Effect of sulphur fertilization on contents of phenolic and sulphuric compounds in onion (*Allium cepa* L.)

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Summary

This study evaluated the effects of different levels of sulphur fertilization on the quality of onions (cultivars Bingo, Boston, Red Matte, Karmen, White Solid and Diamant). The study was conducted as a field experiment. Sulphur was added at doses of 0, 30.00, 40.00 and 50.00 kg·ha^{-1}, respectively. Contents of total sulphur, total polyphenols and quercetin together with their antioxidant activity were determined, as well as possible correlations between the qualitative parameters. Sulphur fertilization increased ($P < 0.05$) the sulphur, quercetin and total polyphenols contents and the value of antioxidant activity in onion. Statistically significant highest contents of polyphenols as well as quercetin were recorded in red cultivars at a sulphur dose of 40.00 kg·ha^{-1}. The highest contents of polyphenols were recorded in yellow cultivars at a sulphur dose of 50.00 kg·ha^{-1}. White cultivars contained the lowest content of polyphenols as well as of quercetin, but the highest values were recorded at the sulphur dose of 40.00 kg·ha^{-1}. The highest values of antioxidant activity were achieved in all cultivars at the sulphur dose of 40.00 kg·ha^{-1}, except for cultivars Boston and Diamant.

Keywords

antioxidant activity; phenolic compounds; total sulphur

Onion (*Allium cepa* L.) is a rich source of biologically valuable substances as well as polyphenols with a positive effect on human health, which induced the interest of scientists for their potential nutritional and therapeutic effects. Many scientific papers suggest that consumption of onion has a wide range of health-promoting effects, in particular antioxidant effects [1–3]. Onions contain bioactive compounds belonging to two main chemical groups, namely, phenolic compounds and alk(en)yl cysteine sulphoxides (ACSO), as well as their degradation products, namely, monosulphide, disulphide, trisulphide, thiosulfinates and thiosulphonates. Some sulphur-containing compounds can be scavengers of reactive oxygen species. Phenolic compounds can play an important role in adsorbing and neutralizing free radicals, e. g. superoxide anion $-O_2^-$, hydroxyl radical $-HO^\bullet$ and hydroperoxyl radical $-HO_2^\bullet$, which may cause cancer and several neurodegenerative disorders [4]. Phenolic compounds are an integral part of the human diet, fulfill an important role and are significantly concerned with organoleptic characteristics (flavanons with flavour, anthocyanidins with colour) [5]. Onions are rich mainly in flavonoids, quercetin compounds being major flavonoids in onions [6, 7]. In onion, quercetin may occur in the free form or in the form of glycosides (quercetin 3-glucoside, quercetin 4-glucoside, quercetin 7,4-diglucoside) [8]. Concerning anthocyanins, onion contains cyanidin-3-glucoside and cyanidin 3-rutinoside [9]. El-Hadidy et al. [10] reported that the other major flavonoids in onion are myricetin, quercetin, rutin and kaempherol conjugates. Polyphenols with quercetin are phytochemicals that are responsible for the antioxidant activity. On the other hand, not all polyphenolic compounds must show antioxidant activity. The activity of antioxidants is dependent on complex factors such as the nature of the antioxidants, properties of the oxidizing substrate and stage of oxidation [11]. ACSO are organosulphur

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substances, which are released through the activity of enzyme allinase and cause the typical flavour and odour of *Allium* vegetables [12]. Fig. 1 represents the formation of various secondary sulphur compounds, which occur in onion in trace amounts [13]. Qualitative and quantitative composition of these compounds depends on plant variety, environmental and climatic factors [14–16]. Liguori et al. [17] reported that the period of growth influences some of the sensory attributes of onion. Fertilization plays an important role in enhancing crop quality, the type and value of fertilizer as well as the level of application directly influencing plant physiology and the biosynthesis of secondary compounds in plants [18, 19]. In addition to basic elements such as nitrogen, phosphorus and potassium, sulphur is essential for the growth and development of onion bulbs.

Sulphur requirement of crops is almost similar to that of phosphorus [20]. The optimal dose of sulphur is important for onions and other species of the genus *Allium* to achieve higher yield and enhanced product quality [21]. Inorganic sulphate in the form of $(SO_4)^{2-}$ enters plants and there it is reduced to sulphide S^{2-}. This form is incorporated into sulphur-containing amino acids and onion flavour precursors ACSOs [22]. Kubec and Dadáková [23] also reported that cysteine sulfoxide content of *Allium* species is an important quality parameter as it determines the taste and sharpness. Sulphur compounds can act as electron donors and react with free radicals, stopping oxidative reactions in food products [24]. Earlier studies [25, 26] indicated that sulphur fertilization during cultivation of onion is important for the formation of bioactive compounds, in particular regarding the sulphur-containing compounds.

The objective of this study was to determine the influence of sulphur fertilization on the total sulphur content, on the total polyphenols content as well as on antioxidant activity in onion.

MATERIALS AND METHODS

Small plot experiment was carried out in the demonstration garden of Slovak University of Agriculture in Nitra, Slovakia (48°18'N, 18°05'E) in years 2015–2016. Nitra belongs to the hot and dry climate zone, average annual air temperature is 9.5 °C, annual rainfall is 539 mm. The soil type of locality Nitra is brown soils.

Plant material

Samples of plant material were collected at full maturity stages from the area of Nitra. The investigated onion cultivars (Bingo, Boston, Red Matte, Karmen, White Solid and Diamant) were conventionally cultivated in the same locality under the same conditions.

In the soil sample, the exchangeable reaction pH/KCl (replaceable acidity at which solution of neutral salt, KCl, extrudes hydrogen ions and other ions from sorption complex of soil) and the contents of available nutrients (potassium, magnesium, phosphorus) were determined.

pH was determined using a pH meter Metrohm 691 (Metrohm, Herisau, Switzerland).

The soil in which the onions were grown was acid to neutral (pH/KCl being 6.58–7.16), with a high level of humus (3.1–4.0 %), potassium (325.57–340.01 mg·kg^{-1}), a very high content of phosphorus (109.83–138.55 mg·kg^{-1}), magnesium (265.24–350.89 mg·kg^{-1}), and a high level of total

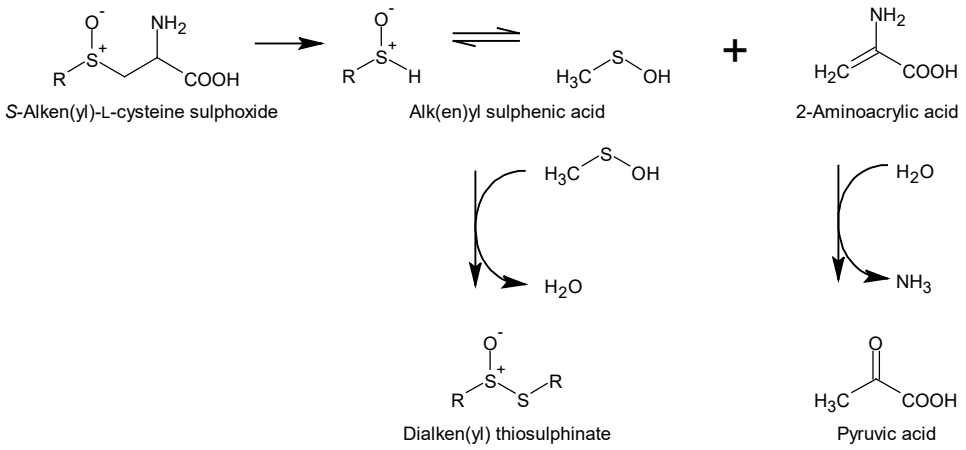

Fig. 1. Formation of flavour compounds in onion [13].

nitrogen (0.2–0.3 %). Contents of nutrients were determined by Mehlich III method [27]. The content of nitrogen in soil was determined by Kjeldahl method [28]. Phosphorus was determined spectrophotometrically [29] at a wavelength of 666 nm using spectrophotometer UV-VIS 1800 (Shimadzu, Kyoto, Japan).

Experimental treatments and design

Three different levels of sulphur (30.00 $kg \cdot ha^{-1}$, 40.00 $kg \cdot ha^{-1}$, 50.00 $kg \cdot ha^{-1}$) and control group without sulphur were evaluated to determine the effective dose to optimize total polyphenol content (*TPC*), content of quercetin (*QC*) and total sulphur content (*TSC*) in onion. The experiment was laid out in a randomized complete block design with four replications. Unit plot size was 2 m × 1 m. The fertilizer (powdered form of K_2SO_4) was incorporated into the soil a week before planting.

Collection of samples and extraction

The samples of 6 varieties of onion were taken at the stage of full maturity once in 4 repetitions (in mentioned locality). From the same places, from the arable layer (0–20 cm), soil samples were taken using a pedological probe GeoSampler (Fisher Slovakia, Levoča, Slovakia). The samples were processed by the modified method according to Azmir et al. [30]. The edible part of the onion bulbs without skin was freeze-dried and then analysed. Samples were pulverized in a mill (Grindomix 200 GD; Retsch, Haan, Germany) and then stored in cleaned polyethylene bottles until subsequent pre-analytical operations. Methanol extracts were prepared by adding 100 ml of 80% methanol to 10 g of a milled sample. The extraction was carried out in the Twisselmann apparatus for 12 h. Samples were then filtered through filter paper (130 $g \cdot m^{-1}$; Filtrak, Thermalbad Wiesenbad, Germany) and kept at 8 °C until further analysis.

Chemicals

High-purity analytical reagents were used for all operations. Ammonium nitrate, hydrochloric acid, nitric acid, Folin-Ciocalteu reagent and gallic acid were purchased from Merck (Darmstadt, Germany). Sodium carbonate, methanol and 2,2-diphenyl-1-picrylhydrazyl radical (•DPPH) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Total polyphenol content

The total polyphenol content (*TPC*) was assessed by the method of Lachman et al. [31] employing the reduction of a phosphowolframate-phosphomolybdate complex to blue oxide products by phenolic compounds. Briefly, an aliquot of the extract, blank or standard was placed in a 50 ml flask, where the Folin-Ciocalteu reagent (2.5 ml) was added and the mixture was allowed to react for 3 min under continuous stirring. Then, a solution of sodium carbonate (7.5 ml) was added and mixed thoroughly. The volume was then made up to 50 ml with distilled water and left to stand at room temperature for 2 h. The absorbance was measured at 765 nm using Shimadzu UV-VIS 1800 spectrophotometer (Shimadzu). Results were expressed as milligrams of gallic acid equivalents (GAE) per kilogram fresh weight (FW).

Determination of quercetin by HPLC

An amount of 10 g of onion slices was homogenized with 40 ml (62.5%) methanol Chromasolv for HPLC grade (Sigma-Aldrich). Then, 10 ml of 6 $mol \cdot l^{-1}$ HCl (Merck) was carefully added to give a total volume of 50 ml. The extraction mixture was thereafter heated to 90 °C in a thermostat for 2 h. After cooling, the homogenate was filtered through "red" filter paper (84 $g.m^{-2}$, Grade 1292, diameter 125 mm; Munktell & Filtrak, Bärenstein, Germany). Subsequently, the extract was filtered through a polytetrafluoroethylene membrane filter (pore size 0.45 μm; Frisenette, Knebel, Denmark). The filtrate was injected into a HPLC system that consisted of an Alliance 2695 chromatograph (Waters, Milford, Connecticut, USA), a LiChroCART Purospher RP C18 column (250 mm × 4 mm; particle size 5 μm; Merck), and a DAD 2996 UV detector (Waters). The column temperature was 30 °C. Gradient elution at a flow rate of 1 $ml \cdot min^{-1}$ of the mobile phase was used. Solvent A was acetonitrile (gradient grade) and solvent B was water solution of 0.1% (v/v) phosphoric acid with pH 2.6. The solvent gradient was as follows: the concentration of solvent A was 40 % for the first 3 min, 5 % for the next 5 min, and 5 % for an additional 2 min. The concentration of solvent B was 60 % for the first 3 min, 95 % for the next 5 min, and 95 % for an additional 2 min. The presence of quercetin was detected at 365 nm. The content of quercetin (*QC*) was calculated on the basis of the calibration curve of quercetin standards (Acros Organics, Waltham, Massachusetts, USA) prepared in methanol (gradient elution grade; Sigma-Aldrich). Results were expressed as milligrams per kilogram FW.

Determination of total sulphur content

Total sulphur content (*TSC*) was determined in freeze-dried samples using elemental analysis by Vario Macro Cube (Elementar Analysensysteme,

Hanau, Germany) and expressed as gram per kilogram of dry matter (DM).

Antioxidant activity

Antioxidant activity (*AA*) was measured by method BRAND-WILLIAMS et al. [32] using •DPPH. To obtain a stock solution, 0.025 g of •DPPH was dissolved in 100 ml of methanol and kept in a cool and dark place. For the analysis, 3.9 ml of the •DPPH working solution was added to a cuvette and the value of absorbance (A_0), which corresponded to the initial concentration of •DPPH solution, was recorded. Absorbance was read at 515 nm with a UV-VIS 1800 spectrophotometer (Shimadzu). Subsequently, 0.1 ml of the extract was added and absorbance was measured after 10 min (A_t). Antioxidants present in the methanol extract of the sample reduced •DPPH and bleached the colour of the solution proportionally to the antioxidant concentration. The percentage of inhibition reflects how antioxidant compound is able to quench •DPPH radical at the given time. The percentage of •DPPH inhibition (*I*) was calculated according to the following equation:

$$I = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

where A_0 is initial absorbance and A_t is absorbance after 10 min.

Statistical analysis

Results were statistically evaluated by analysis of variance, by one-way and multifactor ANOVA (Multiple Range Tests), data being considered significantly different when $P < 0.05$, using statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, The Plains, Virginia, USA). Regression and correlation analyses were done using Excel (Microsoft, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Results on *TPC, QC, TSC* and *AA* for onion cultivated with different levels of sulphur fertilization are shown in Tab. 1. The analysed onion cultivars differed in *TPC*. Statistically significant highest contents of polyphenols ($P < 0.05$) were recorded in red cultivars (Red Matte, Karmen). The values ranged from 1033.74 mg·kg^{-1} to 1493.76 mg·kg^{-1} (expressed as GAE). In the case of both cultivars, the highest values were observed at the level of sulphur fertilization of 40.00 kg·ha^{-1}. In case of yellow cultivars (Bingo, Boston), the values of *TPC* ranged from 375.41 mg·kg^{-1} to 637.63 mg·kg^{-1} (expressed as GAE), and the highest values were observed at the level of sulphur fertilization of 50.00 kg·ha^{-1}. The white cultivars (White Solid, Diamant) contained the least polyphenols (244.37 mg·kg^{-1} and 184.98 mg·kg^{-1} expressed as GAE, respectively) and the highest values were observed, similar to the case of red cultivars, at the level of sulphur fertilization of 40.00 kg·ha^{-1}. Our results are in agreement with previous studies, which reported a significantly higher *TPC* content in red cultivars of onions [33, 34].

The same trend was observed for *QC*, where the analysed onion cultivars showed significant differences. Based on the determined contents of quercetin, onion cultivars were in the order Red Matte > Karmen > Bingo > Boston > White Solid > Diamant. Fig. 2 shows results of chromatographic determination of total quercetin in the onion samples. The highest contents of quercetin in all evaluated cultivars were achieved at the sulphur fertilization level of 40.00 kg·ha^{-1}, except for cultivar Boston with the highest *QC* at the level of sulphur fertilization of 50.00 kg·ha^{-1}.

Several studies [35–37] showed that sulphur fertilization has a positive impact on yield performance of onion and also improves its quality. Our results are in correspondence with the findings of IMEN et al. [16] who suggested that sulphur fertilization increased the content of polyphenols. Antioxidant properties of sulphur are described in several studies [38, 39]. In this study, we observed the effect of sulphur fertilization on the antioxidant activity of onion cultivars (Tab. 1). Both in case of polyphenol content and in this case, the highest values of *AA* were determined in red cultivars (38.5–43.6%), followed by yellow cultivars (21.0–28.4%) and the lowest values of *AA* were observed in white onion cultivars (6.5–10.6%). Statistically significant highest values were recorded in every cultivar at the level of sulphur fertilization of 40.00 kg·ha^{-1}, except for cultivars Boston and Diamant, where the highest values were recorded at the level of sulphur fertilization of 50.00 kg·ha^{-1}.

When evaluating *TSC*, it can be concluded that the higher level of sulphur fertilization, the higher content of sulphur in onion ($P = 6.79 \times 10^{-6}$, $R = 0.44$). Similarly SINGH et al. [40] reported that sulphur fertilization progressively increased the uptake of sulphur in vegetable crops. THANGASAMY et al. [41] reported that sulphur uptake in bulbs was increased with increasing the sulphur fertilization levels up to 50.00 kg·ha^{-1}. Highest levels of sulphur were accumulated by white cultivars, the values ranging from 4.20 g·kg^{-1}

to 5.77 g·kg^{-1} DM, followed by yellow cultivars with the content from 3.80 g·kg^{-1} to 5.50 g·kg^{-1} DM, and the lowest content was accumulated by red cultivars (2.50–3.70 g·kg^{-1} DM). These results are in agreement with studies of JURGIEL-MALECKA et al. [42], who also found the lowest content of sulphur in a red cultivar. KOH and SURH [37] indicated 2.70 g·kg^{-1} and 4.30 g·kg^{-1} DM sulphur in two cultivars of onion. MISHU et al. [36] found the highest content of sulphur in onion using sulphur fertilization of 40.00 kg·ha^{-1}, whereas higher doses (60.00 kg·ha^{-1} and 80.00 kg·ha^{-1}) caused a slight decrease of sulphur content in onion. Our results correspond with findings of SINGH [40] who indicated that sulphur fertilization of 40.00 kg·ha^{-1} was the most appropriate for growing high quality onion. DE SOUZA et al. [26] indicated the optimal sulphur fertilization dose of 45.00 kg·ha^{-1}. On the other hand, TRIPATHY et al. [43] recommended, for higher bulb yield and quality, the sulphur fertilization dose of 30.00 kg·ha^{-1}.

The effect of sulphur fertilization dose on the quality of onion are discussed in other studies [44, 45]. The authors are not unified in their views and results about the sulphur-containing fertilizers. Their results indicate that effect of sulphur fertilization on total polyphenols, antioxidant activity and sulphur content in onion is affected by many factors such as cultivar, growth conditions, sulphur fertilization dose, type of fertilization (organic or inorganic form), soil character and climatic factors.

In this study, relations among *TPC, QC* and *AA* were evaluated. We found positive correlation

Tab. 1. Effect of different levels of sulphur fertilization on parameters of onion cultivars.

Cultivar	Suplhur [kg·ha^{-1}]	*TPC* [mg·kg^{-1}]	*QC* [mg·kg^{-1}]	*TSC* [g·kg^{-1}]	*AA* [%]
Bingo	0 (control)	507.57±29.65^{a}	194.00±21.02^{a}	4.08±0.09^{a}	21.0±0.4^{a}
	30	537.91±34.49^{a}	200.84±22.09^{a}	4.17±0.16^{a}	22.7±0.5^{b}
	40	584.24±16.29^{b}	258.37±16.17^{b}	4.50±0.15^{b}	28.4±0.5^{d}
	50	637.63±34.50^{c}	221.51±1.80^{a}	5.33±0.17^{c}	25.3±0.8^{c}
Boston	0 (control)	375.41±16.18^{a}	172.64±4.37^{a}	3.80±1.30^{a}	23.1±1.3^{a}
	30	461.51±22.39^{b}	176.82±21.42^{b}	4.00±0.12^{b}	23.8±0.2^{a}
	40	506.35±23.23^{c}	184.27±5.99^{c}	4.05±0.05^{bc}	25.8±0.8^{b}
	50	570.21±17.02^{d}	188.63±4.86^{d}	4.20±0.04^{c}	26.7±0.4^{b}
Red Matte	0 (control)	1351.84±36.83^{a}	234.91±10.78^{a}	2.67±0.33^{a}	39.5±0.3^{a}
	30	1312.11±29.74^{a}	269.88±21.74^{b}	3.11±0.11^{b}	40.5±1.2^{b}
	40	1493.76±59.87^{b}	306.72±16.34^{c}	3.34±0.26^{b}	43.6±0.5^{c}
	50	1379.26±86.94^{a}	283.49±31.33^{bc}	3.71±0.20^{c}	43.0±0.2^{c}
Karmen	0 (control)	1033.74±36.85^{a}	203.97±8.41^{a}	2.55±0.17^{a}	38.5±0.6^{a}
	30	1120.99±52.37^{b}	218.55±7.09^{b}	2.89±0.19^{b}	41.9±0.7^{b}
	40	1312.45±51.68^{c}	231.62±4.14^{c}	3.16±0.90^{c}	42.9±0.3^{c}
	50	1248.57±15.49^{c}	223.87±2.22^{bc}	3.60±0.10^{d}	41.6±0.3^{b}
White Solid	0 (control)	205.24±5.73^{a}	4.92±0.30^{a}	4.20±0.05^{a}	8.2±0.5^{a}
	30	215.92±1.45^{ab}	5.29±0.21^{a}	4.50±0.19^{b}	8.7±0.7^{a}
	40	244.37±3.86^{c}	6.96±0.27^{c}	4.90±0.08^{c}	10.6±0.6^{c}
	50	234.77±32.85^{bc}	6.02±0.40^{b}	5.77±0.09^{d}	9.8±0.2^{b}
Diamant	0 (control)	150.61±3.17^{a}	2.87±0.07^{a}	4.13±0.05^{a}	6.5±0.1^{a}
	30	162.38±3.82^{b}	3.13±0.02^{a}	4.37±0.05^{b}	7.1±0.2^{b}
	40	184.98±4.50^{d}	7.64±0.40^{c}	4.84±0.08^{c}	7.3±0.1^{c}
	50	170.34±5.00^{c}	4.17±0.34^{b}	5.77±0.37^{d}	7.7±0.1^{d}

The results are expressed as mean ± standard deviation. Different superscript letters in a column mean significant difference ($P < 0.05$).

TPC – total polyphenol content (expressed as milligrams of gallic acid equivalents per kilogram fresh weight), *QC* – quercetin content (expressed as milligrams per kilogram fresh weight), *TSC* - total sulphur content (expressed as gram per kilogram of dry matter), *AA* – antioxidant activity (expressed as percent of inhibition).

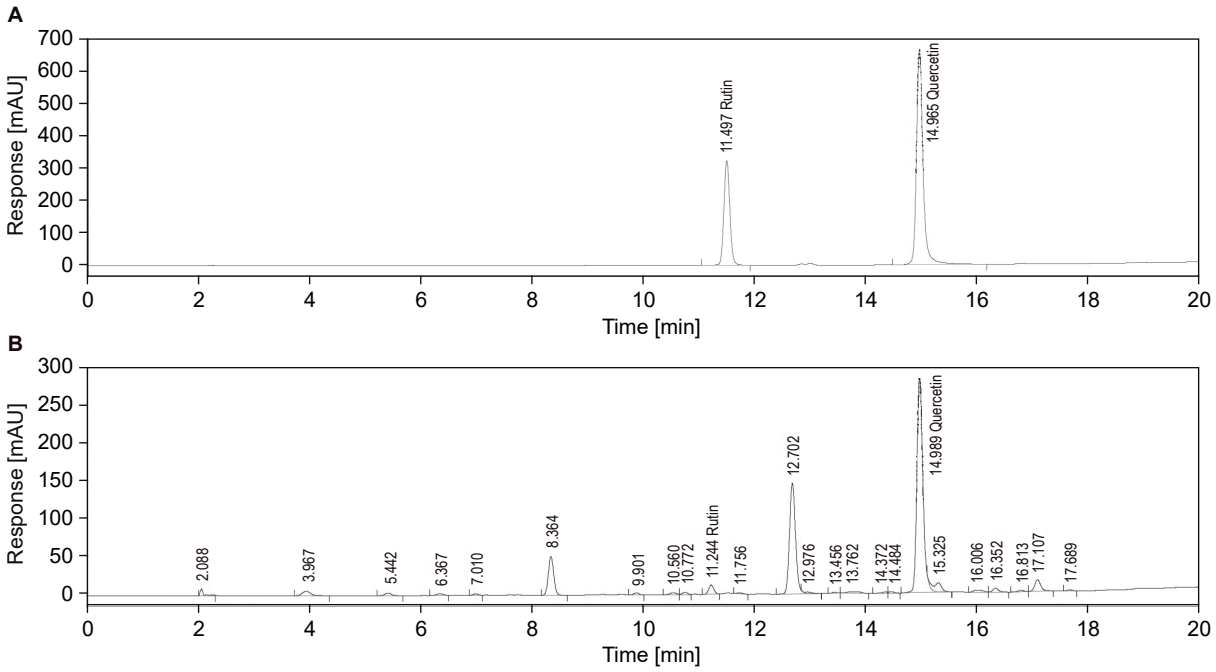

Fig. 2. Chromatogram of quercetin.

A – standard, B – extract from an onion cultivar.

between *TPC* and *AA* in all cultivars, the regression coefficient being statistically significant. For individual cultivars (Bingo, Boston, Red Matte, Karmen, White Solid, Diamant), *P* values were as follows: 3.91×10^{-3}; 2.00×10^{-5}; 2.76×10^{-3}; 3.27×10^{-5}; 1.24×10^{-3}; 1.63×10^{-3}. The results corresponded with those presented by Číž et al. [46] and Musilova et al. [47], who indicated correlations between *TPC* and *AA*. Cheng et al. [48] also referred that higher *TPC* were associated with higher *AA*.

The major flavonoid in onion, quercetin, has potential antioxidant properties. In all cultivars, except cultivar Diamant, a strong positive correlation between *QC* and *AA* was determined. For individual cultivars (Bingo, Boston, Red Matte, Karmen, White Solid), *P* values were as follows: 5.54×10^{-6}; 9.55×10^{-7}; 2.18×10^{-5}; 5.11×10^{-8}; 2.13×10^{-8}. Our results are in agreement with other studies [49, 50], which indicated very high correlation between flavonoids and *AA* in onion.

Our results provide novel information on the effects of sulphur fertilization on *TPC* and *AA*. Two scientific studies [23, 26] dealt with the effect of sulphur-containing fertilizers on the quantity and quality of the onion, but the effects of sulphur fertilization on *TPC* and *AA* were less studied. Antioxidant properties of some sulphur-containing compounds are described by Atmaca et al. [51].

A poor relationship was determined between *TSC* and *AA* in all onion cultivars ($P < 0.05$), except for the cultivar Diamant, for which a high positive correlation was observed. For individual cultivars (Bingo, Boston, Red Matte, Karmen, White Solid, Diamant), *P* values were as follows: 4.64×10^{-2}; 3.77×10^{-5}; 2.11×10^{-4}; 3.99×10^{-3}; 6.37×10^{-3}; 4.49×10^{-7}. Similar results were presented by De-Pascale et al. [52], who reported a positive effect of sulphur fertilization on the antioxidant activity.

CONCLUSIONS

Onions have been extensively studied for their content of bioactive compounds with protective effects against civilization diseases. It is well known that a number of factors, such as agrochemical, climatic, use of fertilizers, storage conditions and cultivar, have an impact on the content of health-beneficial substances in onion. The present study provided data on the effect of different sulphur fertilization doses on bioactive substances and *AA* in several onion cultivars. Our results indicated that sulphur fertilization of 40.00 kg·ha^{-1} led to, in

all cultivars, higher *TPC* and *QC*, and in all cultivars except for Bingo and Boston, higher *AA* (in the latter, the highest *TPC* was reached at sulphur fertilization of 50.00 kg·ha^{-1}). It can be also concluded that onion, mainly red cultivars, are a rich source of polyphenols, which can be used as natural resources for production of functional food.

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