Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties

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ABSTRACT: Polyphenolic antioxidant compounds were studied in three onion varieties (red – cv. Karmen, yellow – cv. Všetana and white – cv. Ala) regarding their total content and qualitative composition of flavonoid complex. The effects of temperature and storage period during onion storage were also studied. The total content of polyphenols was estimated spectrophotometrically with Folin-Ciocalteau's phenolic reagent and individual flavonoid components by HPLC method using Waters™ chromatograph on Watrex 250 × 4 mm Sepharon SGX C18 7µm column with gradient elution. The highest amount of polyphenolic compounds was found in red variety Karmen (108,300 mg/kg DM), the lowest amount in white variety Ala (26,445 mg/kg DM) while the yellow variety Všetana had average content of total polyphenolic compounds 65,210 mg/kg DM. An increase in total polyphenols during storage was found in red and yellow varieties, esp. during storage at a laboratory temperature (22°C). Among the flavonoid and polyphenolic compounds as major constituents spiraeoside (quercetin-4´-*O*-β-*D*-glucoside), rutin and quercetin and three other not identified compounds were found. Significant varietal differences were found. The compound with the highest content was spiraeoside (32,234 mg/kg DM in red cv. Karmen, 23,283 mg/kg DM in yellow cv. Všetana and 265 mg/kg DM in white cv. Ala). Rutin ranged from 15 mg/kg DM in cv. Ala to 157 mg/kg DM in cv. Karmen and similarly quercetin from 1 mg/kg DM in cv. Ala to 163 mg/kg DM in cv. Karmen. During storage an increase in flavonoids could be observed, esp. at a laboratory temperature compared to storage at a lower temperature.

Keywords: onion; red, yellow and white varieties; polyphenols; spiraeoside; rutin; quercetin; varietal differences; changes during storage

A detailed overview of flavonoid and ascorbic acid content in onion was presented by DUKE (1992, 2001) and LACHMAN et al. (1999, 2000). Polyphenolic compounds, esp. flavonoids are effective antioxidants due to their capability to scavenge free radicals of fatty acids and oxygen. One of the richest sources of flavonoids in human diet is common onion (*Allium cepa* L.) or shallot (*Allium ascaloni* L.) as was documented by KOPEC and MINÁROVÁ (1984, 1985). Their flavonol content considerably decreases atherosclerotic processes, inhibits cholesterol accumulation in the blood serum and enhances resistance of vascular walls. Flavonoids decrease a risk of coronary heart disease.

Onion is one of the highly rich sources of main flavonols – quercetin – in human diet (SELLAPPAN, AKOH 2002). Average total quercetin content in onion (347 mg/kg) is 5–10 times higher in comparison with other vegetables. The most frequent flavonol quercetin is present both in the bound and free form (LEIGHTON et al. 1992; RHODES, PRICE 1996). In onion quercetin di- and triglycosides: 3,4´-*O*-β-*D*-diglucoside, 7,4´-*O*-β-*D*-diglucoside, 3,7-*O*-β-*D*-diglucoside, 3-*O*-sophoroside-7-*O*-β-*D*-glucuronide, 3,7,4´-*O*-β-*D*-triglucoside and rutin were found (FOSSEN et al. 1998). Quercetin-monoglycosides spiraeoside (4´-*O*-β-*D*-glucoside), 3-*O*-β-*D*-glucoside, 3´-*O*-β-*D*-glucoside, and 7-*O*-β-*D*-glucoside are very highly manifested (IOKU et al. 2001; TSUSHIDA, SUZUKI 1995). There are also kaempferol-glycosides present at minor amounts:

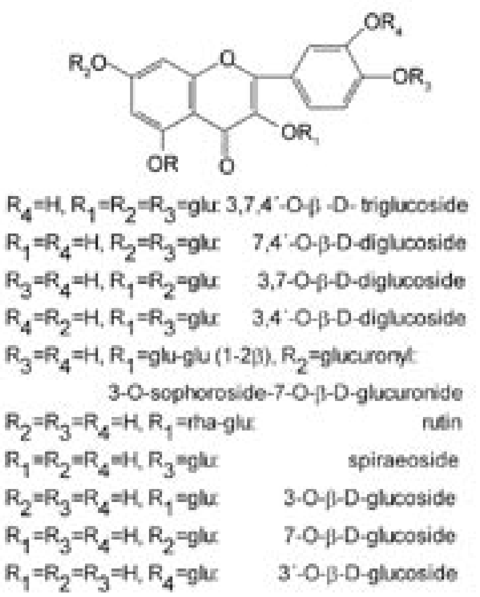

Fig. 1. Glycosides of quercetin

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Table 1. Content of major polyphenols in onion

Compound	Content (mg/kg DM)	Proportion in total polyphenol content (%)
Quercetin	48,000	63.36
Protocatechuic acid	11,020	14.52
Spiraeoside (quercetin-4´-O-β-D-glucoside)	10,650	14.03
Quercetin-3,4´-O-β-D-diglucoside	3,650	4.81
Tyrosine	1,725	2.27
Vanillic acid	228	0.34
Quercetin-7,4´-*O*-β-*D*-diglucoside	160	0.21
p-Hydroxybenzoic acid	258	0.14
Total amount of major polyphenols	75,910	99.68

3,4´-*O*-β-*D*-diglucoside, 7,4´-*O*-β-*D*-diglucoside, 3-*O*-sophoroside-7-*O*-β-*D*-glucuronide, 4´-*O*-β-*D*-glucoside. Another type of flavonols – isorhamnetin – is present only in yellow and red cultivars of onion in the free form and in the bound form in glycosides as: 3,4´-*O*-β-*D*-diglucoside, 4´-*O*-β-*D*-glucoside and 3-*O*-β-*D*-glucoside (Park, Lee 1996). Taxifolin-4´-*O*-β-*D*-glucopyranoside of dihydroflavonol type is referred to as a minor compound. Red onion cultivars have the highest contents of flavonoids, containing other anthocyanins: peonidin-3-*O*-β-*D*-arabinoside and peonidin-3-*O*-β-*D*-glucoside, pelargonidin-3-*O*-β-*D*-glucoside and three cyanidin-glycosides: 3-*O*-β-*D*-glucoside, 3-*O*-β-*D*-glycoside and 3-*O*-β-*D*-laminariobioside (3-*O*-β-*D*-glucopyranosyl-*D*-glucoside). Malonylesters of some of these glycosides, such as cyanidin-3-*O*-malonyl-β-*D*-glucoside and 3-*O*-malonyllaminariobioside and peonidin-3-*O*-malonyl-β-*D*-glucoside are newly reported. As for this rich flavonoid content, the proportions of quercetin-4´-*O*-β-*D*-glucoside and spiraeoside are highest. Yellow and red onion cultivars contain 60–1,000 mg/kg flavonoids. Primary flavonoids identified in shallots are quercetin-4´-*O*-β-*D*-glucoside and free quercetin. The other groups of polyphenolic compounds contained in onion are phenolcarboxylic acids, such as protocatechuic, caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic and vanillic acid. The presence of the amino acid tyrosine is also significant. These acids are represented both in the free and bound form as 1-*O*-β-*D*-glucosides, methyl esters or bound to phloroglucinol units. Among other polyphenols catechol and phloroglucinol are mentioned.

The aim of this study was to estimate the content of total polyphenol antioxidants in three onion varieties (red – cv. Karmen, yellow – cv. Všetana and white – cv. Ala) as well as to determine major flavonoid constituents. Coincidentally, the effect of temperature on their amounts was investigated during a 36-week storage period.

MATERIAL AND METHODS

Three onion varieties (red – cv. Karmen, yellow – cv. Všetana and white – cv. Ala) – harvest 2002, trial field Czech University of Agriculture in Prague-Suchdol.

One half of the samples was stored in a refrigerator (at +4°C) and one half of the samples at a laboratory temperature (+22°C). Analyses were performed after a period of six weeks regularly (in two parallel determinations) in the total period of 36 weeks.

Determination of total polyphenols

Samples were lyophilised before analyses on Lyovac GT-2 (Leybold-Heraeus GmbH) at temperatures between –52°C and +18°C. Lyophilised samples were extracted with 80% ethanol for 7 days. Total polyphenol content was estimated spectrophotometrically according to Lachman et al. (1998) with Folin-Ciocalteau's reagent. For the determination 1mL aliquots were pipetted. After dilution with distilled water to approximately 30 mL volume, 2.5 mL of Folin-Ciocalteau's reagent p.a. (Penta, Chrudim, Czech Republic) were added. After agitation and 3 min standing 7.5 mL 20% Na_2CO_3 p.a. solution was added and the volume was adjusted to the mark with distilled water. After thorough agitation and two hours standing at a laboratory temperature the absorbance of blue solution was measured against blank in cuvettes of 0.5 cm thickness at λ = 765 nm on Heλios γ spectrophotometer (Spectronic Unicam, G.B.). Polyphenol compounds were expressed as gallic acid content on dry matter (DM) basis. Two parallel determinations of each sample were performed and average values were calculated.

HPLC of individual polyphenols and flavonoids

For the determination of individual polyphenolic compounds HPLC method on the chromatograph Waters™ with gradient elution was used (pump Waters™ 600S, autosampler Waters™ 717 plus, detector Waters™ PDA 996-UV-VIS, column Watrex 250 × 4 mm Separon SGX C18 7 μm). Conditions for the determination of individual phenolics (acids) were as follows: mobile phase A – mixture methanol/water 5:95, V/V, mobile phase B – mixture methanol/water 40:60, V/V – both phases were adjusted with H_3PO_4 to pH = 2.5. Flow rate 1 ml/min, elution time 56 min, injection of sample

Table 2. Parameters of gradient elution

Time (min)	% A (V/V)	% B (V/V)
0	100	0
30	15	85
35	15	85
55	0	100
56	0	100
60	100	0

20 µL, detection at λ = 250 nm (parameters of gradient elution are given in Table 2).

For the determination of quercetin and its glycosides mobile phase A – mixture of methanol/water (5:95, V/V) and mobile phase B – mixture of methanol/water (95:5, V/V) were used after addition of acetic acid (to 1%, pure, min. 85%, Lachema Neratovice, Czech Republic). Elution time 42 min, injection of sample 20 µL, detection at λ = 350 nm. Quantitative estimation of the concentration of individual compounds was performed by the method of absolute calibration. Quercetin (> 98%, HPLC, Fluka AG) and rutin (> 99%, HPLC, Fluka AG) were used as standards.

RESULTS AND DISCUSSION

Average total polyphenol content (TP) in cv. Ala (white colour) was 26,445 mg/kg DM, in cv. Všetana (yellow) 65,210 mg/kg DM and in cv. Karmen (red)

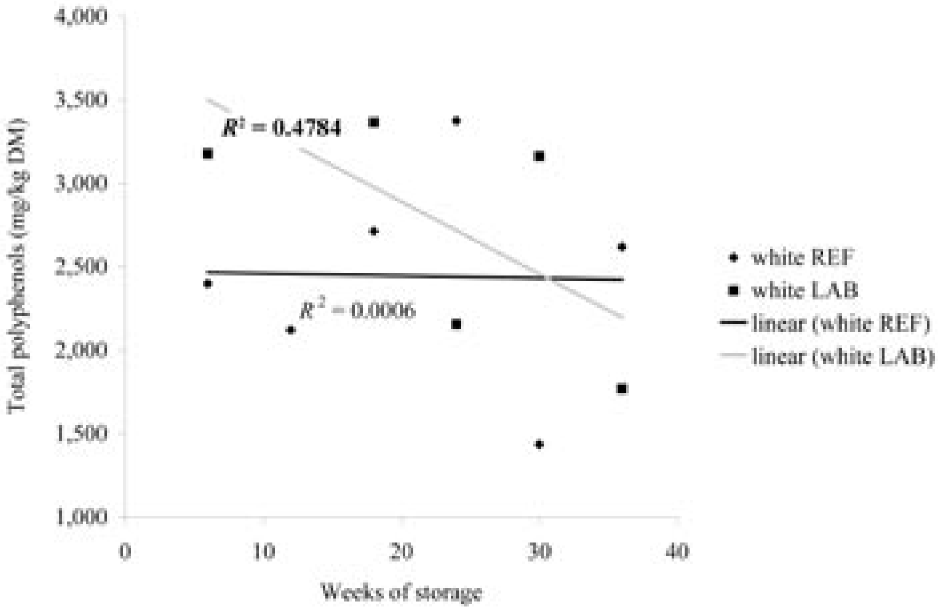

Fig 2. TP-time and TP-temperature storage graphs in cv. Ala. REF – stored in refrigerator (4°C); LAB – stored at laboratory temperature (22°C)

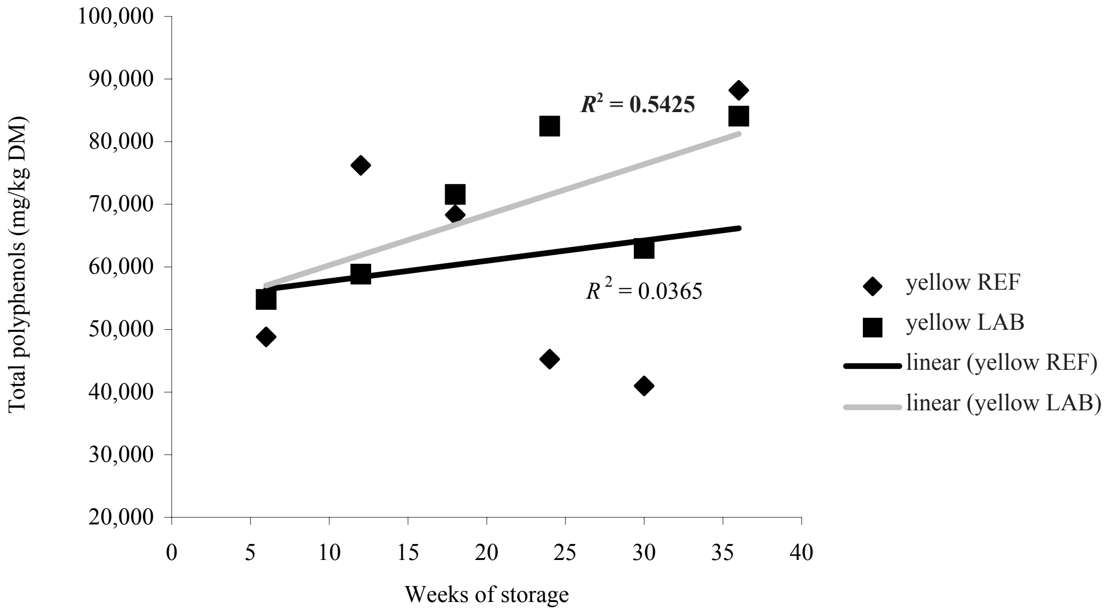

Fig 3. TP-time and TP-temperature storage graphs in cv. Všetana

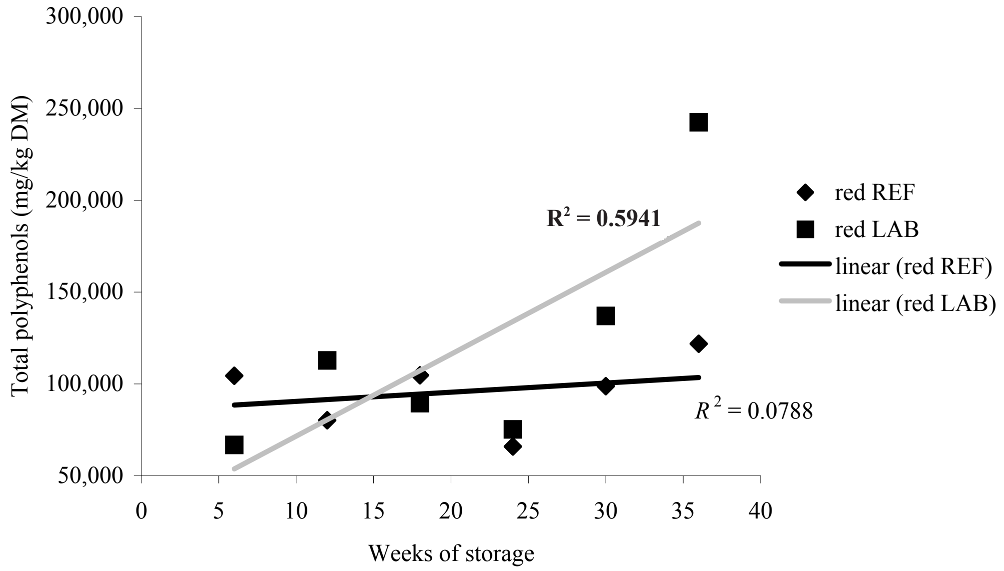

Fig. 4. TP-time and TP-temperature storage graphs in cv. Karmen

108,300 mg/kg DM. These high amounts confirm that among other vegetables onion is a very rich source of flavonoid antioxidants as it was reported elsewhere (KOPEC, MINÁROVÁ 1984, 1985; LACHMAN et al. 1999, 2000; CHU et al. 2000; NUUTILA et al. 2003).

Important are changes in the content of these antioxidant compounds during their storage or conservation of vegetables and fruits (KYZLINK 1988) and regarding this fact, the effect of long storage conditions on the content of polyphenolic antioxidants was investigated. During the storage period TP content increased in cv. Všetana (Fig. 3) and Karmen (Fig. 4), esp. in samples stored at a laboratory temperature. In white cv. Ala TP content was constant or had a reciprocal tendency at higher temperatures and slightly decreased (Fig. 2). The obtained results are in accordance with the observations of PRICE et al. (1997), who observed small changes in the content and composition of major flavonol glucosides over 6 months of storage in two varieties of onion (Red Baron and Crossbow). AWAD and DE JAGER (2003) also confirm the stability of flavonoids in two apple cultivars after harvest and no losses during storage in air and controlled atmosphere conditions and during the shelf life. The average TP contents for both storage methods are given in Table 3. Significant differences between the varieties in average TP contents and in TP contents were

Table 3. Average total polyphenol content in both methods of storage

Variety	Method of storage	Average TP content (mg/kg DM)
Ala (white)	Refrigerator (4°C)	24,428
Ala (white)	Laboratory (22°C)	28,461
Všetana (yellow)	Refrigerator (4°C)	61,296
Všetana (yellow)	Laboratory (22°C)	69,123
Karmen (red)	Refrigerator (4°C)	96,000
Karmen (red)	Laboratory (22°C)	120,665

Table 4. Average contents of individual compounds in analysed varieties

Analysed compound	cv. Ala (white)	cv. Všetana (yellow)	cv. Karmen (red)
Spiraeoside (mg/kg DM)	265	23,283	32,234
Rutin (mg/kg DM)	15	64	157
Quercetin (mg/kg DM)	1.3	56	163
Tr_{20} (mg/kg DM)	881	925	1,266
Tr_{36} (mg/kg DM)	0	2,634	3,402
$Tr_{56.3}$ (mg/kg DM)	0	1,766	1,918

found during onion storage in different systems, and are in accordance with the data given by NUUTILA et al. (2003), who found that red onion is more active than yellow onion.

Among the main flavonoid glycosides and phenolics spiraeoside (quercetin-4´-*O*-β-*D*-glucoside), rutin and quercetin and three other unidentified compounds were detected (Tr_{20}, Tr_{36} and $Tr_{56.3}$, where the indexes indicate retention times). Differences in the contents of these compounds between onion varieties are also significant (Table 4). Regarding the effects of storage period and storage temperature, the content of spiraeoside and rutin increased in yellow and red varieties, esp. in samples stored at a laboratory temperature, whereas quercetin content decreased. In the white Ala variety stored at lower temperatures spiraeoside and Tr_{20} contents decreased while quercetin and rutin contents were not detectable in samples taken during the first period of storage, but in the last period of storage rutin content increased and quercetin content was constant. Whereas the differences between the varieties in flavonoid and polyphenol contents were already mentioned (LACHMAN et al. 1999; SELLAPPAN, AKOH 2002; NUUTILA et al. 2003), the results obtained during storage of onion samples have brought new information about the changes of flavonoid compounds. EWALD et al. (1999) investigated the effect of processing on major flavonoids in processed onions, green beans, and peas and found out that the highest loss of flavonoids in onion occurred during the pre-processing step when the onion was peeled, trimmed and chopped before blanching. Further cooking, frying or warm-holding of the blanched vegetables for up to 2 h did not influence the flavonoid content (quercetin and kaempferol). A preliminary study of flavonols in onions that were finely chopped suggests that in most varieties there is only a small loss of total flavonols but that there is a progressive loss of the diglucoside component with accompanying quantitative accumulation of the monoglucoside and the aglycone (RHODES, PRICE 1996). The accumulation of spiraeoside (quercetin-4´-*O*-β-*D*-glucoside) during the storage period confirms this way of flavonol interconversions and possible formation from quercetin-3,4´-*O*-β-*D*-diglucoside. IOKU et al. (2001) found that the boiling of onion leads to about 30% loss of quercetin glycosides due to their transfer to boiling water.

CONCLUSION

The onion could be evaluated as a major dietary source of flavonoids and polyphenol antioxidants. Three major flavonoid constituents are spiraeoside (quercetin-4´-O-β-D-glucoside), rutin and quercetin. Significant varietal differences in total polyphenol content and individual flavonoids were found: red cv. Karmen > yellow cv. Všetana > white cv. Ala. Red cv. Karmen could be recommended for consumption in fresh state (salads) regarding high levels of polyphenols. During the storage period of 36 weeks an increase of spiraeoside and rutin contents could be observed in red and yellow varieties, esp. at higher temperatures.

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Celkové polyfenolické a dominantní flavonoidní antioxidanty v různých odrůdách cibule (*Allium cepa* L.)

ABSTRAKT: Ve studii byly sledovány polyfenolické antioxidační látky ve třech odrůdách cibule (červená Karmen, žlutá Všetana a bílá Ala) vzhledem k jejich celkovému obsahu a kvalitativnímu složení flavonoidního komplexu. Byl také sledován vliv teploty a doby skladování cibule na obsah těchto látek. Celkový obsah polyfenolů byl stanoven spektrofotometricky s Folin-Ciocalteauovým fenolovým činidlem a jednotlivé látky pomocí HPLC metody za použití chromatografu Waters™ na koloně Watrex 250 × 4 mm Sepharon SGX C18 7 µm s gradientovou elucí. Nejvyšší obsah polyfenolických sloučenin byl nalezen v červené odrůdě Karmen (108 300 mg/kg sušiny), nejnižší obsah v bílé odrůdě Ala (26 445 mg/kg sušiny), zatímco žlutá odrůda Všetana obsahovala průměrné množství celkových polyfenolických látek (65 210 mg/kg sušiny). Byl zjištěn nárůst celkových polyfenolů během skladování u červené a žluté odrůdy, zvláště během skladování při laboratorní teplotě (22°C). Z flavonoidních a polyfenolických sloučenin byly nalezeny jako dominantní konstituenty spiraeosid (4´-*O*-β-*D*-glukosid kvercetinu), rutin a kvercetin a tři další neidentifikované látky. Byly zjištěny významné meziodrůdové rozdíly. Nejvíce zastoupenou látkou byl spiraeosid (32 234 mg/kg sušiny u červené odrůdy Karmen, 23 283 mg/kg sušiny u žluté odrůdy Všetana a 265 mg/kg sušiny u bílé odrůdy Ala). Obsah rutinu se pohyboval od 15 mg/kg sušiny u odrůdy Ala do 157 mg/kg sušiny u odrůdy Karmen a podobně kvercetinu od 1 mg/kg sušiny u odrůdy Ala do 163 mg/kg sušiny u odrůdy Karmen. Během skladování byl zjištěn nárůst flavonoidů zvláště při laboratorní teplotě při srovnání se skladovacími podmínkami při nižší teplotě.

Klíčová slova: cibule; červené, žluté a bílé odrůdy; polyfenoly; spiraeosid; rutin; kvercetin; meziodrůdové rozdíly; změny během skladování

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