivation studies, where neither the source (organic or inorganic) nor the levels of nitrogen fertilization affected the content of flavonoids (*34*, *38*). In contrast, a higher content of myricetin and quercetin-3-rhamnoside has previously been found in organically as compared to conventionally grown onions, but the significance of the growth system was difficult to determine as the crops were grown at two different farms (*39*).

Phenolic Acids in Carrots. The only polyphenol found in carrots was 5-CQA, which is in accordance with previous observations (*23*, *40*), where it accounted for up to 80% of the total phenolic acid content (*23*). The average concentration (±SD, $n = 9$) of 5-CQA in year 1 was 15 ± 4.8 μg q-eq/g fw, which corresponds to 55 ± 18 μg 5-CQA/g fw (refer to **Figure 3**), and the concentration levels were in agreement with previous levels reported for carrots (*23*, *40*). The concentrations were on average 28% lower in year 2, but no significant year-to-year variation was found across the growth systems ($p > 0.05$).

The very different amounts of fertilizer and fertilizer types applied to the three growth systems resulted in a significantly ($p < 0.05$) higher content of nitrogen in conventional carrots (Table 2 in the Supporting Information). However, no significant differences in the harvest yield, dry matter content, or the concentrations of 5-CQA were found ($p > 0.05$). Similar results have been observed in a previous study, where no difference in the content of 5-CQA was found for potatoes cultivated at different fertilization levels (0−300 kg N/ha) (*41*). Furthermore, a large average variation within growth systems was observed for 5-CQA in carrots (RSD, 12%) (**Figure 3**) as was also seen for flavonoids in onions.

Phenolic Acids in Potatoes. 5-CQA was the most abundant phenolic acid identified in potatoes in agreement with previous studies (*22*, *41*), but also small quantities of 4-*O*-caffeoylquinic acid (4-CQA) were found (**Figure 4**). The average concentrations (±SD, $n = 6$) of 5-CQA in year 1 were 21.7 ± 1.8, 19.6 ± 2.5, and 28.2 ± 1.5 μg q-eq/g fw at the three different locations Foulum, Jyndevad, and Flakkebjerg, respectively, which corresponds to 80.2 ± 6.6, 72.2 ± 9.4, and 104 ± 5.4 μg 5-CQA/g fw. The average concentrations in year 2 were 22% lower at the Foulum location and 17% higher at both the Jyndevad and the Flakkebjerg locations. The concentration levels were in accordance to ref *42* or slightly higher (*41*) than previous concentration levels found in

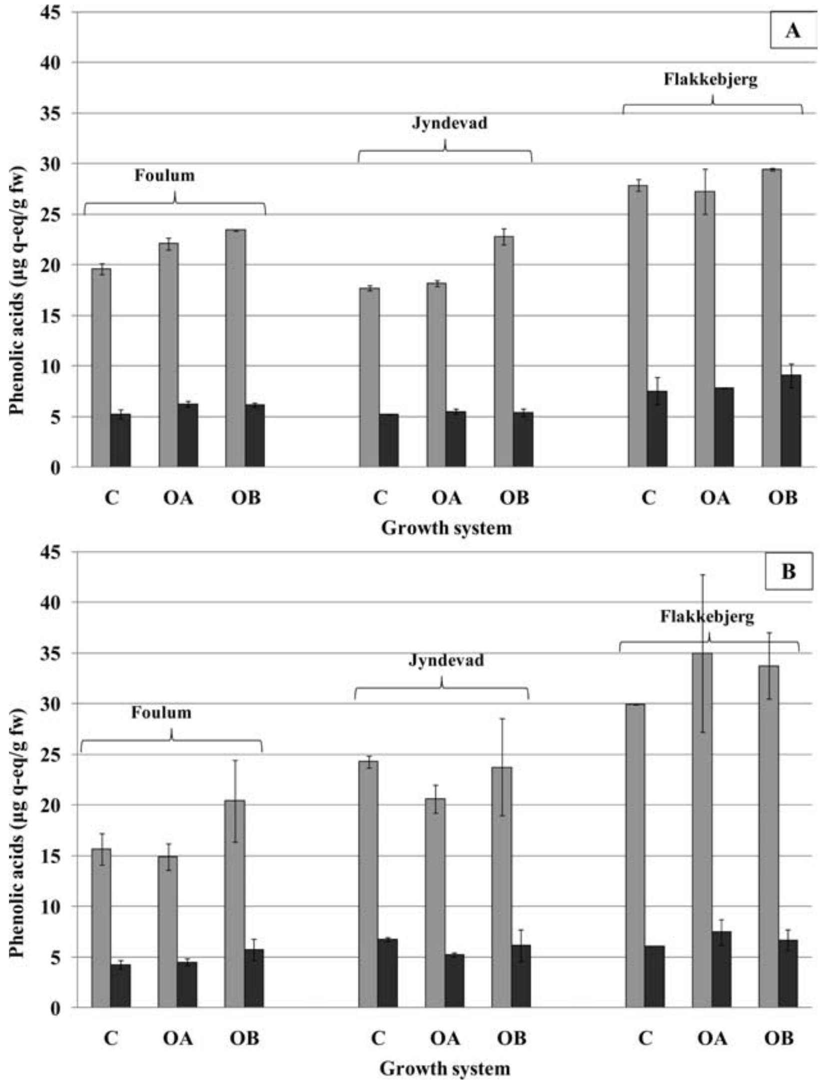


Figure 4. Average concentrations of phenolic acids in potatoes (μg q-eq/g fw) in three different growth systems (C, conventional; OA, organic using animal manure; and OB, organic using cover crops) and in two harvest years (A, year 1; and B, year 2) at three different locations (Foulum, Jyndevad, and Flakkebjerg). Error bars describe the standard deviation of replicates from the field ($n = 2$). Gray square, 5-CQA; and black square, 4-CQA.

potatoes, and the average variation within growth systems (RSD, 7.9%) was lower than previously seen for the carrots (RSD, 12%) and onions (RSD, 16%). The concentration of 4-CQA constituted 20−31% of the concentration of 5-CQA, which was slightly higher than previously found for the sum of 3-*O*-caffeoylquinic acid (3-CQA) and 4-CQA in potatoes of cv. Ranger russet and Norkotah russet (15−20%) (*43*). Traces of CA were also detected in the potatoes, but the concentrations were below the limit of quantification (4 μg q-eq/g fw; data not shown).

A significantly higher content of 5-CQA was found in the OB as compared to the conventional growth system (C) across locations and growth years ($p < 0.05$), despite the fact that no significant differences in nitrogen content were found at harvest (Table 3 in the Supporting Information). This is in agreement with a previous study, where no correlation between nitrogen application rate and production of phenolic acids in potatoes was observed (*41*). Instead, the significantly higher content of 5-CQA in the OB system could be related to a lower potassium fertilization level in comparison with the conventional growth system (Table 1 in the Supporting Information), which has previously been observed for polyphenols in potatoes (*44*, *45*). Hajslova et al. (*46*) also found a higher level of 5-CQA in organically as compared to conventionally grown potatoes. However, the crops were grown at two different farms, which could have induced a variation unrelated to growth system, even though the farms were located relatively close to each other.

The potato sizes and dry matter contents did not differ, but a positive correlation between potato harvest yield and nitrogen application rate was found. Hence, the yield was significantly higher in the conventional as compared to the two organic growth system ($p < 0.05$), C > OA > OB, Table 3 in the Supporting Information), and the nitrogen fertilization was most likely below optimum in the organic growth systems. Furthermore, a significant effect of the geographical location on the content of phenolic acids in potatoes has previously been observed (*46*), but no significant location differences were found in the present study for any of the analyzed parameters. Furthermore, no significant year-to-year variation was seen in the content of phenolic acids in potatoes in contrast to a previous farm study by Hajslova et al. (*46*).

In conclusion, seven flavonoids were detected in onions as well as one and three phenolic acids in carrots and potatoes, respectively. A significant year-to-year variation was observed for Q-3,7,4′-trigly in onions, but no significant differences in the content of flavonoids and phenolic acids between the conventional and the two organic growth systems were found. In the organically grown potatoes fertilized with cover crops, a significantly higher content of 5-CQA was found as compared to the conventional system, which overruled variation caused by geographical location and growth year. On the basis of the present study carried out under well-controlled conditions, it cannot be concluded that organically grown onions, carrots, and potatoes generally have higher contents of health-promoting secondary metabolites in comparison with the conventionally cultivated ones. The ability of crops to synthesize selected secondary metabolites was not systematically affected by the growth system across different growth years and geographical locations.

ABBREVIATIONS USED

C, conventional growth system using pesticides and inorganic fertilizer; CA, caffeic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; fw, fresh weight; I-3,4′-digly, isorhamnetin- 3,4′-diglycoside; I-4′-gly, isorhamnetin-4′-glycoside; MS, mass spectrometry; MS/MS, tandem mass spectrometry; OA, organic growth system using animal manure; OB, organic growth system using cover crops; Q, quercetin; q-eq, quercetin equivalents; Q-3,7,4′-trigly, quercetin-3,7,4′-triglycoside; Q-3,4′-diglu, quercetin-3,4′-diglucoside; Q-7,4′-diglu, quercetin-7,4′-diglucoside; Q-4′-glu, quercetin-4′-glucoside; Q-3-glu, quercetin-3-glucoside; RSD, relative standard deviation; SD, standard deviation.

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Supporting Information Available: Tables of field trial characteristics, onion and carrot harvest yields, potato harvest yields, average temperature and precipitation for periods of potato growth, and planting and harvest time and irrigation of potatoes. This material is available free of charge via the Internet at http://pubs.acs.org.

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