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The effects of plant density and irrigation on phenolic content in cauliflower

Alina Kałużewicz[1], Jolanta Lisiecka[1*], Monika Gąsecka[2], Włodzimierz Krzesiński[1], Tomasz Spiżewski[1], Anna Zaworska[1], Barbara Frąszczak[1]

[1]*Department of Vegetable Crops, Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences, Poznań, Poland*

[2]*Department of Chemistry, Faculty of Wood Technology, Poznań University of Life Sciences, Poznań, Poland*

**Corresponding author: lisica@up.poznan.pl*

Abstract

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This study was conducted to study the influence of plant density and irrigation on the content of phenolic compounds, i.e., phenolic acids and flavonols in cv. 'Sevilla' cauliflower curds. Levels of phenolic acids and flavonols were in the range of 3.0–6.2 mg and 25.4–87.8 mg/100 g of dry weight, respectively, depending on plant density and irrigation. Of the phenolic acids, caffeic acid was detected in the highest amount, followed by *p*-coumaric acid, sinapic acid, gallic acid, and ferulic acid. Of the two flavonols detected, the levels of quercetin were higher than those of kaempferol. The content of the detected phenolic acids (with the exception of ferulic acid) and both flavonols increased with increasing plant density. Furthermore, the concentration of phenolic compounds (with the exception of ferulic acid) was significantly higher under irrigation.

Keywords: *Brassica oleracea* L. var. botrytis; flavonols; phenolic acids

Numerous studies have shown that a diet rich in fruit and vegetables offers several health benefits (Kris-Etherton et al. 2002; Chun et al. 2010). Cauliflower (*Brassica oleracea* L. var. *botrytis*) is a member of the *Brassicaceae* family which is an excellent source of many health-promoting and potentially protective phytochemicals including, among others, phenolic compounds (Podsędek 2007; Cartea et al. 2011). Brassicaceae vegetables are also agriculturally important species that are consumed in high quantities throughout the world (Fahey et al. 2001).

The content of phytochemicals in Brassicaceae vegetables may be influenced by genetics as well as environmental factors (Vallejo et al. 2003; Gliszczyńska-Świgło et al. 2007; Schmidt et al. 2010; Björkman et al. 2011; Fallovo et al. 2011; Pék et al. 2013; Reilly et al. 2014). Agronomic practices can also significantly affect the levels of these compounds (Vallejo et al. 2003; Robbins et al. 2005; Björkman et al. 2011; Fallovo et al. 2011; Pék et al. 2013; Reilly et al. 2014). Several studies have demonstrated that the levels of active compounds in *Brassicaceae* can be increased by the use of appropriate crop management strategies (Schreiner 2005; Krumbein et al. 2010).

A number of biotic and abiotic stresses such as intense light, UV light, water stress, extreme tem-

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peratures and nutrient stress can also affect phytochemical content in plants (Rajashekar et al. 2009; Fortier et al. 2010; Cogo et al. 2011; Cheynier et al. 2013). These factors largely affect the primary and secondary metabolism of brassica plants, resulting in the enhanced production of certain metabolites, e.g., amino acids, sugars, indoles, phenolic compounds and glucosinolates (Jacobo-Velázquez, Cisneros-Zevallos 2012). Phenolic compounds are produced in plants as secondary metabolites (Cartea et al. 2011). Cheynier et al. (2013) reported that plants produce an enormous number of phenolic secondary metabolites, which are of vital importance for their interaction with the environment.

Phenolic compounds, depending on their structure, can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids (Cartea et al. 2011). Brassicaceae are known to contain flavonoids (Soengas et al. 2012), and especially flavonols (Pollastri, Tattini 2011; Agati et al. 2012). Quercetin and kaempferol are the main representatives of the flavonols (Manach et al. 2004).

Many researchers have observed a correlation between climatic conditions (soil moisture and radiation) and the physiological parameters as well as yield of cauliflower plants (Hniličkova, Duffek 2004; Hnilička et al. 2010; Sarkar et al. 2010; Bozkurt et al. 2011; Kashyap 2013; Yanglem, Tumbare 2014). However, reports on the effects of climatic conditions and cultivation practices on phytochemical content in the cauliflower are scarce in the literature (Schreiner 2005; Koudela et al. 2011). Therefore, the aim of the study was to evaluate the influence of plant density and irrigation on the content of phenolic compounds, i.e., phenolic acids and flavonols in cauliflower curds.

MATERIALS AND METHODS

The study was carried out during the 2011, 2012 and 2013 seasons at the 'Marcelin' research station, Poznań University of Life Sciences, Poland. A two-factor experiment was carried out in a randomised block design with four replications. Plants were grown at four different densities (2, 4, 6 and 8 per m^2) and under two water regimes (irrigated and non-irrigated).

Plant material. Cauliflower cv. 'Sevilla' (Bejo Zaden) seeds were germinated in pots (90 cm^3) containing peat substrate for brassica vegetables (Kronen-Klasmann). Seedlings were transplanted to the field on June 29 2011, on the same date in 2012 and on July 3 2013. Each experimental plot contained 30 plants. Depending on plant density, plots were 15, 7.5, 5, or 3.75 m^2 in size. Cauliflower was irrigated using an on-surface system with drip lines (Aqua-traxx), placed next to plant rows. Irrigation was applied when the value of soil water potential at a depth of 25–30 cm was equal to or less than –20 kPa. The value of water potential in the soil was measured using a tensiometer (Irrometer, USA). Each dose of water consisted of 15 mm. The total amount of irrigation water during growth at various plant densities is shown in Table 1. The maximum, minimum and diurnal mean temperatures and the amount of precipitation during the experiment are presented in Table 2. Transplant and harvest dates are shown in Table 3.

Content of phenolic acids and flavonols. The extraction of the phenolic compounds from frozen and homogenated cauliflower curds was carried out in 80% methanol. The content of phenolic compounds was estimated by means of the Folin-Ciocalteu method with some modifications (Dong et al. 2012), using gallic acid as a standard. The quantification of phenolic compounds was determined after alkaline and acid hydrolysis. The methanolic extracts were treated with 2 M NaOH and boiled for 30 minutes. After acidification, phenolic compounds were extracted with diethyl ether. The diethyl fractions were then transferred into vials. Subsequently, extracts were treated with 6 M HCl, boiled for 30 min. and extracted with diethyl ether again. Both diethyl fractions were mixed, dried and redissolved in 1 ml of 80% ethanol before injection into the HPLC column.

The HPLC analysis was performed with a Waters Alliance 2695 Chromatograph with a Waters 2996 Photodiode Array Detector and an RP C-18 column with dimensions of 250 × 4 mm × 5 μm and with

Table 1. Total amount of irrigation water for plant growth at various densities

Plant density (pcs/m^2)	Amount of irrigation water (l/m^2)		
	2011	2012	2013
2	25	53	63
4	83	104	104
6	75	100	109
8	96	119	118

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Table 2. Climatic conditions during the experiment

Year	Mean temperature (°C)			Precipitation (mm)
	average maximum	average minimum	average diurnal	
2011	23.3	13.5	17.7	214
2012	24.5	13.9	19.0	161
2013	21.1	11.1	16.1	151

Table 3. Transplant and harvest dates of cauliflower

Year	Transplant date	Harvest	
		first	last
2011	June 29	September 1	September 13
2012	June 29	August 27	September 3
2013	July 3	September 11	October 19

an acetonitrile: 2% aqueous acetic acid mixture (pH = 2) used as an elution phase (gradient). The concentrations of phenolic compounds were determined by means of an internal standard at wavelengths of 280 nm (gallic, caffeic, *p*-coumaric, sinapic acids) and 320 nm (ferulic acid, quercetin and kaempferol). Compounds were identified by comparison of the retention time of the peak under analysis with the retention time of the standard, and by adding a specific amount of the standard to the analysed samples and repeating the analysis. The limit of detection was 1 μg/g. Gallic, caffeic, *p*-coumaric, ferulic and sinapic acids, quercetin, kaempferol, Folin-Ciocalteu's phenol reagent, sodium carbonate, NaOH and methanol were all purchased from Sigma-Aldrich (St. Louis, USA). Diethyl ether was purchased from Honeywell (Germany). Results were expressed in milligrams per 100 gram of dry weight (mg/100 g d.w.).

Statistical analysis. Statistical analyses of data were carried out using the Stat program. The phenolic content data based on plant density and irrigation were analysed using the *F*-test. Differences between the mean values were estimated with Duncan's test. Significance level was $P = 0.05$.

RESULTS AND DISCUSSION

The results of the present study on the effects of plant density and irrigation on the content of phenolic compounds, i.e., phenolic acids (gallic, caffeic, *p*-coumaric, ferulic, and sinapic) and flavonols (quercetin and kaempferol) in cauliflower curds are shown in Tables 4 and 5. Many researchers have shown that environmental factors and agronomic conditions may influence the content of phytochemicals in cruciferous vegetables (Vallejo et al. 2003; Gliszczyńska-Świgło et al. 2007; Schmidt et al. 2010; Björkman et al. 2011; Fallovo et al. 2011; Pék et al. 2013; Reilly et al. 2014). Our results showed that plant density and irrigation had a significant effect on both phenolic acid and flavonol content in cauliflower curds. Many results suggest that the content of phenolic compounds and flavonoids vary considerably in vegetables (Lin, Tang 2007), even within the same species (Sultana, Anwar 2008; Schmidt et al. 2010).

Phenolic compounds

Phenolic acids. In the present study, the most abundant phenolic acid in cauliflower curds was caffeic acid, followed by *p*-coumaric acid, sinapic acid, gallic acid and ferulic acid (Table 4). These results are in agreement with studies conducted by Pellegrini et al. (2010) and Mazzeo et al. (2011), but not with the study of Sikora et al. (2012). These authors reported *p*-coumaric acid to be most abundant, followed by sinapic acid, caffeic acid and ferulic acid. In a study conducted by Ahmed and Ali (2013) in cauliflower curds, gallic acid was found to be most abundant, followed by *p*-coumaric acid and caffeic acid.

Several publications have reported the phenolic acid contents of cauliflower. The results obtained by the different researchers often vary consider-

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Table 4. Phenolic acid contents in cauliflower curds depending on irrigation and plant density

Irrigation	Plant density (pcs/m^2)				Mean for irrigation
	2	4	6	8	
			Gallic acid (mg/100 g d.w.)		
With irrigation	$3.7^{b*} \pm 0.2$	$3.7^{b} \pm 0.3$	$4.0^{ab} \pm 0.5$	$4.4^{a} \pm 0.7$	$3.9^{A} \pm 0.5$
Without irrigation	$3.3^{b} \pm 0.2$	$3.6^{b} \pm 0.2$	$3.6^{b} \pm 0.3$	$3.6^{b} \pm 0.2$	$3.5^{B} \pm 0.2$
Mean for plant density	$3.5^{B} \pm 0.3$	$3.6^{AB} \pm 0.3$	$3.8^{AB} \pm 0.4$	$4.0^{A} \pm 0.7$	
			Caffeic acid (mg/100 g d.w.)		
With irrigation	$4.8^{d} \pm 0.3$	$5.0^{cd} \pm 0.6$	$5.5^{bc} \pm 0.2$	$6.2^{a} \pm 0.3$	$5.4^{A} \pm 0.6$
Without irrigation	$4.5^{d} \pm 0.2$	$4.8^{d} \pm 0.2$	$5.1^{cd} \pm 0.4$	$5.8^{ab} \pm 0.2$	$5.1^{B} \pm 0.6$
Mean for plant density	$4.6^{C} \pm 0.3$	$4.9^{C} \pm 0.4$	$5.3^{B} \pm 0.4$	$6.0^{A} \pm 0.3$	
			***P*-coumaric acid** (mg/100 g d.w.)		
With irrigation	$4.5^{ab} \pm 0.3$	$4.5^{ab} \pm 0.4$	$4.3^{ab} \pm 0.4$	$4.9^{a} \pm 0.5$	$4.6^{A} \pm 0.4$
Without irrigation	$3.8^{b} \pm 0.4$	$4.0^{b} \pm 0.3$	$4.4^{ab} \pm 0.2$	$4.6^{ab} \pm 0.2$	$4.2^{B} \pm 0.4$
Mean for plant density	$4.2^{B} \pm 0.5$	$4.2^{B} \pm 0.4$	$4.4^{AB} \pm 0.3$	$4.8^{A} \pm 0.4$	
			Ferulic acid (mg/100 g d.w.)		
With irrigation	$3.4^{ab} \pm 0.5$	$3.4^{ab} \pm 0.1$	$3.2^{ab} \pm 0.4$	$3.8^{a} \pm 0.3$	$3.4^{A} \pm 0.4$
Without irrigation	$3.0^{b} \pm 0.3$	$3.3^{ab} \pm 0.4$	$3.5^{ab} \pm 0.2$	$3.3^{ab} \pm 0.4$	$3.3^{A} \pm 0.4$
Mean for plant density	$3.2^{A} \pm 0.4$	$3.4^{A} \pm 0.3$	$3.3^{A} \pm 0.4$	$3.5^{A} \pm 0.4$	
			Sinapic acid (mg/100 g d.w.)		
With irrigation	$3.9^{bc} \pm 0.4$	$.1^{bc} \pm 0.4$	$4.4^{ab} \pm 0.3$	$4.6^{a} \pm 0.4$	$4.3^{A} \pm 0.4$
Without irrigation	$3.5^{c} \pm 0.4$	$3.6^{c} \pm 0.3$	$3.7^{c} \pm 0.4$	$3.9^{bc} \pm 0.3$	$3.7^{B} \pm 0.4$
Mean for plant density	$3.7^{B} \pm 0.4$	$3.8^{B} \pm 0.4$	$4.1^{AB} \pm 0.5$	$4.3^{A} \pm 0.5$	

*mean values and standard deviations, (n = 4); values with different letters are significantly different at P = 0.05; capital letters denote the effect of investigated factors; small letters denote the effect of the interaction of factors

ably. In this study, the content of **gallic acid** ranged from 3.3 to 4.4 mg/100 g of dry weight. Higher values of gallic acid in cauliflower curds were reported by Ahmed and Ali (2013). Levels of **caffeic acid** varied between 4.5 and 6.2 mg/100 g d.w. These data are in agreement with Sikora et al. (2012). In the studies conducted by Mazzeo et al. (2011) and Pellegrini et al. (2010) caffeic acid content was almost 4–fold higher. On the other hand, Ahmed and Ali (2013) found 8–fold lower caffeic acid content. The differences reported by these researchers could be due to the extraction methods used (Jahangiri et al. 2011). The ***p*-coumaric acid** level (3.8–4.9 mg/100 g d.w.) was close to the value observed by Pellegrini et al. (2010) and approximately 2–fold lower than those detected by Ahmed and Ali (2013), Sikora et al. (2012) and Mazzeo et al. (2011). The **ferulic acid** content (3.0–3.8 mg/100 g d.w.) was comparable to the value reported by Sikora et al. (2012). A lower content of ferulic acid in cauliflower was shown by Pellegrini et al. (2010) and Mazzeo et al. (2011). **Sinapic acid** was present at a concentration of 3.5–4.6 mg/100 g d.w. A similar observation was made by Pellegrini et al. (2010). The amount of sinapic acid was relatively low compared with that reported by Mazzeo et al. (2011) and Sikora et al. (2012).

Quercetin and kaempferol. In the review of Podsędek (2007), it is pointed out that quercetin is the predominant flavonol in *Brassica* vegetables. However, data in the literature regarding the content of flavonols in cauliflower are often contradictory. In the present study, quercetin was the predominant flavonol in cauliflower curds (Table 5). This is in agreement with data reported by Puupponen-Pimiä et al. (2003), Bahorun et al. (2004), Ahmed and Ali (2013) and Dos Reis et al. (2015). Other authors obtained contradictory results (Pellegrini et al. 2010; Mazzeo et al. 2011; Sikora et al. 2012). In a study conducted by Sultana and Anwar (2008) quercetin was not detected at all.

The quercetin levels in cauliflower curds ranged between 28.6 and 87.8 mg/100 g of d.w. Much low-

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Table 5. The contents of quercetin and kaempferol in cauliflower curds depending on irrigation and plant density

Irrigation	Plant density (pcs/m^2)				Mean for irrigation
	2	4	6	8	
	Quercetin (mg/100 g d.w.)				
With irrigation	39.2^{d*} ± 3.7	66.1^{c} ± 5.5	76.3^{b} ± 8.0	87.8^{a} ± 5.4	67.4^{A} ± 19.3
Without irrigation	28.6^{e} ± 3.2	38.4^{d} ± 3.9	39.6^{d} ± 4.7	78.4^{b} ± 8.6	46.2^{B} ± 20.3
Mean for plant density	33.9^{C} ± 6.5	52.2^{B} ± 15.5	58.0^{B} ± 20.6	83.1^{A} ± 8.3	
	Kaempferol (mg/100 g d.w.)				
With irrigation	26.1^{e} ± 2.3	31.6^{e} ± 2.3	43.6^{c} ± 1.4	71.4^{a} ± 6.7	43.2^{A} ± 18.4
Without irrigation	25.4^{e} ± 3.8	31.2^{e} ± 3.2	37.8^{d} ± 5	61.9^{b} ± 5.9	39.1^{B} ± 14.9
Mean for plant density	25.8^{D} ± 2.9	31.4^{C} ± 2.6	40.7^{B} ± 4.6	66.7^{A} ± 7.7	

*mean values and standard deviations, (n = 4); values with different letters are significantly different at P = 0.05; capital letters denote the effect of investigated factors; small letters denote the effect of the interaction of factors

er concentrations of this flavonol were confirmed in other experiments (Bahorun et al 2004; Pellegrini et al. 2010; Mazzeo et al. 2011; Sikora et al. 2012). In contrast, Ahmed and Ali (2013) reported very high levels of quercetin.

Levels of kaempferol were in the range of 25.4–71.4 mg/g d.w. The values obtained were similar to those measured by Ahmed and Ali (2013). However, other researchers observed significantly lower kaempferol content (Bahorun et al 2004; Pellegrini et al. 2010; Mazzeo et al. 2011; Sikora et al. 2012). According to Kaur et al. (2007) and Luthria (2009), the differences reported in the levels of phenolic compounds could be due to the methods used.

Effects of plant density and irrigation on phenolic compounds

Studies on plant density and irrigation in cauliflower cultivation are usually concerned with the impact on yield quantity and quality (Stirling, Lancaster 2005; Rahman et al. 2007; Sarkar et al. 2010; Bozkurt et al. 2011; Yanglem, Tumbare 2014). Reports on the relationship between cultivation practices and phytochemical content in cauliflower are scarce in the literature (Schreiner 2005; Koudela et al. 2011).

The effects of plant density on secondary compounds are the result of a combined effect of all factors involved in plant competition, such as decreased availability of light, nutrients and water (Björkman et al. 2011). Plants densities of 16,000 to 40,000 per hectare are generally recommended for cauliflower. In this study, density ranged from 2 to 8 plants per square meter (i.e., 20,000–80,000 per hectare). Levels of phenolic acids and flavonols were in the range of 3.0–6.2 mg and 25.4–87.8 mg/100 g d.w., respectively, depending on plant density and irrigation. The content of phenolic compounds (with the exception of ferulic acid) increased with increasing plant density. Furthermore, the concentration of these compounds (with the exception of ferulic acid) was significantly higher under irrigation.

In the present study, plant density influenced the content of phenolic acids and flavonols in cauliflower curds. According to Ghasemzadeh et al. (2010), phenolic content in shaded plants can be affected by the lower temperatures under these conditions. An increase of total phenolic content with increasing plant density was observed by Lombardo et al. (2009) in globe artichokes and by Danesi et al. (2014) in palm tree kale. In the study conducted by Riad et al. (2009) planting density had no significant effect on the content of phenolic compounds in cabbage. The same results were obtained by Reilly et al. (2014) in broccoli.

Many studies have proven that environmental factors can profoundly influence phenolic content in plants (Rajashekar et al. 2009). However, the results are often conflicting. To date, no studies on the climatic or agronomic basis of the content of phenolic compounds have been reported for cauliflower plants.

Drought is likely to contribute to the accumulation of phenolic compounds in plants. A positive correlation between the total phenolic content and water deficit was observed in lettuce (Oh et al. 2010) and basil (Khan et al. 2012). In studies

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conducted by Pék et al. (2014) and Helyes et al. (2014) irrigation decreased polyphenol content in the tomato. However, Sánchez-Rodríguez et al. (2012) obtained contradictory results. In the present study, cauliflower under irrigation contained more phenolic compounds in comparison to non-irrigated plants.

Studies on broccoli have shown that drought stress can lead to decreased levels of flavonoids (Fortier et al. 2010) and phenolic compounds (Robbins et al. 2005, Cogo et al. 2011). Mao et al. (2004) found that drought stress reduced the content of caffeic acid in sweet potato. The work of Khosh-Khui et al. (2012) showed that long-term drought caused a reduction in total phenolic content in thyme. On the other hand, Pék et al. (2013) did not observed any detectable pattern of the effects of irrigation on total phenolic production in broccoli. Similarly, Rajabbeigi et al. (2013) found that quercetin content in lettuce was not significantly affected by drought stress.

In addition, Lin et al. (2006) reported that flooding and drought stresses increased polyphenol and flavonoid content in sweet potato leaves, while Khan et al. (2011) observed increased kaempferol content in broccoli under flooding and drought stresses.

Our results revealed that phenolic content in cauliflower curds changed depending on varying plant density. Higher plant density led to increased concentrations of phenolic compounds. The present study also demonstrates that irrigation can have a positive effect on phenolic content in cauliflower curds.

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