UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Vlasta CUNJA

VPLIV GENOTIPA IN OKOLJA NA FLAVONOLE IN ANTOCIANE V RAZLIČNIH TKIVIH VRTNIC (*Rosa* spp.)

DOKTORSKA DISERTACIJA

Ljubljana, 2016

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DOKTORSKA DISERTACIJA

INFLUENCE OF GENOTYPE AND ENVIRONMENT ON FLAVONOL AND ANTHOCYANIN CONTENT IN VARIOUS ROSE (*Rosa* spp.) TISSUES

DOCTORAL DISSERTATION

Ljubljana, 2016

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa Komisije za doktorski študij Univerze v Ljubljani z dne 1. julija 2014 je bilo potrjeno, da kandidatka izpolnjuje pogoje za opravljanje doktorata znanosti na Interdisciplinarnem doktorskem študijskem programu Bioznanosti, znanstveno področje hortikultura. Za mentorico je bila imenovana doc. dr. Valentina SCHMITZER.

Praktični del poskusov je bil izveden v rastlinjakih Biotehniške fakultete Oddelka za agronomijo, del vzorčenja pa je potekal v rozariju Arboretuma Volčji Potok in v Botaničnem vrtu Univerze v Ljubljani. Laboratorijske analize in obdelava podatkov je potekala na Katedri za sadjarstvo, vinogradništvo in vrtnarstvo Oddelka za agronomijo Biotehniške fakultete.

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JI sl/en
AI S tekočinsko kromatografijo visoke ločljivosti v povezavi z masno spektrometrijo smo identificirali fenolni profil listov in venčnih listov štirih vrst vrtnic (*Rosa canina, R. glauca, R. rubiginosa, R. sempervirens*) in treh okrasnih sort ('Rosarium Uetersen', 'Ulrich Brunner Fils', 'Schwanensee'). Identificirali smo številne fenolne spojine iz različnih skupin. Vsebnost in profil fenolnih snovi sta se značilno razlikovala med vrstami in sortami ter med rastlinskimi organi. Ugotovljene so bile značilne razlike v porazdelitvi fenolnih spojin v listih, zlasti med vrstami in sortami. Na splošno je bil fenolni profil bolj raznolik v listih vrst, ki so tudi vsebovali večje količine fenolnih snovi kot listi sort. Med vrstami so z veliko in raznoliko vsebnostjo fenolnih spojin izstopali listi *R. canina*. Nasprotno pa so se najbolj zaradi majhne vsebnosti fenolnih komponent razlikovali listi na bolezni občutljive sorte 'Schwanensee'. Glavna sladkorja v plodovih *R. canina* sta bila glukoza in fruktoza, ki predstavljata 92 % skupnih sladkorjev, med kislinami pa citronska (do 58 % vseh organskih kislin). Vsebnost skupnih sladkorjev in askorbinske kisline v plodovih *R. canina* se je po zmrzali značilno zmanjšala. V nasprotju pa se je v šipkih po slani vsebnost β-karotena in likopena povečala. Poleg cianidin-3-glukozida smo v plodovih določili še 45 različnih fenolnih spojin. Vsebnosti katehina, floridzina, flavanonov in več glikozidov kvercetina so bile značilno največje v plodovih nabranih v prvih treh terminih in so se po slani zmanjšale. V tretjem poskusu smo šipkom izbranih vrst *R. canina* (RCA), *R. sweginzowii* (RSW), *R. rugosa* (RUG) in sort 'Fru Dagmar Hastrup' (FDH), 'Repandia' (REP), 'Veilchenblau' (RVB), 'Aloha' (RAL), 'Bonica' (BON) in 'Golden Gate' (RGG) izmerili morfološke parametre (velikost, maso, barvo) in v njih izmerili vsebnosti sladkorjev, organskih kislin, likopena, β-karotena in fenolov. Čeprav so šipki tradicinalno uporabljane RCA vsebovali največ cianidin-3-glukozida in so bili tudi značilno najbolj rdeči, se v drugih merjenih parametrih niso razlikovali. Fenolni profil plodov je bil odvisen od vrste in sorte. Največja raznolikost fenolnih snovi je bila ugotovljena v plodovih RUG in FDH. V večini preučevanih vrst in sort so med fenolnimi spojinami prevladovali flavanoli. Značilno največja vsebnost fenolov je bila izmerjena v šipkih sorte RAL, predvsem zaradi velike vsebnosti hidrolizirajočih taninov v primerjavimi z drugimi vrstami in sortami. Čeprav majhni, so bili plodovi sort BON in REP količinsko najbolj bogati z β-karotenom oz. likopenom v slednji. Škropljenje miniaturnih vrtnic 'Funny Red' s sredstvom Lithovit Forte je značilno povečalo vsebnost skupnih fenolnih snovi v listih le dan po škropljenju, v kasnejših terminih pa se obravnavaja niso razlikovala.

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AB Using high–performance liquid chromatography/mass spectrometry broad leaf and petal phenolic profiles of four *Rosa* species (*R. canina, R. glauca, R. rubiginosa* and *R. sempervirens)* and three modern rose cultivars ('Rosarium Uetersen', 'Ulrich Brunner Fils', 'Schwanensee') were determined. An abundance of phenolic constituents was identified. The content and composition of phenolic compounds varied significantly among species and cultivars and plant organs. Distinct differences in the distribution of leaf phenolic compounds were observed especially between *Rosa* species and modern rose cultivars. In general, leaves of analysed species were richer in the content of most phenolic groups and individual components compared to the cultivars. Among species, leaves of *R. canina* stood out with their high and varied phenolic content. Conversely, leaves of the susceptible 'Schwanensee' appeared most dissimilar due to their low levels of phenolic constituents. Glucose and fructose were the predominant sugars in *R. canina* hips (up to 92 % total sugars), and citric acid was the major organic acid analysed (up to 58 % total organic acids). Total sugar and ascorbic acid content significantly decreased after frost damage. Contrary, β-carotene and lycopene levels were higher in frostbitten rose hips. In addition to cyanidin-3-glucoside, 45 different phenolic compounds have been identified in rose hips. The levels of catechin, phloridzin, flavanones, and several quercetin glycosides were highest on the first three sampling dates and decreased after frost. In the third investigation morphological parameters (size, weight, colour), the content of sugars, organic acids, lycopene, β-carotene and phenolics were determined in hips of *Rosa canina* (RCA), *R. sweginzowii* (RSW), *R. rugosa* (RUG) and selected ornamental *Rosa* cultivars 'Fru Dagmar Hastrup' (FDH), 'Repandia' (REP), 'Veilchenblau' (RVB), 'Aloha' (RAL), 'Bonica' (BON) and 'Golden Gate' (RGG). Although traditionally used RCA hips contained the highest amount of cyanidin-3-glucoside and were the reddest, they did not stand out in other analysed parameters. The phenolic profile was species and cultivar specific. The greatest diversity of phenolic compounds was detected in RUG and FDH hips. Flavanols represented the main phenolic class in most of the investigated species and cultivars. Altogether RAL hips contained the highest quantity of phenolics mainly due to high levels of hydrolysable tannins compared to other species and cultivars. Although small, hips of BON and REP were most abundant in β-carotene and lycopene, respectively. Foliar spraying of miniature 'Funny Red' rose with Lithovit Forte significantly increased total phenolic content in leaves one day after treatment but the effect was not significant on subsequent samplings.

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str.

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Cunja V., Mikulic-Petkovsek M., Stampar F., Schmitzer V. 2014. Compound identification of selected rose species and cultivars: an insight to petal and leaf phenolic profiles. Journal of the American Society for Horticultural Science, 139, 2: 157-166

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KAZALO PREGLEDNIC

str.

Preglednica 1: Vsebnost skupnih fenolnih snovi (povprečje v mg ekvivalentov galne kisline/g sveže mase ± standardna napaka) v listih miniaturne vrtnice 'Funny Red'. Različne črke označujejo statistično značilne razlike med obravnavanji pri posameznem terminu. DPT = dnevi po tretiranju 44

KAZALO SLIK

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Slika 1: Ostanki sredstva Lithovit Forte na vzorčenih listih miniaturne vrtnice 'Funny Red'. Rastlina je bila poškropljena s priporočeno koncentracijo pripravka (5 g/l). 56

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OKRAJŠAVE IN SIMBOLI

BON *Rosa* 'Bonica'

FDH *Rosa* 'Fru Dagmar Hastrup'

RAL *Rosa* 'Aloha'

RCA *Rosa canina*

REP *Rosa* 'Repandia'

RGG *Rosa* 'Golden Gate'

RSW *Rosa sweginzowii*

RUG *Rosa rugosa*

RVB *Rosa* 'Veilchenblau'

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

Vrtnice uvrščamo med najpomembnejše okrasne rastline. Na Kitajskem sega gojenje divjih vrst v vrtovih palač že 2000 let v zgodovino (Guoliang, 2003). V Evropi so vrtnice in njihove divje prednike gojili najprej predvsem v medicinske namene. Gojenje vrtnic v okrasne namene se je razmahnilo šele v 18. stoletju. Takrat se začnejo zaradi kolonizacije in z razvojem pomorske trgovine v Evropi pojavljati nove vrste iz severne Amerike in predvsem vzhodne Azije, ki botrujejo nastanku številnih novih križancev (Joyaux, 2003). Danes poznamo na tisoče sort, ki se razlikujejo po velikosti rasti in cvetov, barvi in vonju kot tudi po sposobnosti uspevanja na različnih rastiščih (Marriott, 2003).

Številne sorte vrtnic že stoletja sadimo v vrtove zaradi njihovih lepih cvetov in vonja. Poleg okrasne vrednosti veljajo šipki tudi za zdravilne rastline. V ta namen se uporabljajo predvsem plodovi, v kitajski medicini pa tudi cvetovi in listi (Coruh in Ercisli, 2010; Fenglin in sod., 2004). Dišavne snovi v cvetovih se uporabljajo v parfumarski industriji (Farooq in sod., 2012), cvetove in plodove pa uporabljajo tudi v kozmetični industriji ter za kulinarične namene (Ercisli in Güleryüz, 2005; Kumar in sod., 2013).

Rod *Rosa* (šipki, vrtnice) vključuje okoli 200 vrst, ki so v glavnem razširjene na severni polobli (Evropa, Bližnji Vzhod, Azija, severna Amerika); od teh jih v srednji Evropi samoniklo uspeva približno 30 vrst, najbolj razširjene so iz sekcije *Caninae* (Wissemann in sod., 2006; Li in sod., 2013). V Sloveniji v naravi raste 22 vrst (Martinčič in sod., 2007), najbolj poznana med njimi pa je *Rosa canina* (navadni šipek), ki jo uvrščamo v prej omenjeno sekcijo.

Taksonomija šipkov je zloglasno zapletena zaradi genetske kompleksnosti, ki ji sledi velika fenotipska raznolikost in na katero vplivajo evolucijski procesi kot je opraševanje oz. hibridizacija (Wissemann, 2003; Wissemann in Ritz, 2005); poleg tega se je šele v zadnjem desetletju začelo preučevati genome in filogenetske povezave med vrstami.

Povzeto po Koopman in sod. (2008) je primarni vir zmede glede taksonomije vrtnic zapletena evolucijska zgodovina divjih vrst v kombinaciji z dolgo zgodovino gojenja in križanja izbranih genotipov. Kompleksnost je posledica številnih faktorjev: obsežne medvrstne hibridizacije, odsotnosti jasnih razlik med številnimi vrstami in poliploidije. Drugi vir zmede pa je dejstvo, da so podlaga za klasifikacijo morfološki znaki, ki pa so pogosto izpostavljeni selekcijskim in okoljskim vplivom. Tako so lahko morfološki znaki podobni pri divergentnih vrstah, ki se razvijajo v enakih življenjskih razmerah, ali pa različni pri sorodnih vrstah, ki se prilagodijo na drugačne rastne razmere.

Cairns (2003) navaja, da je trenutno v uveljavi taksonomski sistem, ki ga je leta 1940 postavil Rehder in dopolnil Wissemann. Rod *Rosa* tako delimo na 4 podredove, od tega

trije vključujejo malo število vrst; to so *Hulthemia, Hesperhodos* in *Platyrhodon.* Večina vrst je vključena v četrti, največji podrod *Rosa* (pred dopolnitvijo *Eurosa*). Ta podrod dalje delimo na 10 sekcij, od katerih je najštevilčnejša sekcija *Caninae* (Koopman in sod., 2008; Cairns, 2003; Wissemann, 2003). Ta je tudi najbolj raznolika, kar še posebej otežkoča njeno taksonomijo (Adamczak in sod., 2012). Sklepajo da je ta sekcija na relativno zgodnji stopnji divergence, saj so razlike med vrstami včasih manjše kot je njihova intraspecifična variabilnost (Adamczak in sod., 2012).

Poleg botanične razdelitve, pa si različna društva in združenja ljubiteljev vrtnic prizadevajo za čim bolj enostavno hortikulturno razvrstitev množice različnih sort vrtnic. Taka delitev temelji deloma na botanični delitvi (razdelitev v skupine glede na 'izvorno' sorto) deloma pa na komercialni razdelitvi vrtnic na stare in moderne sorte ter po načinu rasti. Od leta 2000 naprej je shemo Ameriškega vrtničarskega združenja (American rose society) prevzela tudi Svetovna federacija vrtničarskih združenj (World federation of rose societies) - ta shema sedaj velja tudi pri registraciji novih sort (Cairns, 2003).

Antociani in flavonoli so sekundarni rastlinski metaboliti, ki jih uvrščamo med fenolne snovi (fenilpropanoidi), natančneje v skupino flavonoidov. Pod pojmom antocian združujemo pojma antocianin in antocianidin. Antocianidini predstavljajo osnovno molekulo brez vezanih sladkornih enot. Ko se na to osnovno molekulo veže sladkor, dobimo glikozide, ki jih imenujemo antocianini (Taiz in Zeiger, 2006).

Med flavoidinimi barvili so antociani najpogostejši in najbolj razširjeni. So vodotopni in se v rastlinskih celicah nahajajo v vakuolah (Schwinn in Davies, 2004; Tanaka in sod., 2008). Tanaka in sod. (2008) poročajo o 19 tipih antocianidinov, od katerih so v naravi najpogostejši pelargonidin, cianidin, peonidin, delfinidin, petunidin in malvidin.

Za flavonoide je značilno, da imajo molekule sestavljene iz dveh aromatičnih obročev, ki ju povezuje most treh ogljikovih atomov (C3 enota). Odvisno od stopnje oksidacije tega mostu jih delimo v različne podskupine. Pri antocianih in fenolih trije atomi ogljika skupaj z atomom kisika setavljajo še tretji, heterociklični obroč (Schwin in Davies, 2004; Taiz in Zeiger, 2006; Koes in sod., 1994). Poleg tega pa je lahko vsaka poskupina podvržena še drugim različnim procesom, ki spreminjajo kemične lastnosti, kot npr. metilacija (vezava metilne -CH_3 skupine), hidroksilacija (vezava hidrokislne -OH skupine), acilacija (vezava acilne R-C=O skupine) in glikozilacija (vezava sladkornih enot), zaradi katerih so flavonoidi tako raznolika in barvita skupina (Schwinn in Davies, 2004).

Osnovni ogljični skelet flavonoidov nastane s kondenzacijo treh malonil-CoA enot z p-kumaroil-CoA v naringenin halkon; reakcija poteče s pomočjo encima halkon sintaze. Od tu ob delovanju halkon izomeraze nastane prvi flavonoid, flavonon naringenin. Biosintezna pot se nato razveji, vsaka veja prispeva drugačno podskupino flavonoidov. Naringenin se

pretvori v dihidrokempferol, ta pa je izhodišče za nastanek antocianidinov na eni in flavonolov (kempferol, kvercetin ...) na drugi strani (Jay in sod., 2003; Cooper-Driver, 2001; Falcone Ferreyra in sod., 2012; Koes in sod., 1994).

Flavonoidi so torej fenolne snovi z majhno molekulsko maso in so splošno razširjeni v rastlinskem kraljestvu. So zelo raznolika skupina, ki ima v rastlinah številne biološke funkcije in igra pomembno vlogo pri interakcijah rastline z okoljem. (Koes in sod., 1994).

Od vseh flavonoidov so flavonoli najbolj razširjena podskupina. So evolucijsko najstarejši; njihova sinteza poteka tako v mahovih kot praprotih (Stafford, 1991; Winkel-Shirley, 2002). Distribucija in strukturne razlike med flavonoli so zelo obsežne. Med najpogostejšimi flavonoli najdemo kvercetin, kempferol, isoramnetin in miricetin, na katere so vezani sladkorji. Čeprav je število aglikonov omejeno, lahko v naravi najdemo številne konjugate (Veberič, 2010). Kot barvila dajejo flavonoli bledo rumeno barvo, ki je človeškim očem skoraj nevidna. Zaradi absorpcije UV svetlobe, ki jo zazanavajo določene žuželke, pa vseeno prispevajo k barvi in vzorcem cvetov, ki jih te opraševajejo (Tanaka in sod., 2008).

Zaradi okrasne vloge vrtnic so se prve fitokemične raziskave osredotočile na barvila v venčnih listih. Vsebnost in sestava antocianinov in fenolov je bila preučevana v različnih vrstah in sortah, tudi z namenom olajšati zapleteno taksonomijo rodu *Rosa*. Tako so Biolley in sod. (1994) preučevali flavonoide (flavonole in antociane) v petalih 100 različnih križancev *Rosa* x *hybrida*. Določili so 3,5-diglukozid in 3-monoglukozid cianidina in pelargonidina ter (v le petih križancih) peonidin-3,5-diglukozid, ki je bil prisoten v križancih vrste *Rosa rugosa*. Izmed flavonolov so določili glikozide kempferola in kvercetina, najpogosteje v obliki 3-*O*-glukozida, 3-*O*-ramnozida ter 4'-*O*-glukozida. Mikanagi in sod. (1995) poročajo podobne snovi v venčnih listih 120 taksonov iz različnih sekcij podrodu *Rosa*. Tudi oni so določili glikozide kempferola in kvercetina ter 3,5-diglukozid in 3-glukozid cianidina ter peonidina. Glikozidov pelargonidina niso našli, ti naj ne bi bili značilni za divje vrste. Kljub temu so Cai in sod. (2005) z bolj naprednimi analitičnimi tehnikami in orodji (LC-ESI MS, MALDI-QIT-TOF MS) določili pelargonidin-3,5-di-*O*-glukozid v venčnih listih vrste *Rosa chinensis* (poleg že prej navedenih kvercetin in kempferol mono- in di-glukozidov). Tudi v Sloveniji smo preučevali kemično sestavo venčnih listov vrtnic v povezavi s fiziološkimi procesi v rastlini (Schmitzer in sod., 2010, 2012).

Poleg barve je bilo veliko raziskav osredotočenih na hlapljive snovi, ki dajejo cvetovom vrtnic značilen vonj, ki se med vrstami in sortami močno in značilno razlikuje (Kim in sod., 2000; Cherri-Martin in sod., 2007; Dobson in sod., 1987, 1990; Picone in sod., 2004; Helsper in sod., 1998).

Kemične raziskave so se, poleg cvetov in njihove barve, osredotočile tudi na sestavo birnih plodov vrtnic. Ti se že stoletja nabirajo za prehrano, v zdravilne namene in zaradi njihove okrasne vrednosti. Redko se jedo sveži, običajno se jih suši in/ali predela in uživa v obliki čajev, sokov in drugih napitkov ali marmelad (Uggla in sod., 2005; Yildiz in Alpaslan, 2012; Demir in sod., 2014; Pang in sod., 2009). Pri nas se v te namene najbolj uporabljajo plodovi navadnega šipka (*R. canina*), medtem ko so druge avtohtone vrste manj v uporabi (*R. glauca, R. rubiginosa* in *R. sempervirens*). Drugod uporabljajo različne vrste kot npr. *R. rubiginosa, R. moschata, R. michranta, R. dumalis* in druge.

Obstajajo številne raziskave, ki dokazujejo, da so plodovi šipkov bogati z vitaminom C, fenolnimi snovmi in karotenoidi; te snovi lahko imenujemo tudi fitonutrienti (rastlinske hranljive snovi) in jih povezujemo s krepitvijo zdravja (Beecher, 1999). V zadnjih desetletjih je vse večji interes za funkcionalna živila, ki imajo koristen učinek na zdravje in lahko zmanjšajo možnost za nastanek določenih bolezni (Arai, 1996; Losso, 2003).

Šipki so v prvi vrsti znani po veliki vsebnosti vitamina C (Hvattum, 2002; Roman in sod., 2013; Salminen in sod., 2005). Vitamin C v prehrani ljudi deluje pri aktivaciji encimov, zmanjševanju oksidativenga stresa in pomembno vpliva na imunski sistem. Dodajanje vitamina C prehrani naj bi imelo varovalni učinek pred različnimi bolezenskimi stanji, še posebej pri prehladu, kardiovaskularnih obolenjih in celo pri nekaterih oblikah raka (Schlueter in Johnston, 2011).

Za razliko od cvetov, kjer barvo v glavnem določajo antocianini, pa na značilno oranžno do rdečo barvo plodov vrtnic v veliki meri vpliva prisotnost številnih karotenoidov. Karotenoidi so tetraterpeni, tvorijo verige s 40 ogljikovimi atomi, med katerimi so konjugirane dvojne vezi. Karotenoidom, ki so čisti ogljikovodiki pravimo karoteni, ksantofili pa so karotenoidi, ki imajo poleg atomov ogljika in vodika v molekulah vezan tudi kisik (Veberič, 2010). V plodovih vrtnic so najpogostejši β-karoten, likopen, β-kriptoksantin, rubiksantin, zeaksantin in lutein (Andersson in sod., 2011; Hodisan in sod., 1997; Hornero-Mendez in Minguez-Mosquera, 2000). Böhm in sod. (2003) navajajo, da je vsebnost likopena v nepredelanih plodovih *Rosa canina* celo večja kot v svežih paradižnikih. Karotenoidi so derivati izoprena in so nujni del fotosistemov (Tanaka in sod., 2008), najdemo jih v zelenih delih rastlin (listih) pa tudi v plodovih in cvetovih. Prav tako so karotenoidi pomemben del človeške prehrane, delujejo kot provitamin A in naj bi preprečevali pojav določenih kroničnih bolezni in raka (Mayne, 1996; Landrum in Bone, 2001; Giovannucci, 2002). Nekateri karotenoidi se uporabljajo kot barvila v hrani, v kozmetiki in kot prehranska dopolnila (Tanaka in sod., 2008; Mortensen, 2006).

Antioksidativni učinek plodov pa lahko pripišemo ne samo vsebnosti vitamina C, ampak tudi vsebnosti polifenolov (Daels-Rakotoarison in sod., 2002; Tumbas in sod., 2012). Rezultati številnih raziskav kažejo, da šipki (plodovi) delujejo protivnetno in bi jih lahko

kot take uporabili kot nadomestilo oz. dodatek konvencinalnim zdravilom pri zdravljenju nekaterih vnetnih bolezni kot je npr. artritis (Daels-Rakotoarison in sod., 2002; Rein in sod., 2004; Winther in sod., 2005; Kharazmi in Winther 1999; Willich in sod., 2010). Poleg tega Ninomiya in sod. (2007) poročajo, da vodno-acetonski izvleček birnega plodu oz. semen *Rosa canina* znatno zmanjša pridobivanje telesne teže in/ali trebušne maščobe pri miših. Raziskave kažejo tudi možnost uporabe vodnega izvlečka plodov šipka v živilski industriji, saj inhibira delovanje polifenol oskidaze in tirozinaze, encimov, ki povzročata rjavenje sadja in zelenjave (Zocca in sod., 2011).

V zvezi s fenolno in mineralno sestavo plodov šipkov so bile narejene že številne študije, predvsem v povezavi z njihovo antioksidativno aktivnostjo (Ghazghazi in sod., 2012; Hvattum, 2002; Roman in sod., 2013), ne pa tudi kako se vsebnost fenolnih snovi spreminja med zorenjem. Dolžina in čas zorenja se namreč med različnimi vrstami rodu *Rosa* lahko precej razlikujeta. V nasprotju z nekaterimi drugimi vrstami drobnega sadja (robide, maline), soplodja šipka ob zrelosti ne odpadejo z vej, zato je težko ugotoviti tehnološko oz. fiziološko zrelost plodov. Razlike v vsebnosti posameznih snovi so odvisne od genetske raznolikosti, stopnje zrelosti, razlik med leti, podnebja, skladiščenja in ne nazadnje tudi od analitske metode (Andersson in sod., 2011). Če želimo plodove uporabljati v zdravstvene namene ali kot dodatke prehrani (aditive), je nujno poznavanje vsebnosti in sestave zdravilnih učinkovin v njih. Da zagotovimo optimalno vsebnost teh snovi v plodu pa je treba poznati tudi, kako se vsebnosti teh snovi spreminjajo med zorenjem. Andersson in sod. (2011) so več let preučevali spreminjanje snovi v plodovih različnih vrst šipkov. Ugotovili so, da je vsebnost posameznih snovi odvisna od termina nabiranja. Tako je vsebnost nekaterih karotenoidov (β-karoten, likopen, skupni karotenoidi, skupni karoteni) med zorenjem naraščala, drugih pa ne: zeaksantin in lutein sta z zorenjem neenakomerno upadala, vsebnost skupnih ksantofilov pa se je tekom zorenja le malo spreminjala in ni kazala določenega trenda. Kljub številnim raziskavam na temo karotenoidov in fenolnih snovi v plodovih šipka pa do sedaj še ni bilo preučeno kako se njihova vsebnost spreminja med zorenjem v plodovih navadnega šipka (*Rosa canina*). Poleg tega naj bi soplodja šipka tradicionalno nabirali po prvi slani/zmrzali. V literaturi nismo našli podatkov, kako naj bi to vplivalo na vsebnost bioaktivnih snovi v plodovih.

Čeprav nekateri gojijo vrste, ki jih najdemo v naravi, tudi v vrtovih, gojimo zaradi cvetov predvsem različne moderne sorte vrtnic. Običajno pri teh vrtnicah odcvetele cvetove odstranjujemo, saj tako zagotovimo pocvitanje in lepši videz rastline. Če jih prepustimo naravi pa bodo tudi nekatere okrasne sorte (običajno take z enostavnimi ali polvrstnatimi cvetovi) oblikovale plodove, ki so lahko zanimiv okras v jesenskem vrtu. V literaturi praktično ni podatkov o notranji kakovosti plodov okrasnih, modernih sort. Je vsebnost biološko aktivnih snovi v plodovih okrasnih sort primerljiva z vsebnostmi v plodovih za zdravilne namene tradicionalno uporabljanih vrst šipkov? Ali se v njih nakopičijo

primerljive vsebnosti askorbinske kisline in polifenolov kot v navadnem šipku (*Rosa canina*)? Bi jih lahko uporabljali kot zdrav dodatek k dnevni prehrani?

Za razliko od cvetov in plodov pa je fenolna sestava listov šipkov/vrtnic slabo preučena, čeprav so ti pomemben rastlinski organ preko katerega se rastlina odziva na okolje. Eden od odgovorov na vplive iz okolja je sinteza sekundarnih metabolitov. Različni avtorji navajajo vsebnost skupnih fenolov ali vsebnost skupnih flavonoidov v listnih izvlečkih vrtnic (Ghazghazi in sod., 2012; Nowak in Gawlik-Dziki, 2007). Zelo malo pa je raziskav, ki bi bolj natančno identificirale posamezne fenolne komponente v listih vrtnic. Raziskave polifenolov v listih vrtnic so še vedno redke in nepopolne. Različni avtorji poročajo v ekstraktih listov različnih vrst *Rosa* predvsem skupno vsebnost fenolov ali skupno vsebnost flavonoidnih oz. flavonolskih aglikonov (Ghazghazi in sod., 2012; Nowak in Gawlik-Dziki, 2007), medtem ko je raziskav v katerih bi identificirali in kvantificirali posamezne fenolne spojine še vedno malo.

Številni sekundarni metaboliti fenilpropanoidne poti (fenolne kisline, flavonoidi) so v rastlinah udeleženi pri odgovoru na biotski in abiotski stres (Dixon in Paiva, 1995). Eden od biotskih stresov je napad rastlinskih patogenov. Rastline se branijo s toksičnimi snovmi, ki so v rastlini že sintetizirane in se lahko ob morebitni okužbi pretvorijo v bolj toksične (fitoanticipini, ki so del konstitutivne odpornosti), ali pa jih ob okužbi tvorijo na novo (fitoaleksini, ki so del inducirane odpornosti). Snovi z anti-patogenim učinkom so lahko značilne za posamezno rastlinsko vrsto in pripadajo različnim tipom fenolov (Grayer in Kokubun, 2001).

Glede na to, da so vrtnice ene pomembnejših rastlin, ki se jih goji v okrasne namene ter vse močnejšega okoljskega zavedanja, ki spodbuja čim manjšo rabo pesticidov, je pomembno poznavanje rastlinskih metabolitov, ki imajo lahko antimikrobni učinek. Shetty in sod. (2011) so ugotovili, da foliarna aplikacija klorogenske kisline in rutina pripomore k upadu okuženosti rastlin vrtnic (*Rosa hybrida* 'Smart') z glivo *Podosphaera pannosa* (šipkova pepelovka). V poskusu so ugotovili tudi porast teh snovi v listih po zalivanju rastlin s hranilno raztopino v katero je bil dodan silicij v obliki kalijevega silikata (K_2SiO_3).

V okviru disertacije smo želeli pridobiti sliko fenolne sestave listov in plodov, kar bi omogočilo nadaljne raziskave povezane z odzivom rastlin na stresne dejavnike. To je pomembno predvsem z vidika gojenja in žlahtnenja šipkov in vrtnic. Prav tako je potrebno poznati kako se vsebnost bioaktivnih snovi spreminja med zorenjem, če želimo nabrati plodove z optimalno vsebnostjo nekaterih zdravju koristnih snovi. Do sedaj je bila fenolna sestava plodov navadnega šipka (*Rosa canina*) slabo preučena, malo je znanega o spremembah vsebnosti nekaterih metabolitov med dozorevanjem; o kemični sestavi plodov okrasnih sort pa do sedaj ni bilo objav. Prav tako je bila do sedaj zapostavljena fenolna sestava listov vrtnic, čeprav so listi eden od rastlinskih organov preko katerega se rastlina

odziva na okolje. Mnoge fenolne snovi imajo obrambno delovanje in so del konstitutivne in sistemsko pridobljene odpornosti. Skupaj z vedno večjim okoljskim zavedanjem in željo po čim manjši uporabi pesticidov, raziskovalci iščejo načine kako v rastlini spodbuditi te naravno prisotne mehanizme. Da bi dopolnili manjkajoče znanje smo v okviru doktorske disertacije zastavili štiri poskuse:

1. poskus: Pregled fenolne sestave listov in petalov različnih vrst šipkov in modernih sort

V poskus smo vključili štiri vrste (*Rosa canina, R. glauca, R. rubiginosa, R. sempervirens*) in tri moderne sorte vrtnic ('Rosarium Uetersen', 'Ulrich Brunner Fils', 'Schwanensee'). Vzorce smo nabrali v Aboretumu Volčji Potok in Botaničnem vrtu Univerze v Ljubljani. Vzorčili smo tako cvetove (venčne liste) kot liste. V venčnih listih smo ovrednotili vsebnosti anotcianov in flavonolov, v listih pa flavonolov, flavanolov in vsebnost prostih ter vezanih fenolnih kislin z uporabo HPLC/MS. Namen raziskave je bil ugotoviti fenolni profil venčnih listov izbranih vrst, ki so avtohtone v Sloveniji, in ga primerjati z izbranimi sortami modernih vrtnic. Prav tako smo prvič kvalitativno in kvantitativno določili vsebnosti nekaterih fenolnih snovi v listih izbranih vrst in sort.

2. poskus: Spreminjanje bioaktivnih snovi med zorenjem plodov navadnega šipka

Namen poskusa je bilo ovrednotiti kako se med zorenjem spreminja vsebnost bioaktivnih snovi v plodovih navadnega šipka (*Rosa canina*). Plodove smo začeli nabirati, ko so se obarvali in jih vzorčili vsakih 14 dni do prve slane, zadnje vzorčenje pa smo opravili po njej. Spremljali smo vsebnosti vitamina C, organskih kislin, sladkorjev, določenih karotenoidov in fenolnih snovi. Cilj raziskave je bil identificirati in kvantificirati različne primarne in sekundarne metabolite v soplodjih navadnega šipka (*R. canina*) in analizirati vpliv zmrzali na sestavo plodu. To bo omogočilo lažje določanje pravega časa obiranja za zagotovitev optimalne vsebnosti bioaktivnih snovi in primarnih metabolitov v plodovih navadnega šipka.

3. poskus: Primerjava vsebnosti primarnih in sekundarnih metabolitov v plodovih izbranih vrst in sort vrtnic.

V poskus smo vključili tri vrste *Rosa canina, R. sweginzowii, R. rugosa* in šest modernih, okrasnih sort 'Fru Dagmar Hastrup', 'Repandia', 'Veilchenblau', 'Aloha', 'Bonica' in 'Golden Gate'. Zanimalo nas je ali so plodovi (šipki) okrasnih sort po vsebnostih primarnih in sekundarnih metabolitov primerljivi s šipki izbranih vrst, kar do sedaj še ni bilo preučeno. Poleg nekaterih morfoloških znakov (dolžina, širina, masa in barva plodov), smo v plodovih analizirali še vsebnosti askorbinske in drugih organskih kislin in sladkorjev, fenolnih snovi ter β-karotena in likopena. Vzorce smo nabirali v Arboretumu Volčji Potok od začetka oktobra do konca novembra. Z izjemo *R. canina* in delno *R. rugosa* smo prvič kvalitativno in kvantitativno določili primarne in sekundarne metabolite v plodovih okrasnih vrtnic.

4. poskus: Foliarno tretiranje miniaturnih vrtnic s pripravkom Lithovit Forte

V rastlinjaku Biotehniške fakultete smo rastline sorte miniaturnih vrtnic 'Funny Red' tretirali s pripravkom Lihovit Forte. Uporabili smo priporočeno koncentracijo (5 g sredstva/l vode) in večjo koncentracijo (10 g sredstva/l vode). Kontrolne rastline smo škropili z vodo. Liste smo nabrali pred tretiranjem ter en, dva in sedem dni po škropljenju in v njih spektrofotometrično določili vsebnost skupnih fenolnih snovi. Namen poskusa je bil ugotoviti ali uporaba pripravka Lithovit Forte poveča vsebnost fenolnih snovi v listih, kar bi v prihodnje lahko izrabili za povečanje tolerantnosti rastlin na različne stresne dejavnike.

Skupaj smo za poskuse postavili naslednje delovne hipoteze:

1. Sorte in vrste se med seboj kvantitativno in kvalitativno razlikujejo v vsebnosti fenolnih snovi v listih; prav tako se v vsebnosti bioaktivnih snovi razlikujejo plodovi.
2. Sorte s slabšo odpornostjo na bolezni imajo manjšo vsebnost fenolnih snovi v listih.
3. Stopnja zrelosti plodov navadnega šipka (*Rosa canina*) vpliva na vsebnost fenolov, vitamina C, karotenoidov in njihovo barvo.
4. V plodovih nabranih po prvi slani je vsebnost fenolov, vitamina C in karotenoidov manjša.
5. Foliarno tretiranje s pripravkom Lithovit Forte poveča vsebnost fenolov v listih vrtnic.

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Določitev spojin izbranih vrst in sort vrtnic: vpogled v fenolni profil venčnih listov in listov

Compound identification of selected rose species and cultivars: An insight to petal and leaf phenolic profiles

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S tekočinsko kromatografijo visoke ločljivosti v povezavi z masno spektrometrijo (HPLC/MS) smo identificirali fenolni profil listov in venčnih listov štirih vrst vrtnic (*Rosa canina, R. glauca, R. rubiginosa, R. sempervirens*), ki se tradicionalno uporabljajo za medicinske namene, in treh modernih, okrasnih sort ('Rosarium Uetersen', 'Ulrich Brunner Fils', 'Schwanensee'). Identificirali smo številne fenolne spojine iz različnih skupin: sedem različnih antocianinov in 31 flavonolov v venčnih listih ter 30 flavonolov, 14 fenolnih kislin in njihovih derivatov, 15 flavanolov in 20 hidrolizirajočih taninov v listih. Dodatno smo s kolorimetrom izmerili barvo venčnih listov in z regresijsko analizo ugotovili močno korelacijo med barvnim parametrom *a** in skupno vsebnostjo antocianinov. Vsebnost in sestava fenolnih snovi se je značilno razlikovala med vrstami in sortami ter med rastlinskimi organi. Ugotovljene so bile značilne razlike v porazdelitvi fenolnih spojin v listih, zlasti med vrstami in sortami. Na splošno je bil fenolni profil bolj raznolik v listih vrst, ti so tudi vsebovali večje količine fenolnih snovi kot listi sort. Z multivariatno analizo smo preučevane vrste in sorte razdelili v tri različne skupine. Med vrstami so z veliko in raznoliko vsebnostjo fenolnih spojin izstopali listi *R. canina*. Nasprotno pa so se zaradi majhne vsebnosti fenolnih komponent najbolj razlikovali listi na bolezni občutljive sorte 'Schwanensee'.

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Compound Identification of Selected Rose Species and Cultivars: an Insight to Petal and Leaf Phenolic Profiles

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Additional index words. anthocyanins, flavanols, flavonols, hydrolysable tannins, phenolic acids, *Rosa*

Abstract. **Using high-performance liquid chromatography/mass spectrometry, leaf and petal phenolic profiles of four rose (*Rosa*) species (*R. canina*, *R. glauca*, *R. rubiginosa*, *R. sempervirens*) traditionally used for medicinal purposes and three modern rose cultivars (Rosarium Uetersen, Ulrich Brunner Fils, Schwanensee) were determined. An abundance of phenolic constituents was identified: seven different anthocyanins and 31 flavonols in petals; 30 flavonols, 14 phenolic acids, and their derivatives; 15 flavanols; and 20 hydrolysable tannins in leaves. Additionally, petal color was measured with a colorimeter and regression analysis indicated a strong correlation between color parameter a^* and total anthocyanin content. The content and composition of phenolic compounds varied significantly among species and cultivars and plant organs investigated. Distinct differences in the distribution of leaf phenolic compounds were observed, especially between *Rosa* species and modern rose cultivars. In general, leaves of analyzed species were richer in content of most phenolic groups and individual components compared with cultivars. Multivariate statistical analysis clustered the investigated species and cultivars into three distinct groups. Among species, leaves of *R. canina* stood out with their high and varied phenolic content. Conversely, leaves of the susceptible cultivar Schwanensee appeared most dissimilar as a result of their low levels of phenolic constituents.**

The genus *Rosa* contains more than 200 species distributed in Europe, Asia, the Middle East, and North America (Li et al., 2013). The genus is represented by 22 species in the Slovenian flora, among which *R. canina*, *R. glauca*, *R. rubiginosa*, and *R. sempervirens* are most frequent (Martincic et al., 1999). Numerous rose cultivars are widely planted in gardens and parks for their aesthetic value and native plants are harvested for their fruit and flowers.

Medicinal benefits of the genus *Rosa* have been reported in many studies and specific plant tissue has been used for different purposes (Nowak and Gawlik-Dziki, 2007). Rose hips comprise several biologically active compounds and are famous for their high content of vitamins, particularly vitamin C (Hvattum, 2002; Roman et al., 2013). Fragrance compounds in rose petals are praised in perfumery and food industries (Farooq et al., 2012). Similarly, rose leaves have been used in Chinese and European medicine for centuries as ingredients in common cold remedies (Coruh and Ercisli, 2010; Fenglin et al., 2004). Health-beneficial properties of *Rosa* leaves may be attributed to their content of phenolics, which are known to possess a wide spectrum of bioactive functions such as antioxidant and anti-inflammatory effects (Sroka, 2005). Interspecific variation in the levels of bioactive compounds and their healing potential led to selective harvest of specific rose species for traditional uses. In Slovenia, hips, petals, and leaves of *R. canina* have been favored for their healing powers, but other indigenous rose species have not been used to the same extent.

A number of studies have been published on the phenolic and mineral composition of rose hips in connection to their antioxidant activity (Ghazghazi et al., 2012; Hvattum, 2002; Roman et al., 2013). Petal phenolic antioxidants have also been identified in several rose species and cultivars (Biolley et al., 1994; Cai et al., 2005; Mikanagi et al., 1995; Schmitzer et al., 2010, 2012). However, research on rose leaf polyphenols is still partial and scarce. Different authors report total phenolic content, total flavonoid, or total flavonol aglycone levels in leaf extracts (Ghazghazi et al., 2012; Nowak and Gawlik-Dziki, 2007) of *Rosa* species, but studies targeted at identification of individual phenolic compounds are limited.

The aim of the present study was to identify and quantify phenolic compounds in petals of several indigenous rose species in Slovenia and compare them with the phenolic profiles of certain modern rose cultivars. Moreover, rose leaf phenolic profiles were established and individual phenolic compounds have been identified and quantified for the first time in selected rose species. The qualitative and quantitative differences in leaf phenolic compounds among analyzed rose species and cultivars are discussed.

Materials and Methods

Plant material. Four rose species: *R. glauca* (pink flowers), *R. canina* ssp. *canina* (white to pale pink flowers), *R. sempervirens* (white flowers), and *R. rubiginosa* (pink flowers), and three cultivars: Rosarium Uetersen, Ulrich Brunner Fils (both with pink flowers), and Schwanensee (white flowers with light pink center), were selected for the study. Plants were located at the Botanical Garden and Arboretum Ljubljana (lat. 46.18° N,

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long. 14.61° E, altitude 250 m) and leaves and petals for the analysis were sampled in June 2013. Flowers were collected and analyzed in developmental stage fully open flower (Schmitzer et al., 2010) and petal color measurements were recorded. Leaves were collected at their mature stage and only the first three fully expanded leaves on the branch were analyzed. Plant material was frozen in liquid nitrogen and stored at –20 °C until further analysis.

Petal color measurements. Flower color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In the CIE L^* a^* b^* system of color representation, the L^* value corresponds to a dark–bright scale and represents the relative lightness with a range from 0 to 100 (0 = black, 100 = white). Color parameters a^* and b^* extend from –60 to 60; a^* negative is for green and a^* positive is for red and b^* negative is for blue and positive for yellow. The hue angle ($h°$) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green, and 270° = blue. Color was measured in the middle of each petal (three replicates per flower; 10 flowers per repetition) to ensure equal measurement conditions.

Extraction and high-performance liquid chromatography determination of phenolic compounds. Flower petals (combined samples consisting of five to 15 flowers) and leaves (combined samples consisting of 10 leaves) were ground to a fine powder with liquid nitrogen and 1 g of powder was extracted with 6 mL methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-Di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000 g_n. Supernatant was filtered through a polyamide filter (Chromafil AO-20/25; Macherey-Nagel, Düren, Germany) and transferred to a vial before injection into the high-performance liquid chromatography (HPLC) system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (phenolic acids and their derivatives, hydrolysable tannins, flavanols), 350 nm (glycosides of quercetin, kaempferol, myricetin, and isorhamnetin), and 530 nm (anthocyanins). A HPLC column (150 × 4.6 mm, Gemini 3 µm C18; Phenomenex, Torrance, CA) protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20 µL and the flow rate maintained at 0.6 mL·min^{-1}. The elution solvents were aqueous 0.1% formic acid in double-distilled water (A) and 0.1% formic acid in acetonitrile (B). Samples were eluted according to the linear gradient from 5% to 20% B in the first 15 min followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions (Wang et al., 2002). Phenolics were further identified using a mass spectrometer (LCQ Deca XP MAX; Thermo Scientific) with an electrospray ionization interface operating in negative/positive ion mode using multiple-stage mass spectrometry (MS^n) scanning mode from m/z 115 to 1500. The injection volume was 10 µL and the flow rate maintained at 0.6 mL·min^{-1}. Capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 8 units, respectively, and the source voltage was 4 kV for negative ionization and 0.1 kV for positive ionization. Spectral data were elaborated using Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation. The content of phenolic compounds was assessed from peak areas and quantified with the use of corresponding external standards. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus, galloylquinic acid, methyl gallate hexoside, digalloyl quinic acid, trigalloyl hexose, digalloyl pentose, ellagic acid hexoside, digalloyl glucose isomer, vescalagin, and compounds with a hexahydroxydiphenic (HHDP) moiety were quantified with the calibration curve of ellagic acid, *p*-coumaric acid hexoside, *cis*- and *trans*-5-*O*-*p*-coumaroylquinic acid by *p*-coumaric acid, caffeoyl hexose by caffeic acid, sinapic acid hexoside by sinapic acid, all procyanidins by the standard curve of procyanidin B2, all kaempferol compounds by kaempferol-3-glucoside, and quercetin (Q) compounds (except Q-rutinoside, Q-galactoside, Q-glucoside, Q-rhamnoside, Q-arabinofuranoside, and Q-xyloside) by quercetin-3-galactoside. Anthocyanins were quantified with the standard curves of cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside, respectively. Total anthocyanins, total quercetin and kaempferol glycosides, total flavonols, total phenolic acids, hydrolysable tannins, and flavanols were calculated as the sum of all identified phenolics of the group. All compounds were expressed on a fresh weight basis in micrograms per gram.

Chemicals. The standards used to determine the phenolic compounds in samples were caffeic, ellagic, sinapic and chlorogenic acid (5-caffeoylquinic acid), quercetin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside from Sigma-Aldrich (Steinheim, Germany); and (+)-epicatechin, *p*-coumaric acid, procyanidin B2, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinofuranoside, quercetin-3-*O*-galactoside, and quercetin-3-*O*-rhamnoside from Fluka (Buchs, Switzerland). The chemicals for the sample preparation and mobile phases were methanol, BHT, and acetonitrile from Sigma-Aldrich and formic acid from Fluka. The water used in mobile phase was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

Statistical analysis. The results were analyzed using Statgraphics Plus 4.0 (Manugistics, Rockville, MD) program using one-way analysis of variance. Differences in phenolic content between species/cultivars were estimated with Duncan's multiple range test between means ($P < 0.05$). Multiple-variable analysis with Pearson's correlation coefficient (r) was calculated between color variables a^*, L^*, and C and total and individual anthocyanin content at $P < 0.05$. Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification) was conducted to interpret the differences in secondary metabolites among species and cultivars.

Results and Discussion

Petal color measurements. Color parameter a^* represents the amount of red coloration of plant tissue (Lancaster et al., 1997) and thus increases from white to reddish colored petals. Highest values were measured on the surface of the 'Ulrich Brunner Fils' and 'Rosarium Uetersen' and *R. glauca* petals and lowest on *R. sempervirens* petals (Table 1). Values of color parameter b^*, which indicates yellow (positive values) and blue hues (negative values) (Lancaster et al., 1997), were higher in species and cultivars with (predominantly) white petals (*R. sempervirens*, *R. canina*, and 'Schwanensee') and lower

Table 1. Petal color parameters and anthocyanin content of analyzed rose species and cultivars.

		Color parameter (mean ± SE)				Anthocyanin [mean ± SE (μg·g^{-1} FW)]			
Rosa species/cultivar	Flower color	*a**	*b**	*L**	*h*°	Cy-di-glu[z]	Cy-glu	Pg-di-glu	total AC
R. canina	Pale pink	8.6 ± 0.3 e[y]	4.2 ± 0.1 c	72.3 ± 0.2 e	24.5 ± 0.9 a	19.4 ± 0.9 a	—	5.2 ± 0.5 a	28.6 ± 1.5 a
R. glauca	Pink	49.3 ± 0.7 e	–11.2 ± 0.6 a	36.6 ± 0.5 a	347.0 ± 0.7 c	569.8 ± 8.3 c	26.2 ± 1.8 b	15.4 ± 0.4 b	726.9 ± 11.6 c
R. sempervirens	White	2.9 ± 0.0 a	8.5 ± 0.1 f	76.2 ± 0.2 g	71.3 ± 0.6 b	13.9 ± 0.7 a	—	—	13.9 ± 1.7 a
R. rubiginosa	Pink	35.8 ± 0.9 d	–6.6 ± 0.3 b	55.7 ± 0.6 d	349.7 ± 0.4 d	353.2 ± 8.3 b	31.8 ± 1.4 b	4.8 ± 0.1 a	393.9 ± 8.6 b
Rosarium Uetersen	Pink	50.8 ± 0.4 e	–2.1 ± 0.1 d	49.5 ± 0.3 c	357.3 ± 0.2 f	580.2 ± 20.9 c	48.6 ± 3.4 c	59.0 ± 2.4 c	699.1 ± 24.7 c
Ulrich Brunner Fils	Pink	50.9 ± 0.7 e	–4.6 ± 0.2 c	46.7 ± 0.5 b	354.5 ± 0.5 e	717.2 ± 41.3 d	25.5 ± 1.2 b	—	855.8 ± 45.8 d
Schwanensee	White	6.9 ± 0.2 b	3.9 ± 0.1 c	73.8 ± 0.2 f	23.3 ± 0.6 a	39.7 ± 1.4 a	11.4 ± 1.1 a	—	51.1 ± 2.7 a

[z]Cy-di-glu = cyanidin-3,5-diglucoside; Cy-glu = cyanidin-3-glucoside; Pg-di-glu = pelargonidin-3,5-diglucoside; total AC = sum of all anthocyanins analyzed; — = compound not present in samples.

[y]Different letters (a–g) in rows denote statistically significant differences in color parameters, individual and total anthocyanins by Duncan's multiple range test at $P < 0.05$ among species/cultivars.

in pink-flowered species and cultivars (*R. glauca*, *R. rubiginosa*, and 'Ulrich Brunner Fils'). An inverse relationship between the parameters *a** and *b** was also observed in previous research when comparing flowers of different cultivars of groundcover rose (Schmitzer et al., 2010). Analysis of lightness coefficient (*L**) and hue angle (*h*°) similarly revealed statistically significant differences among studied species and cultivars. The highest values of parameter *L** were observed in flowers of species and cultivars associated with highest *a** value and lowest *b** value (*R. sempervirens*, *R. canina*, and 'Schwanensee'). Flower petals of the latter were also characterized by lowest values of the *h*° parameter.

Anthocyanins and flavonols in rose petals. Anthocyanins are the principal pigments in rose petals attributing intense red to mauve color hues. Flower color investigation of roses so far has shown that four major anthocyanins, 3-glucosides and 3,5-diglucosides of cyanidin (Cy) and peonidin (Pn) can be detected in flowers of wild *Rosa* species and also pelargonidin (Pg) 3-glucoside and Pg-3,5-diglucoside in *Rosa* cultivars (Biolley et al., 1994; Kumar et al., 2008; Mikanagi et al., 1995, 2000). Rarely, Pg-based anthocyanins can be accumulated in *Rosa* plant parts (Cai et al., 2005; Eugster and Märki-Fischer, 1991). In this study two major anthocyanins have been identified in analyzed rose petals, namely Cy-3,5-diglucoside and Cy-3-glucoside [Table 1 (only major anthocyanins presented)], which is in accordance with our earlier studies (Schmitzer et al., 2010). Additionally, Cy-3-rutinoside, Pg-3,5-diglucoside, Pg-3-glucoside, Pn-3,5-diglucoside, and Pn-3-glucoside have been confirmed in petals of specific *Rosa* species or cultivars (Table 2), but their content only accounted several percent of total anthocyanin content level (data not shown). Occurrence of cyanidin, pelargonidin, and peonidin-based anthocyanins has been reported in different red rose cultivars (Biolley et al., 1994; Mikanagi et al., 2000) and higher levels of Cy-3,5-diglucoside quantified in analyzed rose species and cultivars are in agreement with the reported color expression of cyanidins, generally contributing to more intense red hues of plant tissue (Tatsuzawa et al., 2012). Significant differences in anthocyanin composition and content were determined among roses analyzed (Table 1). Highest levels of Cy-3,5-diglucoside, Cy-3-glucoside, and consequently total anthocyanins were quantified in pink-flowering species and cultivars (*R. glauca*, 'Ulrich Brunner Fils', and 'Rosarium Uetersen'). Although the petals of 'Ulrich Brunner Fils' were characterized by highest levels of total anthocyanins, they only accumulated three anthocyanin glycosides compared with the petals of 'Rosarium Uetersen' in which five anthocyanins have been identified. Anthocyanin composition is in tight connection with color expression (Lancaster et al., 1997; Schmitzer et al., 2009) and regression analysis indicated a strong correlation between color parameter *a** and total anthocyanin content (Pearson's correlation coefficient = 0.97, $r^2 = 94.9\%$), similar to the reports of Schmitzer et al. (2010).

Thirty-one flavonols have been detected in rose petals; their content varied significantly among species and cultivars analyzed. As a result of a similar ultraviolet spectrum of individual components of each phenolic group and limited availability of external standards, HPLC–mass spectroscopy was used for reliable peak identification (Table 2). The presence of seven major Q glycosides (Q-rutinoside, Q-glucoside, Q-glucuronide, Q-arabinofuranoside, Q-galactoside, Q-xyloside, and Q-rhamnoside) and five major kaempferol (K) glycosides (K-diglucoside,

Table 2. Phenolic compounds in leaves and petals of *Rosa* species/cultivars, their mass-to-charge ratio (*m/z*) values of the molecular masses and main fragments [second- (MS^2) and third-generation (MS^3) product ion] in positive ($[M-H]^+$), and negative ion mode ($[M-H]^-$) identified with electrospray ionization mass spectrometry (ESI-MS).

Phenolic group	*m/z* $[M-H]^-$ or $[M-H]^+$	(*m/z*) of the main fragments by ESI-MS MS^2 $[M-H]^-$ or $[M-H]^+$	MS^3	Tentative identification	Plant part[y]
Anthocyanins[z]	611	449	287	Cyanidin-3,5-diglucoside	P
	449	287		Cyanidin-3-glucoside	P
	595	449	287	Cyanidin-3-rutinoside	P
	595	433	271	Pelargonidin-3,5-diglucoside	P
	433	271		Pelargonidin-3-glucoside	P
	625	463	301	Peonidin-3,5-diglucoside	P
	463	301		Peonidin-3-glucoside	P
Flavonols	609	301		Quercetin-3-rutinoside	PL
	463	301		Quercetin-3-galactoside	PL
	463	301		Quercetin-3-glucoside	PL
	477	301		Quercetin-3-glucuronide	PL
	433	301		Quercetin-3-arabinopyranoside	L
	433	301		Quercetin-3-arabinofuranoside	PL
	433	301		Quercetin-3-xyloside	PL
	447	301		Quercetin-3-rhamnoside	PL
	609	463, 447	301	Quercetin-hexoside-rhamnoside 1-2	PL
	615	463	301	Quercetin-galloylhexoside 1-5	PL
	585	433	301	Quercetin-galloylpentoside 1-3	L
	599	447	301	Quercetin-galloylrhamnoside	L
	625	463	301	Quercetin-dihexoside	P
	651	609, 447	301	Quercetin-acetyl-hexoside-rhamnoside	P
	595	433	301	Quercetin-hexosyl-pentoside	P
	609	447	285	Kaempferol-di-hexoside	P
	431	285		Kaempferol-3-rhamnoside	PL
	593	285		Kaempferol-3-rutinoside	L
	447	285		Kaempferol-3-galactoside	P
	447	285		Kaempferol-3-glucoside	PL
	461	285		Kaempferol-3-glucuronide	PL
	489	285		Kaempferol-acetylglucoside	PL
	599	447, 285		Kaempferol-galloylhexoside 1-2	PL
	417	285		Kaempferol-pentoside 1-3	PL
	579	447	285	Kaempferol-pentoside-hexoside	P
	593	447	285	Kaempferol-rhamnoside-hexoside 1-4	P
	635	593, 431	285	Kaempferol-acetyl-hexoside-rhamnoside	P
	447	315		Isorhamnetin-3-arabinoside	L
	461	315		Isorhamnetin-3-rhamnoside	L
	493	317		Myricetin-3-glucuronide	L
	463	317		Myricetin-3-rhamnoside	L
Flavanols	289	245		Catechin	L
	289	245		Epicatechin	L
	577	451, 425, 407, 289,		Procyanidin dimer 1-5	L
	865	577, 695, 451, 425, 407, 289		Procyanidin trimer 1-6	L
	1153	865, 695, 575, 577, 407, 289		Procyanidin tetramer 1-2	L
Phenolic acids and their derivatives	353	191, 179		3-caffeoylquinic acid	L
	353	173, 179		*cis*-5-caffeoylquinic acid	L
	353	191, 179		*trans*-5-caffeoylquinic acid	L
	325	163		*p*-coumaric acid hexoside	L
	341	179		Caffeoyl hexose	L
	353	173, 179		4-caffeoylquinic acid	L
	385	223, 205, 152		Sinapic acid hexoside	L
	337	191, 163		*cis*-5-*p*-coumaroylquinic acid	L

Continued next page

Table 2. Continued.

Phenolic group	*m/z* [M-H]⁻ or [M-H]⁺	(*m/z*) of the main fragments by ESI-MS MS² [M-H] or [M-H]⁺	MS³	Tentative identification	Plant part[y]
	337	191, 163		*trans*-5-*p*-coumaroylquinic acid	L
	301	257, 229, 185		Ellagic acid	L
	345	183		Methyl gallate hexoside	L
	463	301		Ellagic acid hexoside	L
	433	301		Ellagic acid pentoside 1-2	L
Hydrolysable tannins	343	191, 169		Galloyl quinic acid	L
	495	343	191, 169	Digalloyl quinic acid	L
	635	483, 465, 423, 271, 193		Trigalloyl hexose 1-2	L
	453	313, 327, 285, 169		Digalloyl pentose	L
	483	439, 331, 313, 271, 169	313, 287	Digalloyl glucose isomer	L
	783	481, 301, 275	257, 229	Di-HHDP glucose 1-2	L
	785	633, 615, 419, 301, 275	257	HHDP digalloyl glucose isomer 1-3	L
	933	631, 451, 301		Vescalagin isomer 1-4	L
	935	633, 301		Galloyl bis HHDP glucose	L
	937	767, 785, 465, 741, 635, 301		Trigalloyl HHDP glucose 1-2	L

[z]Anthocyanins were obtained in the positive ion mode ([M-H]⁺), other phenolic groups in negative ion mode ([M-H]⁻).
[y]Plant part (in which individual compound was determined): P = petals; L = leaves; PL = petals and leaves.
HHDP = hexahydroxydiphenoyl.

K-rhamnoside, K-glucoside, K-glucuronide, and K-galactoside) in addition to an abundance of species/cultivar specific flavonols was confirmed (Table 3; content levels of major flavonols are represented) in rose petals comparable to previous research (Cai et al., 2005; Kumar et al., 2009; Mikanagi et al., 2000).

The highest content of total quercetin glycosides was determined in flowers of 'Ulrich Brunner Fils' and the two major quercetin glycosides identified in the petals were Q-arabinofuranoside and Q-galactoside. In other *Rosa* species and cultivars, the abundance of specific quercetin glycosides varied greatly; Q-glucoside was the major quercetin in petals of *R. canina*, *R. sempervirens*, and *R. rubiginosa*, and Q-rhamnoside in petals of *R. glauca*. Other authors similarly report high levels of quercetin glucoside, arabinoside, rhamnoside, and other glycosides in

Table 3. Flavonols in petals of *Rosa* species and cultivars.

	Rosa species/cultivar						
	R. canina	*R. glauca*	*R. sempervirens*	*R. rubiginosa*	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
Flavonol[z]	[mean ± SE (μg·g⁻¹ FW)]						
1	—	37.6 ± 1.3 a[y]	—	502.7 ± 10.8 d	—	164.2 ± 12.1 b	344.9 ± 6.2 c
2	629.0 ± 9.7 d	259.4 ± 3.6 b	372.8 ± 7.1 c	775.0 ± 12.9 e	12.6 ± 0.4 a	846.8 ± 44.2 f	353.9 ± 1.5 c
3	309.4 ± 7.2 c	92.5 ± 1.8 b	—	—	5.5 ± 0.2 a	93.1 ± 1.9 b	—
4	115.5 ± 0.8 a	178.0 ± 2.2 a	112.9 ± 1.2 a	—	—	1279.7 ± 47.4 c	906.3 ± 22.7 b
5	62.4 ± 1.9 a	—	—	17.4 ± 0.3 a	—	1480.2 ± 41.0 c	207.1 ± 1.8 b
6	17.0 ± 0.9 a	—	16.1 ± 0.6 a	112.4 ± 5.3 c	—	139.9 ± 5.8 d	69.5 ± 1.5 b
7	438.7 ± 5.0 e	573.8 ± 16.3 f	131.7 ± 9.7 b	302.8 ± 3.7 c	37.9 ± 1.7 a	455.4 ± 7.8 e	410.7 ± 5.2 d
8	333.8 ± 3.8 d	126.9 ± 0.6 b	—	51.4 ± 1.7 a	217.3 ± 7.7 c	—	—
9	273.6 ± 4.9 c	249.6 ± 1.3 b	407.6 ± 1.7 d	521.9 ± 10.4 f	123.9 ± 6.1 a	113.9 ± 3.3 a	439.0 ± 5.8 e
10	273.5 ± 1.8 c	40.8 ± 0.5 a	2607.6 ± 27.7 f	2463.0 ± 14.3 e	42.3 ± 1.7 a	130.1 ± 4.8 b	973.7 ± 24.4 d
11	182.0 ± 4.2 c	—	—	—	13.7 ± 0.7 a	22.9 ± 1.4 b	—
12	—	—	35.3 ± 1.4 a	44.5 ± 0.6 b	—	99.4 ± 2.1 c	260.2 ± 5.3 d
Total Q	2125.7 ± 19.4 d	1646.6 ± 16.0 c	684.1 ± 17.6 b	1834.3 ± 21.0 c	90.9 ± 3.0 a	5086.8 ± 170.8 f	2361.9 ± 32.2 e
Total K	1211.9 ± 9.9 c	576.4 ± 9.3 a	3875.8 ± 40.6 e	6512.9 ± 43.3 f	574.6 ± 22.8 a	1040.8 ± 60.1 b	3033.1 ± 52.3 d
% Q	63.7 ± 0.2 e	74.1 ± 0.4 f	15.0 ± 0.3 b	22.0 ± 0.1 c	13.7 ± 0.2 a	83.0 ± 0.8 g	43.8 ± 0.2 d
% K	36.3 ± 0.2 c	25.9 ± 0.4 b	85.0 ± 0.3 f	78.0 ± 0.1 e	86.3 ± 0.2 g	17.0 ± 0.8 a	56.2 ± 0.2 d

[z]Flavonol, quercetin (Q) compounds (1–7): 1 = Q-3-rutinoside, 2 = Q-3-glucoside, 3 = Q-3-glucuronide, 4 = Q-3-arabinofuranoside, 5 = Q-3-galactoside, 6 = Q-3-xyloside, 7 = Q-3-rhamnoside; kaempferol (K) compounds (8–12): 8 = K-di-hexoside, 9 = K-3-rhamnoside, 10 = K-3-glucoside, 11 = K-3-glucuronide, 12 = K-3-galactoside; total Q = sum of quercetin compounds; total K = sum of kaempferol compounds; % Q = percent of total quercetin compounds among flavonols; % K = percent of total kaempferol compounds among flavonols, — = compound not present in samples.
[y]Different letters (a–g) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.
FW = fresh weight.

petals of different rose species (Barros et al., 2013; Kumar et al., 2009; Schieber et al., 2005).

Based on their fragmentation pattern and ultraviolet spectral data, 17 compounds have been tentatively identified as kaempferol glycosides in rose petals (Table 2). Most species contained high levels of K-glucoside, which is in accordance with the results of Schieber et al. (2005) who investigated phenolic compounds in *R. damascena* flowers and Barros et al. (2013) who identified phenolics in *R. micrantha* flower petals. Several species and cultivars in this study also contained high levels of K-rhamnoside similar to *R. damascena*, *R. bourboniana*, and *R. brunonii* petal profiling reported by Kumar et al. (2009). In quantitative terms, the species with the highest total kaempferol content was *R. rubiginosa*; its petals contained predominantly K-glucoside and K-rhamnoside previously identified in petals of *R. damascena* (Schieber et al., 2005) and *R. micrantha* (Barros et al., 2013). Contrary, the petals of *R. glauca* and 'Rosarium Uetersen' contained less than one-tenth the level of total kaempferol glycosides compared with *R. rubiginosa*.

Leaf phenolic composition. Thirty different flavonols have been tentatively identified in leaves of different rose species and cultivars (Table 2). MS^n confirmed the presence of the prevailing quercetin and kaempferol glycosides and in some species/cultivars also isorhamnetin and myricetin glycosides (*R. canina*, *R. sempervirens*, and 'Schwanensee'). Porter et al. (2012) correspondingly reported an abundance of kaempferol and quercetin glycosides in leaves of *R. spinosissima* and quercetin glycosides have been detected in leaves of *R. rugosa* (Hashidoko, 1996) and *R. sericea* (Li et al., 2013). Nowak and Gawlik-Dziki (2007) measured total leaf phenolic content, and quercetin and kaempferol levels of different *Rosa* species including *R. canina* and *R. rubiginosa* but did not study their specific chemical composition. The same applies to research on *R. sempervirens* and *R. canina* leaves by Ghazghazi et al. (2010, 2012) and thus the identification of individual components in leaves of selected *Rosa* species was not comprehensive. In the present research, seven major quercetin glycosides have been identified in rose leaves (Q-arabinofuranoside, Q-galactoside, Q-glucoside, Q-glucuronide, Q-rhamnoside, Q-rutinoside, Q-xyloside). Q-arabinofuranoside, Q-xyloside, and Q-rhamnoside have been determined in all studied species and cultivars. Particularly high levels of the latter were characteristic for *R. sempervirens* and *R. canina* leaves (Table 4). Shetty et al. (2011) similarly detected several quercetin and kaempferol glycosides in leaves of *R. hybrida* 'Smart' asserting Q-rutinoside and Q-rhamnoside occurred in highest concentrations.

Three predominant kaempferol glycosides (K-rhamnoside, K-glucuronide, K-glucoside) have been determined in leaves of selected rose species and cultivars (Table 4) in addition to five minor kaempferol compounds (Table 2). Generally, the content of K-glycosides in rose leaves was considerably lower compared with petals and only K-rhamnoside was present in leaves of all studied species and cultivars. Shetty et al. (2011) similarly identified this glycoside as the prevalent kaempferol in *R. hybrida* leaves. The highest content of K-rhamnoside was again determined in *R. canina* leaves, which was correspondingly the species with the highest content of total kaempferol, quercetin, and flavonol glycosides (Fig. 1A). High levels of K-rhamnoside were also detected in 'Ulrich Brunner Fils' and *R. rubiginosa*. The leaves of 'Schwanensee' contained characteristically low levels of total kaempferol and also quercetin glycosides. The percentage of total quercetin and total kaempferol glycosides varied among the studied species and cultivars (Table 4) but generally, quercetin glycosides were the predominant flavonols in most rose leaves, which is in accordance with the research of Nowak and Gawlik-Dziki (2007) on different *Rosa* species and cultivars.

Table 4. Flavonols in leaves of *Rosa* species and cultivars.

	Rosa species/cultivar						
	R. canina	*R. glauca*	*R. sempervirens*	*R. rubiginosa*	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
Flavonol[z]	[mean ± SE (μg·g^{-1} FW)]						
1	330.7 ± 18.5 c[y]	62.0 ± 5.5 a	377.3 ± 14.1 d	48.6 ± 1.7 a	26.9 ± 1.8 a	334.2 ± 21.6 c	135.5 ± 11.4 b
2	194.9 ± 13.3 b	—	—	292.3 ± 6.8 c	—	194.4 ± 17.4 b	114.4 ± 11.4 a
3	186.7 ± 9.9 bc	54.8 ± 8.4 a	191.0 ± 16.8 c	325.9 ± 6.4 d	80.9 ± 5.7 a	148.7 ± 8.9 b	88.5 ± 7.3 a
4	411.8 ± 4.2 e	56.8 ± 7.7 b	—	316.0 ± 8.0 d	108.1 ± 9.1 c	121.5 ± 8.5 c	18.1 ± 2.5 a
5	2601.8 ± 267.6 d	1428.2 ± 127.1 c	2909.8 ± 118.7 d	1002.4 ± 51.9 b	1021.6 ± 115.7 bc	545.9 ± 59.9 a	175.4 ± 19.8 a
6	37.1 ± 3.6 b	—	—	73.5 ± 3.6 c	5.6 ± 0.4 a	25.9 ± 2.9 ab	172.8 ± 16.7 d
7	125.9 ± 14.4 c	12.2 ± 1.1 a	51.9 ± 6.0 b	14.8 ± 0.6 a	10.6 ± 1.4 a	53.5 ± 4.6 b	48.2 ± 1.3 b
8	160.3 ± 36.0 e	84.9 ± 3.1 bc	88.2 ± 10.4 cd	133.0 ± 5.6 de	29.8 ± 5.4 a	140.5 ± 13.5 e	39.2 ± 3.2 ab
9	158.9 ± 32.4 b	—	—	71.0 ± 2.2 a	90.4 ± 8.2 a	—	—
10	—	—	—	93.5 ± 3.3 b	16.9 ± 2.4 a	—	—
Total Q	4201.1 ± 80.8 e	1515.4 ± 149.5 b	3651.3 ± 168.5 d	2449.9 ± 96.0 c	1451.1 ± 125.3 b	1699.3 ± 128.6 b	776.8 ± 77.6 a
Total K	473.2 ± 34.7 d	157.5 ± 14.6 b	140.9 ± 11.6 b	294.8 ± 10.6 c	132.8 ± 12.2 b	168.6 ± 16.5 b	50.9 ± 4.2 a
% Q	90.9 ± 1.0 b	91.3 ± 0.7 b	96.3 ± 0.3d	89.2 ± 0.3 a	91.8 ± 0.5 b	90.4 ± 0.1 b	93.8 ± 0.2 c
% K	9.1 ± 0.1 c	8.7 ± 0.7 c	3.7 ± 0.2 a	10.8 ± 0.3 d	8.2 ± 0.5 c	9.6 ± 0.1 c	6.2 ± 0.2 b

[z]Flavonol, quercetin (Q) compounds (1–7): 1 = Q-3-arabinofuranoside, 2 = Q-3-galactoside, 3 = Q-3-glucoside, 4 = Q-3-glucuronide, 5 = Q-3-rhamnoside, 6 = Q-3-rutinoside, 7 = Q-3-xyloside; kaempferol (K) compounds (8–10): 8 = K-3-rhamnoside, 9 = K-3-glucuronide, 10 = K-3-glucoside; total Q = sum of quercetin compounds; total K = sum of kaempferol compounds; % Q = percent of total quercetin compounds among flavonols; % K = percent of total kaempferol compounds among flavonols; — = compound not present in samples.

[y]Different letters (a–e) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.

FW = fresh weight.

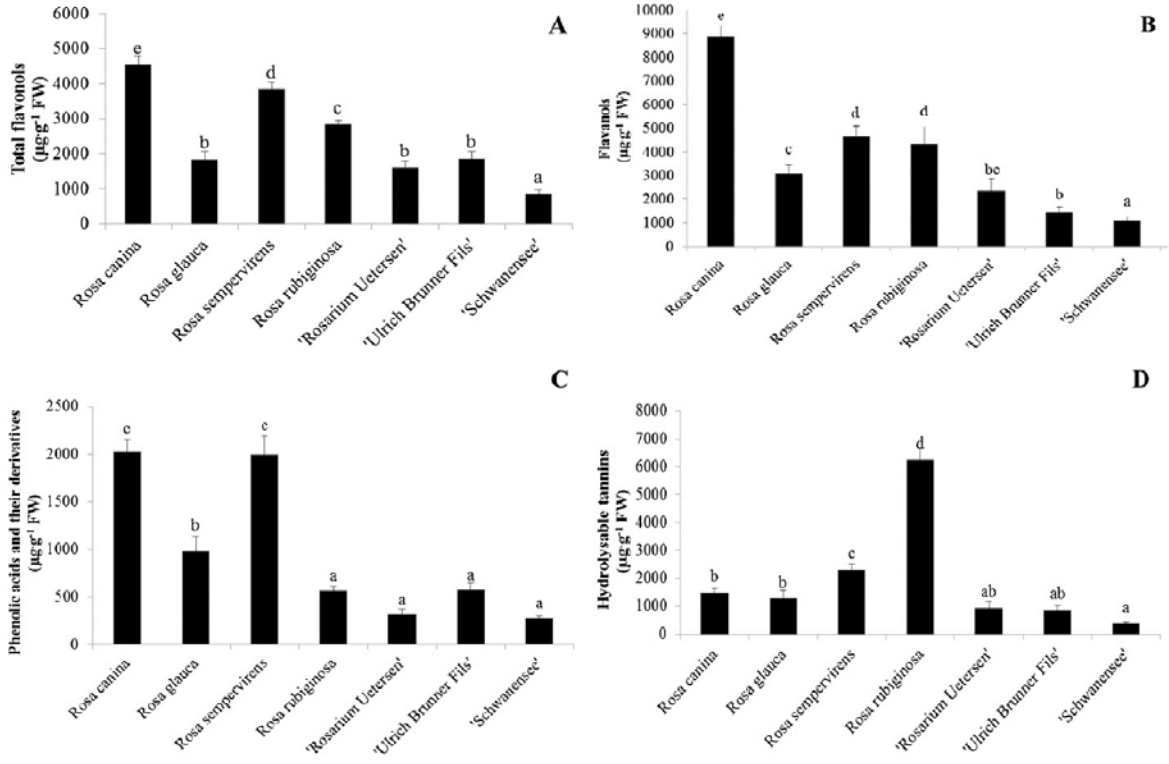

Fig. 1. Content of total flavonols (**A**), flavanols (**B**), phenolic acids and their derivatives (**C**), and hydrolysable tannins (**D**) in leaves of *Rosa* species and cultivars. Bars represent SE. Different letters (**a**–**e**) within individual phenolic groups denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.

In addition to flavonols, an abundance of flavanols, phenolic acids, and their derivatives and hydrolysable tannins were determined in leaves of selected *Rosa* species and cultivars using MS^n (Table 2). Rosaceae can generally be characterized as a family rich in catechin and proanthocyanidin secondary metabolites (Hoffmann et al., 2012). From the group of flavanols, catechin was detected in all studied species and cultivars (Table 5) with the highest content measured in leaves of *R. canina* and the lowest in leaves of 'Schwanensee'. Oppositely, epicatechin could only be identified in 'Schwanensee' leaves. Catechin and epicatechin have previously been determined in extracts of *R. damascena* leaves and different organs of *R. rugosa* (Hashidoko, 1996). High levels of catechin were recently also quantified in root tips of *R.* ×*hybrida* (Hoffmann et al., 2012) and in *R. micrantha* flowers (Barros et al., 2013). According to Baydar and Baydar (2013), catechin and epicatechin represent the most important phenolic constituents of *R. damascena* leaves. *R. canina* leaves contained several procyanidin di- and trimers, and in *R. canina*, *R. glauca*, and *R. sempervirens,* procyanidin tetramers have also been

Table 5. Flavanols in leaves of *Rosa* species and cultivars.

	Rosa species/cultivar						
	R. canina	*R. glauca*	*R. sempervirens*	*R. rubiginosa*	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
Flavanol[z]	[mean ± SE (µg·g⁻¹ FW)]						
1	3655.1 ± 211.6 d[y]	1592.0 ± 210.4 c	1485.4 ± 147.5 c	1215.3 ± 255.0 c	1151.3 ± 272.5 bc	620.0 ± 116.2 ab	517.6 ± 45.2 a
2	1506.2 ± 70.4 c	598.6 ± 61.6 b	664.3 ± 57.9 b	1502.7 ± 246.8 c	176.2 ± 32.8 a	123.8 ± 12.5a	131.5 ± 19.8 a
3	3534.4 ± 180.8 e	750.8 ± 74.6 ab	2336.9 ± 186.8 d	1398.3 ± 224.0 c	1026.5 ± 209.3 bc	697.2 ± 70.5 ab	386.4 ± 58.2 a
4	171.8 ± 14.3 a	151.5 ± 21.0 a	196.0 ± 18.1a	—	—	—	—
5	—	—	—	—	—	—	76.1 ± 10.7

[z]Flavanol: 1 = sum of procyanidin dimers 1, 2, 3, 4, 5; 2 = sum of procyanidin trimers 1, 2, 3, 4, 5, 6; 3 = catechin; 4 = sum of procyanidin tetramers 1, 2; 5 = epicatechin; — = compound not present in samples.
[y]Different letters (a–c) within specific groups of phenolic compounds denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.
FW = fresh weight.

determined. Generally, the rose species in this study contained significantly higher levels of procyanidins and were also richer in total flavanol content compared with the studied cultivars (Table 5; Fig. 1B).

Similar to the flavanol content, diversity and content of phenolic acid (and their derivatives) varied significantly among analyzed species and cultivars (Table 6; Fig. 1C). *Cis*- and *trans*-5-caffeoylquinic acid (chlorogenic acid) have been determined in all studied species and cultivars. Shetty et al. (2011) also detected chlorogenic, neochlorogenic, and an unknown phenolic acid in leaves of *R. hybrida* 'Smart' and reported that chlorogenic acid was the most abundant phenolic acid present. Comparatively, this is in accordance with our results because *cis*-5-caffeoylquinic acid content was highest among all phenolic acids in *R. canina*, *R. glauca*, *R. rubiginosa*, and 'Rosarium Uetersen' leaves; in 'Ulrich Brunner Fils', the predominant phenolic acid was 3-caffeoylquinic acid (neochlorogenic acid). In contrast, in leaves of *R. sempervirens*, ellagic acid was the main phenolic acid constituent and 'Schwanensee' was characterized by high content levels of ellagic acid pentosides.

More than eight minor phenolic acids were tentatively identified in analyzed rose leaves (Table 2). Similarly, Baydar and Baydar (2013) also report low levels of different phenolic acids such as caffeic, chlorogenic, *p*-coumaric, ferulic, and gallic acids in leaves of *R. damascena*.

Twenty hydrolysable tannins have been tentatively identified in rose leaves (Table 2). Despite the research of Miyasaki et al. (2013) who reported ellagic acid as the most important active compound of *R. rugosa* extracts, free ellagic acid was only identified in leaves of *R. sempervirens*, *R. glauca*, and 'Rosarium Uetersen' (Table 6). In leaves of the latter two, it did not represent the major constituent of their leaves. However, numerous conjugated forms and isomers of ellagic acid have been determined in *Rosa* leaves in the present study, many reported for the first time (Tables 2 and 7). Most importantly, hydrolysable tannins (ellagitannins) have been determined in leaves of selected *Rosa* species and cultivars consisting of one or more HHDP moieties esterified to a polyol, usually glucose (Haslam, 1996; Koponen et al., 2007). They are important in plant physiology because they provide protection against microbial decay. This ability is linked to their characteristic feature to form strong complexes with proteins and polysaccharides and consequently inhibiting microbial growth (Ascacio-Valdés et al., 2011). Ellagitannins are widely distributed in the Hamamelidae, Dilleniidae, and Rosidae and have frequently been used as chemotaxonomic markers (Haslam, 1996). As a result of their strong antioxidant properties, they are also important in the human diet, especially in the prevention of degenerative diseases (Ascacio-Valdés et al., 2011; Haslam, 1996; Sroka, 2005). The species with the highest total hydrolysable tannin content was *R. rubiginosa* (Fig. 1D), in which the highest levels of HHDP digalloyl glucose isomers, galloyl bis HHDP glucose and trigalloyl HHDP hexose, have been measured. Along with HHDP digalloyl glucose, also di-HHDP glucose isomers and vescalagin isomers have been determined in all studied species and cultivars. The latter was highest in 'Rosarium Uetersen'. The lowest total hydrolysable tannin content was determined in 'Schwanensee'.

Multivariate statistical analysis clustered the analyzed rose species and cultivars into three distinct groups (Fig. 2). Leaf phenolic profile was the principal classification factor and *R. canina* stood out as the species with the highest content and abundance of most phenolic compounds. This is in accordance with the reports of Nowak and Gawlik-Dziki (2007) who also measured highest levels of phenolics in different *R. canina* cultivars. *R. sempervirens* and *R. rubiginosa* represented the second group based on their high content of free and conjugated forms of ellagic acid, flavanols, and phenolic acids and were thus closest to *R. canina*. *R. glauca* was the most

Table 6. Phenolic acids and their derivatives in leaves of *Rosa* species and cultivars.

Phenolic acids and their derivatives[z]	*Rosa* species/cultivar						
	R. canina	*R. glauca*	*R. sempervirens*	*R. rubiginosa*	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean ± SE (μg·g^{-1} FW)]						
1	—	118.2 ± 20.3	—	—	—	—	—
2	—	—	—	—	58.4 ± 15.0 b	—	28.3 ± 2.4 a
3	473.0 ± 46.2 c[y]	182.9 ± 31.4 a	433.6 ± 37.5 c	154.3 ± 11.0 a	—	306.1 ± 53.5 b	—
4	24.5 ± 2.4	—	—	—	—	—	—
5	1120.9 ± 57.4 c	283.6 ± 28.2 b	130.8 ± 10.5 a	298.2 ± 26.2 b	91.0 ± 18.5 a	104.0 ± 10.5 a	57.7 ± 8.7 a
6	50.0 ± 4.1 d	14.2 ± 2.8 b	29.6 ± 4.5 c				1.1 ± 0.1 a
7	—	—	82.2 ± 12.5 b	—	—	25.3 ± 2.5 a	20.5 ± 2.5 a
8	97.3 ± 8.1 b	108.8 ± 18.2 b	105.0 ± 8.4 b	97.7 ± 5.7 b	26.3 ± 5.0 a	38.8 ± 5.5 a	13.9 ± 1.8 a
9	6.0 ± 0.3 a	9.0 ± 1.5 b	17.1 ± 1.1 c	11.2 ± 0.6 b	4.4 ± 1.0 a	—	8.9 ± 0.9 b
10	68.2 ± 8.7 c	23.5 ± 7.0 b	6.9 ± 1.4 ab	7.0 ± 0.6 ab	24.5 ± 7.8 b	—	3.3 ± 0.5 a
11	—	—	—	—	—	—	20.8 ± 3.1
12	183.9 ± 7.1 b	110.0 ± 19.1 ab	527.2 ± 68.3 c	—	63.1 ± 7.3a	97.9 ± 4.0 ab	125.0 ± 13.5 ab
13	—	135.9 ± 24.6 a	667.7 ± 55.1 b	—	47.5 ± 9.3 a	—	—

[z]Phenolic acid and its derivative: 1 = methyl gallate hexoside; 2 = *p*-coumaric acid hexoside; 3 = 3-caffeoylquinnic acid; 4 = caffeoyl hexose; 5 = *cis*-5-caffeoylquinnic acid; 6 = 4-caffeoylquinnic acid; 7 = sinapic acid hexoside; 8 = *trans*-5-caffeoylquinnic acid; 9 = *cis*-5-*O*-*p*-coumaroylquinnic acid; 10 = *trans*-5-*O*-*p*-coumaroylquinnic acid; 11 = ellagic acid hexoside; 12 = sum of ellagic acid pentoside 1, 2; 13 = ellagic acid.; — = compound not present in samples.

[y]Different letters (a–c) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.

FW = fresh weight.

Table 7. Hydrolysable tannins in leaves of *Rosa* species and cultivars.

	Rosa species/cultivar						
Hydrolysable tannins[z]	*R. canina*	*R. glauca*	*R. sempervirens*	*R. rubiginosa*	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean ± SE (µg·g^{-1} FW)]						
1	109.7 ± 8.3 bcd[y]	92.1 ± 13.6 bc	155.4 ± 14.3 d	104.5 ± 6.6 bcd	67.6 ± 18.0 ab	137.2 ± 32.4 cd	31.0 ± 2.0 a
2	266.3 ± 18.1 d	197.0 ± 46.7 bcd	210.1 ± 27.0 cd	692.0 ± 44.2 e	110.8 ± 19.0 ab	154.1 ± 14.4 bc	41.0 ± 3.4 a
3	289.2 ± 38.0 b	288.6 ± 75.9 b	66.5 ± 9.7 a	117.3 ± 6.4 a	324.9 ± 91.5 b	97.9 ± 26.8 a	20.1 ± 2.1 a
4	92.3 ± 9.8 ab	171.5 ± 40.9 c	—	527.7 ± 21.8 d	121.8 ± 29.4 bc	35.4 ± 7.4 a	32.6 ± 6.1 a
5	381.0 ± 52.5 ab	313.8 ± 80.3 ab	670.9 ± 58.5 b	3375.5 ± 288.0 c	119.4 ± 29.2 a	156.9 ± 34.8 a	129.6 ± 17.6 a
6	354.8 ± 22.9 b	172.8 ± 30.6 a	395.4 ± 41.8 b	655.0 ± 29.4 c	161.8 ± 28.0 a	153.7 ± 26.5 a	115.2 ± 14.5 a
7	—	—	695.1 ± 53.0 b	313.5 ± 20.0 a	—	—	—
8	—	—	—	328.8 ± 21.3 b	—	95.3 ± 13.7 a	—
9	—	38.5 ± 5.4 a	114.9 ± 8.0 b	138.4 ± 7.3 c	37.2 ± 9.4 a	—	—
10	—	—	—	—	—	31.0 ± 7.4	—

[z]Hydrolysable tannin: 1 = sum of di-HHDP glucose 1, 2; 2 = sum of HHDP digalloyl glucose isomer 1, 2, 3; 3 = sum of vescalagin isomer 1, 2, 3, 4; 4 = galloyl bis HHDP glucose; 5 = sum of trigalloyl HHDP glucose 1, 2; 6 = galloyl quinic acid; 7 = digalloyl quinic acid; 8 = sum of digalloyl glucose isomer 1, 2, 3; 9 = sum of trigalloyl hexose 1, 2; 10 = digalloyl pentose; — = compound not present in samples.
[y]Different letters (a–e) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at $P < 0.05$.
FW = fresh weight; HHDP = hexahydroxydiphenoyl.

distinct of all species analyzed and grouped in the third cluster along with rose cultivars. However, 'Schwanensee' appeared most dissimilar of all cultivars analyzed as a result of low levels of phenolic constituents measured in its leaves (Tables 4 to 7). This could potentially be linked to its known susceptibility to diseases because phenolic composition and their antioxidant effects reportedly play a role in plants' defense against various stressors (Dixon and Paiva, 1995; Osbourn, 1996; Shetty et al., 2011; Sroka, 2005).

It seems that species are more suitable as a potential source of leaf phenols with antioxidative activity. Traditional practice of *R. canina* selective use for medicinal purposes also appears

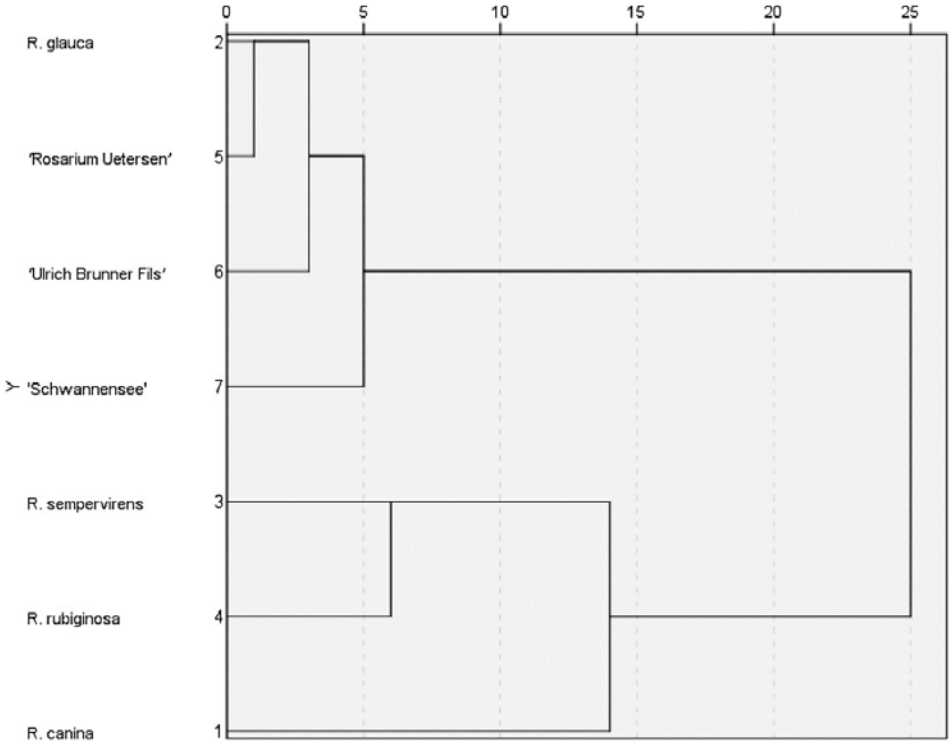

Fig. 2. Dendrogram of *Rosa* species and cultivars using Ward's method based on square Euclidian distance combining biochemical data on petal and leaf phenolic composition.

scientifically justified because it contained significantly more phenolic antioxidants compared with other naturally occurring rose species of the region. Moreover, because specific phenolic compounds display different biological functions in plants themselves, the data on phenolic content in rose species and cultivars may be important in breeding for further research of resistance and susceptibility to plant disease.

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2.1.2 Zmrzal zmanjša vsebnost sladkorjev, askorbinske kisline in nekaterih kvercetin glikozidov ter poveča izbrane karotene v plodovih *Rosa canina*

Frost decreases content of sugars, ascorbic acid and some quercetin glycosides but stimulates selected carotenes in *Rosa canina* hips

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Med zorenjem in po zmrzali smo s tekočinsko kromatografijo visoke ločljivosti v povezavi z masno spektrometrijo (HPLC/MS) identificirali nekatere primarne in sekundarne metabolite v plodovih (šipkih) vrste *Rosa canina*. Šipke smo nabrali šestkrat, od začetka septembra do začetka decembra. Med zorenjem so se barvni parametri *a**, *b**, *L** statistično značilno zmanjšali. Osrednja sladkorja v plodovih sta bila glukoza in fruktoza, ki predstavljata 92 % skupnih sladkorjev, med kislinami pa je prevladovala citronska kislina (predstavljala je do 58 % vseh analiziranih organskih kislin). Vsebnosti skupnih sladkorjev in askorbinske kisline sta se po zmrzali značilno zmanjšali, pri sladkorjih od 42,2 na 25,9 g/100 g suhe mase in pri askorbinski kislini od 716,8 na 176,0 mg/100 g suhe mase. V nasprotju pa se je v šipkih po slani vsebnost β-karotena in likopena povečala na 22,1 oz. na 113,2 mg/100 g suhe mase. Poleg cianidin-3-glukozida (največja vsebnost v šipkih je bila 125,7 µg/100 g suhe mase), smo določili še 45 različnih fenolnih spojin. Od tega je bilo največ procianidinov (njihova vsebnost je predstavljala do 90 % vseh določenh fenolnih spojin), katerih vsebnost se ni značilno spreminjala med dozorevanjem. Vsebnosti katehina, floridzina, flavanonov in več glikozidov kvercetina so bile največje v plodovih nabranih v prvih treh terminih in so se po slani značilno zmanjšale. Podobno se je v vzorcih po zmrzali značilno zmanjšala antioksidativna aktivnost. Vsebnost skupnih fenolov (določena spektrofotometrično) se je v vzorcih do tretjega vzorčenja značilno povečala, nato pa zmanjšala.

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Biochemistry

Frost decreases content of sugars, ascorbic acid and some quercetin glycosides but stimulates selected carotenes in *Rosa canina* hips

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SUMMARY

Primary and secondary metabolites of *Rosa canina* hips were determined by HPLC/MS during ripening and after frost damage. Rose hips were harvested six times from the beginning of September until the beginning of December. Color parameters a^*, b^* and L^* decreased during maturation. Glucose and fructose were the predominant sugars representing up to 92% total sugars, and citric acid was the major organic acid detected in rose hips (constituting up to 58% total organic acids). Total sugar and ascorbic acid content significantly decreased after frost damage: from 42.2 to 25.9 g 100 g^{-1} DW for sugars and from 716.8 to 176.0 mg 100 g^{-1} DW for ascorbic acid. Conversely, β-carotene and lycopene levels increased in frostbitten rose hips to 22.1 and 113.2 mg 100 g^{-1} DW, respectively. In addition to cyanidin-3-glucoside (highest level in hips was 125.7 μg 100 g^{-1} DW), 45 different phenolic compounds have been identified. The most abundant were proanthocyanidins (their levels amounted up to 90% of total flavanol content) and their content showed no significant differences during maturation. The levels of catechin, phloridzin, flavanones and several quercetin glycosides were highest on the first three sampling dates and decreased after frost. Antioxidant capacity similarly decreased in frostbitten rose hips. Total phenolic content increased until the third sampling and decreased on later samplings.

Introduction

Rosa canina L. is a widespread shrub native to Europe and western Asia. It is a common species of dog roses (*Rosa* sect. *Caninae*), a section of *Rosa* most widespread in Central Europe (Wissemann et al., 2006). For centuries rose hips (pseudofruits) of *R. canina* have been used for nutrition, medicinal purposes and their ornamental value. Hips are rarely eaten fresh; usually they are dried and/or processed and consumed in form of tea, nectar, wine or marmalade (Uggla et al., 2005; Yildiz and Alpaslan, 2012).

Rose hips contain significant amounts of biologically active compounds and are considered a particularly rich source of ascorbic acid (Hvattum, 2002; Roman et al., 2013). Vitamin C functions in activation of enzymes, oxidative stress reduction and demonstrates an important immune function. Vitamin C supplementation is known to have a protective effect against several disease conditions, most notably the common cold, cardiovascular disease and some cancers (Schlueter and Johnston, 2011). The antioxidative effects of *R. canina* have not only been ascribed to vitamin C, but also to polyphenolics (Daels-Rakotoarison et al., 2002; Tumbas et al., 2012). The results of several studies indicate that rose hips possess anti-inflammatory properties and might as such be used as a replacement or supplement for conventional drug therapies in some inflammatory diseases such as arthritis (Daels-Rakotoarison et al., 2002; Rein et al., 2004; Winther et al., 2005). Ninomiya et al. (2007) have also observed that aqueous acetone extracts from spurious fruit and seeds of *R. canina* substantially inhibit the gain of body weight and/or weight of visceral fat in mice. Zocca et al. (2011) recommended a potential use of dog rose hip extract in food industry as an anti-browning agent, since it effectively inhibits polyphenol oxidase and tyrosinase activity.

The distinct orange to red color of rose hips is formed as a result of various carotenoids. The most abundant are β-carotene and lycopene, followed by β-cryptoxanthin, rubixanthin, zeaxanthin and lutein (Hodisan et al., 1997; Hornero-Méndez and Mínguez-Mosquera, 2000; Andersson et al., 2011). Carotenoids are an important part of a healthy human diet, as they function as provitamin A and are supposed to prevent certain chronic diseases and even cancer (Mayne, 1996).

Different species of *Rosa* vary in date and duration of hip maturation. In contrast to some other species of small fruit (e.g.

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blackberries, raspberries), rose hips do not abscise from branches when ripe. In order to assure an optimal composition of fruits it is important to know how the content of bioactive compounds changes during fruit maturation. An old Slovenian tradition claims that one is supposed to harvest rose hips after they have been subjected to frost. But do cold temperatures really positively affect the content of bioactive compounds in rose hips? The aim of the present study is to, for the first time, identify and quantify different primary and secondary metabolites in *R. canina* hips during ripening and analyze the impact of frost damage on rose fruit composition. This will provide better information to determine the right harvesting period to optimize the content of bioactive compounds and primary metabolites in *R. canina* hips.

Materials and methods

Plant material

Rosa canina rose hips were harvested from bushes growing in nature in the vicinity of Biotechnical Faculty (central Slovenia, lat. 46,18°N, long. 14,61°E, altitude 250 m) every two weeks, from the beginning of September until the beginning of November and once again in early December when they were subjected to frost. All together there were six sampling dates. For each harvest period, a mixed sample of approximately 75 hips was collected. Plants were identified by morphological key characteristics described in the Mala flora Slovenije (Martinčič et al., 2007). Voucher specimens are deposited in the herbarium of the Chair for Fruit, Wine and Vegetable Growing, Department of Agronomy, Biotechnical Faculty, University of Ljubljana. The material was transported to the laboratory facility and seeds (achenes) and calyces were removed prior to the analysis. The analysis for vitamin C and carotenoid content was performed immediately on fresh pericarp. At this point samples for dry weight analysis were taken. The rest of the sample was frozen in liquid nitrogen and stored at −20 °C until further analysis of phenolic compounds, organic acids and sugars.

Rose hip color measurements

Rose hip color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In CIE $L^* a^* b^*$ system of color representation, the L^* value corresponds to a dark-bright scale and represents the relative lightness with a range from 0 to 100 (0 = black, 100 = white). Color parameters a^* and b^* extend from −60 to 60; a^* negative is for green and a^* positive is for red and b^* negative is for blue and positive for yellow. The hue angle (h°) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 270° = blue. 30 rose hips were measured per sampling date.

Dry matter content determination

Fresh weight (FW) was recorded immediately after harvest after seed removal. Dry weight (DW) was recorded after drying at 105 °C for 48 h in an electrical oven until constant weight was achieved. Five replicates per sampling date were taken, each included approximately five hips. Dry matter (DM) content was determined as the percentage of total pericarp weight (DW × 100/FW) by weighing samples before and after drying.

Determination of sugars, organic acids and vitamin C using high-performance liquid chromatography (HPLC)

Extraction of sugars and organic acids was carried out as reported by Mikulic-Petkovsek et al. (2012) with some modifications. A mixed sample of rosehip pericarp (five replicates per sampling date) was ground to a paste in a mortar and 2.5 g were extracted with 12.5 mL of double distilled water. Extraction of vitamin C was carried out using the same procedure except that 1 g of the paste was extracted with 5 mL of 2% metaphosphoric acid. Samples were left at room temperature for 30 min on an orbital shaking platform (Grant-Bio POS-300, Grant Instruments, Shepreth, England), centrifuged (Eppendorf 5810 R Centrifuge, Hamburg, Germany) at 12,000g_n for 5 min at 4 °C and filtered through a Cromafil A-20/25 cellulose mixed ester filter (Macherey-Nagel, Düren, Germany) into vials. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA). Chromatographic conditions used for determination of sugars and organic acids were the same as described by Mikulic-Petkovsek et al. (2012). The chromatographic conditions for ascorbic acid determination were the same as for the organic acids, only the column temperature was set at 20 °C, and the UV detector at 245 nm. Quantification was assessed from peak areas and calculated by the use of a calibration curve of corresponding standards. Concentrations were expressed on a dry weight basis in g or mg per 100 g of pericarp. From the data of individual sugars and individual organic acids, the sums of sugars (total sugars) and organic acids (total acids) were calculated.

Extraction and determination of phenolic compounds using high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS)

The extraction of phenolic compounds from rose hips was carried out as described by Veberic et al. (2014) with some modifications. Rose hips were ground in a mortar (combined samples of five rosehips in five repetitions per sampling date) and 2.5 g of paste was extracted with 4 mL of methanol containing 3% (v/v) formic acid in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000g_n. The supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred to a vial prior to injection into the HPLC system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (phenolic acids and their derivatives, flavanols), 350 nm (flavones, flavonols, flavanones, phloridzin and ellagic acid pentosides) and 530 nm (anthocyanins). The equipment and chromatographic conditions used for HPLC analysis were the same as described by Veberic et al. (2014). Phenolics were further identified using a mass spectrometer (LCQ Deca XP MAX; Thermo Scientific) with an electrospray ionization interface (ESI). For detailed mass spectrometry conditions, see Veberic et al. (2014). The identification of compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation. The content of phenolic compounds was assessed from peak areas and quantified with the use of corresponding external standards.

For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus methylgallate hexoside was quantified with the calibration curve of gallic acid, proanthocyanidins and their glycosides by procyanidin B2, catechin hexoside by catechin, coumaroylquinic acids and *p*-coumaric acid hexoside by *p*-coumaric acid, sinapic acid hexoside by sinapic acid. All apigenin derivatives were quantified in equivalent of apigenin-7-glucoside, naringenin and eriodyctiol hexosides by naringenin, ellagic acid pentosides, methyl gallate acetyl dihexoside, methyl gallate rutinoside and methyl ellagic acid pentoside by ellagic acid, isorhamnetin hexosides by isorhamnetin-3-glucoside and quercetin-3-galactoside by calibration curve of quercetin-3-galactoside. Total flavonols, flavanons, proanthocyanidin (PA) dimer glycosides, PA aglycons, PAs, flavanols and total

phenolic acids and their derivatives were calculated as the sum of all identified phenolics of the group. All compounds were expressed on a dry weight basis in mg per g of rose hips.

Total phenolic content

Total phenolic content (TPC) of rose hip methanolic extract was determined using the Folin-Ciocalteau (FC) reagent (five samples per sampling date, three replicates per sample) as described by Mikulic-Petkovsek et al. (2010). Gallic acid was used as standard and TPC was expressed in mg of gallic acid equivalents (GAE) per g of dry weight.

Evaluation of antioxidant activity with the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method

The same samples were used for the evaluation of antioxidant activity as for the determination of phenolic compounds using HPLC-MS. Free radical scavenging activity of methanolic extracts was analyzed according to the method by Mikulic-Petkovsek et al. (2010) with some modifications. The samples were diluted in methanol (1:100), 50 μL of the extract was placed in 96-well microplates and 200 μL of 0.1 mM 80% methanolic solution of DPPH was added to the extract. All samples were analyzed in triplicate. The DPPH radical scavenging activity of fruit methanolic extracts was expressed as mg of ascorbic acid equivalents per 100 g of dry fruit in 30 min of reaction time. Evaluation of antioxidant capacity of the samples was performed using the standard curve of ascorbic acid. The ability to scavenge the DPPH radical was calculated using the following equation: $\%\ inhibition = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the blank sample and A_1 the absorbance of the sample measured after 30 min. The concentration of methanol extract where absorbance of DPPH decreased by 50% (EC_{50} values) was calculated from graphs depicting % inhibition against different concentrations of samples.

Extraction and HPLC determination of carotenoids

The extraction procedure for carotenoids was similar to the method described by Orazem et al. (2013). 0.3 g of rosehip pericarp was placed in a round-bottomed centrifuge tube, wrapped in aluminum foil to prevent light access, and homogenized with 10 mL of cold ethanol for 3 min using the T-25 Ultra-Turax (IKA®–Labortechnik, Staufen, Germany) at 8400 rpm. Further extraction follows the method described by Orazem et al. (2013).

For HPLC analysis of the carotenoids, the method described by Andersson et al. (2011) was used. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with detection carried out at 458 nm. A HPLC column (150 × 4.6 mm, Gemini 3 μ C18; Phenomenex, Torrance, CA), protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20 μL and the flow rate maintained at 0.7 mL min^{-1}. The elution solvents were A (80% acetonitrile, 15% MeOH, 5% dichloromethane (DCM), v:v) and B (30% acetonitrile, 20% MeOH, 50% DCM, v:v) eluted according to a binary gradient 0% B (0–2 min), 0–25% B (2–15 min), 25–60% B (15–17 min), 60–90% B (17–29 min), 90% B (29–39 min), 90–0% B (39–41 min) and 0% B (41–47 min).

The identification of compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation. Lycopene and β-carotene were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an atmospheric pressure chemical ionization (APCI) operating in positive ion mode. The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 110 to 1500. The injection volume was 10 μL and the flow rate maintained at 0.7 mL min^{-1}. The capillary temperature was 275 °C, the sheath gas and auxiliary gas were 45 and 10 units, respectively; the source voltage was 16 kV. Spectral data were elaborated using the Excalibur software (Thermo Scientific). The content of carotenoid compounds was assessed from peak areas and quantified with the use of β-carotene as a corresponding external standard. For quantification of lycopene and β-carotene unsaponified samples were used, since this process results in their breakdown.

Chemicals

For the determination of sugars and organic acids, the following standards were used: sucrose, fructose, glucose and ascorbic, citric, malic, fumaric, tartaric, shikimic and quinic acid from Sigma-Aldrich Co. (St. Louis, USA).

The standards used to determine the phenolic compounds in samples were *p*-coumaric acid, procyanidin B2, kaempferol-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, phloridzin dehydrate, apigenin-7-glucoside and (+)-catechin, sinapic acid, ellagic acid, quercetin-3-O-rutinoside, cyanidin-3-O-glucoside, (±)-naringenin, quercetin-3-O-arabinopyranoside from Sigma-Aldrich Co. Isorhamnetin-3-glucoside was obtained from Extrasynthese (Genay, France), gallic acid from Merck (Darmstadt, Germany), and quercetin-3-O-arabinofuranoside from Apin Chemicals (Abingdon, UK). The standard used for the determination of carotenoids was β-carotene from Sigma-Aldrich Co. The chemicals for sample preparation and mobile phases were methanol, BHT, acetonitrile and formic acid from Sigma-Aldrich Co. The water was double distilled and purified with a Milli-Q water purification system by Millipore (division of Merck, Darmstadt, Germany). For the extraction and HPLC analysis of carotenoids additional chemicals were used: n-hexane and sodium chloride from Sigma-Aldrich Co. and ethanol and dichloromethane from Merck.

Statistical analysis

The results were analyzed with the Statgraphics Plus 4.0 (Manugistics, Rockville, USA) program using one-way analysis of variance (ANOVA). Results are given as mean values with standard error (SE). Differences in the content of analyzed compounds among sampling dates were estimated with Duncan's multiple range test ($P < 0.05$). Multiple-variable analysis with Pearson's correlation coefficient (r) was calculated between TPC, antioxidant capacity and percent of inhibition and also between color parameters a^* and L^* and anthocyanin content at $P < 0.05$.

Results and discussion

Rose hip color measurements

As reported by Lancaster et al. (1997), positive values of color parameter a^* indicate red hues and negative green color of the sample. Differences in this color parameter were most evident on the second sampling date in late September and on two later samplings in October, when the reddest hips were recorded (Table 1). A decrease of the a^* value was measured on the last sampling after the hips were frostbitten. Similarly, the b^* parameter decreased with ripening as rose hips gradually lost their yellow coloration indicated by positive values of this parameter. The analysis of the lightness coefficient (L^*) revealed significant differences during rose hip ripening and an increased dark color of fruit on the final samplings. Values were highest on the initial sampling in September (43.1) and lowest after hips were frostbitten (28.2).

Table 1
Fruit color parameters, cyanidin-3-glucoside (Cy-3-glu), lycopene and β-carotene content of *R.canina* hips during ripening.

Sampling	Color parameters (mean ± SE)				Cy-3-glu [mean ± SE (μg g^{-1} DW)]	Lycopene [mean ± SE (mg 100 g^{-1} DW)]	β-carotene [mean ± SE (mg 100 g^{-1} DW)]
	a^*	b^*	L^*	h°			
1	47.7 ± 0.5 b[a]	32.5 ± 0.6 e	43.1 ± 0.4 e	34.2 ± 0.6 d	9.2 ± 2.6 a	NA[b]	NA[b]
2	54.8 ± 0.2 d	24.4 ± 0.5 d	35.0 ± 0.3 d	24.0 ± 0.4 c	71.8 ± 7.4 bc	32.9 ± 8.6 a	6.5 ± 1.7 a
3	54.1 ± 0.4 cd	22.1 ± 0.6 c	32.6 ± 0.4 c	22.1 ± 0.4 b	125.7 ± 19.5 d	57.6 ± 2.9 b	12.0 ± 0.4 bc
4	52.1 ± 0.9 cd	18.6 ± 0.6 ab	29.4 ± 0.4 b	19.5 ± 0.31 a	120.2 ± 9.6 d	52.9 ± 4.3 b	8.9 ± 0.6 ab
5	51.1 ± 1.3 c	20.1 ± 0.7 b	28.9 ± 0.4 ab	21.4 ± 0.5 b	110.0 ± 18.0 cd	61.0 ± 5.8 b	12.6 ± 0.7 c
6	41.5 ± 1.7 a	17.9 ± 0.6 a	28.2 ± 0.4 a	23.6 ± 0.5 c	61.0 ± 18.9 b	113.2 ± 4.7 c	22.1 ± 1.4 d

[a] Different letters (a–e) for each color parameter individually denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.
[b] Analysis not performed.

Values of color parameters a^*, b^* and L^* recorded on the first sampling date are comparable to those reported by Ercişli (2007). Similar changes of color parameters have also been observed by Uggla et al. (2005) during fruit ripening of *Rosa dumalis* and *Rosa rubiginosa*. However, they also reported a decrease of hue angle during fruit ripening of these rose species, which was not confirmed in the present study. Hue angle (h°) of *R. canina* hips decreased from the first to the fourth sampling date and increased again on the last two samplings.

Dry matter

Dry matter (DM) content of *R. canina* hips increased significantly over the sampling period (Table 2). The percentage was lowest on the first sampling (26.4%), increased until the fifth sampling (36.5%) and decreased slightly after frost (35.6%). Similar DM values of ripe *R. canina* hips have been reported by Türkben et al. (2010). According to Ercişli (2007), total dry weight content of ripe hips harvested from different rose species ranges between 33.9% and 40.4%. An increase of dry matter content during ripening has also been observed by Uggla et al. (2005) in *R. dumalis* and *R. rubiginosa* hips.

The content of sugars, organic acids and ascorbic acid

Three different sugars have been quantified in *R. canina* rose hips: glucose, fructose and sucrose (Table 2), previously identified by Barros et al. (2010, 2011). These authors also report the presence of trehalose and raffinose in unripe hips (Barros et al., 2011), which could not be detected in our samples. Glucose and fructose were the predominant sugars in ripe rose hips–their content levels increased until the last two samplings. A significant decrease of glucose and fructose levels was observed after the fruit were subjected to frost. The content of sucrose did not change significantly during ripening with the exception of the first sampling in September. Fruit harvested on that sampling contained significantly higher levels of sucrose compared to fruit collected on all other samplings. Total sugar content was highest on the fourth sampling date (late October), although no statistical differences were observed during maturation except on the last sampling; fructose, glucose and total sugar content decreased twofold after hips were frostbitten. Uggla et al. (2005) reported that the content of glucose and fructose in *R. dumalis* and *R. rubiginosa* fruit varies during the harvesting period; however, in both species no significant changes in glucose content have been observed on the last three samplings. Particularly in *R. rubiginosa*, total sugar content increased during the sampling period. The content of glucose and fructose in *R. canina* fruit determined in our study is similar to the values reported by Demir et al. (2014). Barros et al. (2010, 2011) measured significantly lower values but the reported proportion of sugars was similar to our results. Kovács et al. (2000) determined fructose and glucose as the major sugars in *R. canina* hips and very high levels of this sugars in *R. canina* 'Inermis' fruit compared to other investigated *Rosa* species. The difference in the content of specific and total sugars among different studies can be ascribed to environmental factors, geographic origin, genetic variation and different analytical methods.

Generally, the content of ascorbic acid decreased with the progression of maturation, but the decrease was not linear. The highest content was thus determined on the third sampling (935.0 mg 100 g^{-1} DW); however, a significant drop occurred after frost, when rose hips contained only a fifth of the maximum content (176.0 mg 100 g^{-1} DW ascorbic acid). Numerous studies report diverse content levels of ascorbic acid in *R. canina* hips (Ercişli and Eşitken, 2004; Barros et al., 2011; Adamczak et al., 2012). This could be due to differences in the analytical methods, the maturity stage of the examined fruits, environmental conditions, geographic origin and genetic variation. Compared to other investigated rose species/genotypes, *R. canina* contains relatively low levels of ascorbic acid (Kovács et al., 2000; Adamczak et al., 2012). Adamczak et al. (2012) reported an average amount of ascorbic acid in *R. canina* to be 0.5 g 100 g^{-1} DW, similar to the content measured in our study in hips collected on the 4th sampling in late October. Even lower levels of ascorbic acid have been determined in *R. canina* fruits from Portugal: 262.1 mg 100 g^{-1} DW of ascorbic acid were measured in unripe and as little as 68.0 mg 100 g^{-1} DW in fully ripe rose hips (Barros et al., 2011). Contrary, Ercişli and Eşitken (2004) report higher values of ascorbic acid in ripe rose hips ranging from 1074.0 mg 100 g^{-1} to as much as 1586.0 mg 100 g^{-1} but the analytical method is somewhat unclear. Roman et al. (2013) analyzed the composition of *R. canina* biotypes from different altitudes and measured diverse content levels of ascorbic acid in range of 112.2–360.2 mg 100 g^{-1} fresh fruit. These results correspond to data obtained in our study expressed in fresh weight (data not shown). Similar levels of ascorbic acid have been measured in hips of *Rosa micrantha* (Guimarães et al., 2010); specifically, 580.0 mg 100 g^{-1} DW ascorbic acid in unripe hips, 943.9 mg 100 g^{-1} DW in ripening hips and 693.7 mg 100 g^{-1} DW in fully ripe hips. These results suggest that the content of ascorbic acid is at its peak when hips are not yet fully or overly mature.

The following organic acids were determined in rose hips: citric > malic > quinic > tartaric > shikimic > fumaric acid. The content of citric and quinic acids was lowest on the second sampling and no significant differences have been observed among later samplings. Their levels were thus not affected by frost. Tartaric acid was only present in rose hips harvested on the last three samplings and again, no significant differences have been detected among samples. Malic, shikimic and fumaric acids showed greater variation during the ripening period; however, their levels were also lowest on the second sampling in late September. A significant decline of these organic acids was measured in frostbitten rosehips. Little information is available on rose hip organic acid composition and, to the best of our knowledge, no study has monitored how it varies during the maturation. Adamczak et al. (2012) and Kovács et al.

Table 2
Dry matter (DM), sugar, organic acid and ascorbic acid content in *R. canina* hips during ripening.

Sampling	1	2	3	4	5	6
DM [mean (%) ± SE]	26.4 ± 0.5 a[a]	31.2 ± 0.2 b	30.5 ± 0.8 b	31.4 ± 1.5 b	36.5 ± 1.0 c	35.6 ± 1.3 c
SUGARS [mean (g 100 g^{-1} DW) ± SE]						
Sucrose	5.9 ± 0.8 b	4.5 ± 0.3 a	4.5 ± 0.2 a	4.0 ± 0.1 a	4.2 ± 0.3 a	4.1 ± 0.5 a
Glucose	18.9 ± 1.2 b	21.5 ± 0.7 b	20.9 ± 1.0 b	21.5 ± 1.0 b	18.4 ± 0.9 b	10.0 ± 1.7 a
Fructose	18.2 ± 1.1 b	21.2 ± 0.7 bc	21.7 ± 1.1 bc	22.5 ± 1.1 c	19.6 ± 0.9 bc	11.8 ± 1.6 a
Total sugars	42.9 ± 2.6 b	47.3 ± 1.5 b	47.1 ± 2.3 b	48.0 ± 1.9 b	42.2 ± 1.9 b	25.9 ± 3.7 a
ORGANIC ACIDS [mean (g 100 g^{-1} DW) ± SE]						
Citric acid	18.0 ± 0.3 b	15.0 ± 0.2 a	18.3 ± 0.3 b	18.6 ± 0.4 b	18.4 ± 1.4 b	18.3 ± 1.4 b
Malic acid	8.5 ± 0.3 c	6.1 ± 0.1 ab	7.3 ± 0.2 bc	8.2 ± 0.1 c	7.9 ± 0.7 c	5.8 ± 0.8 a
Quinic acid	6.8 ± 0.2 b	4.8 ± 0.1 a	6.4 ± 0.1 b	7.2 ± 0.1 b	7.0 ± 0.6 b	6.2 ± 0.6 b
Tartaric acid	-	-	-	2.3 ± 0.3 a	2.9 ± 0.2 a	3.5 ± 0.6 a
Shikimic acid[b]	21.2 ± 2.1 bc	10.8 ± 0.9 a	19.0 ± 1.1 bc	23.8 ± 0.7 c	23.9 ± 2.5 c	17.0 ± 3.1 b
Fumaric acid[b]	6.8 ± 0.5 c	4.4 ± 0.2 a	6.3 ± 0.1 ba	8.5 ± 0.1 d	9.5 ± 0.9 d	5.2 ± 0.7 ab
Total organic acids	33.3 ± 0.8 b	26.0 ± 0.4 a	32.0 ± 0.5 b	36.4 ± 0.6 b	36.3 ± 2.7 b	35.0 ± 3.1 b
Ascorbic acid[b]	906.1 ± 20.6 cd	824.4 ± 29.2 cd	935.0 ± 88.0 d	560.3 ± 58.7 b	716.8 ± 96.9 bc	176.0 ± 47.4 a

[a] Different letters (a–d) for each individual compound/group denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.
[b] Expressed in mg 100 g^{-1} DW.

(2000) determined the content of citric acid in hips of different rose species from Poland and Hungary and Demir et al. (2014) quantified citric acid as the predominant acid in rose hips collected in Turkey. The latter also contained malic acid but in far lesser amounts. Zocca et al. (2011) and Mikulic-Petkovsek et al. (2012) similarly reported citric acid as the predominant organic acids in rose hips, followed by malic acid. In addition to these organic acids, lactic acid, oxalic acid and fumaric acid have been detected in rose hips (Zocca et al., 2011). Unfortunately, data comparison is not possible due to different analytical methods and units (mg L^{-1}). Similar levels of fumaric and shikimic acids in rose hips have been reported in a study by Mikulic-Petkovsek et al. (2012); however, the content of citric and malic acids was higher in the present research.

Phenolic compounds

A single anthocyanin has been detected in rose hips (Table 1), namely cyanidin-3-glucoside (cy-3-glu), previously identified in *R. canina* hips by Hvattum (2002) and Guimarães et al. (2013). Rose hips initially contained low levels of cy-3-glu but the content increased in later samplings. The levels were highest on the third and fourth sampling date (in October) and significantly higher than the content reported by Guimarães et al. (2013) in well maturated *R. canina* hips. The content of cy-3-glu declined during the two following sampling dates and dropped significantly in frostbitten rose hips. As reported by Christie et al. (1994) very cold temperatures inhibit the biosynthetic capability of anthocyanins and possibly the accumulated anthocyanins start to degrade. This effect could explain the reduction of anthocyanin levels in frostbitten rose hips. Previous studies have shown that anthocyanin content and composition is in tight correlation with color expression (Lancaster et al., 1997; Schmitzer et al., 2009). Schmitzer et al. (2010) determined a strong correlation between the a^* parameter and total anthocyanidin content in petals of ground cover roses (*Rosa* x *hybrida*). In the present study, the correlation between cy-3-glu and the a^* parameter was not strong ($r = 0.47$, $P = 0.01$), as other pigments (such as carotenoids) define the color of rose hips.

Forty-five different phenolic compounds were identified in maturing rosehips using MS^n. The most abundant phenolic group was the group of flavanols (Table 3). The *Rosaceae* family is characterized by high levels of catechin and proanthocyanidin secondary metabolites (Hoffmann et al., 2012) and several proanthocyanidin glycosides and aglycones (less abundant) were identified in rose hip samples. The statistical analysis generally revealed no significant differences in the content of these compounds during rose hip maturation. In addition to proantocyanidins, catechin and catechin hexoside have been quantified in rose hips and their content levels changed during the process of maturation. The highest content was determined on the third sampling date (124.6 μg g^{-1} DW catechin and 315.9 μg g^{-1} DW catechin hexoside) and the lowest after the hips were frostbitten (79.0 μg g^{-1} DW catechin and 178.3 μg g^{-1} DW catechin hexoside). However, due to a high proportion of proanthocyanidins in rose hips only minor differences have been observed for total flavanol levels during fruit ripening. Salminen et al. (2005) previously identified catechin and proanthocyanidins as the most abundant phenolics in hips of three different dog rose species and Ganhão et al. (2010) and Guimarães et al. (2013) in hips of *R. canina*. Moreover, Türkben et al. (2010) reported catechin as the main phenolic component of *R. canina* hips, observing that its levels were greater in reddish-orange hips than in riper fully red hips. The results of the present study correspond to the catechin levels obtained by Guimarães et al. (2013). Contrary, Fecka (2009) measured higher levels of catechin in *R. canina* hips (average levels of 0.4 mg g^{-1} DW), but no catechin glycosides have been reported in this research. Ganhão et al. (2010) reported significantly higher content levels of catechin in ripe rose hips (1178.9 mg·100 g^{-1} DW).

Several phenolic acids and their derivatives were identified in rose hips (Table 4). The most abundant were gallic and ellagic acid derivatives (methyl gallate hexoside, methyl gallate acetyl dihexoside, methyl gallate rutinoside, methyl ellagic acid pentoside and ellagic acid pentosides). Methyl gallate rutinoside and methyl gallate hexoside have previously been determined in *R. canina* hips by Hvattum (2002). Fecka (2009) further identified the latter as methyl gallate 3-*O*-β-glucoside. In addition to catechin and ellagitannin rugosin A, methyl gallate 3-*O*-β-glucoside is claimed to be the predominant polyphenol in dog rose fruit (Fecka, 2009). Fecka (2009) has determined higher levels of methyl gallate glucoside compared to the results obtained in our study (0.9 mg g^{-1} DW). Guimarães et al. (2013) reported methyl gallate hexoside in *R. micrantha* fruits in similar levels to ours. Ganhão et al. (2010) also reported the presence of hydroxybenzoic and hydroxycinnamic acids in *R. canina* fruit but did not identify specific compounds. Derivatives of sinapic acid hexoside, coumaroylquinic acids and *p*-coumaric acid hexoside have also been quantified in our rose hip samples. *Cis*- and *trans*-5-*p*-coumaroylquinic acid has previously been determined in leaves of several rose species and cultivars (Cunja et al., 2014);

Table 3
Flavanol content in *R. canina* hips during ripening.

Sampling						
Mean (μg g $^{-1}$ DW) ± SE	1	2	3	4	5	6
Catechin	109.3 ± 12.7 bc[a]	110.4 ± 4.1 bc	124.6 ±6.5 c	113.0 ± 3.7 bc	91.0 ± 7.0 ab	79.0 ± 16.5 a
Catechin hexoside	265.2 ± 35.4 bc	274.1 ± 17.7 bc	315.9 ± 12.3 c	273.1 ± 12.1 bc	210.5 ± 19.5 ab	178.3 ±35.7 a
PA[b] dimer monoglycoside 1-3	1186.1 ± 150.7 a	1224.8 ± 65.6 a	1387.0 ± 60.4 a	1325.9 ±44.3 a	1041.0 ±92.2 a	1062.1 ± 193.8 a
PA dimer diglycoside 1-3	1121.6 ± 147.3 ab	1255.4 ± 84.4 ab	1415.6 ± 55.9 b	1421.4 ± 69.4 b	993.9 ± 104.7 a	963.6 ± 195.3 a
PA dimer glycosides total	2307.8 ± 297.5 ab	2480.2 ± 149.0 ab	2802.7 ± 115.0 b	2747.2 ±107.2 ab	2034.8 ± 195.7 a	2025.8 ±388.6 a
PA dimer 1-4	634.8 ± 77.9 a	656.7 ± 39.0 a	736.0 ± 25.6 a	728.9 ± 30.1 a	545.0 ± 46.5 a	638.6 ± 101.4 a
PA trimer 1-3	135.0 ± 19.4 ab	144.0 ± 16.2 ab	157.8 ± 5.3 ab	168.8 ± 7.4 ab	119.6 ±13.5 a	181.4 ± 27.7 b
PA tetramer	63.4 ± 8.0 a	72.7 ± 6.0 a	80.3 ± 3.6 a	82.9 ± 5.6 a	59.9 ± 5.7 a	82.1 ± 13.5 a
PA aglycons total	833.1 ± 105 a	873.5 ± 60.6 a	974.1 ± 33.7 a	980.6 ±42.2 a	724.6 ± 65.2 a	902.1 ± 141.9 a
Total PA	3140.9 ± 402.1 a	3353.6 ± 207.9 a	3776.8 ± 148.3 a	3727.8 ± 148.5 a	2759.4 ± 260.5 a	2927.9 ± 529.3 a
Total flavanols	3515.4 ± 450.0 ab	3738.2 ± 229.1 ab	4217.3 ± 166.3 b	4113.9 ± 164.2 ab	3060.8 ± 286.7 a	3185.2 ± 580.8 ab

[a] Different letters (a–c) for each individual compound/group denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.
[b] PA – proanthocyanidin.

however, to our knowledge this is the first report of 3-*p*-*O*-, 4-*p*-*O*- and 5-*p*-*O*-coumaroylquinic acid in rose hips. Significant differences in levels of several phenolic acids and their derivatives have been determined during rose hip maturation and their levels were generally, lowest after frost. Although higher levels of sinapic acid hexoside have been detected in rose hip fruit no significant differences in this compound have been confirmed during maturation. Demir et al. (2014) also reported the presence of sinapic acid in *R. canina* hips. Several authors identified *p*-coumaric acid in hips of *R. canina* and other rose species (Zocca et al., 2011; Tumbas et al., 2012; Demir et al., 2014).

From the group of flavonols, several quercetin (Q) glycosides, two isorhamnetin-3-hexosides and a kaempferol (K) derivative were determined (Table 5). K derivative, Q-galactoside, Q-glucuronide and Q-arabinofuranoside were the most abundant flavonols in rose hips (Table 5) and minor quantities of Q-rutinoside, Q-glucoside, Q-arabinopyranoside and Q-rhamnoside have been determined. All Q glycoside have previously been reported by Hvattum (2002), Slaminen et al. (2005) and Guimarães et al. (2013) but Q-arabinopyranoside and Q-arabinofuranoside only as Q-pentosides. Fecka (2009) identified astargaline (K-glucoside), Q, hyperoside (Q-galactoside) and rutin (Q-rutinoside) in rose hip samples. In addition to these compounds, Guimarães et al. (2013) also reported isorhamnetin 3-O-rutinoside and kaempferol rhamnosyl-hexoside. The levels of flavonols measured in the present study are comparable to the reports of Guimarães et al. (2013). No significant differences in the content of K derivative were observed during rose hip ripening. On the other hand, levels of almost all Q glycosides decreased during ripening and a significant decrease was also found after frost. This is consistent with the study of Türkben et al. (2010), who report higher values of Q derivatives in reddish-orange compared to fully red *R. canina* hips.

In addition to flavonols, four naringenin hexosides, two eriodictyol hexosides and two apigenin derivatives have been identified in rose hips (Table 5). Apigenin derivatives showed no statistical differences during fruit maturation except on the last sampling date after frost when the lowest levels of these compounds have been recorded. The content levels of naringenin hexosides and phloridzin were similarly highest on the first four samplings and a decrease was recorded on the final two samplings. Hvattum (2002) and Salminen et al. (2005) previously detected phloridzin, an apigenin derivative and eriodictyol hexosides in *R. canina* hips but their levels have not been reported. Eriodictyol hexosides have been quantified by Guimarães et al. (2013), who reported similar levels as in our samples. To the best of our knowledge, this is the first report on naringenin hexoside in rose hips. Naringenin belongs to flavanons – an important group of secondary metabolites and precursors for flavones, (e.g., apigenin), isoflavones and dihydroflavonols, which are intermediates for flavonol and anthocyanidin biosynthesis (Cooper-Driver, 2001). Naringenin is typically found in citrus fruits, but has also been determined in tomato (Erlund, 2004) and fruits of *Mabea caudata* from *Euphorbiaceae* family (Barros et al., 1982).

The decrease of some phenolic compounds on the last sampling date may be linked to frost damage of rose hip tissue. Tissue damage occurs as a result of mechanical injury from ice crystal formation or cell dehydration as liquid water freezes. Consequently, cell membranes are damaged and phenolic oxidative browning occurs due to interactions between the enzymes and their respective substrates (Chalker-Scott, 1999; Morelló et al., 2003). The darker color (L^* parameter) of hips on the last sampling could also be a result of this process.

Total phenolic content

Total phenolic content (TPC) showed a pattern during rose hip ripening similar to cyanidin-3-glucoside (Table 6). In September, values increased and reached the maximum on the third sampling date at the beginning of October (15.7 mg g^{-1} DW). TPC decreased in fruit collected on the final two samplings; however, no statistical differences between frostbitten and fully ripe rose hip fruit have

Table 4
Content of phenolic acids and their derivatives in *R. canina* hips during ripening (only major phenolic acids and their derivatives presented).

Sampling						
Mean (μg g^{-1} DW) ± SE	1	2	3	4	5	6
Methyl gallate hexoside	76.1 ± 8.1 ab[a]	83.1 ± 4.8 ab	90.9 ± 4.4 ab	97.6 ± 4.8 b	67.7 ± 6.2 a	71.7 ± 14.5 a
Sinapic acid hexoside	33.6 ± 4.2 a	38.6 ± 3.2 a	42.6 ± 1.9 a	44.0 ± 3.0 a	31.8 ± 3.0 a	43.6 ± 7.1 a
Ellagic acid pentoside 1-2	30.6 ± 3.7 b	29.3 ± 1.2 b	32.1 ± 1.5 b	29.2 ± 0.9 b	25.5 ± 1.5 ab	20.8 ± 3.5 a
Methyl gallate acetyl dihexoside	21.0 ± 2.6 c	19.7 ± 0.5 bc	22.5 ± 0.9 c	18.1 ± 0.7 bc	15.7 ±1.3 b	9.2 ± 1.8 a
Total	212.1 ± 24.9 ab	221.5 ± 11.9 ab	244.4 ± 10.3 b	238.5 ± 10.4 ab	180.7 ± 14.5 a	185.1 ± 30.9 ab

[a] Different letters (a–c) for each individual compound/group denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.

Table 5
Content of flavons, flavonols (only major presented), flavanones and phloridzin in *R. canina* hips during ripening.

Sampling						
Mean (μg g^{-1} DW) ± SE	1	2	3	4	5	6
FLAVONES						
Sum apigenin derivative 1-2	6.7 ± 1.3 b[a]	8.3 ± 0.4 b	8.5 ± 0.3 b	7.8 ± 0.3 b	7.0 ± 0.6 b	4.1 ± 0.7 a
FLAVONOLS						
Kaempferol derivative	28.0 ± 3.4 a	27.5 ± 1.5 a	30.6 ± 1.2 a	28.1 ± 0.9 a	23.4 ± 1.4 a	29.1 ± 4.5 a
Q-3-glucuronide[b]	17.2 ± 2.1 b	16.7 ± 1.5 ab	17.5 ± 1.4 b	15.4 ± 1.0 ab	11.1 ± 1.1 a	13.7 ± 2.7 ab
Q-3-ara-fur[c]	13.0 ± 1.6 c	11.6 ± 0.7 bc	13.3 ± 0.9 c	10.3 ± 0.5 abc	8.3 ± 0.3 ab	7.8 ± 1.6 a
Sum isorham-3-hex[d] 1-2	7.0 ± 0.8 b	6.1 ± 0.2 ab	6.8 ± 0.4 ab	7.2 ± 0.4 b	7.1 ± 0.7 b	5.0 ± 0.9 a
Flavonols total	112.8 ± 13.6 b	106.4 ± 7.3 ab	117.2 ± 7.6 b	104.0 ± 3.9 ab	83.2 ± 3.9 a	90.8 ± 14.7 ab
FLAVANONES						
Sum naringenin hexosides 1-4	81.7 ± 8.9 b	94.0 ± 4.0 bc	104.9 ± 5.8 c	79.9 ± 4.1 b	60.4 ± 5.0 a	46.2 ± 5.7 a
Sum eriodictyol hexosides 1-2	75.9 ± 9.5 c	69.0 ± 3.6 bc	78.8 ± 2.6 c	66.2 ± 2.3 bc	56.9 ± 3.9 ab	47.0 ± 7.9 a
Flavanons total	157.5 ± 18.3 d	163.0 ± 7.2 d	183.7 ± 8.2 d	146.1 ± 6.0 bc	117.4 ± 8.8 ab	93.1 ± 13.5 a
Phloridzin	44.2 ± 5.4 c	41.7 ± 0.9 bc	47.7 ± 2.0 c	38.2 ± 1.4 bc	33.1 ± 2.7 b	19.4 ± 3.9 a

[a] Different letters (a–d) for each individual compound/group denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.
[b] Q-3-glucuronide = quercetin-3-glucuronide.
[c] Q-3-ara-fur = quercetin-3-arabinofuranoside.
[d] isorham-3-hex = isorhamnetin-3-hexoside.

been observed. Barros et al. (2011) similarly observed an increase of TPC during the ripening of *R. canina* fruit and Guimarães et al. (2010) confirmed this pattern in *R. micrantha* hips. Our results are in agreement with TPC levels previously reported in *R. canina* hips by Mikulic-Petkovsek et al. (2012) and Roman et al. (2013), although several papers report higher TPC levels (Ercişli, 2007; Barros et al., 2010, 2011; Demir et al., 2014). The differences may be due to different extraction procedures, plant genotype, cultivation area as well as differences in fruit ripeness and initial sample preparation as some authors did not remove the seeds prior to the extraction.

Antioxidant capacity

The antioxidant capacity (AC) of rose hip methanolic extracts was expressed as ascorbic acid equivalents (mg 100 g^{-1} DW), as % of inhibition after 30 min of reaction time with DPPH and as EC_{50} values (concentration of sample where absorbance of DPPH decreased by 50%). Parameters showed significant differences during rose hip ripening process (Table 6).

The highest AC was observed on the first sampling (837.3 mg 100 g^{-1} DW) and the lowest on the last sampling of frostbitten rose hips (591.0 mg 100 g^{-1} DW). However, antioxidant capacity did not decrease gradually during fruit maturation. A significant increase has namely been detected on the third and fourth sampling, which could be linked to increased TPC levels and accumulation of specific phenolics in rose hips (cy-3-glu, naringenin and eriodyctiol hexosides, catechin). AC of rose hip samples in the present study corresponds to values obtained by Buřičová and Réblová (2008). EC_{50} values for DPPH scavenging activity show less variation as significantly higher values have only been recorded in hips from the first sampling compared to all other sampling dates. This suggests an increase of antioxidant properties with the progression of maturation (lower values indicating higher antioxidant capacity). Barros et al. (2011) an the other hand report significantly higher EC_{50} values in ripe *R. canina* hips compared to unripe hips. Direct comparison of the results is generally not possible due to great variation of the methods used and differences in maturity stages. Several studies measured antioxidant activity of rose hips with the DPPH method but use (+/−)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox) instead of ascorbic acid for standard. Demir et al. (2014) reported EC_{50} values of rose hips from five different rose species in range from 161.3 μg mL^{-1} to 278.9 μg mL^{-1}. Roman et al. (2013) compared hips of eight *R. canina* biotypes and measured antioxidant activity from 63.4 to 127.8 μM Trolox 100 g^{-1} fruit. Rose hip samples analyzed in the present study had a greater antioxidant capacity compared to apple peel evaluated by Mikulic-Petkovsek et al. (2010) who utilized the same method.

The percent of inhibition demonstrates the consumption of the DPPH reagent compared to the blank sample. In our experiment the change was monitored after 30 min and the initial samples were heavily diluted (1:100). The inhibition values ranged from 49.5% to 61.2% and there were statistical differences among sampling dates. The lowest value vas determined in frostbitten samples and the highest on the fourth sampling date.

There was a strong correlation between TPC and AC ($r = 0.76$, $P = 0{,}00$), TPC and % of inhibition ($r = 0.77$, $P = 0{,}00$) and a negative correlation between TPC and EC_{50} values ($r = -0.69$, $P = 0.00$). A positive correlation between DPPH radical scavenging activity and TPC has also been observed by Nowak and Gawlik-Dziki (2007) in leaves of several *Rosa* species and by Roman et al. (2013) in hips of different *R. canina* biotypes. A negative correlation between EC_{50} values for DPPH scavenging activity and TPC has also been reported by Ganhão et al. (2010).

Table 6
Total phenolic content (TPC), antioxidant capacity (AC), % inhibition and EC_{50} values of DPPH scavenging activity in *R. canina* hips during ripening.

Mean ± SE	Sampling					
	1	2	3	4	5	6
TPC (mg ekv GAE g^{-1} DW)	13.2 ± 1.0 ab[a]	14.1 ± 0.1 ab	15.7 ± 0.5 b	15.5 ± 0.4 b	12.3 ± 0.5 a	12.3 ± 1.1 a
AC (mg ekv AA 100 g^{-1} DW)	837.3 ± 12.1 d	707.8 ± 16.0 b	764.7 ± 9.4 c	780 ± 17.1 c	676.2 ± 18.7 b	591.0 ± 14.3 a
% Inhibtion[b]	55.3 ± 0.9 b	56.6 ± 1.3 b	57.6 ± 0.7 bc	61.2 ± 1.3 c	58.7 ± 1.7 bc	49.5 ± 1.2 a
EC_{50} DPPH scavenging activity (mg DW mL^{-1})	23.5 ± 2.3 b	17.7 ± 0.7 a	17.7 ± 0.4 a	16.2 ± 0.8 a	14.5 ± 0.7 a	17.7 ± 0.8 a

[a] Different letters (a-d) for each measured parameter denote statistically significant differences among sampling dates by Duncan's multiple range test at $P < 0.05$.
[b] % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the blank sample and A_1 the absorbance of the sample measured after 30 min. Ascorbic acid was used as a standard.

Lycopene and β-carotene

In addition to phenolic compounds the content of two carotenoids, namely lycopene and β-carotene has been determined. Their presence was not evaluated on the first sampling. An increase of carotenoid levels was observed from the second sampling onward (Table 1). Interestingly, the highest content of lycopene was measured in frostbitten rosehips (113.2 mg 100 g^{-1} DW). Similarly, the content of β-carotene was lowest on the second sampling (6.5 mg 100 g^{-1} DW), increased on the third sampling and decreased on the fourth sampling date. The content increased again on the fifth and sixth sampling date when highest values have been measured (22.1 mg 100 g^{-1} DW). It can be speculated that the highest carotenoid content in frostbitten hips is due to ice crystal damage of the cells and consequently a better extraction as reported by Veberic et al. (2014) in blackberry fruit. The increase of β-carotene and lycopene in maturing rose hips has also been reported by Türkben et al. (2010) and Barros et al. (2011) in *R. canina*, by Andersson et al. (2011) in hips of different rose species and by Guimarães et al. (2010) in hips of *R. micrantha*. However, studies using HPLC identification and quantification of carotenoids in rose hips are still scarce. Hornero-Méndez and Mínguez-Mosquera (2000), Olsson et al. (2004) and Andersson et al. (2011) analyzed lycopene and β-carotene levels in rose hips with the use of HPLC. Our results are in range with their reports, although higher levels of lycopene have been detected in our samples. Olsson et al. (2004) and Andersson et al. (2011) similarly report higher levels of lycopene compared to β-carotene in *R. canina*; however, in *R. mosqueta* the latter one prevails (Hornero-Méndez and Mínguez-Mosquera, 2000). Böhm et al. (2003) reported the content of lycopene in raw rose hips in range from 12.9 to 35.2 mg 100 g^{-1}, which is in accordance with the levels detected in the present study (expressed in FW, data not shown). The levels of lycopene in *R. canina* hips are higher than in most fresh tomatoes (Böhm et al., 2003). Similar levels of β-carotene and lycopene have been reported by Guimarães et al. (2010), who spectrophotometrically established the content of carotenoids in *R. canina* fruit. Contrary, Barros et al. (2011) spectrophotometricaly determined as much as 100-fold lower levels of lycopene compared to our study but similar contents of β-carotene.

In conclusion, the results obtained in the present study provide insight into the changed levels of primary and secondary metabolites during maturation and after frost damage of *R. canina* hips. This highlights the importance of determining the right harvest period to optimize the content of bioactive compounds and primary metabolites in *R. canina* fruit. The traditional practice of harvesting rose hips after frost does not seem appropriate if high levels of sugars, ascorbic acid, flavons, flavonols, flavanols, catechin and phenolic acids and their derivatives are desired. The antioxidant capacity of methanolic samples was also lowest on the last sampling. Conversely, β-carotene and lycopene are increased after frost damage.

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2.1.3 Sveže iz okrasnega vrta: šipki izbranih sort vrtnic bogati z rastlinskimi hranljivimi snovmi

Fresh from the ornamental garden: Hips of selected rose cultivars rich in phytonutrients

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Šipkom izbranih vrst *Rosa canina* (RCA), *R. sweginzowii* (RSW), *R. rugosa* (RUG) in sort 'Fru Dagmar Hastrup' (FDH), 'Repandia' (REP), 'Veilchenblau' (RVB), 'Aloha' (RAL), 'Bonica' (BON) in 'Golden Gate' (RGG) smo izmerili morfološke parametre (velikost, maso, barvo) in vsebnosti sladkorjev, organskih kislin, likopena, β-karotena in fenolov. Čeprav so šipki tradicinalno uporabljene RCA vsebovali največ cianidin-3-glukozida (83 µg/g suhe mase) in so bili tudi statistično značilno najbolj rdeči ($h°$ = 17,5), v drugih merjenih parametrih niso izstopali. Vzpenjavka RGG je oblikovala najtežje plodove (8,86 g), ki so vsebovali največ sladkorjev (50,9 g/100 g suhe mase). Sorta RAL je izstopala po vsebnosti organskih kislin (33,9 g/100 g suhe mase), predvsem zaradi velike vsebnosti kiniske kisline (17,6 g/100 g suhe mase). V šipkih sorte FDH in vrste RSW smo določili značilno največ askorbinske kisline (4325 mg/100 g suhe mase oz. 4711 mg/100 g suhe mase v slednji). Druge sorte so v primerjavi s preučevanimi vrstami vsebovale značilno manj askorbinske kisline. Fenolni profil je bil odvisen od vrste in sorte. Največja raznolikost fenolnih snovi je bila ugotovljena v plodovih RUG in FDH (55 oz. 54 različnih fenolnih spojin identificiranih s HPLC/MS). V večini preučevanih vrst in sort so med fenolnimi spojinami prevladovali flavanoli; največja vsebnost katehina ter procianidinskih derivatov je bila ugotovljena v plodovih RGG (15855 µg/g suhe mase). Največja količina fenolov je bila določena v šipkih sorte RAL (44746 µg/g suhe mase), predvsem zaradi velike vsebnosti hidrolizirajočih taninov v primerjavi z drugimi vrstami in sortami. Čeprav majhni, so bili plodovi sort BON in REP količinsko najbolj bogati z β-karotenom oz. likopenom v slednji.

Fresh from the Ornamental Garden: Hips of Selected Rose Cultivars Rich in Phytonutrients

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Abstract: Morphological parameters (size, weight, color), the content of sugars, organic acids, lycopene, β-carotene, and phenolics were determined in hips of *Rosa canina* (RCA), *Rosa sweginzowii* (RSW), *Rosa rugosa* (RUG), and selected ornamental *Rosa* cultivars Fru Dagmar Hastrup (FDH), Repandia (REP), Veilchenblau (RVB), Aloha (RAL), Bonica (BON), and Golden Gate (RGG). Although traditionally used RCA hips contained the highest amount of cyanidin-3-glucoside (83 μg/g DW) and were the reddest ($h° = 17.5$), they did not stand out in other analyzed parameters. RGG climber had the biggest hips (8.86 g), which also contained highest sugar levels (50.9 g/100 g DW). RAL stood out as the cultivar rich in organic acids (33.9 g/100 g DW), mainly because of high quinic acid content (17.6 g/100g DW). FDH and RSW hips were characterized by particularly high ascorbic acid levels (4325 mg/100 g DW and 4711 mg/100 g DW). Other ornamental cultivars contained low amounts of ascorbic acid compared to the analyzed species. The phenolic profile was species/cultivars-specific. The greatest diversity of phenolic compounds was detected in RUG and FDH hips (55 and 54 different tentatively identified compounds with HPLC/MS). Flavanols represented the main phenolic class in most of the investigated species/cultivars and RGG hips contained the highest amount of catechin and proanthocyandin derivatives (15855 μg/g DW). Altogether RAL hips contained the highest quantity of phenolics (44746 μg/g DW) mainly due to high levels of hydrolysable tannins compared to other species/cultivars. Although small, hips of BON and REP were most abundant regarding β-carotene and lycopene content, respectively.

Keywords: carotenoids, organic acids, phenolics, rose hip, sugars

Practical Application: Rose hips of some ornamental cultivars could represent an alternative to traditionally utilized *Rosa canina* (RCA) hips as some accumulate particularly high levels of ascorbic acid (cultivar Fru Dagmar Hastrup – FDH), beneficial phenolic constituents (cultivars Golden Gate – RGG and Aloha – RAL) and carotenoids (cultivars Bonica – BON and Repandia – REP). Some of these cultivars (FDH, RGG, RAL) also had bigger hips than RCA, making them interesting for harvesting and processing.

Introduction

In recent decades there is a growing interest in functional foods, that is foods that have a beneficial effect on health and could reduce the possibility of certain diseases (Arai 1996; Losso 2003). Traditionally, rose hips of several wild species are gathered for food or for medicinal purposes and used fresh or dried (Uggla and others 2005; Pang and others 2009; Yildiz and Alpaslan 2012; Demir and others 2014).

There have been numerous studies proving that rose hips are rich in biologically active compounds such as vitamin C, phenolic compounds, and carotenoids (Hodisan and others 1997; Daels-Rakotoarison and others 2002; Böhm and others 2003; Salminen and others 2005). These compounds are also known as phytonutrients and are associated with health promotion (Beecher 1999). Results of several surveys also indicate that rose hips possess anti-inflammatory properties and might as such be used as a prophylactic or a supplement in treating certain maladies (Kharazmi and Winther 1999; Rein and others 2004; Willich and others 2010).

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Roses are considered one of the most important ornamental species, typically cultivated for their beautiful flowers and have been grown in our gardens for centuries. To encourage roses to rebloom and keep plants looking attractive, one usually removes the spent flowers. If left on their own ornamental varieties with simple or semi-double flowers will usually produce hips that can add interesting ornamental value to the autumn garden. But is that all? Could these hips be used as a healthy addition to daily diet? Little is known on inner quality of hips produced by ornamental roses. Is the content of biologically active compounds comparable between hips of selected ornamental cultivars and hips of *Rosa* species, traditionally harvested for their medicinal purposes? Do they accumulate comparable levels of ascorbic acid or polyphenols as RCA hips? To answer these questions, hips of several different types of ornamental roses (ground covers, floribundas, climbers, and selected rose species) were selected at their technological maturity and analyzed. Except for RCA and to a certain extent also for RUG, this is the first time qualitative and quantitative evaluation of primary and secondary metabolites has been performed on hips of ornamental roses.

Materials and Methods

Plant material

Hips from different species and cultivars were gathered in Volčji Potok Arboretum (central Slovenia, 46°11′4.99″N,

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14°36′22.12″E, and altitude 340 m) in 2014. The following species and cultivars (listed in the order the hips were picked) were selected for the analysis: *Rosa canina* (RCA), *Rosa sweginzowii* (RSW), *Rosa rugosa* (RUG), "Fru Dagmar Hastrup" (FDH), "Repandia" = KORsami (REP), "Veilchenblau" (RVB), "Aloha" = KORwesrug (RAL), "Bonica" = MEIdomonac (BON), and "Golden Gate" = KORgolgat (RGG). Hips were gathered consecutively from the start of October (RCA – Oct.10) till the end of November (RGG – Nov. 27); hip ripeness was determined according to their color and firmness. Hips were collected from 5 adjacent plants and at least 200 g of fresh rose hips were gathered for each species/cultivar. Color parameters and external quality characteristics were measured immediately after harvest, seeds (achenes) and calyces were removed prior to the analysis of primary and secondary metabolites. Analysis of ascorbic acid (vitamin C) and β-carotene and lycopene content was performed immediately on fresh pericarp. At this time samples for dry matter content were taken. The rest of the sample was frozen in liquid nitrogen and stored at −20 °C up to 1 mo for further chemical analysis (organic acids, sugars, phenolic compounds).

Morphological parameters of rose hips

Fruit length (from pedicel to calix) and width (at the widest part of the hip, perpendicular to its length) were measured with a digital caliper, measurement were performed on 30 rose hips for each species/cultivar. Fruit shape index was calculated as length to width ratio. Weight of the whole individual fruit was determined as average fruit weight of 5 (or 10, depending on the size) hips, 10 replicates made for each species/cultivar. At the next stage, the seeds were discarded and only the weight of the pericarp (hypanthium) was recorded. Seed weight and flesh ratio (share of pericarp weight compared to whole fruit weight) were calculated.

Color parameters of rose hips were measured with a portable colorimeter (CR-10 Chroma; Minolta, Japan) on 30 hips for each species/cultivar.

Dry matter content was obtained as reported previously by Cunja and others (2015). Five replicates per species/cultivar were taken, each including approximately 2 g of material.

Determination of sugars, organic acids, and vitamin C using high-performance liquid chromatography (HPLC)

Extraction of sugars, organic acid, and vitamin C was carried out as reported by Cunja and others (2015), with some modifications. For each species/cultivar 6 replicates were made. For the extraction of ascorbic acid 1 g of pericarp was extracted with 10 mL of 2% metaphosphoric acid. Content of individual sugars and organic acid was expressed in g/100 g of dry weight (DW) or mg/100 g DW where appropriate (tartaric, shikimic, fumaric, and ascorbic acid). From the data of individual sugars and individual organic acids, the sum of sugars (total sugars), and organic acids (total acids) were calculated.

Extraction and determination of phenolic compounds using HPLC coupled with mass spectrometry (HPLC/MS)

Extraction and determination of phenolic compounds was performed with HPLC/MS as described before by Cunja and others (2015). For each individual species/cultivar 6 repetitions were made.

Compounds were identified by comparing retention times and absorption spectra, by fragmentation and by adding authentic standard solution to the sample. The content was calculated from peak areas and response factors of calibration curves of corresponding external standards. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus proanthocyanidin (PA) derivatives were quantified with the calibration curve of procyanidin B2, ellagic glycosides, methyl ellagic, and methyl gallate derivatives together with hydrolysable tannins (compounds with a hexahydroxydiphenic [HHDP] moiety) with ellagic acid, catechin hexoside with catechin, and apigenin derivatives with apigenin-7-glucoside. For quantification of 3-feruloylquinic acid calibration curve of ferulic acid was used, coumaroylquinic acids and *p*-coumaric acid hexoside were calculated with *p*-coumaric acid, sinapic acid hexoside with sinapic acid. Isorhamnetin glycosides were expresed in equvivalents of isorhamnetin-3-glucoside, and kaempferol derivatives in equivalents of kaempferol-3-glucoside. Content of quercetin (Q)-3-glucuronide together with Q-acetyl hexoside, Q-acetyldihexoside, Q-3-hexoside, Q-galloyl hexoside, and Q-rhamnosyl hexoside was calculated using the calculation curve of Q-3-galactoside. Eriodictyol, naringenin, and taxifolin derivatives were expressed in equivalents of naringenin and resveratrol derivative was calculated using resveratrol as standard. Concentrations of phenolic compounds were expressed in μg/g DW; from individual content levels the sum of particular compound classes and total phenols were calculated.

Extraction and HPLC/MS determination of carotenoids

Carotenoids were determined using a method described in Kacjan Maršić and others (2010) with modifications. In dimmed light conditions 0.1 g of chopped fresh rose hip pericarp was extracted with ice cold acetone and homogenized using T-25 Ultra-Turax (IKA® – Labortechnik, Staufen, Germany) at 8400 rpm. For each species/cultivar 8 replicates were made. Acetone extracts where analyzed using a Thermo Finnigan Accela HPLC system (Thermo Fisher Scientific, Inc., Waltham, Mass., U.S.A.) with a diode array detector at 450 nm. A HPLC column (150×4.6 mm, Gemini 3μ C18; Phenomenex Inc., Torrance, Calif., U.S.A.), protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20 μL and the flow rate maintained at 1 mL/min. The elution solvents were A (acetonitrile : MeOH : H_2O = 100 : 10 : 5, v/v/v) and B (acetone : ethyl acetate = 2 : 1, v/v) eluted according to a linear gradient as described by Šircelj and Batič (2007).

Presence of lycopene and β-carotene was confirmed on a TSQ Quantum Access Max quadrupole mass spectrometer (Thermo Fisher Scientific, Inc.) using an atmospheric pressure chemical ionization (APCI) source in positive ion mode. Argon was used as collision gas to achieve collision-induced dissociation. Capillary temperature was set to 320 °C and vaporizer temperature at 450 °C. Other APCI parameters were set as follows: corona voltage at 4.0 kV, sheat gas flow rate at 55 L/h and auxiliary gas flow rate at 10 L/h. Mass spectra were scanned in range from m/z 70 to 650. Xcalibur 2.2 software was used for data aquisition. Lycopene and β-carotene were identified based on mass spectra scans, fragmentation and comparison of retention times and spectral properties to corresponding external standards.

Chemicals

Chemicals used in extraction of compounds were obtained from Sigma-Aldrich Corp., St Luis, Mo., U.S.A. (methanol, formic acid, sulfuric acid, metaphosphoric acid, acetone, ethyl acetate). Purified water used in extraction was obtained with Milli-Q Direct 8 system by Millipore (Merck KGaA, Darmstadt, Germany).

In analysis of organic acids and sugars standards of citric acid, quinic acid, and ascorbic acid were from Sigma-Aldrich Co.; tartaric acid, shikimic acid, fumaric acid, glucose, fructose, and sucrose from Fluka (Fluka Chemie AG, Buchs, Switzerland); malic acid from Merck (Merck KGaA, Darmstadt, Germany).

For identification and quantification of phenolic compounds the following standards were used from Sigma-Aldrich: ellagic acid, naringenin, 5-caffeolyquinic acid (5-CQA), 4-CQA (cryptochlorogenic acid), 3-CQA (neochlorogenic acid), Q-arabinopyranoside, Q-rhamnoside, Q-rutinoside, Q-xyloside, and resveratrol. Standards of procyanidin B2, catechin, apigenin7-glucoside, ferulic acid, *p*-coumaric acid, sinapic acid, kaempferol-3-glucoside, Q-3-galactoside, Q-arabinofuranoside, Q-glucosideand phloridzin were from Fluka. Isorhamnetin-3-glucoside was from Extrasinthèse (Genay, France). β-carotene was also obtained from Sigma-Aldrich and lycopene from DHI LAB Product (Hørsholm, Denmark).

Statistical analysis

Statistical analysis was performed with the Statgraphics Plus 4.0 (Manugistics, Rockville, Md., U.S.A.) program using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to ($P < 0.05$) determine differences in analyzed parameters and compound concentrations among species/cultivars. Results are given in mean values, expressed in dry weight (DW), with standard error (SE).

Results and Discussion

Morphological parameters of rose hips

Analysis of fruit weight revealed major statistical differences among different *Rosa* species and cultivars analyzed (Table 1). Climbers developed the heaviest fruit; whole hips of RGG weighed 8.86 g, followed by RAL (4.96 g per whole fruit). RGG hips were also the widest (26.7 mm) and had, together with RAL, the lowest fruit shape index (0.88 and 0.92, respectively). Lower index values indicate rounder fruit shape. Contrary, RSW developed the longest hips (34.8 mm), characterized by the greatest fruit shape index (2.01). The smallest hips were collected from REP (groundcover shrub) and RVB (hybrid multiflora) with an average of 0.27 and 0.21 g, respectively. RVB was also characterized by the least favorable flesh to fruit ratio: the flesh constituting 65.5% of the whole fruit. Since their small weight, size, and unfavorable flesh to fruit ratio these hips are difficult to process. In that respect, they are better left on the shrub for their ornamental value and as an invaluable food source for garden wildlife. The most favorable flesh ratio was determined in FDH (a hybrid rugosa) and RGG hips in which 83.8% and 80.0% of the whole fruit weight was represented by the weight of its flesh. Najda and Buczkowska (2013) reported fruit weight of 5 various rose species including RUG (1.58 g), and their results are is in accordance with our data. The second lowest flesh ratio (68.5%) has been determined for RCA hips, traditionally used for food and medicinal purposes. According to Kazankaya and others (2005) fruit flesh ratio of different RCA genotypes varies from 46.8% to 79.9%. Statistical differences among species/cultivars have also been observed in dry matter content (Table 1). FDH pericarp contained the most moisture, while dry matter content was highest in hips of RVB and RSW.

Regarding color parameters (Table 2), RCA hips were the reddest and contained the least yellow pigmentation. The values of color parameters for RCA correspond to our previous research (Cunja and others 2015) and indicate a suitable maturity. The greenest and yellowest technologically mature hips have been collected from RGG bushes. RUG hips were the darkest, while RAL and BON developed lightest hip color. The lowest level of $h°$ parameter, detected on the surface of the RCA hips, additionally confirm, that the fruits of this analyzed cultivar are the reddest amongst the inspected species/varieties. Contrary, RGG hips were characterized by highest $h°$ values indicating a more yellowish color. Rose hip color largely depends on its genotype and maturity stage. Kazankaya and others (2005) reported a significant genotypic variation of RCA hip color; from light orange to dark red. Moreover, the *R. spinosissima* species is known to produce very dark, almost black hips (Andersson and others 2011).

There are several studies that provide information on morphological parameters of rose hips indicating that the data can be very variable depending on the genotype, origin, environmental factors and (Kovács and others 2000; Kazankaya and others 2005; Uggla and others 2005; Dogan and Kazankaya 2006; Guneş and Dolek 2010; Mabellini and others 2011; Najda and Buczkowska 2013) but data on ornamental cultivars are scarce.

Organic acids and sugars

The following organic acids have been determined in investigated rose hips: citric, malic, quinic, tartaric, shikimic, and fumaric acid. Analysis revealed significant differences among species/cultivars. Citric and malic acids were the dominant organic acids in investigated hips. However, in RAL fruit, quinic acid was the most abundant (Table 3). In RCA and RSW hips, citric acid predominated over malic acid, while in other species/cultivars malic acid prevailed. RCA (11.6 g/100 g DW) hips contained highest levels of citric acid and BON hips accumulated the most malic acid (15.0 g/100 g DW). Quinic acid could not be detected in FDH, RUG, and RSW hips. Moreover, the content of quinic acid did not differ significantly among the cultivars, with the exception of RAL. Tartaric, shikimic, and fumaric acids have been quantified in low quantities in all investigated species/cultivars. The greatest variation in organic acids levels has been demonstrated for tartaric acid; RVB hips only contained 51.2 mg/100 g DW of this organic acid and approximately 20-fold higher levels have been detected in RSW fruit (930 mg/100 g DW). In general, RAL hips accumulated highest levels of organic acids (33.9 g/100 g DW) among cultivars/species analyzed. Contrary, RVB hips were characterized by significantly lowest content of total (11.2 g/100 g DW) and individual organic acids. Previous studies have focused their research on organic acids in RCA hips. Various authors reported the predominant citric and malic acids (Kovács and others 2000; Adamczak and others 2012; Mikulic-Petkovsek and others 2012a; Demir and others 2014; Cunja and others 2015) and also detected tartaric, shikimic, and fumaric acid in dogrose hips. Both Demir et al. (2014) and Mikulic-Petkovsek and others (2012a) measured higher content of citric acid compared to malic acid in RCA hips. A similar ratio has also been reported by Zocca and others (2011). The content of citric and malic acids in RCA hips are in range with our previous study (Cunja and others 2015).

Glucose, fructose and sucrose have been identified in hips of all investigated rose cultivars/species (Table 3). Glucose and fructose were the most abundant sugars in rose fruit. Glucose prevailed in BON, RAL, REP, and RGG. The highest content of glucose was detected in RGG hips (25.4 g/100 g DW) while fructose was most abundant in RGG (24.9 g/100 g DW) and RSW (24.3 g/100 g DW) hips. The former was also the species with highest total sugar content (50.9 g/100 g DW). Lowest sugar levels have been

Table 1–Morphological parameters (mean ± SE) of rose hips from selected species/cultivars.

Species/ cultivar	Fruit weight (g)	Seed weight (g)	Flesh ratio (%)	Length (mm)	Width (mm)	Fruit shape index (%)	Dry matter (%)
BON	1.71 ± 0.05b*	0.49 ± 0.02bc	71.6 ± 0.3c	15.7 ± 0.2c	14.3 ± 0.2b	1.10 ± 0.01c	28.5 ± 0.5b
FDH	3.63 ± 0.15d	0.59 ± 0.04c	83.8 ± 0.9f	20.9 ± 0.5dc	21.0 ± 0.4c	1.00 ± 0.02b	21.1 ± 1.1a
RAL	4.96 ± 0.18e	1.22 ± 0.05f	75.4 ± 0.3d	20.1 ± 0.4d	21.8 ± 0.3f	0.92 ± 0.01a	27.7 ± 2.0b
RCA	2.67 ± 0.07c	0.88 ± 0.05e	68.5 ± 1.6b	21.5 ± 0.5e	16.0 ± 0.2c	1.34 ± 0.02e	34.2 ± 0.7c
REP	0.27 ± 0.01a	0.08 ± 0.00a	70.5 ± 0.3bc	10.9 ± 0.1b	7.3 ± 0.1a	1.49 ± 0.02f	32.1 ± 0.8c
RGG	8.86 ± 0.20f	1.77 ± 0.04g	80.0 ± 0.6e	23.4 ± 0.4f	26.7 ± 0.3g	0.88 ± 0.01a	27.9 ± 1.0b
RSW	2.95 ± 0.21c	0.77 ± 0.06d	74.9 ± 0.8d	34.8 ± 0.6g	17.4 ± 0.3d	2.01 ± 0.04g	37.9 ± 0.3d
RUG	1.80 ± 0.09b	0.39 ± 0.03b	78.4 ± 0.7e	15.9 ± 0.4c	15.9 ± 0.4c	1.01 ± 0.02b	27.6 ± 0.9b
RVB	0.21 ± 0.01a	0.07 ± 0.00a	65.5 ± 0.9a	8.6 ± 0.1a	7.3 ± 0.1a	1.19 ± 0.02d	38.9 ± 0.7d

BON, Bonica; FDH, Fru Dagmar Hastrup; RAL, Aloha; RCA, *Rosa canina*; REP, Repandia; RGG, Golden Gate; RSW, *Rosa sweginzowii*; RUG, *Rosa rugosa*; RVB, Veilchenblau.
*Different letters (a to g) for each parameter denote statistically significant differences among species/cultivars by Duncan's multiple range test at $P < 0.05$.

Table 2–Color parameters (mean ± SE) of rose hips from selected species/cultivars.

Species/cultivar	a^*	b^*	L^*	C	$h°$
BON	32.5 ± 0.4b*	21.3 ± 0.4c	33.7 ± 0.3ef	38.9 ± 0.4a	33.3 ± 0.5d
FDH	36.8 ± 1.0c	18.3 ± 1.1b	28.7 ± 0.7b	41.1 ± 1.3abc	25.9 ± 1.1c
RAL	40.8 ± 0.6d	24.3 ± 1.0de	34.7 ± 0.5f	48.1 ± 0.5d	31.4 ± 1.1d
RCA	46.3 ± 0.6e	14.7 ± 0.4a	29.8 ± 0.3bc	48.6 ± 0.7d	17.5 ± 0.4a
REP	36.9 ± 0.5c	15.2 ± 0.6a	30.4 ± 0.5c	40.0 ± 0.6ab	22.3 ± 0.8b
RGG	25.1 ± 0.9a	30.9 ± 1.0f	39.8 ± 0.7g	40.8 ± 0.8ab	51.2 ± 1.8e
RSW	42.6 ± 0.5d	26.0 ± 1.5c	31.4 ± 0.9cd	51.7 ± 1.2e	31.6 ± 1.6d
RUG	38.2 ± 0.7c	16.8 ± 0.9ab	26.9 ± 0.5a	41.8 ± 0.9bc	23.4 ± 0.8bc
RVB	37.1 ± 0.7c	22.0 ± 0.5cd	32.8 ± 0.5de	43.6 ± 0.9c	31.3 ± 0.8d

BON, Bonica; FDH, Fru Dagmar Hastrup; RAL, Aloha; RCA, *Rosa canina*; REP, Repandia; RGG, Golden Gate; RSW, *Rosa sweginzowii*; RUG, *Rosa rugosa*; RVB, Veilchenblau.
*Different letters (a to g) for each parameter denote statistically significant differences among species/cultivars by Duncan's multiple range test at $P < 0.05$.

Table 3–Content of sugars and organic acids (mean (g/100 g DM) ± SE) in rose hips from selected species/cultivars.

Compound	BON	FDH	RAL	RCA	REP	RGG	RSW	RUG	RVB
Glucose	20.8 ± 0.5bc*	13.1 ± 0.8a	18.4 ± 1.8b	20.5 ± 0.6bc	23.3 ± 0.4de	25.4 ± 0.3e	22.0 ± 0.2cd	14.7 ± 1.1a	18.4 ± 0.4b
Fructose	18.6 ± 0.4c	15.0 ± 0.8a	17.4 ± 1.2bc	20.8 ± 0.7d	21.0 ± 0.5d	24.9 ± 0.3e	24.3 ± 0.4e	16.5 ± 0.6ab	16.1 ± 0.2ab
Sucrose	0.80 ± 0.08a	1.82 ± 0.39a	11.4 ± 1.8c	5.14 ± 0.39b	0.79 ± 0.02a	0.58 ± 0.05a	1.04 ± 0.04a	1.31 ± 0.05a	0.84 ± 0.01a
Total sugars	40.2 ± 0.9c	30.0 ± 1.5a	47.2 ± 1.7d	46.4 ± 1.7d	45.1 ± 0.9d	50.9 ± 0.5e	47.3 ± 0.6d	32.5 ± 1.6ab	35.3 ± 0.5b
Citric acid	4.91 ± 0.05c	8.32 ± 0.22de	3.84 ± 0.09ab	11.6 ± 0.3f	4.40 ± 0.05bc	4.76 ± 0.12c	7.77 ± 0.15d	8.55 ± 0.40e	3.30 ± 0.06a
Malic acid	15.0 ± 0.4e	13.8 ± 0.8d	11.4 ± 0.6c	6.33 ± 0.28a	10.3 ± 0.1bc	10.1 ± 0.3b	5.89 ± 0.09a	10.5 ± 0.4bc	6.50 ± 0.09a
Quinic acid	0.98 ± 0.071a	–	17.6 ± 0.8b	1.49 ± 0.06a	1.39 ± 0.03a	1.45 ± 0.14a	–	–	1.11 ± 0.04a
Tartaric acid **	96.1 ± 3.8abc	695 ± 181d	667 ± 49d	341 ± 35bc	65.8 ± 2.0ab	358 ± 30c	930 ± 45d	859 ± 192d	51.2 ± 3.6a
Shikimic acid **	3.26 ± 0.08a	20.1 ± 1.6e	36.5 ± 1.8g	8.50 ± 0.64b	15.9 ± 0.3d	28.6 ± 2.2f	10.0 ± 0.3bc	12.1 ± 0.9c	7.54 ± 0.16b
Fumaric acid **	5.36 ± 0. 13a	24.0 ± 1.7d	4.00 ± 0.74a	9.41 ± 0.42ab	12.4 ± 0.2bc	15.7 ± 0.5c	11.2 ± 0.6bc	24.9 ± 5.0d	9.15 ± 0.12ab
Ascorbic acid**	418 ± 20a	4325 ± 205e	359 ± 22a	1835 ± 84c	808 ± 34b	332 ± 22a	4711 ± 144f	3341 ± 158d	235 ± 7a
Total organic acids	21.4 ± 0.4d	27.2 ± 0.9f	33.9 ± 0.7g	21.6 ± 0.7d	16.9 ± 0.2b	17.0 ± 0.2b	19.3 ± 0.2c	23.3 ± 0.4e	11.2 ± 0.2a

BON, Bonica; FDH, Fru Dagmar Hastrup; RAL, Aloha; RCA, *Rosa canina*; REP, Repandia; RGG, Golden Gate; RSW, *Rosa sweginzowii*; RUG, *Rosa rugosa*; RVB, Veilchenblau.
*Different letters (a to g) for each parameter denote statistically significant differences among species/cultivars by Duncan's multiple range test at $P < 0.05$.
**Expresed in mg/100 g DW.

measured in FDH (30.0 g/100 g DW) and RUG (32.5 g/100 g DW) hips due to their low content of all analyzed sugars. Sucrose was less plentiful; among the analyzed rose hips RAL stood out with significantly highest levels of this sugar (11.4 g/100 g DW), followed by RCA (5.14 g/100 g DW) hips. There have been some previous studies on sugar content in hips of different species, including RCA and RUG (Kovács and others 2000; Uggla and others 2005; Barros and others 2010; Guimarães and others 2010; Barros and others 2011; Mabellini and others 2011; Najda and Buczkowska 2013; Demir and others 2014). Kovács and others (2000) demonstrated a significant seasonal variation in the glucose to fructose ratio in several rose species. In addition to glucose, fructose, and sucrose Barros and others (2011) also detected trehalose and raffinose in RCA hips. The content of individual/total sugars in RCA hips is in range with previous studies, since some variation can be expected due to different geographic origin, environmental factors, genetic variation, and analytical methods.

Rose hips are particularly rich in ascorbic acid and thus appreciated as a potential preventive agent against oxidative stress (Carr and Frei 1999; Schlueter and Johnston 2011). The highest level of ascorbic acid (Table 3) has been detected in RSW (4711 mg/100 g DW) hips where it represented 24% total organic acids, followed by the hybrid rugosa FDH (4325 mg/100 g DW; 16% total organic acids) and RUG (3341 mg/100 g DW, 14% total organic acids). The remaining rose cultivars (RVB, RGG, RAL, BON) contained similar (significantly lower) levels of ascorbic acid. RCA hips accumulated moderate levels of ascorbic acid (1835 mg/100 g DW). A similar finding has been reported by Kovács and others (2000) and Adamczak and others (2012), who stated that RCA hips are not particularly rich in vitamin C. Several authors analyzed the content of vitamin C in hips of different species and obtained diverse results (Ercişli and Eşitken 2004; Kazankaya and others 2005; Barros and others 2010; Barros and others 2011; Hallmann and others 2011; Najda and Buczkowska 2013) due to high

dependency of this metabolite on the genotype, various environmental factors, latitude, maturity stage, and analytical methods.

Phenolic content

More than 100 different phenolic compounds have been identified in investigated rose hips (Table 4). The phenolic profile varied significantly among the analyzed rose species/cultivars (Table 5). Cyanidin-3-glucoside (cy-glu) was the single anthocyanin identified in all hip samples and RCA hips contained highest levels of this compound (83.0 μg/g DW). The ornamental cultivars (BON, RAL, REP, RGG, RVB) accumulated significantly lower levels of this pigment compared to other species analyzed. Cy-glu has previously been identified in RCA hips by Hvattum (2002) and its content in our samples corresponds to earlier reports (Guimarães and others 2013; Cunja and others 2015).

Hips of the RAL climber contained the highest amount of total phenolics. They were the richest in the content of phenolic acids, flavonols and hydrolysable tannins. The latter prevailed in RAL hips. On the other hand, flavanols were the most abundant phenolic group in other cultivars/species. This corresponds to the reports of Ganhão and others (2010), Türkben and others (2010), Guimarães and others (2013), Cunja and others (2015), who focused on determining RCA phenolic profile. Fecka (2009) and Salminen and others (2005) also detected ellagitannins in rose hips. RGG cultivar was the richest in the content of flavanols—as much as 15855 μg/g DW have been measured in its hips, significantly more than in RCA that contained 4483 μg/g DW flavonols. The lowest amount was quantified in RVB hips (1724 μg/g DW). Catechin and proanthocyanidin (PA) dimer and trimer have been identified in all species/cultivars, while PA mono- and diglycosides were only present in RUG, FDH, RCA, RSW, and RAL hips. The greatest diversity of phenolic compounds was determined in hips of RUG and its FDH hybrid; 55 and 54 different phenolic compounds have been tentatively identified in their rose hips. On the contrary, RGG and RVB hips were characterized by distinctly lower number of diverse phenolic compounds (35 and 40 different phenolic compounds, respectively). RVB also contained lowest levels of total phenolics as it contained statistically the lowest amount of flavanols and total phenolic acids (and their derivatives).

From the class of phenolic acids, ellagic, and gallic acid derivatives prevailed over hydroxycinnamic acids in almost all investigated hips, except in RVB. This cultivar predominately accumulated hydroxycinnamic acids. Several authors report phenolic acids and/or their derivatives in rose hips. Ganhão and others (2010) report total hydroxybenzoic and hydroxycinnamic acids in RCA hips. In our study different methyl gallate derivatives have been confirmed in all species/cultivars except in REP. Hvattum (2002) also detected methyl gallate and its derivatives methyl gallate hexoside and methyl gallate rutinoside in RCA hips. The latter has also been reported by Salminen and others (2005) in hips of different *Rosa* species. Methyl gallate hexoside has previously been detected in *R. micrantha* hips by Guimarães and others (2013) in similar quantities than in species/cultivars studied in the present research. Fecka (2009) detected methyl gallate and methyl gallate glucoside in dry RCA hips and Zocca and others (2011) identified ellagic acid in addition to chlorogenic and *p*-coumaric acid in dog rose extracts. The latter 2 compounds have also been reported by Demir and others (2014) in 5 different *Rosa* species in addition to gallic, caffeic, ferrulic, and sinapic acid. Some of those phenolic acids have been found in rose hip extracts by Tumbas and others (2012). In RUG hips Mattila and others (2006) and Hallmann and others (2011) determined gallic acid.

Flavonols have been determined in all investigated rose species/cultivars. Isorhamnetin, kaepferol, and quercetin (Q) derivatives, usually in the form of glycosides, have been detected in different hip samples and Q-glycosides were generally the most abundant from this phenolic class. Isorhamnetin and Q-glycosides were typically found in BON and RAL hips and kaempferol and quercetin derivatives in RVB hips. In RGG only Q-glycosides have been identified. RCA hips contained significantly lowest levels of flavonols among the investigated species/cultivars. Contrary, RAL hips, followed by RGG and REP, accumulated highest levels of flavonols. Flavonol content of RCA hips corresponds to our previous research (Cunja and others 2015) and reports by Guimarães and others (2013). These authors also identified quercetin, kaempferol, and isorhmanetin glycosides in *R. micrantha* hips. Several authors have studied flavonol content of rose hips: Olsson and others (2004), Hvattum (2002), Ganhão and others (2010), Fecka (2009), Pang and others (2009), Hallmann and others (2011), and Mikulic-Petkovsek and others (2012b) and reported diverse levels of these compounds. The specific phenolic profile of the hips can be ascribed to numerous factors such as genetic predisposition of a rose species/cultivar, the impact of the environment (pedoclimatic conditions of a specific site, elevation, etc.) and stress. Differences in flavonol content among the research reports can also be due to diverse analytical methods used.

Eriodictyol hexoside, naringenin hexoside, taxifolin pentoside, and di-pentoside have been determined in investigated rose species/cultivars. However no flavanones could be detected in RAL hips. RGG fruit were characterized by the highest content of flavanones, even though only taxifolin derivatives have been identified from this phenolic group. The only species containing all identified flavanones (eriodyctiol, naringenin, and taxifolin derivatives) was REP. The content of flavanones in RCA hips corresponds to data reported by Cunja and others (2015). Hvattum (2002), Salminen and others (2005), and Guimarães and others (2013) also detected taxifolin pentoside and eriodictyol hexoside in RCA and the latter in *R. micrantha* fruits. Phloridzin has been quantified in all species/cultivars except in RGG. RAL hips accumulated the highest amount of phloridzin, and no statistical differences among RUG, BON, RCA, FDH, and RSW could be detected. Apigenin derivatives were present in 4 rose species/cultivars. RCA and RSW had statistically the lowest content and RUG and FDH the highest. Both Hvattum (2002) and Salminen and others (2005) reported phloridzin and apigenin derivatives in hips of different *Rosa* species. In addition to all phenolic constituents, resveratrol derivative was determined in 3 species/cultivars; in RSW the content was most abundant, while in RUG and FDH the content was statistically lower. Demir and others (2014) have additionally identified *t*-resveratrol in hips of *R. gallica* and *R. hirtissima*.

Lycopene and β-carotene content

The investigated species/cultivars showed statistical differences in content and ratio of the 2 carotenoids (Table 5). β-Carotene usually prevailed, except in REP where lycopene content was predominant (3731 μg/g DW). Species/cultivars with the lowest β-carotene content were FDH (681 μg/g DW), RUG (765 μg/g DW), and RSW (1017 μg/g DW). Together with RGG, they also contained the lowest amounts of lycopene. The highest content of β-carotene was observed in cultivar BON (11158 μg/g DW), followed by cultivars RAL and RGG. Besides REP, BON,

Table 4–Phenolic compounds, stilbenoid, lycopene and *β*-carotene in hips of 9 *Rosa* species/cultivars. Mass to charge ratio (*m/z*) values of the molecular masses and main fragments (MS^2 – second generation product ion, MS^3 – third generation product ion) in positive (M^+) and negative ion mode ($[M-H]^-$) identified with ESI-MS (phenolic compounds and stilbenoid) or APCI probe (for carotenes).

Compound class	Tentative identification[a]	*m/z* $[M-H]^-$ or M^+	MS^2 (*m/z*)	MS^3 (*m/z*)	Species/ cultivar[c]								
					BON	FDH	RAL	RCA	REP	RGG	RSW	RUG	RVB
Anthocyanin	**Cyanidin-3-glucoside**[b]	449	287		x	x	x	x	x	x	x	x	x
Phenolic acids and derivatives	*Hydroxycinnamic acid and derivatives:*												
	3-feruloylquinic acid	368	193		x	x	x					x	x
	3-*p*-coumaroylquinic acid	337	163		x	x	x	x	x			x	x
	4-*p*-coumaroylquinic acid 1, 2	337	173					x				x	
	5-*p*-coumaroylquinic acid 1, 2	337	173, 191, 163		x	x	x	x	x	x	x		x
	p-coumaric acid hexoside 1 – 3	325	163			x	x	x	x		x	x	x
	Sinapic acid hexoside 1, 2	431	385	223, 153, 205		x	x	x	x		x	x	x
	***trans*-5 -caffeoylquinic acid**	353	191		x		x			x	x		x
	***cis*-5 -caffeoylquinic acid**	353	191		x					x	x		x
	4-caffeoylquinic acid	353	173, 191, 161		x		x			x	x		
	3-caffeoylquinic acid	353			x					x			
	Hydroxybenzoic acids and derivatives:												
	Ellagic acid pentoside 1 – 3	433	301		x	x	x	x		x		x	x
	Ellagic acid hexoside 1, 2	463	301		x	x	x		x		x	x	
	Methyl ellagic acid 1, 2	433	313			x		x	x		x		x
	Methyl ellagic acid pentoside	461	328, 446	313		x		x	x		x		
	Methyl gallate acetyl dihexoside	551	345	183, 168, 124	x	x		x			x	x	
	Methyl gallate hexoside	345	183, 168, 124		x	x	x	x		x	x	x	x
	Methyl gallate rutinoside	491	345, 183, 163		x	x		x					x
	Methyl gallate pentoside	491	345, 183, 163								x		

(*Continued*)

Table 4–Continued.

Compound class	Tentative identification[a]	*m/z* $[M-H]^-$ or M^+	MS^2 (*m/z*)	MS^3 (*m/z*)	Species/ cultivar[c]							
					BON	FDH	RAL	RCA	REP	RGG	RSW	RUG	RVB
Hydrolysable tannins	Digalloyl HHDP hexoside 1 – 4	785	683, 615, 483, 419, 301			x						x	
	Trigalloyl hexoside 1, 2	635	465, 483, 423, 271, 193				x						
	Trigalloyl HHDP hexoside	937	767, 785, 465, 741, 635, 301				x						
	Trigalquinic acid	647	495, 343				x						
	Di-HHDP glucose 1, 2	783	481, 301, 275		x		x			x			
	Digalloyl hexoside 1, 2	483	331	271, 169, 211			x						
	Digalloylquinic acid 1 – 3	495	343	191, 169, 325			x						
	Galloyl bis HHDP glucose 1, 2	935	633, 301				x						
	HHDP digalloyl glucose isomer 1 – 3	785	301, 483, 615, 419, 633, 275				x						
	HHDP galloyl hexoside	633	463, 301		x		x						
	HHDP glucose isomer	433			x		x						
Flavanols	**Catechin**	335	289	245	x	x	x	x	x	x	x	x	x
	Catechin hexoside	451	271, 289, 137			x	x	x			x	x	x
	PA dimer 1 – 5	577	425, 451, 407, 289		x	x	x	x	x	x	x	x	x
	PA trimer 1 – 8	865	577, 451, 407, 289, 695, 739		x	x	x	x	x	x	x	x	x
	PA tetramer	1153	865, 577, 407, 983				x	x	x				
	PA dimer monoglyc 1 – 3	739	587, 407, 449, 559, 612, 289, 269			x	x	x			x	x	
	PA dimer diglyc 1 – 4	1189	899, 739, 1063, 575, 719, 857, 843, 449			x		x			x	x	
	Dimer PA monogallate	729	577, 559, 407, 425, 451, 289				x			x			
Flavonols	Isorhamnetin-pentoside 1, 2	447	315		x	x	x	x	x		x	x	
	Isorhamnetin-3-rhamnoside	461	315					x					
	Isorhamnetin-hexoside	477	315						x				
	Kaempferol derivative	465	285			x		x	x		x	x	x
	Kaempferol-acetyl-hexoside	489	285									x	
	Kaempferol-acetyl-hexoside-rhamnoside	635	285						x				

(*Continued*)

Table 4-Continued.

Compound class	Tentative identification[a]	*m/z* [M-H]$^-$ or M$^+$	MS^2 (*m/z*)	MS^3 (*m/z*)	Species/ cultivar[c]								
					BON	FDH	RAL	RCA	REP	RGG	RSW	RUG	RVB
	Kaempferol-3-glucoside	447	285									x	
	Kaempferol-3-glucuronide	461	285									x	
	Quercetin-3-arabinofuranoside	433	301		x	x	x	x	x	x	x	x	x
	Quercetin-3-arabinopyranoside	433	301			x		x	x		x	x	x
	Quercetin-3-galactoside	463	301			x	x	x	x	x	x	x	x
	Quercetin-3-glucoside	463	301		x	x	x	x	x	x	x	x	x
	Quercetin-3-glucuronide	477	301		x	x	x	x	x	x	x	x	x
	Quercetin-3-rhamnoside	447	301		x	x	x	x	x	x		x	x
	Quercetin-3-rutinoside	609	301		x	x	x	x	x	x	x	x	x
	Quercetin-3-xyloside	433	301		x	x		x	x	x	x	x	x
	Quercetin-acetyl-hexoside	505	463, 301	301	x				x				x
	Quercetin-hexoside	463	301									x	
	Quercetin-galloylhexoside 1, 2	615	463	301	x		x		x	x			x
	Quercetin-rhamnosyl-hexoside	609	301		x								x
Flavanones	Eriodictyol hexoside 1, 2	449	287	151, 135		x		x	x		x		
	Naringenin hexoside 1 – 5	433	271			x		x	x		x	x	x
	Taxifolin pentoside 1 – 3	435	285, 303, 151, 125		x				x	x			
	Taxifolin di-pentoside	597	465	303, 285, 151, 129	x								x
	Dihydrokaempferol hexoside	449	287, 265						x				x
Flavone	Apigenin derivative 1,2	449	269			x		x			x	x	
Dihydrochalcone	**Phloridzin**	481	435	273	x	x	x	x	x		x	x	x
Stilbenoid	Resveratrol derivative	551	505	373, 273		x					x	x	
Carotenes	**Lycopene**[b]	535, 537			x	x	x	x	x	x	x	x	x
	β-carotene[b]	535, 537			x	x	x	x	x	x	x	x	x

[a]Compounds in bold were identified with appropriate authentic standards, other compounds were tentatively identified.
[b]Anthocyanin and carotenes were determined in positive ion mode M$^+$, other phenolic classes in negative ion mode [M-H]$^-$
[c]*Rosa* species/cultivars in which compounds were detected: BON, Bonica; FDH, Fru Dagmar Hastrup; RAL, Aloha; RCA, *R. canina*; REP, Repandia; RGG, Golden Gate; RSW, *R. stergiana*; RUG, *R. rugosa*; **RVB**, Veilchenblau.

Table 5–Phenolic, stilbenoid, lycopene, and β-carotene content (mean (μg/g DW) ± SE) of rose hips from selected species/cultivars.

Compound	BON	FDH	RAL	RCA	REP	RGG	RSW	RUG	RVB
Cyanidin glucoside	3.50 ± 0.32a*	28.5 ± 4.5b	7.73 ± 1.90a	83.0 ± 7.8c	1.53 ± 0.13a	2.72 ± 0.59a	24.4 ± 1.7b	54.2 ± 8.2d	6.33 ± 0.42a
Ellagic acid derivatives	293 ± 7abc	502 ± 49c	8762 ± 360c	50.6 ± 3.2a	250 ± 15abc	1973 ± 122d	87.3 ± 7.3ab	474 ± 72bc	30.2 ± 1.1a
Methyl gallate derivatives	241 ± 4c	565 ± 46e	4.35 ± 0.23a	449 ± 26d	–	130 ± 5b	400 ± 23d	603 ± 66e	39.4 ± 1.6a
Total hydroxybenzoic acids	534 ± 9b	1067 ± 75c	8766 ± 360e	499 ± 29b	250 ± 15ab	2104 ± 123d	488 ± 29b	1077 ± 127c	69.6 ± 2.5a
Hydroxycinnamic acids	172 ± 5cd	149 ± 8c	622 ± 16f	195 ± 13d	29.1 ± 1.2a	145 ± 4c	348 ± 14c	183 ± 25cd	96.7 ± 4.9b
Total phenolic acids and derivatives	706 ± 13bc	1216 ± 84d	9389 ± 366f	695 ± 42bc	279 ± 16ab	2249 ± 124e	835 ± 42cd	1260 ± 151d	166 ± 7a
Hydrolysable tannins	342 ± 4a	1770 ± 101a	23516 ± 1343b	–	–	245 ± 11a	–	1756 ± 202a	–
Catechin and derivatives	1221 ± 12de	1040 ± 87bcd	853 ± 21ab	1111 ± 60cde	1296 ± 41ef	5694 ± 57g	777 ± 49a	1479 ± 147f	984 ± 22bc
Sum PA aglycones (di-, tri-, tetramers)	2738 ± 22b	2748 ± 181b	4199 ± 131c	1162 ± 76a	878 ± 47a	9257 ± 165d	946 ± 55a	3993 ± 500c	739 ± 33a
Sum PA dimer glycosides (mono- and diglycosides)		3041 ± 227c	26.3 ± 2.1a	2209 ± 114b	–	–	1969 ± 113b	3410 ± 358c	–
Total flavanols	3959 ± 32b	6829 ± 484c	9022 ± 149d	4483 ± 247b	2174 ± 87a	15855 ± 235c	3691 ± 213b	8883 ± 987d	1724 ± 55a
Isorhamnetin glycosides	8.57 ± 0.20b	12.0 ± 1.0c	43.8 ± 1.6f	11.1 ± 0.7bc	23.1 ± 1.1c	–	17.0 ± 1.3d	1.70 ± 0.16a	–
Kaempferol derivatives	–	6.43 ± 0.64a	–	3.07 ± 0.24a	41.4 ± 1.6c	–	5.73 ± 0.44a	25.6 ± 2.8b	38.8 ± 2.9c
Quercetin glycosides	453 ± 11c	181 ± 14b	1699 ± 61e	68.0 ± 4.0a	721 ± 29d	796 ± 33d	263 ± 19b	198 ± 15b	492 ± 14c
Total flavonols	462 ± 11d	200 ± 15b	1743 ± 62f	82.2 ± 4.7a	785 ± 31c	796 ± 33c	286 ± 21c	225 ± 18bc	531 ± 16d
Eriodictyol derivatives	–	3.25 ± 0.19a	–	77.7 ± 4.0d	21.0 ± 1.2b	–	56.0 ± 5.1c	–	–
Naringenin derivatives	–	19.6 ± 1.1b	–	98.9 ± 5.7d	56.2 ± 3.4c	–	4.45 ± 0.33a	13.6 ± 1.6b	51.7 ± 2.7c
Taxifolin derivatives	181 ± 5a	–	–	–	113 ± 5a	698 ± 117b	–	–	60.4 ± 2.0a
Total flavanones	181 ± 5bc	22.8 ± 1.3a	–	177 ± 10bc	296 ± 14c	698 ± 117d	60.4 ± 5.4ab	13.6 ± 1.6a	206 ± 9c
Apigenin derivative	–	12.2 ± 0.9b	–	6.03 ± 0.34a	–	–	7.60 ± 0.72a	11.1 ± 1.2b	–
Phloridzin	55.4 ± 1.7a	69.1 ± 7.8a	1068 ± 64d	59.2 ± 2.9a	251 ± 9b	–	84.9 ± 8.3a	43.9 ± 3.9a	332 ± 9c
Resveratrol derivative	–	10.7 ± 1.0a	–	–	–	–	31.0 ± 1.9b	11.3 ± 1.4a	–
Total phenols	5708 ± 45b	10158 ± 663c	44746 ± 1639c	5584 ± 311b	3787 ± 147ab	19846 ± 279d	5021 ± 283ab	12258 ± 1359c	2965 ± 90a
Lycopene	2825 ± 218d	351 ± 39a	935 ± 47b	1550 ± 61c	3731 ± 207e	88.3 ± 8.6a	42.7 ± 14.7a	356 ± 63a	2578 ± 134d
β-carotene	11158 ± 777d	681 ± 49a	7271 ± 393c	2126 ± 58b	2303 ± 118b	6304 ± 138c	1017 ± 143a	765 ± 107a	2692 ± 236b

BON, Bonica; FDH, Fru Dagmar Hastrup; RAL, Alohs; RCA, *Rosa canina*; REP, Repandia; RGG, Golden Gate; RSW, *Rosa sweginzowii*; RUG, *Rosa rugosa*; RVB, Veilchenblau.
*Different letters (a to g) for each parameter denote statistically significant differences among species/cultivars by Duncan's multiple range test at $P < 0.05$.

and RVB exhibited high lycopene content (2825 μg/g DW and 2578 μg/g DW, respectively), higher than RCA, which is suggested by Böhm and others (2003) as a rich source of this antioxidant. Previous research on carotenoid content of rose hips using HPLC methods include: Hodisan and others (1997) for *R. canina* hips (no quantitative evaluation); Hornero-Mendez and Minguez-Mosquera (2000) identified carotenoids in *R. mosqueta*; Olsson and others (2004) report carotenoid composition of *R. villosa* x *villosa* hips; Hallman and others (2011) analyzed content of β-carotene and lycopene in RUG hips and Andersson and others (2011) report carotenoid composition of hips of 4 different *Rosa* species. In all studies β-carotene and lycopene were the predominant carotenoids.

Conclusion

Thought RCA hips proved to be the richest in anthocyanin content, some modern rose cultivars stand out in terms of their favorable fruit composition. FDH hips proved to be richer in ascorbic acid content than the traditionally used RCA hips, contrary other ornamental cultivars accumulated lower levels of ascorbic acid compared to the investigated rose species. Conversely, hips of specific ornamental cultivars are characterized by their rich phenolic composition. RAL hips contained the highest amount of hydrolysable tannins, compounds that were not determined in RCA hips, while RGG hips were abundant in flavanols (almost 4-fold higher level than in RCA). Hips of FDH, RAL, and especially RGG were also the biggest and had high flesh to whole fruit ratio making them interesting for harvesting and processing. Species/cultivars varied in the content and ratio of β-carotene and lycopene. The richest in β-carotene content were the hips of BON cultivar that, together with REP and RVB hips, also contained high amounts of lycopene. Hips of REP and RVB were the smallest and as such difficult to gather and process. It can be concluded, that selected ornamental cultivars represent an interesting alternative to traditionally utilized RCA hips as they accumulate particularly high levels of ascorbic acid and beneficial phenolic constituents, making them not only decorative but functional. To fully exploit the phytonutrients, further investigations are needed to assess the optimum harvest time for different cultivars.

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Conflict of Interest

The authors have no conflict of interest to declare.

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2.2 POVEZOVALNO ZNANSTVENO DELO

2.2.1 Foliarno tretiranje vrtnic 'Funny Red' s sredstvom Lithovit Forte

V okviru doktorske disertacije je bil narejen še poskus foliarnega tretiranja miniaturnih vrtnic s sredstvom Lithovit Forte.

Tvorba sekundarnih metabolitov fenil propanoidne poti v rastlinskih tkivih je eden od načinov odzivanja rastline na biotske in abiotske strestne dejavnike (Dixon in Paiva, 1995). Številne raziskave do sedaj so pokazale, da se sinteza fenolov poveča po napadu patogena, povezujejo pa jih tudi z odzivi na abiotski stres kot npr. sušo, nizke temperature in UV-sevanje (Treutter, 2005; Slatnar in sod., 2013).

Z aplikacijo določenih snovi ali biotičnih pripravkov lahko povečamo vsebnost fenolov v rastlinskih tkivih že pred napadom patogena oz. pred stresnim dogodkom, kar pripomore k boljši tolerantnosti na bolezni oz. zakasni njen potek (Treutter, 2005; Karthikeyan in sod., 2007). Povečanje akumulacije fenolov lahko sprožimo na različne načine, eden od načinov modifikacije sekundarnega metabolizma je tudi z dodajanjem ali odvzemom določenih makro in mikro hranil (Treutter, 2005).

Povečana vsebnost fenolnih snovi v listih lahko zmanjša pojavnost določenih rastlinskih glivičnih bolezni; tako so Shetty in sod. (2011) ugotovili da foliarna aplikacija klorogenske kisline ali rutina (kvercetin rutinozid) poveča odpornost miniaturnih vrtnic 'Smart' na šipkovo pepelovko (*Podosphaera pannosa*). Ugotovili so tudi, da zalivanje rastlin s hranilno raztopino, ki ji je bil dodan silicij povzroči večjo sintezo in akumulacijo fungitosičnih fenolnih spojin in tako zakasni pojav simptomov šipkove pepelovke in zmanjša intenzovnost bolezni (Shetty in sod., 2012).

Lithovit Forte je foliarno gnojilo iz mletega apnenca, ki vsebuje tudi lahko dostopna mikrohranila. Delci gnojila preko listnih rež vstopajo v rastlino, kjer sproščajo ogljikov dioksid. Tako se poveča obseg fotosinteze, kar skupaj z mikrohranili, poveča odpornost, rast in vitalnost rastlin (Lithovit, 2009; dokument je v prilogi A).

S poskusom smo želeli ugotoviti, ali foliarno tretiranje rastlin s pripravkom Lithovit Forte vpliva na povečanje fenolnih snovi v listih teh rastlin, kar bi lahko pripomoglo k boljši tolerantnosti rastlin na glivične bolezni in k zmanjšanju uporabe fungicidov pri njihovi pridelavi.

Materiali in metode

V poskusu smo uporabili miniaturne vrtnice sorte 'Funny Red'. Sredi januarja 2016 so bile dobavljene cvetoče rastline, povprečno so bili v enem loncu potaknjeni po štirje potaknjenci. Rastline smo presadili v večje plastične lonce (premer 15 cm), porezali smo jim cvetove in prikrajšali poganjake, da smo spodbudili vegetativno rast. Miniaturne vrtnice so imele urejeno poplavno namakanje in so bile tri dni pred začetkom poskusa enkrat tretirane s sistemičnim fungicidom, da ne bi prišlo do razvoja glivičnih bolezni. V steklenjaku je bilo urejeno gretje, da minimalna temperatura ni padla pod 8 °C. Konec februarja 2016, ko so bili novi poganjki dovolj razviti, smo rastline z ročno pršilko tretirali s pripravkom Lithovit Forte. Uporabili smo priporočen odmerek 5 g/l vode ter dvojno koncentracijo 10 g/l vode. Kontrolne rastline smo škropili z vodo. Za vsako obravnavanje smo tretirali rastline v desetih loncih. Vzorčili smo polno razvite liste pred tretiranjem (0 DPT) in nato en (1 DPT), dva (2 DPT) in sedem dni po škropljenju (7 DPT). Iz vsakega lonca smo nabrali po dva lista (ne iz iste rastline), jih oplaknili, osušili in zamrznili s tekočim dušikom ter shranili na -20 °C do analize skupnih fenolnih snovi. Skupne fenolne snovi smo določali spektrofotometrično. Za ekstrakcijo smo liste s tekočim dušikom zmleli v prah ter 0,5 g prahu prelili s 4 ml metanola s 3 % mravljinčne kisline. Preliti material smo ekstarhirali v ultrazvočni kopeli 1 h, nakar smo vzorce centrfugirali in prefiltrirali čez poliamidni filter v mikrocentrifugirko (epico). Za določitev skupnih fenolnih snovi smo prilagodili metodo, ki jo navajata Singleton in Rossi (1965). V centrifugirko smo nalili 7,9 ml bidestilirane vode, dodali 100 µl vzorca (iz epice) in 500 µl Folin-Ciocalteu reagenta (Sigma-Aldrich, St. Louis, Združene države Amerike) . Po pretečnih petih minutah smo dodali 1,5 ml 20 % raztopine natrijevega karbonata (Na_2CO_3) ter vzorce postavili na 40 °C za 30 minut. V tem času se je razvila modra barva, vzorce smo prelili v polistirenske kivete (Brand, Wertheim, Nemčija) in s spektrofotometrom (Labda Bio 20, Perkin Elmer, Waltham, Združene države Amerike) določili absorbcijo pri valovni dolžini 765 nm. Vsebnost skupnih fenolnih snovi v vzorcu smo izračunali iz umeritvene krivulje galne kisline in rezultate izrazili v mg ekvivalentov galne kisline (GAE) na g sveže mase.

Statistična analiza je bila narejena s programom Statgraphics Centurion XV. Razlike med obravnavanji pri posameznem terminu smo ugotavljali z enosmerno analizo variance (ANOVA), značilne razlike med obravnavanji pa smo ovrednotili z Duncan-ovim testom pri 5 % tveganju. Z različno črko smo označili obravnavanja, ki se med seboj statistično razlikujejo (statistične razlike pri $p < 0,05$).

Rezultati in razprava

Vsebnost skupnih fenolnih snovi v listih miniaturne vrtnice 'Funny Red' se pred tretiranjem ni razlikovala (Preglednica 1). Statistično značilno razliko v vsebnosti skupnih fenolnih snovi smo izmerili en dan po tretiranju (1 DPT), kjer so imeli listi kontrolnih rastlin manjšo vsebnost skupnih fenolnih snovi v primerjavi z listi rastlin škropljenimi s pripravkom Lithovit Forte, pri katerih pa uporaba različnih koncentracij ni pokazala značilnih razlik.

Listi kontrolnih rastlin so 1 DPT vsebovali 4,1 mg GAE/g sveže mase, medtem ko so listi tretiranih rastlin vsebovali 5,0 mg GAE/g sveže mase (tretirani s koncentracijo 5 g/l) oz. 4,9 1 mg GAE/g sveže mase (tretirani z 10 g/l). Pri ostalih terminih nismo izmerili značilnih razlik v vsebnosti skupnih fenolnih snovi med kontrolo in obravnavanji z Lithovitom.

Preglednica 1: Vsebnost skupnih fenolnih snovi (povprečje v mg ekvivalentov galne kisline/g sveže mase ± standardna napaka) v listih miniaturne vrtnice 'Funny Red'. Različne črke označujejo statistično značilne razlike med obravnavanji pri posameznem terminu. DPT = dnevi po tretiranju.
Table 1: Content of total phenolic compounds (mean in mg gallic acid equivalents/g fresh weight ± standard error) in leaves of miniature rose 'Funny Red'. Different letters denote statistically significant differences between observations at individual sampling dates. DPT = days after treatment.

Termin	Kontrola	Lithovit Forte 5 g/l	Lithovit Forte 10 g/l
0 DPT	4,5 ± 0,2 a	4,4 ± 0,1 a	3,9 ± 0,2 a
1 DPT	4,1 ± 0,2 a	5,0 ± 0,3 b	4,9 ± 0,2 b
2 DPT	4,8 ± 0,2 a	5,2 ± 0,2 a	5,4 ± 0,3 a
7 DPT	4,4 ± 0,2 a	4,7 ± 0,2 a	4,5 ± 0,2 a

Sredstvo Lithovit Forte se ni izkazalo za učinkovito z vidika povečanja skupnih fenolnih snovi v listih vrtnice 'Funny Red', saj je bilo izmerjeno značilno povečanje le dan po tretiranju. Estiarte in sod. (1999) so pri pšenici, ki je bila gojena v atmosferi s povečano koncentracijo ogljikovega dioksida (CO_2), ugotovili povečano vsebnost flavonoidov v listih. Sredstvo Lithovit Forte naj bi zaradi svoje sestave v rastlini sproščalo CO_2 in s tem vplivalo na večjo stopnjo fotosinteze (Lithovit, 2009; dokument je v prilogi A). Domnevamo lahko, da je do povečanja skupnih fenolnih snovi prišlo zaradi učinka CO_2, podobno kot poročajo Estiarte in sod. (1999).

3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V prvem poskusu smo določili fenolni profil listov in venčnih listov različnih sort in vrst vrtnic. Izbrali smo štiri vrste šipkov, ki so pri nas avtohtoni: navadni šipek (*Rosa canina* L.), rdečelistni šipek (*R. glauca* Pourr.), rjastordeči šipek (*R. rubiginosa* L.) in vednozeleni šipek (*R. sempervirens* L.). Poleg njih smo v raziskavo vključili tudi tri moderne sorte: 'Rosarium Uetersen', 'Ulrich Brunner Fils' in 'Schwanensee'.

Vzorčenje je potekalo poleti 2013. Ob vzorčenju smo nabrali venčne liste polno odprtih cvetov (Schmitzer in sod., 2010) in polno razvite liste brez znakov bolezni. Venčnim listom smo izmerili barvo s prenosnim kolorimetrom, z visokoločljivostno tekočinsko kromatografijo in masno spektrometrijo pa smo v tkivu določili še antocianine in flavonole. V listih smo poleg flavonolov določili tudi druge fenolne snovi: flavanole, fenolne kisline in njihove derivate ter hidrolizirajoče tanine.

Tako rjastordeči šipek, rdečelistni šipek kot sorti 'Rosarium Uetersen' in 'Ulrich Brunner Fils' imajo rožnato obarvane venčne liste. Navadni šipek razvije bele do rahlo rožnate petale, vednozeleni šipek in sorta 'Schwanensee' pa oblikujetabele venčne liste, ki so pri sorti v sredini cveta rahlo rožnati.

Pri vrstah z rožnatimi cvetovi smo izmerili največje vrednosti parametra *a**, ki predstavlja delež rdeče barve v vzorcu. Pri sorti in obeh vrstah z bolj belimi cvetovi smo izmerili majhne vrednosti parametra *a**, najmanjši je bil pri *R. sempervirens*, ki ima popolnoma bele cvetove. Iz analize barvih parametrov smo ugotovili, da imajo sorte z (večinoma) belimi cvetovi večje vrednosti parametra *b**, kar pomeni, da je barva njihovih petalov bolj rumena, kot pri rožnatih sortah: pri slednjih je bila vrednost parametra *b** negativna, kar nakazuje bolj modrikaste tone. Podobno obratno sorazmernost med parametroma *a** in *b** so pri venčnih listih belo, rožnato in rdeče cvetočih prekrovnih vrtnic ugotovili tudi Schmitzer in sod. (2010).

Parameter *L** se uporablja za izražanje svetlosti barve. Vrednosti so lahko med 0 in 100, kjer 0 pomeni črno in 100 popolnoma belo barvo. Pričakovano smo pri venčnih listih *R. sempervirens*, *R. canina* in 'Schwanense' izmerili največje vrednosti tega parametra. Ostale sorte in vrste z rožnatimi cvetovi so imele negativne vrednosti parametra *b**, kar pomeni bolj modrikasto barvo. To lahko opazimo tudi pri parametru *h°*, ki je bil pri teh vrstah in sortah okoli 350, kar na barvnem krogu pomeni barvo med rdečo (0 oz 360) in modro (270). Vrednosti barvnih parametrov smo lahko dobro korelirali z vsebnostjo antocianinov v venčnih listih. Podobno so v svojem članku ugotovili že Schmitzer in sod. (2010) - večjo vsebnost antocianinov lahko torej povežemo z večjim parametrom *a**.

V venčnih listih smo določili mono- in di-glukozide treh antocianidinov: cianidina, pelargonidina in peonidina; poleg njih pa še rutinozid cianidina. O teh snoveh v venčnih listih vrtnic poročajo že Biolley in sod. (1994), Mikanagi in sod. (1995), Cai in sod. (2005), ter Schmitzer in sod. (2009). Kot poročajo te raziskave se je tudi v naši izkazalo, da je cianidin-3,5-diglukozid prevladujoč, določili smo ga v petalih vseh preučevanih vrst in sort, tudi pri belocvetnih, le da ga je bilo tam statistično značilno najmanj. *R. canina, R. sempervirens* in 'Schwanensee' se značilno niso razlikovale v njegovi vsebnosti, medtem ko smo pri ostalih sortah in vrstah zaznali statistično značilne razlike. Poleg razlik v vsebnosti posameznih antocianinov, so se sorte in vrste razlikovale tudi po vrsti vsebujočih antocianinov. Sestava antocianinov je bila najbolj raznolika pri sorti 'Rosarium Uetersen'. V njenih petalih smo lahko določili 5 različnih antocianinov, kljub temu pa je bila skupna vsebnost antocianinov največja v petalih sorte 'Urlich Brunner Fils', v kateri smo določili le tri različne antocianine.

Še večja variabilnost med sortami in vrstami se je izkazala pri vsebnosti flavonolov v petalih. Določili smo lahko kar 31 različni flavonolov: 14 glikozidov kvercetina in 17 različnih glikozidov kempferola. Najpogostejši so bili naslednji glikozidi kvercetina: rutinozid, glukozid, glukuronid, arabinofuranozid, galaktozid, ksilozid in ramnozid. Med glikozidi kempferola so prevladovali diglukozid, ramnozid, glukozid, glukouronid ter galaktozid. Poleg omenjenih smo v venčnih listih potrdili še druge derivate kvercetina in kempferola, med drugim tudi acetilirane in take z vezano galoilno skupino. Identificirane snovi se ujemajo s prejšnimi raziskavami venčnih listov vrtnic (Cai in sod., 2005; Kumar in sod., 2009; Mikanagi in sod., 2000).

Vrste in sorte so se razlikovale tako v določenih flavonolih kot tudi v njihovi vsebnosti v venčnih listih. Tako so imele vrste *R. canina* in *R. glauca* ter sorta 'Ulrich Brunner Fils' večje vsebnosti kvercetinov v primerjavi s kempferolom, ostale preučevane vrste in sorte pa so imele bolj ali manj izrazito večjo vsebnost kempferolov v primerjavi z glikozidi kvercetina. Petali vrste *R. rubiginosa* so imele največjo vsebnost konjugatov kempferola. Največjo vsebnost kempferola v primerjavi z vsebnostjo kvercetina, gledano v odstotkih, smo določili v petalih sorte 'Rosarium Uetersen', čeprav je količinsko gledano vsebovala desetkrat manj kempferolov kot *R. rubiginosa.* Biolley in sod. (1994) so na podlagi vsebnosti kempferola in kvercetina določili tri različne kemotipe križancev vrtnic: ugotovili so, da so križanci, kjer v petalih prevladuje kvercetin, manj pogosti.

Tudi v fenolnem profilu listov so se pokazale značilne razlike med vrstami in sortami. Ugotovili smo še večjo raznolikost kot v petalih, saj smo poleg derivatov kempferola in kvercetina v *R. sempervirens* in 'Schwanesee' določili glikozide izoramnetina, pri vrsti *R. canina* pa glikozide miricetina. Pri vseh preučevanih vrstah in sortah se je izkazalo, da prevladujejo glikozidi kvecetina v primerjavi s kempferolom, kar se sklada z raziskavo

Nowak in Gawlik-Dziki (2007), ki sta pri različnih sortah in vrstah ugotovili enako. Li in sod. (2013) ter Hashidoko (1996) poročajo o kvercetin glikozidih v listih *R. sericea* in *R. rugosa*, Porter in sod. (2012) pa so detektirali glikozide kvercetina in kempferola v listih *R. spinossisima*.

Določili smo sedem prevladujočih glikozidov kvercetina (arabinofuranozid, galaktozid, glukozid, glukuronid, ramnozid, rutinozid, ksilozid) in tri glikozide kempferola (ramnozid, glukuronid, glukozid); od tega smo v vseh vrstah in sortah potrdili kvercetin arabinofuranozid, ksilozid in ramnozid ter kempferol ramnozid.

Poleg flavonolov smo v listih določili tudi številne druge spojine, ki jih uvrščamo v skupine flavanolov, hidrolizirajočih taninov (elagitaninov) in fenolnih kislin ter njihovih derivatov. Iz skupine flavonolov je bil katehin določen pri vseh vrstah in sortah, v največji meri se je pojavil v listih *R. canina,* najmanj pa so ga vsebovali listi sorte 'Schwanensee'. Le v slednji smo lahko identificirali tudi epikatehin. Da so *Rosaceae* na splošno bogate s katehinom in procianidini, poročajo Hoffmann in sod. (2012). Drugi avtorji so potrdili prisotnost katehina v listih *R. damascena* (Baydar N. G. in Baydar H., 2013), kjer naj bi bil najbolj zastopana fenolna spojina. Identificirali so ga tudi v petalih *R. micrantha* (Barros in sod., 2013) ter v koreninskih laskih *R.* x *hybrida* (Hoffmann in sod., 2012) in različnih delih *R. rugosa* (Hashidoko, 1996). Poleg katehina smo v sortah in vrstah določili še procianidin dimere in trimere, v *R. canina, R. glauca in R. sempervirens* pa tudi tetramere procianidina. Spet se je izkazalo, da sorte v splošnem vsebujejo manjše količine omenjenih snovi kot vrste.

Kljub temu da Miyasaki in sod. (2013) navajajo elagno kislino, kot najbolj pomembno aktivno sestavino ekstraktov *R. rugosa*, smo lahko prosto elagno kislino določili le v listih *R. sempervirens, R. glauca* in pri sorti 'Rosarium Uetersen'. V listih slednjih dveh tudi ni predstavljala ene od glavnih komponent. Smo pa v listih lahko določili njene številne konjugate. Še posebej obilno so bili zastopani hidrolizirajoči tanini, ki jih poimenujemo tudi elagitanini. Ti so sestavljeni iz vsaj ene HHDP enote (heksahidroksidifenol), ki je zaestrena s sladkorno enoto, običajno glukozo (Haslam, 1996; Koponen in sod., 2007). Najbolj so bili elagitanini zastopani v listih *R. rubiginosa* v kateri smo določili izomer HHDP galoil glukoze, galoil bis HHDP glukozo in trigaloil HHDP heksozo. Od elagitaninov smo lahko v vseh vrstah in sortah določili HHDP digaloil glukozo, di HHDP glukozni izomer in izomer elagitanina veskalagina. Najmanjše vsebnosti smo spet določili pri sorti 'Schwanensee'. Ascacio-Valdez in sod. (2011) navajajo, da so elagitanini pomembni v fiziologiji rastlin, saj ščitijo (celico) pred mikrobnim razpadom. To lastnost povezujejo z lastnostjo elagitaninov, da s proteini in polisaharidi tvorijo močne komplekse in tako zavirajo rast mikrobov. Zaradi svojih močnih antioksidativnih lastnosti, naj bi bili pomembni tudi v človeški prehrani, zlasti pri preprečevanju degenerativnih bolezni (Ascacio-Valdez in sod., 2011; Haslam, 1996; Sroka, 2005).

Od fenolnih kislin smo v vseh vrstah in sortah določili *cis*- in *trans*-klorogensko kislino. Vrste in sorte so se ponovno razlikovale tako v posameznih spojinah iz te skupine, kot tudi v vsebnosti teh snovi. Da je klorogenska kislina najbolj zastopana fenolna kislina v listih *R. x hybrida* 'Smart' poročajo Shetty in sod. (2011). V naši raziskavi (Cunja in sod., 2014) je bila njena vsebnost največja tako v listih vrst *R. canina*, *R. glauca* in *R. rubiginosa* ter v listih sorte 'Rosarium Uetersen'; v listh sorte 'Urlich Brunner Fils' je bila najbolj zastopana neoklorogenska kislina. Listi vrste *R. sempervirens* so izkazali največjo vsebnost proste elagne kisline, medtem ko so v listih sorte 'Schwanensee' prevladovali njeni pentozidi. Poleg omejenih spojin smo določili več kot osem različnih prostih fenolnih kislin oz. so bile te vezane s sladkorji v obliki pentoze ali heksoze. Tudi Baydar N. G. in Baydar H. (2013) poročata o majhnih vsebnostih različnih fenolnih kislin (kavina, klorogenska, p-kumarna, ferulna, galna kislina) v listih *R. damascena*.

V raziskavi smo opravili Wardovo metodo razvrščanja v skupine (multivariatno statistično analizo), s katero smo preučevane vrste in sorte lahko razdelili v tri skupine. Izstopala je *R. canina* z veliko vsebnostjo raznolikih fenolnih snovi. Njej najbližje sta bili sorti *R. sempervirens* in *R. rubiginosa*. Vrsto *R. glauca* je analiza uvrstila k sortam, med katerimi se je zaradi majhne vsebnosti fenolov v listih najbolj razlikovala sorta 'Schwanesee'. Izmed obravnavanih sort je bila ta najbolj občutljiva na bolezni. To bi lahko povezali prav z majhno vsebnostjo fenolnih snovi, ki jih avtorji povezujejo z obrambnimi mehanizmi rastline pred različnimi stresnimi dejavniki (Dixon in Paiva, 1995; Osbourn, 1996; Shetty in sod., 2011; Sroka, 2005).

V prvi raziskavi (Cunja in sod., 2014) se je izkazalo, da je tradicionalna uporaba *R. canina* upravičena, saj vsebuje velike vsebnosti različnih snovi, v primerjavi z drugimi avtohtonimi vrstami. Tradicionalno se v zdravilne in prehranske namene uporablja predvsem birni plod, točneje omesenelo cvetišče v katerem najdemo plodove – oreške. Vsebnosti bioaktivnih snovi v šipkih so že precej raziskane, nas pa je zanimalo, kako se vsebnosti spreminjajo med dozorevanjem ploda. V Slovenji pogosto slišimo, da naj bi se šipke nabiralo po prvi jesenski slani. Zanimalo nas je, kaj bi to doprineslo k vsebnosti nekaterih primarnih in sekundarnih metabolitov. Plodove smo postopno nabirali od začetka septembra do začetka novembra ter še enkrat po prvi slani zgodaj decembra. V njih smo določili vsebnosti sladkorjev, organskih kislin, vitamina C, fenolnih snovi ter β-karotena in likopena. Plodovom smo s prenosnim kolorimetrom vsakič izmerili tudi barvne parametre, saj je lahko barva indikator zrelosti kot so pri *R. dumalis* in *R. rubiginosa* ugotovili Uggla in sod. (2005), Perkins-Veazie in Nonnecke (1992) pa barvo pri malinah povezujeta z vsebnostjo topne suhe snovi.

Ker so bili plodovi ob prvem obiranju že precej obarvani, se vrednosti parametra *a** niso močno spreminjale. Najbolj rdečkasti so bili plodovi ob drugem obiranju konec septembra,

vrednosti pa so naknadno upadale, tako da je bila najmanjša vrednost zabeležena po zmrzali. Bolj statistično značilno so upadale vrednosti parametra *b**, zmanjšanje katerega nakazuje, da so plodovi zgubljali rumenkasto barvo. Z zorenjem je značilno upadal tudi parameter *L**, kar kaže na to, da so bili plodovi z zorenjem vse temnejši. Enako so pri *R. dumalis* in *R. rubiginosa* ugotovili Uggla in sod. (2005). Podobne vrednosti barvnih parametrov *a**, *b** in *L**, kot smo jih določili pri prvem vzorčenju, navaja tudi Ercişli (2007). Slednji navaja tudi delež suhe snovi, ki naj bi se pri plodovih različnih vrst šipkov gibal med 33,9 in 40,4 %, kar se ujema z našimi ugotovitvami in ugotovitvami Türkben-a in sod. (2010). Med zorenjem smo podobo kot Uggla in sod. (2005) ugotovilil statistično značilno povečanje vsebnosti suhe snovi.

V plodovih šipka smo izmerili vsebnost treh sladkorjev: glukoze, fruktoze in saharoze. Glukoza in fruktoza sta predstavljali glavnino sladkorjev v plodu; izmerjene vrednosti se ujemajo s tistimi, ki jih poročajo Demir in sod. (2014). Kovács in sod. (2000) navajajo ta dva prevladujoča sladkorja v plodovih navadnega šipka. Barros in sod. (2010, 2011) so ugotovili podobno, vendar navajajo poleg teh treh še vsebnosti trehaloze in rafinoze v nezrelih šipkih, poleg tega pa so izmerili precej manjše vsebnosti sladkorjev. Pri fruktozi in glukozi smo ugotovili značilno zmanjšanje po zmrzali, medtem ko je bila vsebnost saharoze največja v času prvega obiranja, nato pa v njeni vsebnosti ni bilo značilnih razlik med termini.

Šipki so znani po veliki vsebnosti vitamina C. Znano je, da je to precej nestabilna snov, ki pod vplivom temperature hitro razpade, zato nas je še posebej zanimalo, kaj se z vitaminom C zgodi v plodovih nabranih po slani. Največja vsebnost vitamina C je bila izmerjena ob tretjem obiranju v začetku oktobra. Vsebnost vitamin C je v plodovih nabranih po slani močno upadla, saj so vsebovali samo še petino izmerjene največje vsebnosti. Vitamin C je v plodovih šipka pogosto preučevan, raziskave navajajo precej različne vsebnosti. Tako Barros in sod. (2011) navajajo vsebnosti vitamin C v šipkih s Portugalske od 68 (v zrelih) do 262 mg/100 g suhe mase (v nezrelih plodovih), Ercişli in Eşitken (2004) pa sta v šipkih iz Turčije določila od 1074 do 1586 mg vitamina C na 100 g plodov, vendar metoda določanja ni povsem jasna. Kovács in sod. (2000) ter Adamczak in sod. (2012) poleg tega poročajo, da *R. canina* vsebuje relativno malo vitamina C v primerjavi z drugimi vrstami in genotipi, ki so jih preučevali. Podobno smo ugotovili tudi mi (Cunja in sod., 2016). Vsebnosti vitamina C, ki smo jih določili, se ujemajo z navedbami Adamczak in sod. (2012), Roman in sod. (2013), ter Guimarães in sod. (2010). Slednji so preučevali vsebnosti vitamina C v nezrelih, dozorevajočih in zrelih plodovih *R. micrantha*. Kot v naši raziskavi (Cunja in sod., 2015) so tudi ti avtorji ugotovili največjo vsebnost v dozorevajočih, še ne povsem zrelih oz. prezorenih plodovih. Yahia in sod. (2001) so podobno upadanje vsebnosti vitamina C z dozorevanjem ugotovili v plodovih paprike in paradižnika. Zmanjšanje vitamina C povezujejo s porabljanjem askorbata za delovanje encima 1-aminociklopropan-1-karboksilat oksidaze, ki katalizira nastanek

etilena; tik pred zmanjšanjem vsebnosti vitamina C pa so zaznali tudi povečano delovanje encima osidaze askorbinske kisline.

Citronska kislina je bila najbolj zastopana organska kislina v analiziranih plodovih, sledila je jabolčna kislina. Poleg teh organskih kislin smo določili še kinisko, vinsko, šikimsko in fumarno kislino. Da sta citronska in jabolčna kislina najbolj zastopani organski kislin v plodovih, poročajo Zocca in sod. (2011), Mikulic-Petkovsek in sod. (2012a) ter Demir in sod. (2014). Adamczak in sod. (2012) in Kovács in sod. (2000) pa poročajo le o vsebnosti citronske kisline v plodovih različnih vrst šipkov. Po naših podatkih je malo informacij o kompoziciji organskih kislin v plodovih vrtnic (šipkov) in nobena raziskava do sedaj še ni poročala kako se ta spreminja med zorenjem. Zocca in sod.(2011) poleg citronske in jabolčne navajajo še vsebnosti mlečne, oksalne in fumarne kisline. Iz naše študije lahko razberemo, da zmrzal ni imela vpliva na vsebnost citronske in kininske kisline, saj se njuna vsebnost v zadnjih terminih vzorčenja ni razlikovala, najmanjša pa je bila ob drugem vzorčenju. Vinsko kislino smo določili le v vzorcih zadnjih treh obiranj, vsebnosti pa se med vzorčenji niso značilno razlikovale. Vsebnosti jabolčne, šikimske in fumarne kisline so med zorenjem bolj variirale. Tudi pri njih smo ugotovili značilno najmanjše vsebnosti ob drugem vzorčenju, značilno zmanjšanje pa smo lahko ugotovili ob vzorčenju po zmrzali. V primerjavi z Mikulic-Petkovsek in sod. (2012a) smo določili nekoliko večje vsebnosti citronske in jabolčne kisline, medtem ko se vsebnosti fumarne in šikimske kisline ujemajo z omenjeno raziskavo.

V plodovih smo določili le en antocianin, cianidin-3-glukozid. Tudi drugi avtorji so določili oz. navajajo njegovo vsebnost v šipkih *R. canina* (Hvattum, 2002; Guimarães in sod., 2013). Največje vsebnosti smo določili ob tretjem oz. četrtem vzorčenju. Naši vzorci so vsebovali več omenjenega antocianina, kot so ga določili Guimarães in sod. (2013) v zrelih plodovih iste vrste. Vsebnost cianidin-3-glukozida je nato upadala, še posebej v vzorcih nabranih po zmrzali. Kot navajajo Christie in sod. (1994), nizke temperature zavirajo nastajanje anocianinov, predvidevajo pa tudi da se že nakopičeni antocianini začnejo razgrajevati. To bi lahko razložilo manjše vsebnosti v plodovih po zmrzali. V petalih rožnato in rdeče cvetočih sort in vrst smo lahko določili močno korelacijo med barvnim parametrom *a** in skupno vsebnostjo antocianinov (Cunja in sod., 2014), kar so ugotovili tudi Schmitzer in sod. (2010). V plodovih ta korelacija ni bila močno izražena, saj dajejo barvo plodovom predvsem drugi pigmenti (karotenoidi).

Poleg antocianina smo v plodovih s tekočinsko kromatografijo visoke ločljivosti v povezavi z masno spektrometrijo (HPLC/MS) določili še 45 različnih fenolnih snovi. Po vsebnosti so bili najbolj zastopani flavanoli (katehin, katehin heksozid ter številni glikozidi ter aglikoni procianidina), kar se ujema tudi z drugimi raziskavami plodov vrtnic. Vsebnosti katehina ter procianidinov v *R. canina* navajajo Ganhão in sod. (2010) in Guimarães in sod. (2013). Prvi navajajo večje vsebnosti katehina, kot smo jih določili v

naših vzorcih, medtem ko slednji navajajo vsebnosti katehina podobne našim. Tudi Fecka (2009) navaja večje vsebnosti katehina, vendar ne poroča o prisotnosti katehin glikozidov. Salminen in sod. (2005) navajajo procianidine in katehin kot glavne fenolne komponente tudi v treh drugih vrstah sekcije *Caninae.* Türkben in sod. (2010) pa so ugotovili večje vsebnosti katehina v manj zrelih oranžnordečih plodovih kot v bolj zrelih rdečkastih plodovih. Največjo vsebnost katehina in katehin heksozida smo določili ob tretjem vzorčenju, vsebnost pa je bila najmanjša v plodovih po zmrzali. Medtem ko sta se vsebnosti katehina in katehin heksozida med zorenjem spreminjali, pa med različnimi termini nismo zaznali razlik v vsebnosti procianidinov.

Fecka (2009) v svoji raziskavi navaja, da je poleg katehina ter elagitanina rugosidina A, med glavnimi polifenolnimi komponentami v plodovih navadnega šipka še metil galat 3-O-β-glukozid. Tudi v naši raziskavi smo ugotovili številne derivate (glikozide) galne in elagne kisline (metil galat heksozid, metil galat acetil diheksozid, metil galat rutinozid, metil elagna kislina pentozid, pentozid elagne kisline). Tudi Hvattum (2002) je v plodovih *R. canina* določila metil galat rutinozid ter metil galat heksozid; slednjega je Fecka (2009) bolj natančno določila kot prej omenjeni galat 3-O-β-glukozid, navaja pa tudi večje vsebnosti, kot smo jih določili v naših analizah. Podobne vsebnosti metil galat heksozida navajajo v plodovih *R. micrantha* Guimarães in sod. (2013). V plodovih *R. canina* so Ganhão in sod. (2010) določili prisotnost hidroksibenzojskih in hidroksicimetnih kislin, vendar niso bolj natančno določili posameznih snovi. V naših vzorcih smo poleg prej omenjenih določili majhne vsebnosti derivatov heksozida sinapinske kisline, kumaroilkininske kisline in heksozid *p*-kumarne kisline. *Cis* in *trans* 5-*p*-kumaroilkinisko kislino smo določili že v listih različnih vrst in sort vrtnic (Cunja in sod., 2014), kolikor vemo pa smo prvič določili 3-*p*-*O*-, 4-*p*-*O*- in 5-*p*-*O*-kumaroilkinisko kislino v plodovih. Demir in sod. (2014) prav tako navajajo vsebnost sinapinske kisline v šipkih *R. canina*; drugi avtorji pa navajajo tudi vsebnost *p*-kumarne kisline v plodovih navadnega šipka in drugih vrst (Zocca in sod., 2011; Tumbas in sod., 2012; Demir in sod., 2014). Ugotovili smo lahko razlike med termini v vsebnosti številnih fenolnih kislin ter njihovih derivatov; večinoma so bile vrednosti najmanjše v vzorcih nabranih po zmrzovanju.

Iz skupine flavonolov smo določili različne glikozide kvercetina, dva heksozida izoramnetina ter derivat kempferola. Derivat kempferola ter kvercetin galaktozid, glukuronid in arabinofuranozid so bili zastopani v največji meri, medtem ko so bili kvercetin rutinozid, glukozid, arabinopiranozid in ramnozid določeni v manjših količinah. Tudi drugi avtorji poročajo prisotnost prej naštetih glikozidov kvercetina v plodovih (Hvattum, 2002; Salminen in sod., 2005). Kvercetin arabinofuranozid in arabinopiranozid so, kot pentozida, v plodovih potrdili Guimarães in sod. (2013). Fecka (2009) je identificirala kempferol glukozid, kvercetin galaktozid in kvercetin rutinozid, Guimarães in sod. (2013) pa še izoramnetin 3-O-rutinozid in kempferol ramnozil-heksozid. Vsebnost kempferol derivativa, ki smo ga določili, se med zorenjem ni spreminjala, medtem ko so

vrednosti večinoma vseh glikozidov kvercetina med zorenjem upadale, še posebej po zmrzali. To se ujema tudi z navedbami Türkbena in sod. (2010), ki poročajo večje vsebnosti derivatov kvercetina v nedozorelih oranžnordečih šipkih kot v polno zrelih rdeče obarvanih plodovih. Vrednosti flavonolov, ki smo jih določili v naši raziskavi se ujemajo s poročanji Guimarães in sod. (2013).

V vzorcih plodov šipka smo poleg že naštetih snovi določili še 4 naringenin heksozide, 2 eriodiktiol heksozida, 2 apigenin derivata in floridzin. Koncentracija apigenin derivatov se med zorenjem ni spreminjala, je pa upadla v vzorcih po zmrzali. Hvattum (2002) in Salminen in sod. (2005) poročajo o prisotnosti floridzina, eriodiktiol heksozida in apeginin derivata v plodovih šipka *R.canina,* vednar ne navajajo vsebnosti. Guimarães in sod (2013) poročajo vsebnosti eriodiktiol heksozida primerljive našim. Drugi avtorji niso potrdili naringenin heksozida v tkivih vrtnic. Naringeni uvrščamo med flavanone, ki so prekurzorji flavonom (npr. apigenin), izoflavonom in dihidroflavonolom; iz slednjih se sintetizrajo flavonoli ter antocianidini (Cooper-Driver, 2001). Naringenin običajno najdemo v plodovih citrusov, določili pa so ga tudi v paradižniku (Erlund, 2004) in plodovih vrste iz družine *Euphorbiaceae* (Barros in sod., 1982).

Zmanjšanje vsebnosti nekaterih fenolnih snovi po zmrzali je verjetno povezano s poškodbami tkiv. V tkivih ob zmrzali pride do mehanskih poškodb zaradi tvorbe ledenih kristalov oz. zaradi dehidracije celic, ko tekoča voda zamrzne. Posledično pride do poškodb celičnih membran, kar sproži oksidativno rjavenje fenolnih snovi zaradi interakcije med encimi in substratom (Chalker-Scott, 1999; Morelló in sod., 2003). Temnejša barva šipkov po zmrzali (manjše vrednosti parametra *L**) so prav tako lahko posledica teh procesov.

Poleg fenolnih snovi smo v plodovih določili tudi vsebnost β-karotena in likopena, ki sta najpomembnejša karotenoida v šipkih (Andersson in sod., 2011; Hodisan in sod., 1997; Hornero-Mendez in Minguez-Mosquera, 2000). Njuna vsebnost je bila največja v vzorcih nabranih po zmrzali. Sklepamo, da je zaradi tvorbe ledenih kristalov prišlo do poškodb celic posledično pa do boljše ekstrakcije karotenoidov kot predvidevajo Veberic in sod. (2014) pri plodovih robide. O povečanju vsebnosti β-karotena in likopena med zorenjem plodov navadnega šipka poročajo Türkben in sod. (2010) ter Barros in sod. (2011). Andersson in sod. (2011) so ugotovili podobno v plodovih drugih vrst šipkov, Guimarães in sod. (2010) pa v plodovih *R. micrantha.* V naši raziskavi smo ugotovili podobne vsebnosti β-karotena, kot jih navajajo Hornero-Mendez in Minguez-Mosquera (2000), Olsson in sod. (2004) in Andersson in sod. (2011). Slednji dve raziskavi tudi navajata, da je vsebnost likopena večja od vsebnosti β-karotena, kar kažejo tudi naši rezultati. Vsebnosti likopena se ujemajo z rezultati Böhm in sod. (2003).

V drugi raziskavi (Cunja in sod., 2015) smo dobili dobro predstavo o sestavi plodov navadnega šipka in kako se ta spreminja med zorenjem. Nato pa nas je zanimalo kakšna je sestava plodov okrasnih sort v primerjavi z botaničnimi vrstami. V Arboretumu Volčji Potok smo nabrali plodove različnih vrst (*Rosa canina* (RCA), *R. sweginzowii* (RSW), *R. rugosa* (RUG)) in sort vrtnic ('Fru Dagmar Hastrup' (FDH), 'Repandia' (REP), 'Veilchenblau' (RVB), 'Aloha' (RAL), 'Bonica' (BON) in 'Golden Gate' (RGG)). Ker plodovi različnih vrst in sort ne dozorevajo istočasno, smo se pri določanju zrelosti plodov opirali na barvo in trdoto. Prve vzorce smo tako nabrali v začetku oktobra (RCA), zadnje pa konec novembra (RGG). Nabranim plodovom smo izmerili morfološke parametre (dolžina, širina, masa celega plodu ter masa semen, barva plodov, delež suhe snovi) in s HPLC ter HPLC/MS določili vsebnosti organskih kislin, sladkorjev, fenolnih snovi in dveh karotenoidov (likopen, β-karoten).

Vrste in sorte so se zelo razlikovale v velikosti plodov in njihovi masi. Najtežje in najbolj okrogle plodove sta imeli sorti RGG in RAL, obe vzpenjalki. Vrsta RSW je imela izmed vseh najdaljše in tako najbolj podolgovate plodove (razmerje med dolžino in širino plodov je bilo največje). Najmanjše plodove sta imeli prekrovna sorta REP in hibridna multiflora RVB. Plodovi slednje so imeli izmed analiziranih vrst in sort tudi najbolj neugodno razmerje med maso perikarpa in maso celega plodu. Predelava tako majhnih plodov se je izkazala za izredno zamudno, zato bi jih bilo s tega vidika bolje pustiti na grmu kot hrano za divje živali oz. v okras. Za najbolj mesnate so se izkazale FDH (križanec rugoze) in RGG. Plodovi RCA niso izstopali po velikosti ali masi, imeli so celo drugo najmanjše razmerje med maso perikarpa in maso celega plodu, kar se ujema z navedbami Kazankaya in sod. (2005), ki poročajo o deležu mesa birnih plodov različnih genotipov *Rosa canina* v vrednostih od 46,8 % do 79,9 %. Šipki RCA so dosegli največje vrednosti le pri barvnih parametrih. Izkazali so se kot najbolj rdeči (največja vrednost parametra *a** in najmanjši parameter *h*°). Najbolj zeleni in rumenkasti so bili ob nabiranju plodovi RGG. Raziskav o morfoloških parametrih plodov različnih vrst šipkov je precej in kažejo, da so ti lahko izredno variabilni, odvisno od genotipa, izvora, okoljskih dejavnikov in zrelosti (Kovács in sod., 2000; Kazankaya in sod., 2005; Uggla in sod., 2005; Dogan in Kazankaya, 2006; Guneş in Dolek, 2010; Mabellini in sod., 2011; Najda in Buczkowska, 2013), medtem ko podatkov o plodovih okrasnih sort v znanstveni literaturi ni.

Glukozo, fruktozo in saharozo smo določili v plodovih vseh analiziranih vrst in sort. Pri vseh sortah in vrstah sta v plodovih prevladovali glukoza in fruktoza, saharoza je bila običajno zastopana v manjši meri. Največjo vsebnost glukoze smo ugotovili v plodovih RGG, prevladovala pa je še v plodovih BON, RAL in REP. Po vsebnosti saharoze so izstopali plodovi RAL in RCA. Plodovi RGG so se izkazali za najbolj bogate s sladkorji, medtem ko so jih plodovi FDH in RUG vsebovali najmanj. Kovács in sod. (2000) so ugotovili, da je razmerje v vsebnosti med glukozo in fruktozo pri številnih vrstah šipkov močno odvisno od leta. Predhodne raziskave navajajo predvsem vsebnost sladkorjev

plodov RCA pa tudi RUG (Kovács in sod., 2000; Uggla in sod., 2005; Barros in sod., 2010, 2011; Guimarães in sod., 2010; Mabellini in sod., 2011; Najda in Buczkowska, 2013; Demir in sod., 2014), podatkov o plodovih okrasnih sort pa ni.

Kot v predhodni raziskavi (Cunja in sod., 2015) sta se tudi v plodovih analiziranih vrst in sort za prevladujoči organski kislini izkazali citronska in jabolčna kislina. Izjema so bili plodovi sorte RAL, kjer je prevladovala kininska kislina. Te kisline nismo določili v plodovih FDH, RUG in RSW, ostali plodovi (z izjemo RAL) pa se po vsebnosti te organske kisline niso razlikovali. V plodovih RCA in RSW je prevladovala citronska kislina, medtem ko so imeli ostali plodovi večjo vsebnost jabolčne kisline (z izjemo RAL). Vinska, šikimska in fumarna kislina so bile določene v manjših količinah v vseh analiziranih vrstah in sortah. Vsebnosti vinske kisline so bile precej raznolike, saj je bila razlika med plodovi RVB, ki so vsebovali najmanj vinske kisline in plodovi RSW, ki so vsebovali največ te kisline, skoraj 20-kratna. Večina raziskav o organskih kislinah v šipkih se je osredotočila na plodove RCA. Številni avtorji navajajo prevladujočo citronsko in jabolčno kislino ter druge v manjših količinah (Adamczak in sod., 2012; Cunja in sod., 2015; Demir in sod., 2014; Kovács in sod., 2000; Mikulic-Petkovsek in sod., 2012a). RSW je med analiziranimi vrstami vsebovala največ vitamina C, ki je predstavljal kar 24 % vsebnosti vseh določenih organskih kislin. Veliko količino vitamina C sta imeli tudi FDH in RUG, RCA plodovi pa so vsebovali srednjo količino vitamina C. Tako Kovács in sod. (2000) kot Adamczak in sod. (2012) poročajo, da RCA ni posebej bogata z vitaminom C. Plodovi sort RVB, RGG, RAL in BON se med seboj značilno niso razlikovali v vsebnosti vitamina C in so ga vsebovali najmanj. Ker se vsebnosti vitamina C lahko precej razlikujejo glede na genotip, okoljske dejavnike, zrelost in analitične metode so vsebnosti, ki jih navajajo predhodniki, posledično raznolike in težko primerljive (Ercişli in Eşitken, 2004; Kazankaya in sod., 2005; Barros in sod., 2010, 2011; Hallmann in sod., 2011; Najda in Buczkowska, 2013).

Kot v raziskavi Cunja in sod. (2015) je bil edini določen antocianin v plodovih vseh sort in vrst šipkov cianidin-3-glukozid. Največ ga je bilo v plodovih RCA, okrasne sorte (BON, RAL, REP, RGG, RVB) pa so ga nakopičile precej manj kot vrste. Plodovi sorte RAL so vsebovali med drugim največje vsebnosti fenolnih snovi, fenolnih kislin, flavonolov in hidrolizirajočih taninov. Pri omenjeni vrsti so prevladovali hidrolizirajoči tanini, v plodovih ostalih vrst in sort pa flavanoli. To je skladno tudi s prej navedenimi raziskavami plodov RCA (Ganhão in sod., 2010, Türkben in sod., 2010, Guimarães in sod., 2013, Cunja in sod., 2015). Fecka (2009) in Salminen in sod. (2005) so v plodovih šipkov določili tudi elagitanine. Dimeri in trimeri katehina in procianidina so bili določeni v plodovih vseh preučevanih vrst in sort, v RUG, FDH, RCA, RSW in RAL pa smo določili tudi mono- in di-glikozide procianidina. Sorta RGG je bila po vsebnosti najbolj bogata s flavanoli, količinsko najmanj pa smo jih določili v plodovih RVB. Slednje so vsebovale tudi najmanj fenolnih kislin (in njihovih derivatov) in posledično najmanjšo skupno

količino fenolnih snovi. Najbolj raznoliko fenolno sestavo smo določili v plodovih RUG in FDH (55 oz. 54 različnih fenolnih snovi), medtem ko smo v plodovih RGG določili le 35 različnih fenolnih snovi.

V plodovih so med fenolnimi kislinami prevladovali derivati elagne, galne kisline in hidrokiscimetne kisline. Slednje so bile prevladujoče med fenolnimi kislinami le v plodovih sorte RVB. Z izjemo plodov sorte REP smo lahko pri vseh vrstah in sortah določili derivate metil galata. Drugi avtorji so predhodno določili metil galat (tudi v obliki glikozidov) v plodovih šipkov (Hvattum, 2002; Salminen in sod., 2005; Fecka, 2009; Guimarães in sod., 2013), poleg tega pa poročajo tudi o drugih fenolnih kislinah, ki smo jih potrdili v plodovih analiziranih vrst in sort (Zocca in sod., 2011; Demir in sod., 2014; Tumbas in sod., 2012; Mattila in sod., 2006; Hallmann in sod., 2011).

V plodovih vseh preučevanih vrst in sort smo določili fenolne snovi iz skupine flavonolov. Določili smo derivate izoramnetina, kempferola in kvercetina, večinoma v obliki glikozidov. Glikozidi kvercetina so bili najbolj zastopani. V plodovi BON in RAL smo določili tako kvercetin kot izoramnetin glikozide, medtem ko so plodovi RVB vsebovali le glikozide kvercetina in kempferola, RGG pa le glikozide kvercetina. Največje vsebnosti flavonolov smo analizirali v plodovih RAL, ki jim sledijo RGG in REP. Plodovi RCA so vsebovali najmanj flavonolov, vrednosti se ujemajo s prejšnjimi objavami (Cunja in sod., 2015; Guimarães in sod., 2013). Več raziskovalcev je preučevalo flavonolno sestavo šipkov. Olsson in sod. (2004), Hvattum (2002), Ganhão in sod. (2010), Fecka (2009), Pang in sod. (2009), Hallmann in sod. (2011) in Mikulic-Petkovsek in sod. (2012b) so objavili raznolike rezultate, saj je fenolna sestava močno odvisna od mnogih dejavnikov, tako genotipa preučevane rastline, vpliva okolja (pedoklimatske razmere, nadmorska višina), kot tudi vpliva stresnih dejavnikov. Razlike med posameznimi študijami pa lahko pripišemo tudi uporabi različnih analitičnih metod.

Poleg že naštetih snovi smo lahko v skoraj vseh plodovih določili tudi flavanone (eriodiktiol heksozid, naringenin heksozid, taksifolin pentosid in di-pentosid), le plodovi RAL niso vsebovali teh snovi. Plodovi RGG so vsebovali največ flavanonov, vendar le derivate taksifolina, medtem ko so bili plodovi REP edini, ki so vsebovali derivate vseh prej naštetih oblik. Hvattum (2002) in Salminen in sod. (2005) poročajo o taksifolin pentozidu in eriodiktiol heksozidu v plodovih RCA, kot tudi o floridzinu in derivatih apigenina. Slednji so bili prisotni tudi v plodovih drugih vrst in sort v naši raziskavi. V vsebnosti floridzina je izstopala sorta RAL, med plodovi RUG, BON, RCA, FDH in RSW pa ni bilo statistično značilnih razlik v vsebnosti tega dihidrohalkona. V štirih vrstah/sortah smo določili tudi dva derivata apigenina; plodovi RUG in FDH so ga vsebovali več kot plodovi RCA in RSW. Demir in sod (2014) so v plodovih *R. hirtissima in R. gallica* določili tudi *t*-resveratrol, katerega derivat smo določili le v plodovih RSW (največ) in (manj) v RUG ter FDH.

V plodovih preučevanih sort in vrst smo določili tudi likopen in β-karoten. Slednji je bil prevladujoč karotenoid, razen v REP, ki je bila bolj bogata z likopenom. Vrste in sorte z majhno vsebnostjo β-karotena so bile FDH, RUG in RSW, ki so skupaj z RGG vsebovale tudi najmanj likopena. Bogati z β-karotenom pa so bili plodovi BON, RAL in RGG. Po drugi strani so veliko likopena vsebovale sorte REP, BON in RVB, celo več kot RCA, ki je glede na navedbe Böhm-a in sod. (2003) bogata s tem antioksidantom. Prejšnje raziskave karotenoidov v plodovih šipkov z metodo HPLC vključujejo naslednje avtorje: Hodisan in sod. (1997) so določili karotenoidno sestavo plodov RCA (brez kvantifikacije); Hornero-Mendez in Minguez-Mosquera (2000) sta identificirala karotenoide v *R. mosqueta;* Olsson in sod. (2004) poročajo o sestavi *R. villosa* x *villosa;* Hallmann in sod. (2011) so določili vsebnost β-karotena in likopena v plodovih *R. rugosa;* Andersson in sod. (2011) pa v plodovih 4 različnih *Rosa* vrst. Vsi avtorji navajajo, da sta glavna karotenoida v plodovih likopen in β-karoten.

Slika 1: Ostanki sredstva Lithovit Forte na vzorčenih listih miniaturne vrtnice 'Funny Red'. Rastlina je bila poškropljena s priporočeno koncentracijo pripravka (5 g /l).
Figure 1: Residue of product Lithovit Forte on sample leaves of miniature rose 'Funny Red'. The plant was sprayed with the recommended concentration (5 g/l).

V prvem poskusu smo analizirali fenolni profil listov vrtnic (Cunja in sod., 2014). Izmed obravnavanih vrst in sort je bila najbolj občutljiva na bolezni sorta 'Schwanensee', ki je imela v listih najmanjšo vsebnost fenolnih snovi, ki so ene izmed snovi, ki jih povezujemo z odpornostjo rastlin na bolezni. Sinteza fenolov se običajno poveča po napadu patogena, raziskovalci pa iščejo različne načine kako bi vsebnost fenolnih snovi v rastlinskih tkivih

povečali že pred napadom patogena in tako pripomogli k večji tolerantnosti rastline na bolezen ali vsaj zakasnili potek bolezni. Izveden je bil tipalni preiskus s katerim smo želeli izvedeti ali tretiranje rastlin vrtnic s pripravkom Lithovit Forte poveča vsebnost skupnih fenolnih snovi v listih. Ugotovili smo značilno povečanje vsebnosti skupnih fenolnih snovi en dan po tretiranju. Rastline, ki smo jih poškropili s pripravkom (ne glede na koncetracijo pripravka) so imele v listih značilno večjo vsebnost skupnih fenolnih snovi kot kontrolne rastline. Lithovit Forte sestavljajo v glavnem kalcijev karbonat, silicijev dioksid in magnezijev karbonat. Posebej obdelani mineralni delci naj bi preko por vstopali v rastlino, kjer naj bi sproščali ogljikov dioksid (CO_2), s tem pa vplivali na večjo stopnjo fotosinteze (Lithovit, 2009; dokument je v prilogi A). Večjo vsebnost skupnih fenolnih snovi en dan po aplikaciji lahko povezujemo s sproščanjem CO_2 iz mineralov. Estiarte in sod. (1999) so ugotovili večje vsebnosti flavonoidov v listih pšenice, ki je bila izpostavljena atmosferi z večjo koncentracijo CO_2, kar je v skladu s hipotezo, da se viški ogljika vgrajujejo v ogljikove skelete sekundarnih metabolitov kot so npr. fenolne snovi. Dva in sedem dni po tretiranju nismo več izmerili značilnih razlik med obravnavanji. Z vidika vsebnosti skupnih fenolnih snovi se sredstvo ni izkazalo za učinkovito. Na površini listov so bili ostanki sredstva močno opazni, kar bi tudi zmajšalo tržno vrednost takih rastlin (Slika 1).

S poskusi, ki jih zajema doktorska disertacija smo pridobili pomembne informacije o fenolnih profilih listov in plodov izbranih vrst in sort vrtnic, med drugim tudi avtohtonih slovenskih vrst. Poskus primerjave vsebnosti primarnih in sekundarnih metabolitov v plodovih izbranih vrst in sort vrtnic je osvetlil kemično sestavo plodov, še posebej v izbranih sortah, kjer do sedaj ta še ni bila preučena. Fenolne snovi v različnih tkivih smo kvalitativno in kvantitativno določili z uporabo HPLC/MS. To bo omogočilo nadaljne raziskave povezane z odzivom rastlin na stresne dejavnike, kar je pomembno predvsem z vidika gojenja in žlahtnenja šipkov in vrtnic. Prav tako smo preučili spreminjanje fenolnih snovi v plodovih navadnega šipka (*Rosa canina*) med zorenjem in po slani, to pa je lahko v pomoč farmacevtski in prehrambeni panogi pri nabiranju plodov z optimalno vsebnostjo zdravju koristnih snovi.

3.2 SKLEPI

S poskusi, ki so del te doktorske disertacije, smo lahko potrdili hipotezo, da se sorte in vrste med seboj kvantitativno in kvalitativno razlikujejo v vsebnosti fenonih snovi v listih kot tudi v plodovih. Hipoteze, da imajo sorte s slabšo odpornostjo na bolezni tudi manjšo vsebnost fenolnih snovi v listih ne moremo zagotovo potrditi. Sorta 'Schwanensee' je bila, izmed preučevanih vrst in sort v poskusu, najbolj občutljiva in je imela najmanjšo vsebnost fenolov v listih, vendar bi bile za potrditev hipoteze potrebne nadaljne raziskave na več sortah. Potrdimo lahko hipotezo, da stopnja zrelosti plodov navadnega šipka (*Rosa canina*) vpliva na vsebnost fenolov, vitamina C, karotenoidov in na njihovo barvo. Poskus je pokazal, da je v plodovih nabranih po prvi slani vsebnost večine fenolnih snovi in vitamina

C značilno manjša, medtem ko se je vsebnost β-karotena in likopena značilno povečala. Foliarno tretiranje s pripravkom Lithovit Forte je v listih miniaturne vrtnice 'Funny Red' povzročilo povečanje vsebnosti skupnih fenolnih snovi le prvi dan po tretiranju, nato pa med kontrolo in ostalimi obravnavanji ni bilo značilnih razlik. V prihodnje bi veljalo razširiti poskus tudi na druge sorte in preučiti delovanje skupaj z glivično okužbo rastline.

- Venčni listi sort in vrst so se razlikovali tako v količini kot prisotnosti posameznih antocianinov.

- Vrste *R. canina* in *R. glauca* ter sorta 'Ulrich Brunner Fils' so vsebovale v venčnih listih večje vsebnosti glikozidov kvercetina v primerjavi z glikozidi kempferola. Pri ostalih preučevanih vrstah in sortah pa je bolj ali manj izrazito prevladovala vsebnost glikozidov kempferola.

- V listih vseh preučevanih vrst in sort prevladujejo glikozidi kvercetina nad glikozidi kempferola.

- V listih vseh preučevanih vrst in sort smo določili še procianidin di- in trimere, pri *R. canina*, *R. glauca* in *R. sempervirens* pa tudi tetramere. Sorte v splošnem vsebujejo manjše količine omenjenih snovi kot vrste.

- Zaradi majhne vsebnosti skoraj vseh fenolnih komponent so izstopali listi na bolezni občutljive sorte 'Schwanensee'.

- Vrste in sorte so se zelo razlikovale v velikosti plodov in njihovi masi. Najtežje in najbolj okrogle plodove sta imeli sort RGG in RAL; RSW je imela izmed vseh najdaljše in tako najbolj podolgovate plodove. Najmanjše plodove sta imeli prekrovna sorta REP in hibridna multiflora RVB. Za najbolj mesnate plodove so se izkazali plodovi FDH (križanec rugoze) in RGG.

- Plodovi RCA so bili najbolj rdeči (največja vrednost parametra *a** in najmanjši parameter *h*°); medtem ko so bili, med preučevanimi vrstami in sortami, ob nabiranju najbolj zeleni in rumenkasti plodovi RGG.

- V plodovih preučevanih vrst in sort smo določili le en antocianin, cianidin-3-glukozid. Ugotovili smo, da se je vsebnost te snovi v plodovih *R. canina* po zmrzali značilno zmanjšala. Med različnimi vrstami in sortami ga je bilo največ v plodovih *R. canina*, okrasne sorte (BON, RAL, REP, RGG, RVB) pa so ga nakopičile precej manj kot preučevane vrste.

- Pri vseh sortah in vrstah sta v plodovih prevladovali glukoza in fruktoza, saharoza je bila običajno zastopana v manjši meri. Plodovi RGG so se izkazali za najbolj bogate s sladkorji, medtem ko so jih plodovi FDH in RUG vsebovali najmanj.

- Izmed analiziranih vrst in sort so največ vitamina C vsebovali plodovi RSW ter FDH in RUG, medtem ko so plodovi RCA kopičili zmerne vsebnosti vitamina C. Plodovi sort RVB, RGG, RAL in BON se značilno niso razlikovali v vsebnosti vitamina C in so ga vsebovali najmanj. Vsebnost vitamina C je v plodovih nabranih po slani močno padla, saj so vsebovali samo še petino izmerjene največje vsebnosti ob tretjem obiranju.

- V plodovih sta prevladovali citronska in jabolčna kislina. Izjema so bili plodovi sorte RAL, kjer je prevladovala kininska kislina. Vinska, šikimska in fumarna kislina so bile določene v manjši meri v vseh izbranih vrstah in sortah.

- Od fenolnih snovi so bili po vsebnosti v plodovih večine vrst in sort najbolj zastopani flavanoli. Izjema so bili plodovi sorte RAL, kjer so prevladovali hidrolizirajoči tanini. Ta sorta je imela od vseh preučevanih vrst in sort količinsko največjo vsebnost fenolnih snovi.

- Najbolj raznoliko fenolno sestavo smo določili v plodovih RUG in FDH (55 oz. 54 različnih fenolnih snovi), medtem ko smo v plodovih RGG določili le 35 različnih fenolnih snovi.

- V plodovih preučevanih vrst in sort smo določili derivate kvercetina, izoramnetina in kempferola, večinoma v obliki glikozidov. Glikozidi kvercetina so bili najbolj zastopani. Največje vsebnosti flavonolov so izkazali plodovi RAL, ki jim sledijo RGG in REP. Plodovi RCA so vsebovali najmanj flavonolov izmed preučevanih vrst in sort.

- Med dozorevanjem plodov RCA se vsebnost kempferol derivata ni značilno spreminjala, medtem ko so se vrednosti večinoma vseh glikozidov kvercetina med zorenjem zmanjšale, še posebej po zmrzali.

- Rastline miniaturnih vrtnic 'Funny Red' tretirane s sredstvom Lithovit Forte so izkazale značilno večjo vsebnost skupnih fenolnih snovi v listih le dan po tretiranju.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V okviru doktorske naloge smo želeli opraviti temeljne raziskave, ki bi pripomogle k boljšemu poznavanju primarnih in sekundarnih metabolitov v različnih tkivih tako navadnega šipka (*Rosa canina*), kot tudi drugih slovenskih avtohtonih sort ter jih primerjati z drugimi vrstami in sortami vrtnic.

Z tekočinsko kromatografijo visoke ločljivosti v povezavi z masno spektrometrijo (HPLC/MS) smo v različnih tkivih vrtnic določili vsebnosti sladkorjev, organskih kislin in fenolnih snovi pa tudi β-karotena in likopena. V venčnih listih in listih različnih vrst (*R. canina, R. glauca, R. sempervirens, R. rubiginosa*) in sort vrtnic ('Rosarium Uetersen', 'Urlich Brunner Fils', 'Schwanesee') smo določili fenolne komponente iz raličnih skupin; v venčnih listih vsebnost antocianinov in flavonolov, v listih pa flavonolov, flavanolov ter vsebnost fenolnih kislin in njihovih derivatov. Ugotovili smo lahko, da so se venčni listi sort in vrst razlikovali tako v vsebnosti kot tudi v sestavi antocianinov, glavni pri vseh sortah in vrstah pa je bil cianidin-3,5-diglukozid. Poleg njega smo določili še glikozide pelargonidina in peonidina. Flavonolni profil venčnih listov je razkril, da ti vsebujejo različne glikozide kvercetina in kempferola. Vrste *R. canina* in *R. glauca* ter sorta 'Ulrich Brunner Fils' so v venčnih listih vsebovale večje vsebnosti glikozidov kvercetina v primerjavi s kempferolom, pri ostalih preučevanih vrstah in sortah pa so bolj ali manj izrazito prevladovali glikozidi kempferola. V listih preučevanih vrst in sort smo poleg glikozidov kvercetina in kempferola določili tudi glikozide izoramnetina (*R. sempervirens* in 'Schwanesee') ter glikozide miricetina (*R. canina*). Pri vseh sortah in vrstah so v listih prevladovali glikozidi kvercetina. Pomemben del fenolne sestave listov je bila vsebnost katehina in njegovih derivatov, procianidinov ter hidrolizirajočih taninov. Listi sort so večinoma vsebovali manjše količine omenjenih snovi kot vrste, kjer je bila tudi raznolikost teh snovi večja. V manjši meri smo v listih določili tudi različne fenolne kisline in njihove derivate. Izkazalo se je, da so listi vrst bolj primeren potencialen vir različnih fenolnih snovi, kot listi sort. Listi na glivične bolezni najbolj občutljive sorte 'Schwanesee' so vsebovali najmanj fenolnih snovi izmed preučevanih sort in vrst.

V naslednjih dveh poskusih smo se osredotočili na sestavo plodov. V njih smo določili sladkorje, organske kisline vključno z vitaminom C, fenolne snovi ter β-karoten in likopen. V prvem od dveh poskusov nas je zanimalo, kako se v plodovih navadnega šipka (*R. canina*) vsebnost določenih primarnih in sekundarnih metabolitov spreminja med zorenjem, še posebej kaj se s temi snovmi dogaja, če plodove naberemo po slani, kar nekateri priporočajo. V drugem pa smo analizirali plodove več vrst (*Rosa canina, R. sweginzowii, R. rugosa*) in sort ('Fru Dagmar Hastrup', 'Repandia', 'Veilchenblau', 'Aloha', 'Bonica' in 'Golden Gate'), saj nas je zanimalo kako se sestava plodov okrasnih sort vrtnic

lahko primerja s plodovi šipkov. Pri vseh sortah in vrstah sta v plodovih med sladkorji prevladovali glukoza in fruktoza, saharoza je bila običajno zastopana v manjši meri. V plodovih *R. canina* smo ugotovili statistično značilno zmanjšanje glukoze in fruktoze v vzorcih nabranih po zmrzali, medtem ko je bila vsebnost saharoze največja v času prvega obiranja, nato pa v njeni vsebnosti ni bilo statistično značilnih razlik med termini. Po vsebnosti saharoze so izstopali tudi plodovi okrasne sorte 'Aloha', medtem ko so plodovi *R. rugosa* in 'Frau Dagmar Hastrup' (hibridna rugoza) vsebovali najmanjše količine določenih sladkorjev.

Med dozorevanjem je bila največja vsebnost vitamin C v plodovih *R. canina* izmerjena ob tretjem obiranju v začetku oktobra. Vsebnost vitamina C je v plodovih nabranih po slani močno upadla, saj so vsebovali samo še petino izmerjene največje vsebnosti. Izmed analiziranih vrst se je za najbolj z vitaminom C bogato izkazala *R. sweginzowii*: vitamin C je predstavljal kar 24 % vsebnosti vseh določenih organskih kislin. Veliko vsebnost vitamina C sta izkazali tudi *R. rugosa* in 'Frau Dagmar Hastrup', medtem ko so plodovi *R. canina* vsebnovali zmerne vsebnosti vitamina C v primerjavi z ostalimi vrstami in sortami. Plodovi sort 'Veilchenblau', 'Golden Gate', 'Aloha' in 'Bonica' se značilno niso razlikovali v vsebnosti vitamina C in so ga vsebovali najmanj. V plodovih so od organskih kislin prevladovale citronska in jabolčna kislina, izjema so bili plodovi sorte 'Aloha', ki so vsebovali največi delež kininske kisline. Slednje nismo določili v plodovih *R. rugosa*, 'Frau Dagmar Hastrup' in *R. sweginzowii*. V vseh plodovih smo lahko določili še vinsko, fumarno in šikimsko kislino. Zmrzal ni imela vpliva na vsebnost citronske in kininske kisline v plodovih *R. canina*, smo pa po njej v plodovih ugotovili zmanjšanje jabolčne, šikimske in fumarne kisline.

V plodovih preučevanih vrst in sort smo določili le en antocianin, cianidin-3-glukozid. Ugotovili smo, da je bila vsebnost te snovi v plodovih *R. canina* največja ob tretjem oz. četrtem vzorčenju in je po zmrzali upadla. Med različnimi vrstami in sortami so bili z njim najbolj bogati plodovi *R. canina*, okrasne sorte pa so ga nakopičile precej manj kot preučevane vrste.

Od fenolnih snovi so bili po vsebnosti v plodovih večine vrst in sort najbolj zastopani flavanoli. Izjema so bili plodovi sorte RAL, kjer so prevladovali hidrolizirajoči tanini. Katehin ter procianidin di- in trimeri so bili določeni v plodovih vseh preučevanih vrst in sort, v nekaterih (*R. rugosa*, 'Frau Dagmar Hastrup', *R. canina*, *R. sweginzowii*, 'Aloha') pa smo določili tudi mono- in di-glikozide procianidina. V plodovih *R. canina* se je med zorenjem vsebnost katehina spreminjala in je bila najmanjša po zmrzali. V vsebnosti procianidinov med različnimi termini nismo zaznali razlik. Od flavonolov smo v plodovih preučevanih vrst in sort določili derivate kvercetina, izoramnetina in kempferola, večinoma v obliki glikozidov. Glikozidi kvercetina so bili najbolj zastopani. Največje vsebnosti flavonolov smo določili v plodovih sorte 'Aloha', ki jim sledijo plodovi sort 'Golden Gate'

in 'Repandia'. Plodovi *R. canina* so vsebovali najmanj flavonolov izmed preučevanih vrst in sort. Med dozorevanjem plodov te vrste so vrednosti večinoma vseh glikozidov kvercetina med zorenjem upadale, še posebej so se zmanjšale po zmrzali. Izkazalo pa se je, da nabiranje po slani ugodno vpliva na vsebnost β-karotena in likopena, saj je bila največja vsebnost izmerjena v vzorcih nabranih po njej. V raziskavi plodov več vrst in sort so se za bogate z β-karotenom izkazali plodovi sort 'Bonica', 'Aloha' in 'Golden Gate', veliko likopena pa so vsebovale tudi 'Repandia', 'Bonica' in 'Veilchenblau'.

Pri uporabi in predelavi samih plodov pa ni zanemarljiva velikost plodov in njihova mesnatost (razmerje med perikarpom in semeni). Najtežje in najbolj okrogle plodove sta imeli sorti 'Golden Gate' in 'Aloha', najmanjše pa sta imeli prekrovna sorta 'Repandia' in hibridna multiflora 'Veilchenblau'. Za najbolj mesnate plodove so se izkazali srednje težki plodovi 'Frau Dagmar Hastrup' (križanec rugoze) in plodovi že omenjene sorte 'Golden Gate'. Ugotovili pa smo lahko razlike tudi v obarvanosti plodov.

Tretiranje miniaturnih vrtnic sorte 'Funny Red' s sredstvom Lithovit Forte je v listih značilno povečalo vsebnost skupnih fenolnih snovi le dan po tretiranju, ne glede na uporabljeno koncentracijo škropljenja (5 g/l oz. 10 g/l). Nato pa med kontrolo in ostalimi obravnavanji ni bilo značilnih razlik.

4.2 SUMMARY

Within the framework of the doctoral thesis, we carried out basic research, which would help to improve the understanding of primary and secondary metabolites in various tissues of *Rosa canina*. Additionally, leaf, petal and fruit profiles of indigineous Slovenian rose species have been compared to modern rose cultivars.

Using high pressure liquid chromatography coupled with mass spectrometry (HPLC/MS) the content of sugars, organic acids, and phenolics as well as β-carotene and lycopene was determined in various rose tissues. Phenolic compounds were investigated in petals and leaves of different species (*R. canina, R. glauca, R. sempervirens, R. rubiginosa*) and rose cultivars ('Rosarium Uetersen,' 'Ulrich Brunner Fils', 'Schwanesee'). The content of anthocyanins and flavonols was determined in petals and flavonols, flavanols and phenolic acids and their derivatives were identified in leaves. The investigation exposed that petals of cultivars and species differ both in the content and in composition of anthocyanins. However, the main anthocyanin in all investigated cultivars and species was cyanidin-3,5-diglucoside. Additionally, pelargonidin and peonidin glycosides have been determined in rose petals. Flavonol composition of petals revealed that they contain various quercetin and kaempferol glycosides. In petals of *R. canina*, *R. glauca* and 'Urlich Brunner Fils' quercetin glycosides prevailed over kaempferol glycosides.Contrary, kaempferol glycosides were the main flavonol constituents in other investigated cultivars and species. In addition to

quercetin and kaempferol glycosides, isorhamnetin glycosides (*R. sempervirens* in 'Schwanensee') and myricetin glycosides (*R. canina*) were determined in leaves of analysed cultivars and species. In all species and cultivars quercetin glycosides were predominant. Catechin and its derivatives, procyanidins and hydrolyzable tannins were important constituents of leaf phenolic profile. Leaves of cultivars generally contained lower levels of these compounds than leaves of the investigated species; diversity of the compounds was also greater in leaves of rose species. In leaves, phenolic acids and their derivatives were determined in lower amounts. It seems that species are more suitable as a potential source of leaf phenols with antioxidative activity. In addition, leaves of the susceptible 'Schwanensee' contained lowest levels of phenolic constituents.

In subsequent experiments, we focused on the composition of rose hips in which we determined sugars, organic acids including vitamin C, phenolic compounds, β-carotene and lycopene. In the first experiment, we investigated different primary and secondary metabolites in *R. canina* hips during ripening and after frost damage. We aimed to determine if the traditional recommendation for rose hip harvest after frost has any merit in their improved composition. In the second experiment we investigated fruit composition of several rose species (*R. canina, R. sweginzowii, R. rugosa*) and cultivars ('Fru Dagmar Hastrup', 'Repandia', 'Veilchenblau', 'Aloha', 'Bonica' and 'Golden Gate'). We compared the content of biologically active compounds among hips of selected ornamental cultivars and hips of *Rosa* species, traditionally harvested for their medicinal purposes. Glucose and fructose were the main sugars in all investigated species and cultivars, and sucrose was determined in lower amounts. A significant decrease of glucose and fructose levels has been observed in *R. canina* fruits after hips were subjected to frost. Sucrose content was highest on the first sampling and no statistical differences have been measured among later samplings. 'Aloha' stood out as the cultivar with high sucrose content in the hips. Contrary, *R. rugosa* and 'Fru Dagmar Hastrup' fruit were characterized by lowest levels of determined sugars.

During maturation of *R. canina* fruits the highest level of vitamin C was determined on the third sampling at the beginning of October. The content of vitamin C dropped significantly after frost, when rose hips contained only a fifth of the maximum content. Hips of *R. sweginzowii* contained the highest amounts of vitamin C; its content represented 24 % of all determined organic acids. High in vitamin C were also fruits of *R. rugosa* and 'Fru Dagmar Hastrup', while the fruit of *R. canina* showed a moderate level of vitamin C compared to other investigated species and cultivars. Fruit of 'Veilchenblau', 'Golden Gate', 'Aloha' and 'Bonica' cultivars did not statistically differ in vitamin C content and also contained the lowest amounts. Citric and malic prevailed from the group of organic acids, except in 'Aloha' hips, which accumulated highest proportions of quinic acid. Conversely, this organic acid was not determined in *R. rugosa*, 'Frau Dagmar Hastrup' and *R.*

sweginzowii hips. Additionally, in fruit of all investigated species and cultivars tartaric, fumaric and shikimic acid were determined.

A single anthocyanin was determined in investigated rose hips, namely cyanidin-3-glucoside. In *R. canina* hips the highest content of this compound has been measured on the third and fourth sampling dates and its levels dropped significantly after frost. Hips of modern cultivars showed significantly lower levels of cyanidin-3-glucoside compared to the species - *R. canina* accumulating the highest amount of this compound.

Flavanols were the major phenolic compounds in hips of almost all analysed cultivars and species. However, hydrolysable tannins prevailed in 'Aloha' fruit. Catechin and procyanidin dimers and trimers were determined in hips of all investigated species and cultivars. In *R. rugosa*, 'Frau Dagmar Hastrup', *R. canina*, *R. sweginzowii*, and 'Aloha' fruit procyanidin mono- and di-glycosides have additionally been determined. Catechin levels changed during maturation of *R. canina* hips and were lowest in samples gathered after frost. Procyanidin content showed no statistical differences during hip ripening. In hips of different investigated species and cultivars different derivatives of quercetin, kaempferol and isorhamnetin were determined, usually in the form of glycosides. Quercetin glycosides were the most abundant. The highest flavonol content was determined in hips of 'Aloha', followed by 'Golden Gate' and 'Repandia' fruit. *R. canina* hips contained the lowest levels of flavonols among all investigated species and cultivars. Levels of almost all Q glycosides decreased during ripening and a significant decrease has also been determined after frost. On the other hand, frost damaged hips contained higher levels of β-carotene and lycopene. Investigation showed that the following cultivars were rich in β-carotene: 'Bonica', 'Aloha' in 'Golden Gate' and hips of cultivars 'Repandia', 'Bonica' and 'Veilchenblau' were abundant with lycopene.

When gathering and processing rose hips, fruit size and flesh ratio (the ratio between the pericarp and seeds) of the fruit is also important. Fruits of 'Golden Gate' and 'Aloha' were characterized by the highest mass and were also roundest, while groundcover 'Repandia' and hybrid multiflora 'Veilchenblau' developed the smallest hips. The highest flesh to whole fruit ratio was determined in medium-sized hips of 'Fru Dagmar Hastrup' and 'Golden Gate'. Rose hips of different species and cultivars also showed differences in fruit colour.

Foliar spray of miniature rose 'Funny Red' with Lithovit Forte significantly increased total phenolic content in leaves only one day after treatment, regardles of the concentration used (5 g/l or 10 g/l). In subsequent samplings no significant differences in levels of total phenolic content have been detected between the leaves of the control and treated plants.

5 VIRI

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ZAHVALA

Vsak konec je nov začetek…

Hvala mentorici doc dr. Valentini SCHMITZER, prof. dr. Franciju ŠTAMPARJU ter vsem sodelavcem na katedri, ki so pomagali, tako in drugače, pri nastanku tega doktorata.

Hvala družini in Klemnu za vso podporo in ljubezen.

PRILOGA A

Sestava in delovanje sredstva Lithovit (Lithovit, 2009)

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LITHOVIT

NARAVNO CO_2 FOLIARNO GNOJILO

Sestava:	
79,19 % Kalcijev karbonat ($CaCO_3$)	0,33 % Sulfat (SO_4)
11,41 % Silicijev dioksid (SiO_2)	0,21 % Kalijev oksid (K_2O)
4,62 % Magnezijev karbonat ($MgCO_3$)	0,06 % Nikel (Ni)
1,31 % Železo (Fe)	0,01 % Fosfor pentoksid (P_2O_5)
0,97 % Aluminijev oksid (Al_2O_3)	0,014 % Mangan (Mn)
0,55 % Natrijev oksid (Na_2O)	0,005 % Cink (Zn)
	0,002 % Baker (Cu)

Kalcijev karbonat, z vsebnostjo ogljikovega dioksida, ki se pridobiva iz naravnih depozitov apnenca in vsebuje tudi lahko dostopna mikrohranila. Lithovit je naravno CO_2 foliarno gnojilo za uporabo na odprtih površinah. Je visoko kakovosten nanotehnološki proizvod pridobljen z tribodinamično aktivacijo in mikronizacijo. Visoko energijski delci Lithovita, fino poškropljeni na površino lista, preko listnih por direktno vstopajo v rastlino kjer sproščajo CO_2. S tem lahko Lithovit občutno poveča obseg fotosinteze, saj je na prostem pomemben omejevalni dejavnik fotosinteze količina naravno prisotnega CO_2 v zraku. To ima za posledico povečanje pridelka in zmanjšanje potreb po vodi, saj lahko rastline, ki so bile tretirane z Lithovitom , dlje časa držijo pore zaprte, v primeru vodnega stresa. Ob tem mikrohranila kot so Mangan, Baker, Cink itd., ki jih vsebuje produkt, vplivajo na fiziologijo rastline in povečujejo odpornost, rast, vitalnost in kakovost. Primerno za ekološko pridelavo glede na odlok Evropske skupnosti (EEC) No.2092/91

Povečuje pridelek, kakovost in skladiščno sposobnost kulture; Pospešuje rast in povečuje zeleno obarvanost; Povečuje odpornost, rast in vitalnost; Poveča odpornost na pozebo, sušo in bolezni; Poveča preskrbljenost rastline s pomembnimi mikrohranili; Zmanjšuje potrebo po vodi; Ni primerno za rastline, ki uspevajo na kisli zemlji; Neškodljivo za ljudi in živali. Ne ogroža voda. V bistvu se Lithovit uporablja v treh glavnih terminih: Od razvoja prvih lističev; V času cvetenja; V času rasti plodov in zorenja

LITHOVIT NARAVNO CO_2 FOLIARNO GNOJILO za uporabo na odprtih površinah lahko uporabimo samo enkrat ali večkrat v razmakih 10-15 dni. Uporablja se samostojno ali v kombinaciji s sredstvi za zaščito rastlin. Doza 1,5-2,0 kg/ha kot 0,5% raztopina (500 g Lithovita v 100 lit vode). Za aplikacijo se lahko uporabljamo vse vrste škropilnic, za zaščitna sredstva, ki so dovoljene v prodaji.

Primeri in priporočila za uporabo:

Sladkorna pesa: v stadiju med 4 in 6 listom ter ponovno čez 2 in 4 tedne.

Žitarice: Ozimno žito 1 krat jeseni ko se pojavijo 2-3 poganjki

Žita in ozimna žita enkrat po razvoju zadnjih lističev

Koruza: 1 krat v stadiju med 4 in 8 listom in ponovno v stadiju 10 listov

Oljna repica: ozimna repica 1 krat v jeseni, stadij 2 listov. Ozimna in letna repica v stadiju med 6 in 8 listom, ter pred cvetenjem.
Krompir: 15 dni po prvih poganjkih in ponovno po 15 dneh
Solate in zelenjava: Prvič ob presajanju in potem še 2 krat v razmaku 15 dni
Trta: ob cvetenju, pojav prvih sadov, ob razvoju prvih jagod na grozdih
Paradižnik: 3-5 krat v presledkih 15 dni, prvič v stadiju 2 listov ali presajanju.
Jagode in jagodičevje: med cvetenjem, ob pojavu prvih plodov in po 15 dneh.
Sadjarstvo – okrasne olesenele rastline: 3-5 krat v intervalih po 15 dni z začetkom 10 dni po prvih poganjkih.
Okrasne rastline, zelišča in več letne rastline: 2-4 aplikacije v intervalih 15-20 dni z začetkom v stadiju 3 listov.
PROIZVAJALEC: ZEOVITA GmbH Roter Muhlenweg 28, D 08340 Schwarzenberg, Nemčija

PRILOGA B

Dovoljenje za uporabo članka Cunja in sod. (2014)

From: Michael Neff <mwneff@ashs.org>
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On Jan 11, 2016, at 6:43 AM, Cunja, Vlasta <Vlasta.Cunja@bf.uni-lj.si> wrote:

****permission request****

Dear Sir or Madam,

I am a PhD student currently finalizing my doctoral thesis. My dissertation is composed of several scientific articles published during my research work at the faculty. This is one of

the established forms of PhD dissertation in our program. As the thesis will be published in paper form as well as in electronic format I kindly ask for a letter of permission for the following paper:

Cunja, V., M. Mikulic-Petkovsek, F. Stampar, and V. Schmitzer. 2014. Compound Identification of Selected Rose Species and Cultivars: an Insight to Petal and Leaf Phenolic Profiles. J. Amer. Soc. Hort. Sci. 139 (2):157-166.

Thank you for your time and consideration.
I look forward to your reply.

Sincerely,
Vlasta Cunja

PRILOGA C

Dovoljenje za uporabo članka Cunja in sod. (2015)

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