

UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Jan REŠČIČ

**VPLIV SORTNE AMPELOTEHNIKE NA VSEBNOST
METABOLITOV GROZDJA IN VINA ŽLAHTNE
VINSKE TRTE (*Vitis vinifera* L.)**

DOKTORSKA DISERTACIJA

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**IMPACT OF VARIETAL AMPELOTECHNICS ON THE
METABOLITE CONTENTS IN GRAPE AND WINE OF *Vitis vinifera*
L.**

DOCTORAL DISSERTATION

Ljubljana, 2017

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in Komisije za doktorski študij z dne 7. 7. 2015 je bilo potrjeno, da kandidat izpolnjuje pogoje za opravljanje doktorata znanosti na Interdisciplinarnem doktorskem študijskem programu Bioznanosti, znanstveno področje hortikultura. Za mentorja je bil imenovanizr. prof. dr. Denis RUSJAN.

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IJ	sl
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AI	V študiji smo raziskali vplive redčenja grozdja, razlistanja vinske trte, dvojnega namenskega zorenja grozdja in delnega sušenja grozdja v komori na kakovost grozdja in vina izbranih udomačenih sort žlahtne vinske trte (<i>Vitis vinifera</i> L.). Vplive ukrepov smo vrednotili z analizo kakovostnih parametrov grozdja in vina, s posebnim poudarkom na fenolne spojine v grozdju in vinu, ki smo jih analizirali z metodama HPLC in HPLC-MS. Z redčenjem grozdja rdeče sorte 'Portugalka' sta se povečali vsebnost topne suhe snovi v grozdju in intenziteta barve vina. Pri najbolj intenzivnem redčenju (CT2) smo izmerili značilno večjo vsebnost skupnih in posameznih hidroksicimetnih kislin, večine flavanолоv ter nekaterih flavonolov. Razlistanje sorte 'Istrska malvazija' v coni grozdja v fenofazi nastavka grozdov je značilno zmanjšalo vsebnost alkohola v vinu, a hkrati povečalo vsebnost fenolnih spojin, predvsem hidroksicimetnih kislin. Vino, pridelano iz grozdja trt z zgodnjim razlistanjem, je bilo ocenjeno kot lažje, izrazitejšega okusa in kot bolj harmonično. Pri razlistanju mlajših, fotosintezno aktivnejših listov na trtah sorte 'Istrska malvazija', se niso pokazale značilne razlike v vsebnosti sladkorjev, potrdili smo lahko le značilno večjo vsebnost jabolčne kisline v moštu. Ukrep dvojnega namenskega zorenja grozdja (DMR) je vplival na hitrejšo zorenje grozdja, hkrati pa so se ob izhlapevanju vode, v jagodah koncentrirali sladkorji. DMR je značilno povečal vsebnost fenolnih spojin, identificiranih v kožici in vinu sorte 'Istrska malvazija'. Vino iz grozdja DMR je bilo ocenjeno kot bolj polno vino, a manj harmonično. Značilen vpliv DMR smo opazili pri sortah 'Rebula' in 'Vitovska grganja', pri katerih smo glede na kontrolo izmerili večjo vsebnost posameznih hidroksicimetnih kislin in flavanолоv, predvsem derivatov procianidina in epikatehina. DMR je glede na sušenje grozdja sorte 'Refošk' v komori značilno povečal vsebnost antocianov, flavanолоv in stilbenov v kožici grozdja. Rezultati poskusov potrjujejo, da se posamezne sorte žlahtne vinske trte različno odzivajo na ampelotehnične ukrepe, kar pa ne predpisujemo samo vplivu sorte, ampak tudi vremenskim razmeram med zorenjem grozdja.

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AB In this study we investigated impacts of cluster thinning, leaf removal, Double Maturation Raisonnée and partial dehydration in chamber on grape and wine quality of certain local grapevine varieties (*Vitis vinifera* L.). The impacts were studied by measuring grape and wine quality parameters, with a special emphasis on phenolic compounds, which were analysed by HPLC and HPLC-MS methods. Early cluster thinning at phenophase berry set of red grapevine variety 'Portugalka' significantly increased the content of total soluble solids in berries and the colour intensity of the wine. Significantly higher contents of total and individual hydroxycinnamic acids, flavanols and certain flavonols were measured at the severe cluster thinning (CT2). Leaf removal in bunch zone at fruit set significantly decreased an alcohol content in wine, but on the other hand significantly increased phenolic contents, especially hydroxycinnamic acids. The wine from early leaf removal was evaluated as a lighter wine with improved taste and harmony. At the leaf removal of younger, photosynthetically more active leaves on 'Istrska malvazija' vines, we did not find significant differences in contents of total sugar, but we confirmed only a significantly higher content of malic acid in the must. DMR caused a faster berry ripening and through evaporation also a sugar concentration. DMR influenced the phenolics, as significantly higher contents of mentioned compounds were measured in 'Istrska malvazija' skin and wine. The DMR wine was evaluated as full wine, but with less harmony. An impact of DMR was especially noticed in 'Rebula' and 'Vitovska grganja' grape and wine, where significantly higher contents of individual hydroxycinnamic acids and flavanols (procyanidin derivates and epicatechin) were observed in comparison to the control. Regarding to the partial dehydration in chamber, DMR increased the content of anthocyanins, flavanols and stilbenes in the 'Refošk' berry skin. The results confirm that individual grapevine varieties respond differently on ampelotechnic measures, which cannot be ascribed only to the influence of variety, but also to weather conditions during grape ripening.

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KAZALO PRILOG

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PRILOGA B: Dovoljenje za uporabo članka Reščič in sod. (2016) v reviji Journal of the Science of Food and Agriculture

PRILOGA C: Dovoljenje za uporabo članka Reščič in sod. (2016) v reviji European Journal of Horticultural Sciences

PRILOGA D: Dovoljenje za uporabo članka Mikulič-Petkovšek in sod. (2017) v reviji Turkish Journal of Agriculture and Forestry

OKRAJŠAVE IN SIMBOLI

Okrajšava/simbol	Pomen
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
DMR	Double Maturation Raisonnée
PTP	Priznано tradicionalno poimenovanje
UV	Ultravijolična

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

Vinogradništvo je razširjena, ekonomsko in socialno pomembna kmetijska panoga, saj površina, posajena z vinsko trto v svetovnem merilu obsega več kot sedem milijonov hektarjev (FAO, 2016), in povprečna letna pridelava vina znaša okrog 25 milijard litrov (Wine Institute, 2014). Konkurenčnost vin na trgu je zelo velika, zato so se pridelovalci primorani prilagajati potrebam in zahtevam potrošnikov (Bisson in sod., 2002). Nekateri večji pridelovalci vin se zato, preden pridelajo določeno vino, poslužujejo predhodnih raziskav o preferencah potrošnikov za določen stil vina (Lesschaeve in Findlay; 2004, Landrault in sod., 2001; Francis in Williamson, 2015). Obenem pa se tako imenovani »trend« pridelave glede na preference in zahteve potrošnikov usmerja predvsem v dve smeri; v lažja, sveža vina z majhno vsebnostjo alkohola, ter na drugi strani v bolj kompleksna vina z večjo vsebnostjo alkohola in ekstrakta, polnega okusa in »telesa«, primerna tudi za staranje (Bindon in sod., 2014).

Kemijska sestava grozdja in vina je kompleksna, saj jo zaokrožuje veliko različnih spojin (Conde in sod., 2007). Med razvojem in zorenjem grozdnih jagod se odvija veliko kemijskih procesov in sintez različnih spojin, ki neposredno vplivajo na sestavo in vsebnost določenih metabolitov v grozdju (Conde in sod., 2007).

Sladkorji so primarni metaboliti in kot ogljikovi hidrati imajo pomembno vlogo pri rasti in razvoju vinske trte, kot vir ogljika in energije so udeleženi v številnih procesih (Conde in sod., 2007). Za kakovost grozdja sta najpomembnejša sladkorja heksozi, glukoza in fruktoza, ki se nahajata v grozdnem soku (Liu in sod., 2006). Poleg heksoz so v manjših količinah prisotni še ostali sladkorji, npr. disaharid saharoza ter pentoze (Liu in sod., 2006). Vsebnost sladkorjev v grozdju vpliva na vsebnost alkohola v nastalem vinu, saj kot poročajo Liu in sod. (2006) in Conde in sod. (2007), vsebnost glukoze in fruktoze, ki med vinifikacijo preide v mošt, predstavlja glavni vir hrane za kvasovke.

Organske kisline so poleg sladkorjev v grozdju in vinu pomembna skupina primarnih metabolitov. Prevladujoči vinska in jabolčna kislina, v manjši meri tudi citronska, preidejo v vino iz grozdnih jagod, druge, kot so mlečna in očetna nastajajo med procesi alkoholne in jabolčno-mlečno kislinske fermentacije, oksidacije etanola itd. (Mato in sod., 2005). Njihova vsebnost pomembno vpliva na senzorične lastnosti (okus, vonj, barva) grozdnega mošta in vina, na stabilnost ter razvoj neželenih mikroorganizmov (Ribéreau-Gayon in sod., 2006), obenem pa služi kot indikator za potek določenih procesov (npr. jabolčno-mlečno kislinska fermentacija) (Bauer in Dicks, 2004).

Neposredno z organskimi kislinami je povezan tudi pH. Le-ta vpliva na uspešnost poteka alkoholne fermentacije, nekoliko nižji pH (med 3,0 in 3,5) namreč zavira razvoj neželenih bakterij med samo fermentacijo (Bauer in Dicks, 2004). Nasprotno pa Conde in sod. (2007) navajajo, da višji pH (med 4,0 in 6,0) spodbudno vpliva na delovanje kvasovk. Pomembno vlogo ima pH tudi pri barvi vin, predvsem rdečih, saj je izražanje antocianov v smislu barvnih odtenkov odvisno tudi od pH (Heredia in sod., 1998).

Na kemijsko sestavo vplivajo številni dejavniki. Najpomembnejši so zagotovo sorta, podlaga, talne in klimatske značilnosti (vrsta in tip tal, lega, nagib, izpostavljenost soncu,

količina padavin, število sončnih ur idr.), bolezni in škodljivci ter praktični ukrepi; agrotehnični in ampelotehnični (ampelotehnika), ki se izvajajo v vinogradu (Prajtna in sod., 2007; Bramley in sod., 2011; Rusjan in sod., 2012).

Redčenje grozdja je pogost ukrep v vinogradih. Običajna praksa je, da se redčenje izvede, ko se grozdi povesejo in so jagode velikosti zrn graha (fenološka faza BBCH 75) (Guidoni in sod., 2002; Keller in sod., 2005) ali v času začetka zorenja (Di Profio in sod., 2011; Gil in sod., 2013). Z redčenjem grozdja se sicer zmanjša količina pridelka, zmanjšanje obremenitve trte pa vodi v boljše zorenje jagod (Gil in sod., 2013). Dami in sod. (2006) poročajo, da se ob večji intenziteti redčenja oziroma manjšemu številu grozdov na trti, grozdom poveča masa, saj se spremeni ravnotežje med viri in ponori hranil, predvsem sladkorjev (Guidoni in sod., 2002; Tardaguila in sod., 2010). Gil in sod. (2013) poročajo, da je redčenje grozdja pri sorti 'Syrah' vplivalo na povečano vsebnost topne suhe snovi, ob sočasnem zmanjšanju vsebnosti titracijskih kislin. Potrdili so tudi povečanje vsebnosti alkohola v vinih, pridelanih iz grozdja s trt, kjer je bilo opravljeno redčenje, kar je povezano z večjo vsebnostjo sladkorjev v grozdju. Obenem pa se ob manjšem številu grozdov poveča velikost jagod in s tem tudi razmerje med kožico in mesom jagode. Pospeši se tudi zorenje jagod, kar pomeni, da vpliva na sestavo in vsebnost sekundarnih metabolitov (fenolov) v grozdju in vinu (Bubola in sod., 2011).

Fenoli predstavljajo veliko in raznoliko skupino spojin v grozdju in vinu. V grozdju so, kot navajajo številni avtorji (Mazza in sod., 1999; Montealegre in sod., 2006; Ali in sod., 2010; Garrido in Borges, 2013), najpomembnejše in najbolj pogosto zastopane hidroksicimetne kisline, flavanoli, flavonoli in antociani. Antociane v večini najdemo v jagodah rdečih sort, saj predstavljajo barvila, ki dajejo grozdju in vinu rdečo barvo (Garrido in Borges, 2013). Prav zaradi antocianov je vsebnost fenolnih spojin v rdečih sortah precej večja v primerjavi z belimi (Montealegre in sod., 2006). Pri belih sortah pa je v grozdju več ne flavonoidnih fenolov, npr. hidroksicimetnih kislin in flavanolov (Fernández-Zurbano in sod., 1998).

Fenolne spojine so pomembne za senzorične in kemijske lastnosti vina, saj vplivajo na barvo, aromo, okus, tudi na grenkobo in trpkost oziroma astringenco. Pomembno vlogo imajo tudi pri staranju vin, pri antioksidativnih lastnostih in stabilnosti vina ter oksidacijskih reakcijah (Waterhouse, 2002). Antociani se nahajajo predvsem v kožici jagode in z maceracijo preidejo v mošt in vino (Mazza in sod., 1999), medtem ko je flavanolov v kožici nekoliko manj (Montealegre in sod., 2006). Fenolne kisline (hidroksibenzojske in hidroksicimetne) najdemo prav tako v kožici jagod, vendar so prisotne v precej manjših vsebnostih kot antociani (Ramos in sod., 1999). V grozdju in vinu iz belih sort pa Ramos in sod. (1999) in Mozetič in sod. (2006) navajajo, da so hidroskibenzojske in hidroksicimetne kisline najbolj zastopane fenolne spojine.

Večina študij z redčenjem grozdja je bila opravljena na rdečih sortah, z namenom povečanja intenzivnosti in kakovosti barve v rdečih vinih. Dokazali so, da je redčenje v fazi jagod velikosti graha ali pa v fazi zorenja grozdja vplivalo na večjo vsebnost antocianov, kar je posledično vplivalo na večjo intenziteto barve vina (Guidoni in sod., 2002; Prajtna in sod., 2007; Bubola in sod., 2011). V raziskavah so se osredotočali tudi na vsebnost skupnih fenolov v grozdju in vinu. Številčno malo pa je raziskav, ki bi

podrobneje opisale vpliv redčenja na celoten fenolni profil tako grozdja kot vina, in ne samo na skupne antociane in njihove osnovne aglikone.

Različna intenziteta redčenja različno vpliva na kakovost in sestavo grozdja. Dami in sod. (2006) so v raziskavi ugotovili, da se je povečala masa 100 jagod v primerjavi s kontrolo in manjšo intenziteto redčenja, kot posledica povečanja razmerja med listi in grozdi (viri in porabniki hranil). Podobne ugotovitve so podali tudi Prajitna in sod. (2007) v raziskavi na grozdju sorte 'Chambourcin' in različnih intenzitetah redčenja grozdja, pri čemer so bile pri obravnavanju, pri katerem so pustili samo 10 grozdov na trti, vsebnosti resveratrola, skupnih antocianov ter skupnih fenolov največje. Navsezadnje je končni vpliv redčenja odvisen tudi od odziva posamezne sorte na ta ukrep, kar so potrdili tudi Tardaguila in sod. (2010) v raziskavi z redčenjem grozdja na sortah 'Tempranillo' in 'Grenache'.

Podobno kot redčenje, je delno razlisanje ali odstranitev listov prav tako pogosta vinogradniška praksa (Poni in sod., 2006; Poni in sod., 2009; Tardaguila in sod., 2010). Del listne stene se običajno v coni grozdja, odstrani z namenom izboljšanja mikroklimе grozdov in izboljšanja učinka varstva pred boleznimi in škodljivci (Bavaresco in sod., 2008). Z delno odstranitvijo listov se namreč zmanjša možnost razvoja sive plesni (*Botrytis cinerea* Pers.) in drugih bolezni (Sternad Lemut in sod., 2013). Običajno se razlisanje izvede ob začetku zorenja grozdja (Tessarini in sod., 2014). Omenjen ampelotehnični ukrep je odvisen tudi od vremenskih razmer med zorenjem grozdja, saj je neposredno povezan s fotosintezno aktivnostjo rastline (Bubola in Peršurić, 2012). Listi so organ, v katerih poteka fotosinteza in ob odstranitvi le-teh se namreč spremeni razmerje med viri in ponori hranil, kar praktično pomeni, da trta ne more vseh grozdov optimalno oskrbovati z asimilati. Vendar je vpliv razlisanja na kakovost grozdja odvisen od intenzivnosti in cone, kjer se liste odstrani. To so potrdili tudi Bavaresco in sod. (2008) v raziskavi o vplivu razlisanja v coni grozdja na treh sortah in odkrili, da razlisanje ni imelo značilnega vpliva na vsebnost topne suhe snovi, titracijskih kislin in pH v vseh štirih letih poskusa. Prav tako so se Feng in sod. (2015) v raziskavi osredotočili na razlisanje v coni grozdja na sorti 'Modri pinot' in ugotovili, da se vsebnosti topne suhe snovi, titracijskih kislin ter pH niso značilno razlikovale med tremi leti pri štirih različnih načinih razlisanja.

Poleg tega je vpliv razlisanja na kakovost grozdja in vina posebej povezan s časom (terminom), kdaj oziroma, v kateri fenološki fazi je odstranitev listov izvedena. Nekatere novejšje raziskave (Poni in sod., 2006; Poni in sod., 2009; Tardaguila in sod., 2010; Bubola in Peršurić, 2012; Sternad Lemut in sod., 2013) so se osredotočile na t.i. zgodnje razlisanje, ki se opravi v času okrog cvetenja in do nastanka grozdov, fenološka faza BBCH 71– nastavek grozdov (BBCH ..., 2001). Odstranitev listov v zgodnejšem času je bila do sedaj raziskana predvsem v smislu zmanjšanja pridelka, saj rastlina uspe dovesti v socvetja manj ogljikovih hidratov, posledično je manjši nastavek pridelka (Bubola in Peršurić, 2012). Hkrati pa so Tardaguila in sod. (2010) v študiji pokazali, da zgodnje razlisanje vpliva na večjo vsebnost topne suhe snovi in titracijskih kislin. Prav slednje pa je pomembno pri pridelavi bolj svežih in aromatičnih vin. Ugotovili so tudi, da zgodnje razlisanje vpliva na večjo vsebnost skupnih fenolov v grozdni jagodi.

Obenem pa odstranitev listov po navedbah nekaterih avtorjev (Intrieri in sod., 2008; Poni in sod., 2009) vpliva na povečanje vsebnosti fenolov iz skupine flavonoidov, predvsem

flavonolov. Flavonoli so spojine z dvojno vezjo na drugem in tretjem ogljikovem atomu, skupaj z hidroksilno skupino na tretjem atomu ogljika. Najpogostejši aglikoni flavonolov v grozdju so kvercetini, kempferoli in miricetini, na katere so lahko vezani različni glikozidi (Garrido in Borges, 2013). Poleg tega so v rdečih vinih pomembni kopigmenti, ki vplivajo na barvo vina, v belih vinih pa predstavljajo pomembne antioksidante (Castillo-Muñoz in sod., 2007). Lastnost flavonolov je ta, da absorbirajo presežke UV sevanja in ščitijo rastlino pred škodljivimi vplivi le-tega. Sinteza flavonolov je še dodatno stimulirana s svetlobo (Castillo-Muñoz in sod., 2007). Ob odstranitvi listov v coni grozdja, je le-to bolj izpostavljeno soncu in svetlobi kar vpliva na povečano sintezo flavonolov v kožici. Predvsem pa z razlistanjem med zorenjem v coni grozdja, pride zaradi izpostavljenosti soncu do višjih temperatur v jagodi, kar vodi v manjšo vsebnost kislin v jagodah in posledično pomeni manj svežine v vinu (Tardaguila in sod., 2010). Po mnenju nekaterih avtorjev (Bavaresco in sod., 2008; Bubola in Peršurić, 2012) je vpliv razlistanja večji pri bujnih sortah, ki dajejo tudi večji pridelek.

Vsebnost alkohola je pogojena z vsebnostjo sladkorjev v grozdnih jagodah. Sladkorji se tvorijo pri procesu fotosinteze v listih. Najbolj fotosintezno aktivni so zgornji ali apikalni listi, manj aktivni so listi v sredini listne stene, najmanj pa spodnji listi, ki so tudi najstarejši. Ob razlistanju zgornjih, apikalnih listov pride do zmanjšanja fotosintezne aktivnosti in s tem bistveno manjše sinteze sladkorjev (Poni in sod., 2006). Kljub manjši sintezi sladkorjev pa ni raziskav, ki bi poročale, da se zmanjša tudi vsebnost kislin. Posledično gre pričakovati, da bi bilo vino iz grozdja z razlistanih trt bolj sveže.

Hkrati pa razlistanje zgornjih, mlajših listov za trto, posebno med zorenjem povzroči stres, ki sproži različne mehanizme. En izmed odgovorov rastline na stres je tudi sinteza fenolnih spojin, kar se posledično odraža v povečanju njihove vsebnosti v grozdnih jagodah. Znano je, da je sinteza fenolnih spojin v rastlini precej povezana z delovanjem okoljskih dejavnikov (Jackson in Lombard, 1993). Fenoli se pogosto sintetizirajo kot odziv rastline proti okužbam s patogenimi organizmi, mehanskim poškodbam ali fiziološkim spremembam in stresnim situacijam (Teixeira in sod., 2013), kar vodi v pospešeno sintezo fenolnih spojin preko fenilpropanoidne biosintezne poti (Dixon in Paiva, 1995).

Na drugi strani trg narekuje k pridelavi bolj kompleksnih in polnih vin, z večjo vsebnostjo alkohola in ekstrakta, primernih tudi za staranje. Takšna vina imajo običajno tudi večjo vsebnost primarnih metabolitov (sladkorjev in kislin) ter sekundarnih metabolitov (fenolnih spojin). Večina fenolnih spojin preide iz grozdja v mošt in vino med predelavo in stiskanjem (Gil-Muñoz in sod., 1999; Netzel in sod., 2003; Adams, 2006).

Peršurić in sod. (2000) in Cargnello in sod. (2005) so raziskovali ampelotehnični ukrep, ki se izvede med zorenjem jagod, tako imenovano dvojno zorenje grozdja ali Double Maturation Raisonnée (DMR) že v letih 1996 in 1997. Ta ukrep je namenjen predvsem povečanju vsebnosti primarnih metabolitov (predvsem sladkorjev) ter sekundarnih metabolitov (fenolnih spojin) v jagodah. Do povečanja vsebnosti omenjenih spojin pride posredno zaradi prekinitve toka vode, hranil in asimilatov med matično rastlino in grozdi, saj se osnovno rozgo z mladikami in grozdi, v fazi, ko je grozdje v tehnološki zrelosti odreže kot pri zimski rezi. Odrezana rozga skupaj z grozdi ostane na žici do trgatve. Posledično, grozdje izgublja vodo in se zato, podobno kot pri suši, koncentrirajo sladkorji.

Poleg tega pa se vinska trta na rez rozge odzove kot na stres (Corso in sod., 2013) in kot odgovor sproži mehanizme sinteze fenolnih spojin (Bonghi in sod., 2012). Corso in sod. (2013) so v študiji na sorti 'Raboso Piave' ugotovili, da se je v jagodah, ki so bile na trtah z ukrepom DMR značilno zmanjšala vsebnost titracijskih kislin ter vinske in jabolčne kisline, pri tem pa se pH ni značilno spremenil, značilno pa se je povečala vsebnost topne suhe snovi ter skupnih fenolov.

Poročali so tudi, da ukrep DMR vodi v hitrejšo fenolno zrelost. Pojem fenolne zrelosti je bolj ali manj povezan z rdečimi sortami, saj ima grozdje, ki je fenolno zrelo, največjo vsebnost antocianov in boljše barvne lastnosti (Kontoudakis in sod., 2011). Ob fenolni zrelosti grozdja se, kot navajajo Kontoudakis in sod. (2011), v vinu pričakuje večja intenziteta barve, vina so strukturnejša, z večjo vsebnostjo mehkejših taninov in vonja, ki spominja na sadje. Antociani se v jagodni kožici nahajajo v vakuolah in ob določeni zrelosti grozdja se celične stene zmehčajo ob delovanju encimov hidrolaz, kar omogoča večje sproščanje (ekstrabilnost) omenjenih fenolnih spojin (Hernández-Hierro in sod., 2014).

Po poročanju nekaterih raziskav (Franco in sod., 2004; Frangipane in sod., 2012; Bellincontro in sod., 2016) se vsebnost fenolnih spojin v grozdju poveča tudi s sušenjem grozdja, ki se lahko izvaja na trti ali pa v zaprtih prostorih pod kontroliranimi razmerami (Figueiredo-Gonzalez in sod., 2013). Ukrep sušenja grozdja je sicer v osnovi bolj ali manj namenjen koncentriranju sladkorjev v jagodi, ki se zgodi ob dehidraciji oziroma izhlapevanju vode. Zato je večina dosedanjih raziskav s sušenjem grozdja usmerjena v pridelavo sladkih vin, vin ledene trgatve in podobno. Sočasno z dehidracijo, jagodi upada masa in volumen, kar posredno vpliva tudi na povečanje fenolnih snovi v grozdju (Franco in sod., 2004).

Kljub vsemu pa raziskav o vplivih razlistanja na kakovost grozdja in vina, predvsem o preučevanju vplivov na zeleno kakovost za določen stil vina ni veliko. Raziskave so sicer posredno osredotočene na določene pomembne in lokalne sorte, vendar se ampelotehnični ukrepi ne vpeljujejo v smislu pridelave zelenega stila vina. Manj raziskav je bilo tudi narejenih na odzivu trte na določen ampelotehnični ukrep pri posamezni sorti skozi celoten fenolni profil. Raziskav na temo fenolnih snovi v grozdju in vinu je veliko, vendar malo je napisanega o vplivu ukrepa DMR na vsebnost fenolnih spojin v grozdju ali vinu. Dosedanje raziskave v smeri zgodnjega razlistanja so bile narejene na različnih sortah, predvsem na rdečih, saj je bil cilj, da se raziše vpliv razlistanja na vsebnost rdečih barvil, antocianov, in s tem morebitno izboljšanje barve pri rdečih vinih. Poleg antocianov pa grozdje belih sort vsebuje še številne druge flavonoide, ki bolj kot k barvi pripomorejo k okusu (Garrido in Borges, 2013).

V okviru doktorske disertacije smo želeli pridobiti informacije o vplivu različnih ampelotehničnih ukrepov na vsebnost določenih metabolitov v grozdju in vinu predvsem lokalnih sort žlahtne vinske trte (*Vitis vinifera* L.), ki so bile do sedaj manj poznane in raziskane. Namen je bil tudi raziskati odzive trte na posamezen ukrep pri določeni sorti, s ciljem, da se podajo smernice za praktične ukrepe, ki bi zagotavljale kakovost grozdja oziroma vina, kakršno narekuje današnji trg oziroma potrošniki. Redčenje grozdja je bilo v okviru znanstvenih raziskav širom po svetu že precej raziskano, vendar v smislu

zagotovitve stalne kakovosti na bujnih sortah ni veliko objav. Prvič smo predstavili vpliv različnih intenzitet redčenja na celoten fenolni profil avtohtone rdeče sorte 'Portugalka'. Namreč raziskave do sedaj so bile osredotočene zgolj na antociane v grozdju in vinu. Prav tako so bili raziskani vplivi različnih terminov in intenzitet razlistanja vinske trte. Največ raziskav je bilo narejenih na odstranitvi listov vinske trte v coni grozdja v fazi začetka zorenja grozdja ter tudi v fazi pred, med, ter takoj po cvetenju. Zato je bil namen raziskati tudi razlistanje listov na mladikah nad drugim parom žic in pridobiti informacije o vplivu le tega na kemijsko sestavo in vsebnost metabolitov, predvsem fenolnih spojin. Podobno, zanimal nas je odziv vinske trte na ne-stresne in stresne razmere, ki jih povzročajo različni ampelotehnični ukrepi, skozi profil primarnih in sekundarnih metabolitov. Zato smo v okviru doktorske disertacije postavili štiri poskuse:

Poskus 1: Vpliv redčenja grozdja na kakovost grozdja in vina pri sorti 'Portugalka'

V poskus smo vključili avtohtono sorto 'Portugalka' (*Vitis vinifera* L.), pri kateri smo izvedli dve intenziteti redčenja grozdja. Bločni poskus je bil postavljen v vinorodnem okolišu Bela krajina, na vinorodni legi Radovica. Za določitev mere redčenja smo najprej ovrednotili rastni in rodni potencial trt, in sicer v fenofazi BBCH 53, ko so bili kaberniki na mladikah jasno vidni. Posamezen blok v poskusu je zajemal tri obravnavanja (kontrola, manj intenzivno redčenje in intenzivno redčenje), ki so bila naključno dodeljena štirim vzporednim vrstam. Vsakemu obravnavanju je pripadalo dvajset zaporednih trt. Ob tehnološki zrelosti grozdja smo na jagodah glede na obravnavanje izmerili barvne parametre (L^* , C^* in h) in izračunali CIRG indeks ter parametre dozorevanja grozdja v grozdnem soku, to so vsebnost topne suhe snovi, titracijskih kislin in pH. Pridelano vino, ločeno po obravnavanjih, smo analizirali na glavne kakovostne parametre (vsebnost alkohola, skupnega ekstrakta, sladkorja prostega ekstrakta, reducirajočih sladkorjev, skupnih in posameznih ter hlapnih kislin in pH). S HPLC in HPLC-MS metodama smo analizirali vsebnosti hidroskicimetnih kislin, flavanolov, flavonolov in antocianov ter s spektrofotometrično analizo skupnih fenolov v jagodni kožici in vinu, smo ugotavljali vpliv intenzitete redčenja na celoten fenolni profil pri sorti 'Portugalka', kar do sedaj še ni bilo narejeno. Poskus je tudi prva raziskava, ki vključuje sorto 'Portugalka' in redčenje grozdja. Poskus je nadaljevanje dela diplomske in magistrske naloge na sorti 'Portugalka' (Kordič, 2009; Kordič, 2014).

Poskus 2: Vpliv različnega termina razlistanja in dvojnega namenskega zorenja grozdja na vsebnost primarnih in sekundarnih metabolitov pri sorti 'Istrska malvazija'

V bločni poskus smo vpeljali dva različna termina razlistanja oziroma odstranitve listov: zgodnje razlistanje v coni grozdja (ko so bile jagode v fazi velikosti prosenih zrn (fenofaza BBCH 53), smo odstranili vse liste na mladikah pod prvim parom žic) in razlistanje zgornjih, mlajših listov v času zorenja (ko so bile jagode v fenofazi BBCH 81, smo odstranili vse liste na mladikah nad drugim parom žic) ter tehniko dvojnega namenskega zorenja (DMR) grozdja pri sorti 'Istrska malvazija' (*Vitis vinifera* L.). Na trtah, vključenih v poskus smo izmerili rastni in rodni potencial (število oces, mladik, število in maso grozdov). Dinamiko zorenja jagod smo spremljali z meritvami mase 100 jagod, vsebnosti topne suhe snovi, titracijskih kislin, pH, vsebnosti skupnih fenolov ter z merjenjem vsebnosti posameznih sladkorjev, kislin ter posameznih fenolov s HPLC in HPLC-MS metodo. Za vsako obravnavanje je bilo pridelano tudi vino, katerega se je analiziralo z napravo WineScan FT 120. Namen poskusa je primerjati in ovrednotiti vpliv ukrepov

razlistanja ter DMR glede na kontrolo, kot možnosti pridelave različnega stila vina, ki bi odgovarjal potrošniku. Poskus je nadaljevanje dela magistrske naloge (Babić, 2016).

Poskus 3: Vpliv dvojnega namenskega zorenja (DMR) na kakovost grozdja in vina sort 'Rebula' in 'Vitovska grganja'

Bločni poskus je bil zasnovan na belih lokalnih sortah 'Rebula' in 'Vitovska grganja' (*Vitis vinifera* L.), in sicer prvič se je pri naštetih sortah ugotavljal vpliv DMR na rast in rodnost trt ter kakovost grozdja in vina v danih pridelovalnih razmerah. V vzorcih iz tehnološke zrelosti (trgatev) smo potencialni vpliv obravnavanih ukrepov analizirali z merjenjem vsebnosti topne suhe snovi, titracijskih kislin in pH ter z analiziranjem vsebnosti primarnih metabolitov (sladkorji, organske kisline) in sekundarnih metabolitov (fenolne spojine) z metodama HPLC in HPLC-MS. Namen poskusa je bil preveriti, ali bi bil ukrep DMR lahko zanimiv za lokalne sorte, predvsem v smislu pridelave bolj polnega vina, z večjo vsebnostjo alkohola in ekstrakta, kar je trenutno iskano s strani potrošnikov.

Poskus 4: Vpliv tehnik delnega sušenja na sestavo metabolitov grozdja in vina sorte 'Refošk'

V poskus smo vključili rdečo sorto 'Refošk' (*Vitis vinifera* L.), kateri smo priredili dve tehniki delnega sušenja jagod, to sta DMR in delno sušenje (dehidracija) v sušilni komori. Analizirali smo parametre dozorevanja grozdja, posamezne sladkorje in organske kisline v grozdnih jagodah ter osnovne parametre kakovosti pridelanega vina. V jagodni kožici in vinu smo s HPLC in HPLC-MS sistemom analizirali tudi posamezne fenolne spojine. Namen te raziskave je bil primerjati vpliv obeh tehnik, predvsem zaradi potencialne nadomestitve sušenja v komori s tehniko DMR, saj je proces sušenja v komori kljub kontroliranim razmeram dražji in dolgotrajnejši. Prvič je bil tudi podan celoten fenolni profil vinske sorte 'Refošk'.

V okviru doktorske disertacije smo postavili naslednje raziskovalne hipoteze:

1. Fenolne snovi so odgovorne za okus in barvo grozdja in vina. Ob večji intenziteti redčenja se bo pri izbrani sorti tvorilo številčno več fenolnih snovi, katerih tudi vsebnost bo večja. Grozdje in vino pridelano iz grozdja z redčenih trt bo imelo večjo intenziteto barve.
2. Čas oziroma fenofaza, pri kateri se opravi razlistanje, vpliva na kemijsko sestavo grozdja in vina. Zgodnje razlistanje po cvetenju bo vplivalo na večjo vsebnost sladkorjev in fenolnih spojin ter manjšo vsebnost kislin v grozdju in vinu. Vino bo posledično imelo večjo vsebnost alkohola, ocenjeno pa bo kot vino z več okusa.
3. Razlistanje zgornjih, mlajših listov na mladikah med zorenjem grozdja upočasni sintezo in zmanjša vsebnost skupnih in posameznih sladkorjev in kislin v grozdju izbrane sorte. Grozdje in vino iz grozdja s trt tovrstnega razlistanja bo vsebovalo manjšo vsebnost posameznih in skupnih fenolov, ter bo ocenjeno kot sveže vino z manj telesa.
4. Dvojno namensko zorenje bo povečalo vsebnost skupnih in posameznih sladkorjev, kislin in sestavo ter vsebnost posameznih in skupnih fenolnih spojin v grozdju izbrane sorte.

5. Vino pridelano iz grozdja z DMR trt bo imelo večjo vsebnost alkohola, ekstrakta, skupnih kislin ter nižji pH in bo senzorično ocenjeno kot manj sveže, kompleksno z velikim telesom.
6. Grozdje in vino iz DMR trt bo imelo večjo vsebnost fenolnih spojin, predvsem antocianov, v primerjavi z sušenjem v komori.

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Vpliv redčenja grozdja na rodnost ter sestavo grozdja in vina sorte 'Portugalka' (*Vitis vinifera* L.)

Reščič J., Mikulič-Petkovšek M., Štampar F., Zupan A., Rusjan D. 2015. The impact of cluster thinning on fertility and berry and wine composition of 'Blauer Portugieser' (*Vitis vinifera* L.) grapevine variety. Journal international des sciences de la vigne et du vin, 49, 4: 275-291

Dve različni intenziteti redčenja grozdja sta bili izvedeni z namenom, da se razišče njun vpliv na rast in rodnost ter sestavo grozdja in vina sorte 'Portugalka'. Manj intenzivno CT1 (20-30 % odstranjenih grozdov/trto) in intenzivno CT2 (40-50 % odstranjenih grozdov/trto) redčenje je bilo opravljeno v fenološki fazi, ko so bile jagode velikosti zrn graha (BBCH 75) v letih 2007, 2008 ter 2011. Intenziteta redčenja je bila določena z merjenjem ravnega in rodnega potenciala, potencialni vpliv na kakovost pa z analizami mase in barve jagod, vsebnosti topne suhe snovi, titracijskih kislin, pH ter vsebnosti skupnih fenolov. Prvič so bile s HPLC in HPLC-MS metodama identificirane in kvantificirane posamezne fenolne spojine v grozdju in vinu sorte 'Portugalka'. V splošnem je redčenje grozdja na sorti 'Portugalka' zmanjšalo vsebnost titracijskih kislin v grozdju in vinu, kot tudi je vplivalo na povečanje pH ter barvnih parametrov v grozdju na eni strani ter večjo vsebnost alkohola ter hlapnih kislin na drugi strani. Značilno zmanjšanje pridelka (za 0,92 kg grozdja na trto), skupaj s povečanjem vsebnosti topne suhe snovi (za 2,8 °Brix) v grozdju ter pH in skupnega ekstrakta v vinu je bilo opaziti samo pri intenzivnejšem redčenju (CT2). Nadalje, CT2 je značilno vplivalo na večjo vsebnost skupnih antocianov, flavonolov in hidroksicimetnih kislin v grozdju in vinu, a neznačilno na vsebnost flavanolov. Značilen vpliv na pridelek ter sestavo grozdja in vina smo posebno opazili samo pri CT2, kjer je bilo zmanjšanje pridelka nadomeščeno z večjo vsebnostjo topne suhe snovi v grozdju in alkohola v vinu ter fenolnih snovi v grozdju in vinu. Ta študija prvič poroča o vplivu različnih intenzitet redčenja grozdja na sestavo grozdja in vina sorte 'Portugalka'. Sestava grozdja in vina je bila ovrednotena s poudarkom na natančnem fenolnem profilu ter vsebnostjo skupnih fenolov. Rezultati so zagotovo uporabni za vinogradnike in vinarje, katerim je do sedaj primanjkovalo tehnoloških smernic v smislu optimiziranja kakovosti grozdja in vina sorte 'Portugalka'.

THE IMPACT OF CLUSTER THINNING ON FERTILITY AND BERRY AND WINE COMPOSITION OF 'BLAUER PORTUGIESER' (*VITIS VINIFERA* L.) GRAPEVINE VARIETY

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Abstract

Aim: Two different yield reductions based on cluster thinning (CT) were performed to determine their impact on vine growth, yield, and grape and wine composition of 'Blauer Portugieser' grapevine variety.

Methods and results: Two levels of cluster thinning (limited CT1 – 20-30 % and severe CT2 – 40-50 % cluster reduction) were applied at the pea-size berry (BBCH 75) phenological stage in 2007, 2008 and 2011. The potential impact of CT was determined by measurements of vine growth and fertility potential, berry weight, berry colour, soluble solids content, titratable acidity, pH and total phenolics. Additionally, for the first time, individual phenolic compounds were identified and quantified in berry skin and wine by HPLC-MS. In general, CT of 'Blauer Portugieser' significantly decreased titratable acidity in grape and wine, and increased pH and chromatic parameters in grape and alcohol content and volatile acidity in wine. A significant decrease in yield per vine (of 0.92 kg of grape/vine), together with an increase in soluble solids (of 2.8 °Brix) in grape and pH and total extract content in wine was only observed in severe CT (CT2). Furthermore, CT2 significantly increased the content of total anthocyanins, flavonols and hydroxycinnamic acids, but not total flavanols, in grape and wine.

Conclusion: A significant impact on yield and grape and wine composition was observed, particularly in the CT2 treatment, in which the yield loss was compensated by higher contents of soluble solids in grape, alcohol in wine, and phenolic compounds in grape and wine.

Significance and impact of the study: The present study is the first report on the impact of different levels of cluster thinning on yield and grape and wine composition of 'Blauer Portugieser' variety. Grape and wine composition has been evaluated with an emphasis on a detailed profile of individual and total phenolic contents. The results are undoubtedly useful for winegrowers, who, until now, lacked technological guidelines to optimize 'Blauer Portugieser' yield and wine quality.

Key words: yield, grape quality, hydroxycinnamic acids, flavonols, anthocyanins, HPLC-MS

Résumé

Objectif : Deux réductions de rendement par éclaircissage ont été effectuées afin de déterminer leur incidence sur la croissance et le rendement des plantes ainsi que sur la composition du raisin et du vin de la variété 'Portugais Bleu'.

Méthodes et résultats : Deux niveaux d'éclaircissage (réduction de grappe limitée CT1 – 20-30 % et intense CT2 – 40-50 %) ont été effectués au stade phénologique BBCH 75 (grains de la taille d'un petit pois) en 2007, 2008 et 2011. L'incidence potentielle de l'éclaircissage a été déterminée par des mesures du potentiel de croissance et de fertilité des plantes ainsi que du poids et de la couleur des baies, de la concentration en solides solubles, de l'acidité titrable, du pH et des substances phénoliques totales. En outre, pour la première fois, les composés phénoliques individuels ont été identifiés et quantifiés par CLHP-SM dans la peau des baies et dans le vin. En général, l'éclaircissage du 'Portugais Bleu' a considérablement diminué l'acidité titrable du raisin et du vin, mais il a augmenté le pH et les valeurs des paramètres chromatiques du raisin ainsi que la teneur en alcool et en acidité volatile dans le vin.

Conclusion : Des incidences significatives sur le rendement et la composition du raisin et du vin ont été observées, en particulier suite à l'éclaircissage intense, où la perte de rendement a été compensée par une teneur en solides solubles plus élevée dans le raisin de même qu'une teneur plus élevée en alcool dans le vin et en composés phénoliques dans le raisin et dans le vin.

Signification et impact de l'étude : Cette étude rend compte pour la première fois de l'incidence de différents niveaux d'éclaircissage sur le rendement et la composition du raisin et du vin de la variété 'Portugais Bleu'. L'évaluation de la composition du raisin et du vin s'est concentrée sur le détail des teneurs en composés phénoliques individuels et totaux. Les viticulteurs qui, jusqu'à présent, manquaient d'orientations technologiques en vue d'optimiser le rendement et la qualité du vin de la variété 'Portugais Bleu' trouveront une utilité certaine dans ces résultats.

Mots clés : rendement, qualité du raisin, hydroxycinnamates, flavonols, anthocyanes, CLHP-SM

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INTRODUCTION

Grape and wine production in Europe is an important and traditional agricultural sector, although its extent in EU has decreased by around 15 % in the past decades (O.I.V., 2013). This could be attributed to the high cost of grape production, especially in areas with small fragmented vineyards on high slopes where manual labour still predominates (Preszler *et al.*, 2010). Winegrowers are constantly trying to optimize the ratio between quantity and quality and to raise the price of wine. Yield and vegetative growth affect grape and wine quality and can be controlled by grape cluster and shoot thinning (Šuklje *et al.*, 2013), berry thinning (Gil *et al.*, 2013), shoot topping and defoliation after blooming (Reynolds *et al.*, 2004; Šuklje *et al.*, 2013). Cluster thinning (CT) is undoubtedly one of the most commonly used vineyard practices for achieving the optimal ratio between yield and quality (Naor *et al.*, 2002; Prajitna *et al.*, 2007; Preszler *et al.*, 2010), balancing crop and improving grape and wine composition (Naor *et al.*, 2002). Entire clusters or their parts are usually removed by hand at different phenological stages during grape ripening before harvest (Prajitna *et al.*, 2007; Di Profio *et al.*, 2011; Gil *et al.*, 2013). Although this is a costly and labour-intensive procedure (time-consuming and requiring skills, experience and precision), the enhanced product quality makes it economically feasible (Preszler *et al.*, 2010). However, timing and intensity of cluster thinning depend on the purpose and goal of the wine style. Prajitna *et al.* (2007) demonstrated that cluster removal at the pea-size stage significantly increased pH, total phenolic content and anthocyanins and decreased titratable acidity in grape. Bubola *et al.* (2011) reported that in 'Merlot' yield reduction by

60 % CT at véraison increased leaf area/fruit weight ratio from 1.96 m²/kg in the control treatment to 2.08 m²/kg. They demonstrated that CT significantly increased the amount of total phenolics by 510 mg/L and anthocyanins by 182 mg/L, compared to the control vines. Furthermore, they also reported an impact of CT on colour intensity, which reached 1.84 at 60 % CT, but only 1.05 in the control. Guidoni *et al.* (2002) showed that CT performed at the pea-size phenological stage on 'Nebbiolo' variety significantly increased soluble solids content by 1 to 2 °Brix. Furthermore, Gil *et al.* (2013) and Prajitna *et al.* (2007) demonstrated that, compared to the control, CT of 'Syrah' and 'Chambourcin' varieties significantly increased anthocyanin contents by 24 % and 52 %, respectively. However, CT could result in a significant yield reduction (Gil *et al.*, 2013; Gamero *et al.*, 2014), although the produced grape quantity usually meets winegrowers expectations. On the other hand, Bubola *et al.* (2011) reported an increased size of the remaining berries after CT. Specifically, berries were 34 % bigger in the 60 % CT treatment compared to the control, but a yield loss of 19 to 40 % was measured. In the last decades, landmark publications regarding the impact of CT on grape and wine quality parameters focused mainly on world-wide cultivated grapevine varieties (Bubola *et al.*, 2011; King *et al.*, 2012; Gil *et al.*, 2013; Šuklje *et al.*, 2013) and less on minor varieties such as 'Blauer Portugieser'. However, local varieties are important for many grape and wine producers, especially when the wine production is targeted and specialized.

'Blauer Portugieser' (*Vitis vinifera* L.) is a red grapevine variety grown in Central and South-Eastern Europe and known for its high yield and vigour (Hrček and Korošec-Koruza, 1996). In Slovenia, the

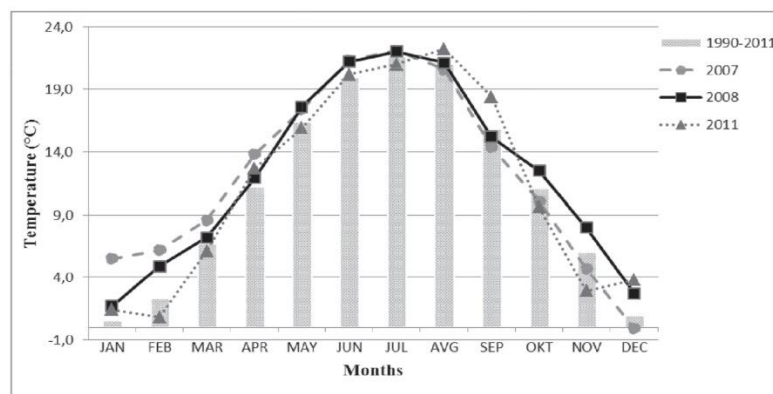


Figure 1 - Average month temperature (°C)
of weather station Črnomelj for years 2007, 2008, 2011 and a longterm average between 1990 and 2011.

'Blauer Portugieser' variety (syn. 'Portugalka') is mostly cultivated in the Bela krajina winegrowing district and is one of the most important varieties for the production of a Slovenian wine with a recognised traditional denomination – PTP Metliška črnina (Rules of wine denomination - Metliška črnina, 2008). The high yield of 'Blauer Portugieser' is often reflected in a poor quality (low sugar content, high acidity) and poor colour intensity of grape and wine (Gamero *et al.*, 2014). A long-term monitoring (2002-2012) of grape quality parameters indicated that the 'Blauer Portugieser' variety has, on average, a low sugar content from 67 to 85 °Oe, quite variable total acidity from 4.4 to 7.3 g/L, a pH from 3.14 to 3.62, and an average berry weight from 185 to 268 g/100 berries (Chamber of Agriculture and Forestry of Slovenia, 2014). The Blauer Portugieser wine is known as a light-bodied, light-coloured dry wine with an alcohol content around 11 %, acidity 7 g/L, total extract 25 g/L, pH 3.4 and volatile acidity around 0.2 g/L (Nemanič, 1999). Thus, CT could represent one of the possible practices to improve and standardize grape and wine quality (Šuklje *et al.*, 2013). The aim of the present study was to monitor for the first time the potential impact of different levels of cluster thinning of 'Blauer Portugieser' on yield and growth potential and grape and wine composition.

The research provides, in particular, information about the phenolic profile of 'Blauer Portugieser' which greatly affects grape and wine quality. The results will be useful to winegrowers of 'Blauer Portugieser', who, until now, lacked information on the effect of thinning on grape and wine quality characteristics.

MATERIALS AND METHODS

1. Experimental vineyard

The experiment was conducted in 2007, 2008 and 2011 on 'Blauer Portugieser' vines trained on single Guyot, planted at the distance of 2.3 m x 0.9 m, and grafted on SO4 rootstock (*Vitis berlandieri* × *Vitis riparia*). The vineyard was maintained with a permanent green cover and managed according to integrated pest management measures; it was located in South-Eastern Slovenia in the Bela krajina winegrowing district, in the Radovica winegrowing area (latitude: 45°41' N, longitude: 15°20' E) at 375 m above sea level. The soil was classified as sandy clay loam (> 45 % sand, 20-40 % clay and < 30 % silt), its depth was more than 70 cm, containing 1 to 2 % of organic matter with a medium water-holding capacity. Soil analysis at the depth 0-20 cm showed 5.4 mg P₂O₅/100 g of soil, 21.3 mg K₂O/100 g of soil, 7.8 mg Mg/100 g of soil, 1.6 % organic matter and a pH value of 7.0, and at the depth 20-40 cm 1.5 mg P₂O₅/100 g of soil, 20.1 mg K₂O/100 g of soil, 4.6 mg Mg/100 g of soil, 0.8 % organic matter and a pH value of 7.2. The soil characteristics are typical for anthrosol, which has been formed or heavily modified due to long-term human activity, such as deep ploughing, cultivation or irrigation (Pedological map of Slovenia, 2007). The climate in the area is subpanonic with an average annual air temperature of 10.1 °C and rainfall not exceeding 1100 mm per year, according to the average temperature (Fig. 1) and precipitation (Fig. 2) from 1990 to 2011 (Slovenian Environment Agency, 2015).

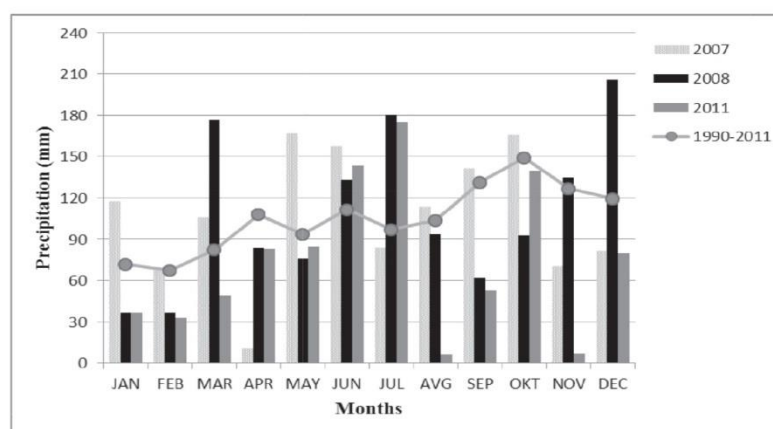


Figure 2 - Average month precipitation (mm) of weather station Črnomelj for years 2007, 2008, 2011 and a longterm average between 1990 and 2011.

2. Experimental design

The study was conducted in a vineyard as a block experiment with three treatments and four replicates per treatment. Each block was built of three randomly set treatments, where each treatment was set on twenty consecutive vines in a single row: control (C - no cluster thinning), cluster thinning 1 (CT1 - all third clusters on the shoot and every second cluster on each second shoot were removed; limited 20-30 % cluster reduction) and cluster thinning 2 (CT2 - only one cluster left on each shoot; severe 40-50 % cluster reduction). The treatment replicates were organized by four parallel and adjacent rows. The initial growth potential was measured - the buds and shoots on studied vines were counted and the yield potential at BBCH 53 (BBCH, 2001) phenological stage was estimated by counting the inflorescences. The level of cluster thinning was set on the basis of recorded yield potential. The cluster thinning was always performed at the pea-size berry phenological stage (BBCH stage 75): in 2007 on 17th of July, in 2008 on 19th of July, and in 2011 on 1st of July. The experiment started in 2007 and was upgraded in 2008 and 2011 with a detailed analysis of secondary metabolites in grape and wine. Approximately 0.5 kg of grapes (4-5 clusters per replicate) were harvested at appropriate ripeness (BBCH 89) and berries were collected from different parts of the cluster for further analyses. Five individual microvinifications per treatment were conducted under equal conditions: 200 kg of grapes per treatment were first divided into five equal parts, the grapes were destemmed, berries crushed and the maceration lasted for 10 days. At the start of the maceration, 20 g/hL of rehydrated yeast *Saccharomyces cerevisiae* Mycoferm ROUGE was added into each vessel, according to the producer guidelines (INRA, Bordeaux, France). During the maceration, the cap of grape marc was punched-down by hand twice per day, and at the end of the maceration process the marc was pressed separately per treatment and replicate. The obtained semi-fermented must was decanted into individual 30-L tanks, where wine fermentation continued for an additional fourteen days. After that, the wine was decanted and 25 mL/hL of 5 % sulphuric acid (H_2SO_4) was added into individual tanks.

3. Measurements and analysis

All measurements of berry characteristics were conducted immediately after harvest, and chemical analyses were carried out during the following months. The growth (vegetative) potential of vines was assessed by counting the total number of

unfertile and fertile buds and shoots before flowering in all three years. In the first year (2007) of the study, the weight of 100 berries, contents of soluble solids and titratable acidity were measured; in the next year (2008), the experiment was upgraded with additional measurements of colour parameters of berry skin and must. In the third year (2011), the study focused on secondary metabolites with measurements of the contents of total and individual phenolics in grape and wine. Additionally, colour parameters of berries, chemical parameters of berries and/or wine (pH value, content of soluble solids and titratable acidity) and wine composition parameters were measured.

4. Berry and wine composition

The content of soluble solids was assessed with a digital refractometer (ATAGO PAL87S) and expressed in Brix scale. To determine the content of titratable acidity, 0.1 M NaOH was added to the sample with a semiautomatic titrator till pH value reached 7.0 (Ough and Amerine, 1988a) and the required volume was recorded. The content of titratable acidity was expressed in g/L. Before the titration, initial pH value of the must was measured. The chemical analyses of the produced wines were performed one month after sulphuring. A single sample of wine per tank (five wines/replicate per treatment) was decanted and the chemical characteristics were evaluated using a WineScan FT120 (Foss, Denmark).

5. Colour parameters

Twenty berries per replicate for each individual treatment were randomly sampled and used to determine colour parameters with a Minolta CR-300 Chroma colorimeter (Minolta Co.; Osaka, Japan). Two measurements at opposite sites of the same berry were taken and CIELAB values a^* , b^* , L^* , C^* and h were recorded to calculate the CIRG index (Carreño *et al.*, 1995).

The must colour was measured according to the method reported by Ough and Amerine (1988b). The pH value of each must sample was recorded and samples were diluted with a solvent solution of sulphuric acid (H_2SO_4) and bidistilled water of the identical pH value (1:10, v/v). Absorbance was measured on a UV-VIS spectrophotometer (Lambda Zobio) at 420 nm, 520 nm and 620 nm. Colour intensity was calculated as the sum of absorbances A_{420} , A_{520} and A_{620} for each sample and tonality (hue) as the ratio of A_{420} and A_{520} (Glories, 1984).

Table 1 - Identification of phenolic compounds in grape skin and wine of 'Blauer Portugieser' variety in positive and negative ionization with HPLC-MS and MS2.

Tentative identification	M ⁺ or [M-H] ⁻ (m/z) ^a	MS ² (m/z)	Analyzed in	
			skin	wine
Hydroxycinnamic acids				
Caftaric acid	311	179	x	x
<i>p</i> -Coumaroyl pentose	295	163	x	x
<i>trans</i> fertaric acid	325	193	x	x
<i>trans</i> coutaric acid	295	163	x	x
Flavanols				
Catechin	289	245	x	x
Epicatechin	289	245		x
Procyanidin dimer 1	577	289		x
Procyanidin dimer 2	577	289		x
Procyanidin trimer	865	407, 289	x	x
Flavonols				
Myricetin-3-glucoside	479	317	x	x
Myricetin-3-glucuronide	493	317	x	x
Quercetin-3-rutinoside	609	391	x	
Quercetin-3-galactoside	463	301	x	x
Laricitrin-3-glucoside	493	331	x	x
Quercetin-3-glucoside	463	301	x	x
Quercetin-3-glucuronide	477	301	x	x
Laricitrin-3-glucuronide	507	331	x	
Kaempferol-3- <i>O</i> -(6- <i>O</i> - <i>p</i> -coumaroyl) glucoside	593	447, 285	x	
Isorhamnetin-3-rutinoside	623	315	x	x
Kaempferol-3-rutinoside	593	285	x	x
Kaempferol-3-galactoside	447	285	x	x
Kaempferol-3-glucoside	447	285	x	x
Isorhamnetin-3-glucoside	477	315	x	x
Syringetin-3-glucoside	507	345	x	x
Kaempferol-3-glucuronide	461	285	x	
Laricitrin-3-rutinoside	639	331		x
Anthocyanins				
Delphinidin-3-glucoside	465	303	x	
Cyanidin-3-glucoside	449	287	x	
Petunidin-3-glucoside	479	317	x	x
Peonidin-3-glucoside	463	301	x	
Malvidin-3-glucoside	493	331	x	x
Delphinidin-3-(6'' acetyl) glucoside	507	303	x	
Petunidin-3-(6'' acetyl) glucoside	521	317	x	
Malvidin-3-(6'' acetyl) glucoside	535	331	x	x
Delphinidin-3-(6''- <i>p</i> -coumaroyl) glucoside	611	303	x	
Petunidin-3-(6''- <i>p</i> -coumaroyl) glucoside	625	317	x	x
Peonidin-3-(6''- <i>p</i> -coumaroyl) glucoside	609	301	x	x
Malvidin-3-(6''- <i>p</i> -coumaroyl) glucoside	639	331	x	x
Malvidin-3-glucoside acetate	517	355		x
Malvidin-3-glucoside pyruvate	561	399		x
Malvidin-3-(6'' acetyl) glucoside pyruvate	603	399		x
Peonidin-3-(6'' acetyl) glucoside	505	301		x
Malvidin-3-(6'' caffeoyl) glucoside	655	331		x
Malvidin-3-(6''- <i>p</i> -coumaroyl) glucoside pyr	707	399		x

^aM⁺ (m/z) anthocyanins were obtained in positive ion mode, other phenolics in negative ion mode.

6. Phenolic compounds

Phenolic compounds were extracted from berry skin according to the method of Mikulič-Petkovšek *et al.* (2012). Briefly, the skin was separated from the pulp with a scalpel when the berries were still frozen, and the skin samples were ground to a fine powder with liquid nitrogen. Approximately 0.5 g of the skin powder (exact weight was recorded for each sample) was put into a 10-mL centrifuge tube, and 10 mL of methanol containing 3 % (v/v) formic acid and 1 % (w/v) 2,6-di-tert-butyl-4-methylphenol (BHT) were added. BHT was added to the samples to prevent oxidation. The extraction was performed in a cooled ultrasonic bath for 1 h. After extraction, the obtained extracts were centrifuged (Eppendorf Centrifuge 5810R) at 10.000 rpm for 7 min at 4 °C. The supernatant was filtered through a 0.20-µm injection filter (Chromafil A-20/25, Macherey-Nagel) into a vial. Phenolic analyses were performed on a HPLC-MS system (Thermo Finnigan Surveyor with quaternary pump, San Jose, USA); a Phenomenex Gemini C18 (150 mm x 4.5 mm, 3 µm) column operating at 25 °C was used. Phenolics were detected at 280 nm (flavanols, hydroxycinnamic acids derivatives), 350 nm (flavonols) and 530 nm (anthocyanins). The elution solvents were aqueous 1 % formic acid (A) and 100 % acetonitrile (B). Samples were eluted according to the linear gradient described by Marks *et al.* (2007), with an injection amount of 20 µL and flow rate of 1 mL/min. All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative (all phenolic groups except anthocyanins) and positive (anthocyanins) ion mode. The analyses were carried out using full scan data-dependent MSⁿ scanning from *m/z* 115 to 1500. The injection volume was 10 µL and the flow rate maintained at 1 mL/min. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, respectively, the source voltage was 3 kV for negative ionization and 4 kV for positive ionization, and normalized collision energy was between 20-35 %. Spectral data were elaborated using the Excalibur software (Thermo Scientific).

The identification of phenolic compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation (Table 1). The content of total hydroxycinnamic acids, flavanols, flavonols and anthocyanins was calculated as the sum of all identified and quantified individual phenolics of the corresponding phenolic group.

7. Total phenolic content (TPC)

Total phenolic content was assessed with the Folin-Ciocalteu reagent method described by Singleton *et al.* (1999). The extraction of phenolics from berry skin was made according to the protocol reported by Mikulič-Petkovšek *et al.* (2012), but without adding the BHT into the extraction solution to avoid interferences with phenolics that may indicate an increase of the analytical value. 100 µL of the extract was placed into a centrifuge tube and 6 ml of bidistilled water and 500 µL of Folin-Ciocalteu reagent were added to the mixture. After 1 to 8 minutes, 1.5 mL of sodium carbonate (Na₂CO₃) was added and the mixture was diluted with bidistilled water until reaching a volume of 10 ml. The samples were mixed and heated in a drying oven at 40 °C for 30 min. The samples were poured into 1.5-mL cuvettes and the absorbance was measured on a spectrophotometer (Perkin-Elmer, UV/visible Lambda Bio 20) at 765 nm. Water and corresponding reagents were used as a blank. Different concentrations of gallic acid were measured to obtain an absorbance standard curve. The absorbance of each sample was measured in three replicates. TPC was expressed as gallic acid equivalents (GAE) in mg/kg FW of berry skin/wine in mg/L.

8. Chemicals

The following standards were used for the quantification of phenolic compounds: cyanidin-3-*O*-galactoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, caffeic acid, catechin, *p*-coumaric acid and procyanidin B2 from Fluka Chemie GmbH (Buchs, Switzerland), delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and kaempferol-3-glucoside from Sigma-Aldrich Chemical (St. Louis, MO, USA). To prevent the oxidation of individual phenolics, 1 % (w/v) BHT from Sigma-Aldrich GmbH was used. The Folin-Ciocalteu reagent from Fluka Chemie GmbH (Buchs, Switzerland) was used to determine TPC. Methanol for extraction of phenolics was from Sigma-Aldrich GmbH. The chemicals used for the mobile phases were acetonitrile HPLC-MS grade and formic acid from Fluka Chemie GmbH. Water was purified and twice distilled with a Milli-Q-system (Millipore, Bedford, MA, USA).

9. Statistical analysis

Data were analyzed using the Statgraphics Centurion XV program (Statpoint Technologies Inc., Warrenton, Virginia, USA). Differences in yield, grape and wine composition among treatments were assessed with one-way analysis of variance

Table 2 - Growth potential and yield characteristics of 'Blauer Portugieser' grapevines at different cluster thinning levels in three years

Growth potential						
Treatment	Buds			Shoots		
2007	Total	Unfertile	Fertile	Total	Unfertile	Fertile
C	13.3±0.4	1.0±0.2	12.9±0.7	13.6±0.6	0.3±0.1	13.3±0.5
CT1	14.0±0.5	1.3±0.3	13.5±0.5	14.3±0.5	0.6±0.2	13.7±0.5
CT2	14.4±0.4	1.4±0.2	12.9±0.3	13.8±0.4	0.3±0.1	13.4±0.4
2008						
C	14.0±0.4	0.8±0.3	12.7±0.5	15.4±0.8	0.3±0.1	14.8±0.7
CT1	13.8±0.7	0.7±0.3	12.4±0.7	14.6±0.8	0.7±0.2	13.7±0.8
CT2	13.7±0.3	1.0±0.3	12.4±0.5	13.7±0.6	0.8±0.2	12.9±0.6
2011						
C	12.8±0.6	1.1±0.3	11.4±0.7	11.2±0.4	0.5±0.3	10.7±0.4
CT1	12.3±0.4	1.5±0.3	11.1±0.5	10.4±0.7	0.5±0.2	9.9±0.7
CT2	13.3±0.4	1.3±0.3	11.4±0.5	10.6±0.5	0.1±0.1	10.7±0.5
Yield characteristics						
Treatment	No. of clusters per vine at flowering	No. of removed clusters per vine	No. of clusters per vine at harvest	Yield (kg/vine)	Cluster weight (g)	Yield potential (t/ha)
2007						
C	24.3±1.0	0±0 a	23.8±1.0 c	3.0±0.2 b	127±7.9	14.3±0.9 b
CT1	24.4±0.9	5.5±0.6 b	19.9±0.9 b	2.8±0.3 b	142±11.5	13.5±1.4 b
CT2	24.8±1.0	10.3±0.7 c	15.4±0.7 a	2.1±0.2 a	135±9.2	10.1±1.0 a
2008						
C	24.8±1.2	0±0 a	24.2±1.1 c	3.8±0.2 b	161±8.1	18.1±0.7 b
CT1	23.7±0.9	5.6±0.8 b	18.3±0.8 b	3.3±0.2 ab	183±12.2	15.7±0.9 ab
CT2	22.0±0.9	9.6±0.9 c	14.0±0.8 a	2.7±0.3 a	194±15.5	13.0±1.3 a
2011						
C	18.8±0.6	0±0 a	18.3±1.1 b	2.6±0.2 b	142±7.6	12.3±1.0 b
CT1	16.1±1.0	5.3±0.7 b	11.3±1.2 a	1.8±0.1 a	133±9.0	7.7±1.4 a
CT2	18.3±1.6	8.3±0.9 c	11.0±0.9 a	1.5±0.2 a	126±14.1	7.0±0.7 a

C: control (no cluster thinning); CT1: 20-30 % cluster reduction; CT2: 40-50 % cluster reduction. The results are presented as mean ± standard error and different letters in columns denote statistically significant differences (LSD test, $p < 0.05$) among treatments in each individual year.

(ANOVA). Significant treatment means were determined using Fisher's least significant difference (LSD) and Duncan multiple range test at 95 % confidence level ($p \leq 0.05$), and significant differences are given by different letters.

RESULTS

1. Growth and yield potential

No significant differences in growth potential were observed between the different levels of CT in a single year, confirming equal growing conditions in the vineyard (Table 2). Irrespective of the year, an average of 12.3 to 14.4 buds/vine were left at winter pruning, which resulted in an average of 10.4 to 15.4

shoots/vine, of which 0.1 to 0.8 were unfertile. No significant differences in cluster number/vine were observed among treatments at flowering, which reached 16.1 to 24.8 clusters/vine, irrespective of the year. However, the reduction of clusters (CT1 and CT2) was made according to the recorded total number of clusters per individual vine. All clusters were left on the vines in the control treatment and, in accordance with the experimental design, the number of removed clusters was significantly higher in the CT2 treatment (8.3 to 10.3 clusters/vine) compared to the CT1 treatment (5.3 to 5.6 clusters/vine), irrespective of the year. As expected, different levels of CT resulted in significantly different yields/vine, especially in CT2 compared to the control. Taking into account all three years, vines subjected to the

Table 3 - Characteristics of 'Blauer Portugieser' grape and wine composition at different cluster thinning levels in three years

Parameters	2007			2008			2011		
	C	CT1	CT2	C	CT1	CT2	C	CT1	CT2
Grape									
Soluble solids (°Brix)	17.2±0.4 a	18.5±0.7 ab	18.6±0.4 b	15.4±0.3 a	16.3±0.3 a	18.3±0.8 b	18.8±0.3 a	19.1±0.9 a	21.5±0.3 b
Titrateable acidity (g/L)	5.3±0.05 b	4.2±0.13 a	3.7±0.08 a	4.2±0.05 b	3.5±0.04 a	3.6±0.01 a	4.2±0.06 b	4.1±0.01 b	3.3±0.04 a
pH	3.4±0.04 a	3.8±0.02 b	3.8±0.02 b	3.3±0.04 a	3.7±0.03 b	3.8±0.05 b	3.2±0.04 a	3.7±0.03 b	3.8±0.03 b
Weight of 100 berries (g)	165±6.5	172±4.8	168±6.2	161±6.9	170±13.0	163±7.5	170±7.7	176±4.4	174±5.2
CIRG index	-	-	-	6.3±0.2 a	6.9±0.1 b	7.0±0.2 b	6.1±0.2 a	7.2±0.3 b	7.2±0.2 b
Colour Intensity	-	-	-	5.7±0.1 a	6.7±0.1 b	8.7±0.3 c	8.8±0.1 a	10.8±0.9 b	11.6±0.3 b
Colour Tonality (Hue)	-	-	-	1.1±0.1 a	1.3±0.1 b	1.3±0.3 ab	1.3±0.1	1.3±0.1	1.3±0.1
Wine									
Alcohol (vol.%)	-	-	-	-	-	-	10.3±0.1 a	11.5±0.2 b	11.8±0.1 b
Total acidity (g/L)	-	-	-	-	-	-	6.9±0.05 c	6.4±0.03 b	6.2±0.01 a
pH	-	-	-	-	-	-	3.56±0.01 a	3.56±0.02 a	3.60±0.01 b
Total extract (g/L)	-	-	-	-	-	-	27.6±0.2 a	28.3±0.1 ab	29.0±0.2 b
Volatile acidity (g/L)	-	-	-	-	-	-	0.28±0.01 a	0.31±0.01 b	0.32±0.02 b

"-"not analyzed; C: control; CT1: 20-30 % cluster reduction; CT2: 40-50 % cluster reduction. The results are presented as mean ± standard error and different letters in rows denote statistically significant differences (LSD test, $p < 0.05$) among treatments in each individual year.

CT2 treatment produced 1.5 to 2.7 kg of grapes, but no significant reduction in yield/vine was observed when compared to CT1 in 2008 and 2011 (Table 2). CT did not significantly affect average cluster weight, which ranged from 126 to 194 g. The results indicate that limited cluster removal (CT1) in 'Blauer Portugieser' variety does not generally reduce yield/ha when compared to the control. This was confirmed in 2007 and 2008. On the other hand, severe cluster removal (CT2) significantly decreased yield/ha compared to the control treatment in all three years of the study by an average of 4.2 to 5.3 t/ha. However, taking into account the average cluster weight, yield per vine and number of vines/ha, the yield/ha in CT2 was quite high, reaching 7.0 to 13.0 t/ha, also in 2011 when the number of buds/vine at winter pruning was further reduced.

2. Grape and wine composition

CT affected many of the studied grape and wine parameters (Table 3). Only the most severe CT (CT2) significantly increased the average content of soluble solids by 1.4 to 2.9 °Brix in comparison to the control, whereas the average content of soluble solids in CT1 was not significantly higher compared to the control in all three years. A greater impact of CT was observed on the content of titrateable acidity (TA) and pH value: irrespective of the year, TA significantly decreased, while pH significantly increased (Table 3). Although the average berry weight was not affected by CT, the berries from CT vines reached

significantly higher CIRG index and colour intensity, but not tonality, in 2011. In 2011, wine composition was additionally studied. Compared to the control, CT significantly increased the alcohol content by 1.2 to 1.5 vol. % and volatile acidity (which reached 0.31 and 0.32 g/L in CT1 and CT2, respectively), but decreased total acidity by 0.5 to 0.7 g/L. Moreover, CT2 significantly increased wine pH and the content of total extract.

3. Phenolic composition of grape skin

Thirty-four different phenolic compounds were identified and quantified in 'Blauer Portugieser' berry skin (Table 4a and b). Flavonols (16 compounds) were the most important group of identified phenolics in grape skin, followed by anthocyanins (12), hydroxycinnamic acids (4) (HCA) and flavanols (2). Generally, CT2 significantly increased the content of most phenolic compounds. Only CT2 significantly increased the content of total hydroxycinnamic acids (THCA) by an average of 16 mg/kg compared to the control. Catearic acid was the most abundant among HCA. Its content was significantly increased by CT2 compared to the control, but its relative abundance was not affected. CT2 significantly increased the content and the proportion of *p*-coumaroyl pentose, which reached 12.8 mg/kg and its proportion was 1.2 % higher than in control. CT did not affect the content of total flavanols (TFA), but the proportion of catechin and procyanidin trimer significantly

Table 4a - Concentration of phenolic compounds in berry skin (mg/kg) and wine (mg/L) and relative abundance (%) according to the total amount of each phenolic group of 'Blauer Portugieser' subjected to different cluster thinnings

Phenolic compounds	Treatment									
	Skin (mg/kg)					Wine (mg/L)				
	C	%	CT1	%	CT2	PI	C	%	CT1	%
Gallic acid	105±2.8 a	82	114±5.1 ab	81	122±3.2 b	82	9.7±0.2 a	56	8.7±0.9 a	54
p-cou pentose	9.7±0.4 a	6:00 AM	8.9±0.6 a	7:00 AM	12.8±1.1 b	9 b	3.5±0.7 a	19 a	3.3±1.1 a	20 a
t-fertaric acid	11.2±5.6	8	10.4±5.0	8	7.5±4.0	5	2.7±0.5	15	2.9±1.0	18
t-coutaric acid	6.4±0.2 b	4	5.4±0.3 a	4	5.6±0.3 ab	4	1.7±0.1	10 b	1.3±0.4	8 ab
Total hydroxycinnamic acids (THCA)	132±5.2 a	100	139±4.6 a	100	148±3.3 b	100	17.5±1.5 a	100	16.1±5.4 a	100
Catechin	167±3.3 a	40 a	143±9.5 a	46 b	209±6.2 b	48 b	39.0±1.2	16	39.1±1.3	15
Procyanidin dimer 1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	29.3±3.7 a	12:00 AM	31.0±1.2 a	12:00 AM
Procyanidin dimer 2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	112±4.6 a	45 b	113±4.4 a	43 ab
Procyanidin trimer	254±15.5	60 b	191±48.3	54 a	271±82.8	52 a	26.9±2.2 a	11	27.1±9.1 a	11
Epicatechin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	41.1±1.3 a	17 a	48.7±1.8 a	19 ab
Total flavanols (TFA)	421±15.5	100	334±57.0	100	480±78.1	100	248±10.7	100	259±92.9	100
Myricetin-3-glu	90.6±8.4 a	10:00 AM	82.2±11.1 a	13 ab	124±8.4 b	16 b	4.2±0.3 a	8:00 AM	5.1±0.2 ab	9 b
Myricetin-3-gluc	22.7±1.4 a	3	29.3±2.1 b	3	31.0±0.5 b	3	0.6±0.2	1	0.7±0.1	1
Quercetin-3-rut	25.9±2.9 a	4	26.8±1.8 b	4	35.0±1.5 b	3	N.D.	N.D.	N.D.	N.D.
Quercetin-3-gal	46.4±5.4	6	55.5±1.4	6	65.2±10.0	7	4.1±0.1	8:00 AM	4.9±1.7	9 b
Laricitrin-3-glu	4.6±0.2	0.5	4.1±0.5	0.6	5.6±0.7	1	4.2±0.1 a	13 a	5.1±1.7 ab	17 b
Quercetin-3-glu	137±4.7	15 a	123±15.9	17 ab	167±21.7	18 b	1.6±0.2 a	4:00 AM	2.7±0.9 ab	5 b
Quercetin-3-gluc	283±20.6 a	43 b	327±33.7 ab	40 b	397±16.2 b	36 a	21.8±0.2 a	44 b	20.0±0.7 a	37 a
Laricitrin-3-gluc	2.8±0.2 a	0.4	3.3±0.3 ab	0.4	3.9±0.2 b	0.4	N.D.	N.D.	N.D.	N.D.
Kaempferol-3-(6-p-cou)	25.0±2.0 a	4	27.9±1.7 b	3	35.0±1.1 b	3	N.D.	N.D.	N.D.	N.D.
Isorhamnetin-3-rut	35.3±1.8	4	25.2±2.2	4	61.5±38.1	6	2.6±0.2	5	1.7±0.8	12
Kaempferol-3-rut	2.9±0.5	0.4	2.9±0.4	0.4	3.9±0.1	0.3	0.1±0.01	0.2	0.1±0.04	0.2
Kaempferol-3-gal	17.0±0.5	2	12.4±2.0	2	12.5±1.6	1	0.9±0.1	2	1.1±0.3	2
Kaempferol-3-glu	50.6±8.3	6	57.7±3.7	7	61.5±3.4	6	0.03±0.01	0.1	0.04±0.02	0.1
Isorhamnetin-3-glu	2.9±0.1	0.3	3.4±0.3	0.5	3.6±0.9	0.4	6.7±0.1	13 c	6.7±2.2	12 b
Syringetin-3-glu	1.8±0.1	0.2	2.2±0.2	0.3	2.4±0.6	0.3	0.9±0.01	2	0.9±0.03	2
Kaempferol-3-gluc	5.1±0.2	0.6 a	5.9±0.5	0.8 a	6.4±1.6	1 b	N.D.	N.D.	N.D.	N.D.
Laricitrin-3-rut	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.0±0.1	2	1.4±0.5	3
Total flavonols (TFO)	755±18.7 a	100	789±16.9 a	100	1017±19.3 b	100	50.0±0.6 a	100	54.2±1.1 a	100

Table 4b - Concentration of phenolic compounds in berry skin (mg/kg) and wine (mg/L) and relative abundance (%) according to the total amount of each phenolic group of 'Blauer Portugieser' subjected to different cluster thinnings

Phenolic compounds	Treatment										Wine (mg/L)			
	C	%	CT1	%	CT2	%	PI	C	%	CT1	%	CT2	%	PI
Delphinidin-3-glu	588±7.4 b	7:00 AM	505±6.9 a	7:00 AM	814±25.1 c	12 b	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cyanidin-3-glu	733±21.0 a	11	660±32.5 a	10	848±22.1 b	10	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Petunidin-3-glu	718±15.03 a	9:00 AM	628±25.7 a	10:00 AM	906±57.7 b	13 b	↑**	9.2±0.2 a	3	14.7±0.9 b	4	13.9±0.2 b	4	↑*
Peonidin-3-glu	459±22.8 a	6:00 AM	405±34.2 a	7:00 AM	553±12.9 b	8 b	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Malvidin-3-glu	1765±41 a	29 b	2105±44 b	30 b	2244±76 b	27 a	↑*	182±1.7	65 c	156±5.2	64 b	199±3.3	62 a	↑*
Malvidin-3-glu ac	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		1.1±0.1 a	0.5 a	1.4±0.2 b	0.4 a	1.9±0.3 c	1 b	↑*
Malvidin-3-glu py	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		22.3±0.6 a	8:00 AM	30.0±0.2 b	9 b	28.8±0.3 b	9 b	↑*
Malvidin-3-(6''acetyl) glu	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		7.4±0.2 a	9	9.5±0.4 b	8	9.9±0.1 b	9	↑*
Peonidin-3-(6''acetyl) glu	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		4.1±0.2	1	4.5±0.1	1	4.5±0.1	1	↑*
Malvidin-3-(6''caffeoyl) ξ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		2.4±0.3 a	1	2.7±0.1 b	1	2.9±0.5 c	1	↑*
Malvidin-3-(6''-p-cou) gl	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		4.1±0.1 a	1:00 AM	5.1±0.2 b	2 b	5.7±0.1 c	2 b	↑*
Delphinidin-3-(6''acetyl)	53.6±1.4 a	0.7	46.6±2.5 a	0.8	91.7±5.5 b	1	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Petunidin-3-(6''acetyl) gl	219±5.4 b	3	187±1.3 a	3	243±1.2 c	3	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Malvidin-3-(6''acetyl) gl	299±18.9 a	6	318±28.4 a	5	442±15.8 b	5	↑*	25.2±0.4 a	9 b	26.8±0.1 ab	8:00 AM	28.3±0.6 b	8:00 AM	↑**
Delphinidin-3-(6''-p-cou)	186.9±5.8 a	3	175±12.4 a	3	232±7.7 b	3	**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Petunidin-3-(6''-p-cou) gl	273±14.1 a	4	261±15.8 a	4	368±11.9 b	4	↑**	3.1±0.4 a	1	3.5±0.1 b	1	3.9±0.2 c	1	↑*
Peonidin-3-(6''-p-cou) gl	307±33.9 a	5	290±21.2 a	5	413±30.1 b	5	↑**	3.3±0.1 a	1	4.2±0.3 b	1	4.5±0.6 c	1	↑*
Malvidin-3-(6''-p-cou) gl	729±47.9 a	14 c	791±73.0 a	12 b	1140±53.0 b	#####	↑*	13.8±0.4 a	1:00 AM	18.01±0.4 b	5 ab	19.9±0.1 c	6 b	↑*
Total anthocyanins (TA)	6334±75 a	100	6376±68 a	100	8299±196 b	100	↑**	279±3.1 a	100	328±1.9 b	100	323±4.1 b	100	↑*
TPC	8069±157 a		8102±127 a		9339±159 b		↑**	880±17.3 a		1045±28.8 b		1075±11.3 b		↑*

Abbreviations of phenolic names: t: trans, p: para, glu: glucoside, gluc: glucuronide, rut: rutinose, cou: coumaroyl, ac: acetate, py: pyruvate.
N.D. - not detected; C: control (no cluster thinning); CT1: 20-30 % cluster reduction; CT2: 40-50 % cluster reduction. TPC - total phenolic content (mg GAE/kg or mg GAE/L). PI - potential impact: ↑* or ↓* - potential increase or decrease among control and thinning treatments, ↑** or ↓** - potential increase or decrease in CT2 compared to C or CT1. The results are presented as mean ± standard error and as proportion (%) of each phenolic compound according to total value of associated phenolic group within individual treatment. Different letters in rows denote statistically significant differences (LSD test, $p < 0.05$) among treatments.

changed after CT. CT significantly increased the accumulation of less than half of the identified flavonols in berry skin. However, the content of total flavonols (TFO) increased after CT2, reaching 1017 mg/kg of fresh weight (Table 4). The most abundant flavonols in 'Blauer Portugieser' berry skin, irrespective of the treatment, were quercetin-3-glucuronide, quercetin-3-glucoside, myricetin-3-glucoside and quercetin-3-galactoside. CT2 only significantly increased quercetin-3-glucuronide and myricetin-3-glucuronide levels in comparison to the control. Interestingly, CT2 significantly increased the content of quercetin-3-glucuronide, but decreased its proportion regarding the total flavonol (TFO) content. Furthermore, a significant increase in the proportion of myricetin-3-glucoside, quercetin-3-glucoside and kaempferol-3-glucuronide was observed following severe CT. In agreement with the results obtained for flavonols, the most severe cluster thinning (CT2) had a significant impact on the total anthocyanin content (TAC) and on most of the identified individual anthocyanins in berry skin (Table 4). The most abundant of them was malvidin-3-glucoside, representing 27 to 30 % of TAC, followed by malvidin-3-(6''-p-coumaroyl) glucoside (10-14 % TAC), petunidin-3-glucoside (9-13 % TAC), cyanidin-3-glucoside (10-11 % TAC), delphinidin-3-glucoside (7-12 % TAC) and peonidin-3-glucoside (6-8 % TAC). CT2 significantly increased the content and proportion of delphinidin-, cyanidin-, petunidin-, and peonidin-3-glucoside, compared to the control. However, the content of malvidin-3-glucoside and malvidin-3-(6''-p-coumaroyl) glucoside also significantly increased in CT2, but the treatment decreased their proportion regarding TAC. Furthermore, TPC in CT1 vines was comparable to the control vines, suggesting that only a severe cluster reduction (CT2) may affect the accumulation of phenolics in berry skin of 'Blauer Portugieser'.

4. Phenolic composition of wine

Phenolic compounds were assessed in wines of the 2011 vintage (Table 4). CT significantly increased the content of several individual phenolic compounds in all four identified phenolic groups in comparison to the control. Caftaric acid was the major hydroxycinnamic acid (8.7 to 11.8 mg/L) identified in wine, followed by *p*-coumaroyl pentose (3.3 to 5.4 mg/L); the content of both significantly increased in CT2 vines, compared to the control (Table 4). On the other hand, the proportion of caftaric acid, regarding THCA, remained stable also in CT2, whereas that of *p*-coumaroyl pentose significantly increased by 6 % and *t*-coumaric acid decreased by 4 %.

Flavanols were the second most prevalent phenolic group in Blauer Portugieser wine, representing almost 43 % of analyzed phenolics. The major flavanol identified in wine was procyanidin dimer 2 (112 to 146 mg/L), representing 40 to 45 % of total flavanols, irrespective of the treatment (Table 4). Among flavanols, only procyanidin dimer 1 and epicatechin responded to CT, and their content and proportion significantly increased only in CT2 compared to the control. The content of procyanidin dimer 2 and procyanidin trimer was similarly increased in CT2; however, the proportion of procyanidin trimer remained stable and that of procyanidin dimer 2 significantly decreased.

In total, thirteen flavonols were identified and quantified in Blauer Portugieser wine, the most abundant being quercetin-3-glucuronide, laricitrin-3-glucoside, isorhamnetin-3-glucoside and myricetin-3-glucuronide. Only CT2 had a positive impact on the content of myricetin-3-glucoside, laricitrin-3-glucoside, quercetin-3-glucoside and quercetin-3-glucuronide, with CT1 showing less marked increases, and their proportion significantly increased in comparison with the control, with the only exception of quercetin-3-glucuronide, which decreased significantly after CT.

In addition, CT had a great impact on the content of individual and total anthocyanins (TAC) in wine (Table 4). TAC was significantly higher in both CT treatments compared to the control. The composition of individual phenolics in berry skin was slightly different to that of wine; five additional malvidin glycosides and peonidin-3-(6''acetyl) glucoside were identified in Blauer Portugieser wine. On the contrary, delphinidin- and cyanidin-glycosides were not detected in wine. Malvidin-3-glucoside was the main anthocyanin, ranging from 156 to 199 mg/L and representing 62 to 65 % of TAC. Although CT did not affect the content of malvidin-3-glucoside, its relative proportion significantly decreased in CT vines. TPC in Blauer Portugieser wine was also affected by CT, with a significant increase in CT1 (1045 mg/L) and CT2 (1075 mg/L) treatments compared to the control wine (880 mg/L).

DISCUSSION

1. Growth and yield potential

'Blauer Portugieser' is a vigorous variety and Reynolds *et al.* (2004) recommend a careful control of the number of buds and clusters per vine at winter pruning and in the growing season to ensure optimal yield and quality of grapes and wine. Growth and yield potential also depend on climatic conditions

(temperature, precipitation, and sunshine hours), which delay or alter phenological stages, shorten their duration or interrupt differentiation (Jones and Davis, 2000). Bubola *et al.* (2011) reported that a smaller number of clusters left on the vine resulted in 18-57 % greater bunch weight of the remaining grapes of the 'Merlot' variety. This was not confirmed for our variety. Moreover, the same authors reported a 34 % decrease in average yield after cluster thinning. A similar pattern was observed in our study: yield decreased significantly by 27-43 % in CT2, especially in 2011 when the vines formed less buds and clusters. Calonnec *et al.* (2004) reported that pests and diseases greatly reduce the yield, but no pathogenic organisms have been recorded in the experimental vineyard. Therefore, the yield decrease in 2011 could also be due to lower average temperatures (21 °C) and above-average precipitation in July (approximately 175 mm), followed by extremely high temperatures and low rainfall (approximately 5 mm) in August (Fig. 1 and Fig. 2), which can cause economic losses (Jackson and Lombard, 1993; Gil *et al.*, 2013). According to the rules of wine denomination - Metliška črnina (2008), the maximum yield of 'Blauer Portugieser' is limited to 11 t/ha as a five-year average yield, which seemed to be achieved only in the severe CT treatment (40-50 % cluster reduction).

2. Grape composition

Cluster thinning generally involves a considerable reduction in yield which potentially improves grape and wine quality and composition (Dokoozlian and Hirschfeld, 1995; Di Profio *et al.*, 2011). Soluble solids content, titratable acidity and pH value still represent the main and basic parameters determining the optimal harvest time (Prajitna *et al.*, 2007), which can be affected by climatic conditions in the growing season (Jones and Davis, 2000). Higher temperatures and precipitation during the ripening process (July and August) in our study might have affected soluble solids content and titratable acidity, especially in 2008 and 2011, when precipitation was above average in July (around 180 mm), compared to the long-term average (approximately 95 mm) (Fig. 1 and Fig. 2). Soil type can also impact vine development, soluble solids content, acidity and pH (Koundouras *et al.*, 2006). Clay soil increases sugar accumulation and speeds the ripening process in comparison to gravel or sandy soil and may influence fruit ripening through mineral supply and water-holding capacity (Jackson and Lombard, 1993). A severe P₂O₅ deficit has been observed in the experimental vineyard (Leskošek, 1993) but Jackson and Lombard (1993) could not determine any direct

relation between soil mineral supply and grape quality parameters. In general, CT affected grape composition of 'Blauer Portugieser', where soluble solids were generally higher in CT treatments in comparison to the control. On the contrary, titratable acidity in grape significantly decreased as a result of cluster reduction in two years of the study, which is in accordance with the report by Di Profio *et al.* (2011). Jackson and Lombard (1993) reported that the pH value of berry juice impacts the suitability and fermentation conditions of must, which was expected also in our study with CT, where significantly higher pH values were measured, compared to the control. Prajitna *et al.* (2007) showed an insignificant impact of cluster thinning on pH value, but it is known that a lower content of titratable acidity also results in higher pH value (Reynolds, 1989), which is in accordance with our results. Titratable acidity of 'Blauer Portugieser' grapes was much lower in all years/treatments compared to data reported by Pavloušek and Kumšta (2011). This could be explained by different climatic conditions, especially higher temperatures, which lead to acid degradation (Jackson and Lombard, 1993) or by the use of a different clone (Pavloušek and Kumšta, 2011). Berry skin colour is an important characteristic, especially for red grapevine varieties. It is directly affected by composition and content of phenolic compounds (Mazza *et al.*, 1999) and is usually represented by colour intensity, tonality (hue) and CIRG index (Carreño *et al.*, 1995). Colour intensity and tonality (hue) mostly depend on the variety and agronomical practices (Di Profio *et al.*, 2011), cluster thinning (Ough and Nagaoka, 1984; Guidoni *et al.*, 2002), water stress and terroir (Jones and Davis, 2000). The colour intensity of 'Blauer Portugieser' in the CT2 treatment reached values higher than that of 'Sangiovese' (Parpinello *et al.*, 2015) and 'Tempranillo' (Gamero *et al.*, 2014).

3. Wine composition

Many factors affect wine composition, the most important is certainly grape quality at harvest (Gil *et al.*, 2013). Nowadays, the demand for less acidic, extract-rich wines is rising (Parpinello *et al.*, 2015). Blauer Portugieser wine is characterized by an intense dark red colour and lower acidity and is mostly drunk young (Nemanič, 1999). On the other hand, a relatively high proportion of 'Blauer Portugieser' grape harvested in Slovenia is used for the production of PTP Metliška črnina (Rules of wine denomination - PTP Metliška črnina, 2008).

Although the Blauer Portugieser wine was produced only in 2011, we observed some interesting and

significant differences in wine composition which could be attributed to CT, especially the content of alcohol, total acidity, total extract and volatile acidity. Moreover, alcohol content in CT2 exceeded the limit defined by the rules (11.5 vol. %), but the difference in alcohol content between the control and CT2 was just 1.5 vol. %. However, wine from the CT2 treatment still meets the current preferences for wines with lower alcohol content (Parpinello *et al.*, 2015). Total acidity seemed to be significantly reduced by thinning, which is in accord with the report of Bubola *et al.* (2011) for Merlot wine. The Blauer Portugieser wine is known as less acidic (Nemanič, 1999; Shellie, 2007), a trait not observed in our study, where 6.2 g/L of total acidity was measured in the severe CT2 wine. However, all analyzed wine samples met the limits set for PTP Metliška črnina (5.5-7 g/L). Contents of total extract and volatile acidity significantly increased in CT2 and met the limits set for PTP Metliška črnina, where a maximum of 0.8 g/L of volatile acidity is permitted. The study suggests that CT, especially with 40-50 % cluster removal, has a remarkable impact on wine composition.

4. Phenolic composition of grape berries and wine

Phenolics are important bioactive compounds in berries and wine, and they play a great role in sensorial characteristics such as berry colour, aroma, flavour, taste and nutritional value (Rodríguez-Montealegre *et al.*, 2006; Castillo-Muñoz *et al.*, 2007). Moreover, these compounds are involved in preventing oxidation and stabilizing the colour of wine (Gómez-Plaza *et al.*, 2000). Phenolic composition and contents are influenced by variety and vineyard practices (Mazza *et al.*, 1999), plant status (Rusjan *et al.*, 2012) and pedoclimatic conditions during ripening (Koundouras *et al.*, 2006). In the present study, higher temperatures in August and September (Fig. 1) and low precipitation in August 2011 (Fig. 2) could have caused stress conditions to the vines, which usually result in increased phenolic accumulation (Koundouras *et al.*, 2006).

Among the HCA group, caftaric acid was the most abundant in 'Blauer Portugieser' berry skin, followed by *p*-coumaroyl pentose. This is not in accordance with the report by Rodríguez-Montealegre *et al.* (2006), who found *t*-coumaric acid as the main HCA in several other red grapevine varieties. In general, higher contents of some individual and total HCA have been measured in 'Blauer Portugieser' berry skin compared to 'Cabernet Sauvignon', 'Merlot' and 'Shiraz' (Rodríguez-Montealegre *et al.*, 2006). This can be attributed not only to the variety but also to the

analytical methods used in the studies (Rusjan *et al.*, 2012). Only the most severe CT significantly increased the content of caftaric acid and THCA and the content and proportion of *p*-coumaroyl pentose in berry skin and wine.

Flavanols are a group of phenolic compounds, contributing mostly to wine taste (Rodríguez-Montealegre *et al.*, 2006). Total flavanols contents (TFA) seemed not to be affected by cluster thinning. The most abundant flavanols in grape skin were procyanidin trimer and catechin and in wine procyanidin dimer 2 and epicatechin (Table 4). This is not in agreement with Francesca *et al.* (2014). The content of catechin in 'Blauer Portugieser' berry skin was 8-fold higher than reported for 'Merlot' (Rodríguez-Montealegre *et al.*, 2006), 5-fold higher than for 'Syrah' (Peña-Neira *et al.*, 2007), and approximately 10-fold higher than for 'Cabernet Sauvignon' (Lorrain *et al.*, 2011), which can be assigned to varietal characteristics. Only CT2 significantly affected catechin content in berry skin. In wine, CT2 significantly decreased the percentage of catechin and procyanidin trimer among TFA. Furthermore, higher contents of TFA have been measured in grape compared to wine of 'Blauer Portugieser', which can be linked to the degradation of the skin cell wall by pectolytic enzymes and greater permeability of the cell wall during vinification (Ortega-Regules *et al.*, 2006). Moreover, a lower TFA in wine could be also attributed to lower alcohol contents and shorter maceration time, which, according to González-Manzano *et al.* (2004), has a great impact on flavanol extraction from skin to wine. The contents of flavanols in Blauer Portugieser wine are comparable to the data on Blauer Portugieser wine reported by Pour-Nikfardjam *et al.* (2006).

Flavonols are a group of phenolic compounds with a role in antioxidant activity and as UV screening copigments (UV-A and UV-B). In red wines they are usually masked by anthocyanin pigments (Castillo-Muñoz *et al.*, 2007). Fang *et al.* (2013) reported that the presence and amount of individual flavonols depend on berry development and maturity, and that ripe berries contain higher levels of quercetin and myricetin. This was partly confirmed in our study. Among flavonols, quercetin-3-glucuronide, quercetin-3-glucoside and myricetin-3-glucoside were the most abundant in grape skin. In Blauer Portugieser wine, quercetin-3-glucuronide, laricitrin-3-glucoside and myricetin-3-glucoside prevailed. This is comparable to the data of Castillo-Muñoz *et al.* (2007) for 'Cabernet Sauvignon', 'Merlot' and 'Syrah'. According to the proportion of TFO, CT

significantly increased three of the sixteen identified flavonols in grape and four of the thirteen identified flavonols in wine of 'Blauer Portugieser'. On the other hand, we have to underline that flavonol accumulation in berry skin mostly depends on the exposure of clusters to sunlight and that shaded clusters usually contain lower levels of flavonols, as reported by Price *et al.* (1995) and Castillo-Muñoz *et al.* (2007). Additionally, Price *et al.* (1995) reported a 10-fold higher flavonol content in sunlight-exposed clusters of 'Pinot Noir', compared to shaded ones. CT provides higher sunlight exposure of clusters, as leaves in the bunch zone are removed (Reynolds *et al.*, 2004). Consequently, flavonol contents are higher in exposed clusters, which was also observed in our study, especially in CT2. Furthermore, a 1- to 2-fold higher TFO content has been determined in berries of 'Blauer Portugieser' compared to 'Malbec' after CT at the pea-size phenological stage (Fanzone *et al.*, 2011). Similarly, Makris *et al.* (2006) reported lower levels of quercetin and myricetin glycosides (by 16 to 35 mg/kg) in red berry skin compared to our results, especially in grapes from CT vines.

Anthocyanins are the main group of red pigments and copigments in ripening grape of red grapevine varieties (Mazza *et al.*, 1999) directly affecting the colour and sensorial attributes of wine (Gao *et al.*, 1997; Heredia *et al.*, 1998). In general, the contents of the analyzed anthocyanins in grape and wine are in agreement with the anthocyanin content reported in Portugieser wine by Pour-Nikfardjam *et al.* (2006) and in other varieties by Lorrain *et al.* (2011). Glycosylated malvidin, petunidin, peonidin, cyanidin and delphinidin compounds are often present in *V. vinifera* L. grapes and wine (Mazza *et al.*, 1999; García-Beneytez *et al.*, 2002). This was confirmed in 'Blauer Portugieser' berry skin, but in wine delphinidin and cyanidin glycosides were not identified, which can be attributed to varietal characteristics (García-Beneytez *et al.*, 2002). On the other hand, five different malvidin glycosides were identified and quantified in Blauer Portugieser wine that were not present in berry skin. Similarly, Revilla *et al.* (1999) reported several anthocyanin compounds in berry skin but not in wine. Gao *et al.* (1997) reported that the most abundant anthocyanins in grape and wine are malvidin-3-glucoside and their acetylated and coumaroylated glycosides, which was also observed in our study, especially in control grape and wine from CT treatments. Furthermore, King *et al.* (2012) claimed that the proportion of malvidin-3-glucoside regarding TAC significantly increased in wine from CT vines, which is not in accordance with our results. Nevertheless, we have to underline that the proportion of malvidin-3-glucoside acetate,

malvidin-3-glucoside pyruvate, malvidin-3-(6"-*p*-coumaroyl) glucoside and malvidin-3-(6"-*p*-coumaroyl) glucoside pyruvate was significantly increased in wine produced from CT treatments. Severe CT significantly increased TAC (8.3 g/kg) in 'Blauer Portugieser' berry skin. It contained higher levels compared to 'Syrah' (Peña-Neira *et al.*, 2007) or 'Merlot' (Bubola *et al.*, 2011) berry skin subjected to CT. Río Segade *et al.* (2008) reported a decline of phenolic concentration in berry skin after phenolic maturity, which has been observed also in our study with CT. A reduction in wine anthocyanin content was observed compared to berry skin, which is in accordance with a report by Rodríguez-Montealegre *et al.* (2006). The amount of extracted phenolics during vinification mostly depends on berry skin cell wall composition and thickness, pectin degradation, alcohol content and maceration period (González-Manzano *et al.*, 2004). Pectin and cellulose, the main components of the cell wall, are degraded by pectinase during the ripening and vinification processes and the result is the release of phenolics into the must (Río Segade *et al.*, 2008). CT could have modified the ripening process of the berries and indirectly influenced the cell wall composition of berry skin. The 'Blauer Portugieser' variety is characterized by high contents of phenolics; therefore, the produced wines are richer in health-promoting compounds (Pour-Nikfardjam *et al.*, 2006). Cluster thinning significantly altered TPC of Blauer Portugieser wine and a similar response has also been reported by Fanzone *et al.* (2011). Heredia *et al.* (1998) reported that wine colour is firstly conditioned by the composition and the content of anthocyanins and secondly by pH, and that anthocyanins in a pH lower than 4.0 yield red to blue tonality (malvidin – red-purple hues, petunidin – purple hues, delphinidin – pink hues, and peonidin – blue-purple hues). Hermosín-Gutiérrez *et al.* (2005) reported that the intense purple-blue tonality of Cabernet Sauvignon wine could be influenced by delphinidin and peonidin contents in grape skin. Anthocyanin polymers are namely less resistant to browning and red colour fading caused by higher pH (Mazza *et al.*, 1999). Acetylated and coumaroylated forms of malvidin and peonidin have been detected in the skin of 'Blauer Portugieser' and in a wider range in wine, which influences the colour of wine (Lorrain *et al.*, 2011). The changes in the proportion of anthocyanins in terms of TAC of 'Blauer Portugieser' suggest that severe CT causes a more purple-blue grape and wine colour compared to the control. However, anthocyanins in berry skin are usually found as monoglycosides, but in must and wine they form into polymeric complexes.

Consequently, the tonality of wine is difficult to predict (Gao *et al.*, 1997).

CONCLUSIONS

Research findings indicated that the impact on yield and grape and wine composition of cluster thinning should not be generalised to all grapevine varieties. Only severe cluster thinning significantly reduced yield (per vine and per hectare) and increased soluble solids content in grape and pH and total extracts in wine. The first report of the detailed profile of phenolics in 'Blauer Portugieser' suggests that anthocyanins prevail in berry skin and wine, followed by flavonols and flavanols in berry, and flavanols and flavonols in wine. Only severe cluster thinning significantly increased the contents of total anthocyanins, flavonols, hydroxycinnamic acids, but not flavanols, in grape and wine. On the other hand, severe cluster thinning modified the proportion of individual phenolics in the corresponding phenolic group, especially of anthocyanins in grape and wine, flavonols in grape, and flavanols in wine. The most abundant hydroxycinnamic acids in 'Blauer Portugieser' grape and wine were caftaric acid and *p*-coumaroyl pentose. Their contents significantly increased following CT2. Severe cluster thinning significantly increased the content and the proportion of catechin in berry skin, and epicatechin and procyanidin dimer 1 in wine. Severe cluster thinning caused several alterations in the flavonol group, especially in wine, where contents and proportions of 3-glucosides of myricetin, laricitrin and quercetin significantly increased. An interesting response of the most abundant flavonol in berry skin and wine (i.e., quercetin-3-glucuronide) was detected after CT2. The content of quercetin-3-glucoside significantly increased, but the proportion regarding TFO decreased. CT also affected the levels of individual anthocyanins, but the response was quite different in berry skin compared to the wine. Severe cluster thinning significantly increased the contents and proportions of 3-glucosides of delphinidin, petunidin and peonidin in berry skin but not in wine. However, cluster thinning significantly increased the amounts and proportions of most identified malvidin-acetylated and coumaroylated glycosides in wine. The results of the study encourage producers of 'Blauer Portugieser' grape and wine to perform severe cluster thinning at pea-size berry phenological stage, so as to enhance grape and wine quality, setting aside the lower yield per vine.

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2.1.2 Vpliv ukrepov na listni steni na sestavo grozdja in vina sorte 'Istrska malvazija' (*Vitis vinifera* L.)

Reščič J., Mikulič-Petkovšek M., Rusjan D. 2016. The impact of canopy managements on grape and wine composition of cv. 'Istrian Malvasia' (*Vitis vinifera* L.). Journal of the Science of Food and Agriculture, 96, 14: 4724-4735

Preference potrošnikov za lažja, na drugi strani pa bolj polna vina z večjo vsebnostjo alkohola, spodbujajo potrebo po izboljšanju ukrepov, s katerimi bi se lahko omenjene zahteve dosegalo. Cilj te raziskave je bil oceniti učinke treh ampelotehničnih ukrepov na listni steni trte, (1) zgodnje razlistanje v coni grozdja, (2) odstranitev mladih listov nad drugim parom žic, (3) dvojno namensko zorenje grozdja (DMR – Double Maturation Raisonnée) na količino pridelka in kemijsko sestavo grozdja in vina sorte 'Istrska malvazija'. DMR grozdja je značilno vplivalo na večjo vsebnost fenolnih spojin v grozdju in vinu, medtem ko je bila največja vsebnost topne suhe snovi (24,3 in 23,5 °Brix) in titracijskih kislin (7,0 in 7,1 g/l) v grozdju, izmerjena pri zgodnjem razlistanju. Odstranitev listov v začetku zorenja je povzročila nepričakovano povečanje vsebnosti flavonolov v jagodni kožici. Rezultat zgodnjega razlistanja je bila značilno manjša vsebnost ekstrakta v vinu. Kljub temu, so vina pridelana iz grozdja z zgodnjim razlistanjem na senzorični analizi dosegla višjo oceno (16,5 od 20,0 točk) v primerjavi z vini iz razlistanja v začetku zorenja in DMR grozdja (15 točk) ter kontrolo (16 točk). Razlistanje v začetku zorenja in DMR sta povečala vsebnost fenolov v vinu, kar vodi v vino z več telesa. Na drugi strani pa je zgodnje razlistanje značilno povečalo pridelek na trto, vsebnost titracijskih kislin, hidroksicimetnih kislin in flavanolov v vinu.

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The impact of canopy managements on grape and wine composition of cv. 'Istrian Malvasia' (*Vitis vinifera* L.)

Jan Rescic,* Maja Mikulic-Petkovsek and Denis Rusjan

Abstract

BACKGROUND: The interest in producing wines preferred by consumers increases the need for improving practices to modify grape and wine composition. The aim of this study was to assess the impacts of three different canopy management measures, (1) early leaf removal in the cluster zone, (2) removal of young leaves above the second pair of wires and (3) Double Maturation Raisonnée, on the yield and chemical composition of 'Istrian Malvasia' grape and wine.

RESULTS: Double Maturation Raisonnée had a significantly greater impact on phenolic compounds, while the highest soluble solids (24.3 and 23.5 °Brix) and titratable acidity (7.0 and 7.1 g L⁻¹) were measured at early leaf removal. Leaf removal at véraison caused an unexpected augmentation of flavonols in the berry skin. Early leaf removal resulted in significantly lower extracts of wine. Nevertheless, they reached the highest mark (16.5 out of 20.0 points) in sensory evaluation compared with leaf removal at véraison and Double Maturation Raisonnée (15.0 points) and control (16.0 points).

CONCLUSION: Leaf removal at véraison and Double Maturation Raisonnée improved the phenolic composition of wine, producing a full-bodied wine. On the other hand, early leaf removal significantly augmented the yield and titratable acidity, hydroxycinnamic acids and flavanols of wine, which might have led to a fresher but less-bodied wine.

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Keywords: canopy; grape; wine; composition; 'Istrian Malvasia'

INTRODUCTION

Achieving improved grape and wine quality and meeting consumer demands and preferences are the main aims of global wine production today.¹ Nowadays, consumer 'demands' advocate young, fresh wines with lower alcohol content and expressed varietal and aromatic characteristics on one hand and complex, full-bodied wines richer in extract and alcohol on the other.² Winegrowers are trying to achieve adequate chemical composition and organoleptic characteristics for the production of a certain wine style.^{2,3}

The most commonly used vineyard practices for improving grape and wine composition are cluster and shoot thinning,^{4–6} berry thinning⁷ and defoliation in the cluster zone.^{5,8} Traditional leaf removal in the cluster zone is mostly carried out from fruit set to the beginning of ripening,^{9,10} but as an innovative practice it could also be conducted earlier in the growing season (around bloom and fruit set).^{1,11} Removing basal and fully functional leaves at pre-bloom reduces the carbohydrate supply to the flowers compared with non-defoliated vines, leading to a reduction in fruit set.¹⁰ Consequently, lower titratable acidity but higher soluble solids and pH are usually obtained in comparison with non-defoliated vines.^{12,13} Furthermore, noticeable freshness, higher alcohol content and improved flavour could be the main characteristics of wine obtained after leaf removal, as reported by Suklje *et al.*¹³ in the case of 'Sauvignon Blanc' (growth stage E-L 29). In a cluster zone defoliation study on 'Istrian Malvasia', Bubola and Persurić¹¹ did not find any significant differences in yield,

which reached 3.51 kg per vine after post-bloom leaf removal. An augmentation in soluble solids (22.1 vs 22.6 °Brix for control vs leaf removal) was shown; on the other hand, titratable acidity and pH were slightly decreased in comparison with the control. Feng *et al.*¹² demonstrated the influence of leaf removal on the grape phenolic profile: quercetin glycosides, flavan-3-ols as well as hydroxycinnamic acids were significantly higher after 100% cluster zone defoliation treatment compared with the control in a three-year study on 'Pinot noir'. Removing the upper leaves on shoots at the time of véraison causes a delay in photosynthesis,¹⁰ as a consequence of which sugar accumulation in berries is limited, since the upper young leaves are more photosynthetically active than the leaves in the lower part of the canopy.⁶ Moreover, removing the upper leaves on shoots allows the basal leaves to intercept more light and therefore increases their photosynthetic activity.¹⁰ However, the clusters are shaded by the remaining leaves, thus the degradation of malic acid is reduced and the grapes are consequently richer in acidity. Additionally, the wines are lower in alcohol and extract but abundant in freshness.¹⁰ Furthermore, the removal of leaves could increase the synthesis of phenolics, mostly procyanidins from flavanols and quercetins from flavonols.^{14,15}

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To improve the chemical composition of grapes and wines, a vineyard practice based on on-plant withering, called Double Maturation Raisonnée (DMR), has been developed.^{16,17} This technique is characterized by a deliberate cane cut similar to that of winter pruning, 2–3 weeks preharvest.^{17,18} The water from the clusters on the cut cane evaporates and consequently the concentration of sugars increases in the berries.¹⁶ Furthermore, the acidity in the berries remains high, as reported by Corso *et al.*,¹⁸ while the catabolism of the two main acids (malic and tartaric) is reduced. Grapevines react to DMR as to a stress condition and hence defence mechanisms are activated via increased synthesis of secondary metabolites.^{19,20} In addition, DMR accelerates the maturation of the berries as well as of phenolics, present mostly in grape skin,¹⁹ which later results in improved skin texture, permitting easier release of the phenolic compounds into the must.²¹ Therefore wines are richer in extract and alcohol content and are usually evaluated as rather full-bodied.²

'Istrian Malvasia', known locally as 'Istrska Malvazija', is a white grapevine variety frequently reflecting high sugars and lower acids, resulting in wines richer in alcohol but less harmonious and balanced in the mouth. Being the most commonly cultivated white grapevine variety in the Slovenska Istra winegrowing area (738 ha),²² 'Istrian Malvasia' is characterized as a high-yielding (4.7 kg per vine) and vigorous variety with dense canopy and excessive vine growth. Long-term variety observations show that it reaches an average sugar content of up to 20.5 °Brix, while total acidity varies from 5 to 8 g L⁻¹ and the average weight of 100 berries amounts to 225 g.^{23,24} 'Istrian Malvasia' wines reach an average alcohol content of 12.8 vol%, total extract 22.1 g L⁻¹, pH 3.3–3.5 and total acidity 5.3–6.2 g L⁻¹.²³

The aim of this study was to observe the impacts of three different ampelotechnic treatments, (1) leaf removal in the cluster zone at groat-size berry stage, (2) leaf removal above the second pair of wires at véraison and (3) DMR at véraison, on 'Istrian Malvasia' yield and grape and wine chemical composition for the potential production of wines differing in style. The research focuses on sugars and organic acids and especially on phenolic compounds, which are responsible for the sensory characteristics of wines. The results are useful for winegrowers and winemakers, who until now lacked information on the impact of varietal ampelotechnics that allow modification of grape and wine composition, especially in terms of white grapevine varieties.

MATERIALS AND METHODS

Experimental vineyard

The experiment was conducted on non-irrigated 'Istrian Malvasia' (*Vitis vinifera* L.) vines, trained on a vertical shoot-positioned single guyot with two pairs of catching wires, planted at a distance of 2.7 m × 0.8 m and grafted on 5BB rootstocks. The vineyard was located in southwest Slovenia, the country's coastal part, Slovenska Istra winegrowing region, in the location of Izola Ricorvo (45° 32' N, 13° 39' E), at an altitude of 20 m above sea level, with a N–S orientation, and was managed according to the guidelines of integrated pest management with row space tillage. The climate of the area is Mediterranean with an average annual temperature of 13.4 °C and average annual precipitation of no more than 950 mm.²⁵

Experimental design

The experiment was conducted in the growing seasons 2013 and 2014 as a randomized block design. Each block was divided into

four different treatments: leaf removal 1 (LR1 – all leaves under the first pair of wires (four or five leaves, ~0.5 m²) were manually removed at phenological stage BBCH 73; berries up to that point were about groat size), leaf removal 2 (LR2 – all leaves above the second pair of wires (three or four leaves, ~0.3 m²) were manually removed at BBCH 81), (DMR – in the middle of ripening, BBCH 85, the cane with shoots and clusters was cut off manually using scissors, leaving only two or three shoots for further growth) and control (C – no leaf removal or cut). Each treatment was carried out in four randomized replications (adjacent rows) of 20 consecutive vines. At first, growth potential was measured on the studied vines before blooming: buds and shoots were counted and fertility and yield potential were estimated by counting the inflorescences at BBCH 53. Sampling was done when the grapes were ripe for harvest (BBCH 89). Berries were manually sampled from different parts of grape clusters and yield area and carefully collected (~0.5 kg) per treatment and replication and brought to the laboratory for further analysis. Four individual vinifications per treatment were conducted under the same conditions. Grapes were harvested manually (~200 kg per treatment) and divided into four equal parts per treatment; grapes were destemmed, berries were crushed, protected with 5 g kg⁻¹ potassium metabisulfite (K₂S₂O₅) and then the must was cold-soaked for 15 h in a cooling chamber set at 10 °C. The must without sediment was racked into 50 L stainless steel experimental tanks (Mühlfellner Tankbau GmbH, Ehrenhausen, Germany) in a chamber set at 17 °C, where the fermentation lasted 14 days until the residual sugar content was below the limit for dry wines.²⁶ At the start of fermentation, 30 g hL⁻¹ of rehydrated yeast *Saccharomyces cerevisiae* Anchor VIN 13 (Anchor Yeast, Industria, South Africa) and 30 g hL⁻¹ (one half at the start and the other half 7 days after the start of fermentation) of yeast nutrient OptiMUM WHITE (Lallemand, Milwaukee, WI, USA) were added to each tank according to the instructions of the producers. After the fermentation was completed, i.e. when the concentration of residual sugars was lower than 2 g L⁻¹, the wine was racked into 10 L stainless steel tanks (Mühlfellner Tankbau GmbH) and 2.5 g kg⁻¹ K₂S₂O₅ was added to each tank.

Measurements of berry and wine composition

Berry characteristics were determined after harvest, while chemical analyses were carried out during subsequent months. First, the weight of 100 berries was recorded. To obtain ~30 mL of must needed for analysis, an amount of berries was manually destemmed, crushed and pressed. Soluble solids in the must were assessed with a digital refractometer (Atago PAL-87S, Tokyo, Japan) and expressed as °Brix. Approximately 25 mL of berry must was used to determine the titratable acidity with a semiautomatic titrator, adding 0.1 mol L⁻¹ NaOH to the sample until pH 7.0 was reached. Before titration, the initial pH of the must was measured. A sample of wine (50 mL) was taken from each tank to determine its relative density, alcohol, total and sugar-free extract, residual sugar, total acidity, pH and organic acids. The sample was analysed 1 month after sulfuring, and chemical analysis was carried out using a WineScan FT120 (Foss, Hillerød, Denmark).

Sensory evaluation of wine

Sensory evaluation of experimental wines was carried out using the 20-point Buxbaum method²⁰ with some modifications. Six panellists (official tasters, highly trained and with long-term experience in wine sensory analysis) applied the evaluation with the following score ranking: maximum two points for clearness, maximum two points for colour, maximum four points for aroma,

maximum six points for taste and maximum six points for harmony (overall impression). The purpose of sensory evaluation was to assess the impact of applied canopy managements on the sensory characteristics of individual wines produced under each treatment, especially on taste and harmony (overall impression). Statistical analysis of the sensory evaluation results was carried out by one-way analysis of variance (ANOVA), and significant differences between treatment means were determined using the least significant difference (LSD) and Duncan multiple range tests at 95% confidence level ($P \leq 0.05$).

Extraction and determination of primary metabolites (individual sugars and organic acids) in grape juice

For the extraction of primary metabolites, the method described by Mikulic-Petkovsek *et al.*²⁷ was used. First, 1 mL of juice was topped with 9 mL of doubly distilled water and left for 30 min at room temperature. After 30 min of extraction, the sample was centrifuged (Eppendorf Centrifuge 5810R, Hauppauge, USA) at $10\,000 \times g$ for 7 min at 4 °C. The supernatant was filtered through a 0.45 µm cellulose ester filter (Macherey-Nagel, Düren, Germany) and transferred to a vial; 20 µL of sample was used for analysis. Further procedure was carried out using an Accela high-performance liquid chromatography (HPLC) system (Thermo Scientific, Waltham, MA, USA). The separation column for individual sugars was a Rezex RCM-Monosaccharide Ca + 2 column (300 mm $\times$ 7.8 mm; Phenomenex, Torrance, USA) operated at 65 °C. The mobile phase was doubly distilled water at a flow rate of 0.6 mL min⁻¹. The total run time was 30 min and a refractive index (RI) detector was used to determine eluted carbohydrates as described by Mikulic-Petkovsek *et al.*²⁷. The same HPLC system equipped with a UV detector set at 210 nm was used to analyse individual organic acids. A Rezex ROA column (Phenomenex) was employed according to the methods described by Mikulic-Petkovsek *et al.*²⁷

Extraction and determination of secondary metabolites (phenolic compounds) in berry skin and wine

The extraction of phenolic compounds from berry skins was performed according to the method of Mikulic-Petkovsek *et al.*²⁸. Berries were peeled while still frozen and the skins were ground to a fine powder with liquid nitrogen. Then 0.5 g of skin sample was placed in a 10 mL centrifuge tube and topped with 10 mL of methanol containing 30 mL L⁻¹ formic acid and 10 g L⁻¹ 2, 6-di-*tert*-butyl-4-methylphenol (BHT). BHT was added to the sample only to prevent oxidation during extraction. Extraction was performed in a cooled ultrasonic bath for 1 h. The extract was then centrifuged (Eppendorf Centrifuge 5810R) at $10\,000 \times g$ for 7 min at 4 °C and the supernatant was filtered through a 0.20 µm injection filter (Chromafil A-20/25, Macherey-Nagel) into a vial. Phenolics were analysed using an HPLC/mass spectrometry (MS) system (Thermo Finnigan Surveyor, San Jose, CA, USA) with a quaternary pump and detected at 280 nm (hydroxybenzoic acids, hydroxycinnamic acids, flavanols) and 350 nm (flavonols). The column was a Gemini C18 column (150 mm $\times$ 4.5 mm, 3 µm; Phenomenex) operated at 25 °C. The elution solvents were aqueous 10 mL L⁻¹ formic acid (A) and pure acetonitrile (B). Samples were eluted according to the linear gradient described by Marks *et al.*²⁹ with an injection amount of 20 µL and a flow rate of 1 mL min⁻¹. Spectral data were elaborated using Excalibur (Thermo Scientific). The identification of phenolic compounds was confirmed by comparison of retention times and spectra

as well as by fragmentation (Table 1). The contents of individual phenolic compounds were quantified from peak areas with the help of corresponding standards and expressed as mg kg⁻¹ fresh weight and mg L⁻¹ of grape and wine respectively. Similar standard compounds were used for quantification of compounds lacking standards. The content of total hydroxybenzoic acids, hydroxycinnamic acids, flavanols and flavonols was calculated as the sum of all identified and quantified individual phenolics of the corresponding phenolic group. The following standards were used for the quantification of sugars, organic acids and phenolic compounds: glucose, fructose, malic acid, citric acid, lactic acid, *p*-coumaric acid, procyanidin B1, catechin, epicatechin, caffeic acid, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, myricetin-3-*O*-rhamnoside and isorhamnetin-3-*O*-glucoside from Fluka (Fluka Chemie GmbH, Buchs, Switzerland) and kaempferol-3-*O*-glucoside and resveratrol from Sigma-Aldrich (Sigma-Aldrich Chemical, St Louis, MO, USA). The numbers (1 and 2) after the names of some phenolic compounds (*p*-coumaric acid, ferulic acid, procyanidin trimer) indicate the order of their detection in the HPLC system and recording on the chromatogram. BHT (10 g L⁻¹) from Sigma-Aldrich was used to prevent the oxidation of individual phenolics. Methanol was from Sigma-Aldrich. The chemicals used for the mobile phases were acetonitrile (HPLC/MS grade) and formic acid from Fluka. Water was purified and distilled twice with a Milli-Q-system (Millipore, Bedford, MA, USA).

Statistical analysis

Data were analysed using Statgraphics Centurion XV (StatPoint Technologies Inc., Warrenton, VA, USA). Differences in grape and wine composition among treatments were assessed by ANOVA. Significant differences between treatment means were determined using the LSD and Duncan multiple range tests at 95% confidence level ($P \leq 0.05$). Only statistical differences among treatments within each year were investigated owing to the highly distinctive years (seasons).

RESULTS

Growth and yield potential

The growth potential of 'Istrian Malvasia' was not significantly affected by the studied treatments, which confirms equal growing conditions during the experiment (Table 2). As an average of both years, 10.8–16.4 buds were left on each vine at winter pruning, which later resulted in 9.7–14.3 total shoots per vine, of which 7.8–12.7 were fertile and 1.4–2.2 were unfertile. The number of clusters per vine was much lower in 2014 (10.1–14.1 clusters per vine) in comparison with the first year of the experiment (20.1–22.3 clusters per vine) despite there being no significant differences among treatments within each year. Taking into account an evidently lower number of clusters in 2014, this resulted in a greater bunch weight ranging from 287.7 to 297.8 g, although significant differences were not observed. The obtained results suggested that leaf removals and double maturation had significantly different impacts on the weight of a single bunch, but only in certain years. In 2013 the average bunch weight reached a maximum of 187.1 g with LR1, but with DMR a significantly lower bunch weight (140.1 g only) was measured compared with the control. Different treatments significantly affected the yield per vine: leaf removal at berry set (LR1) significantly increased while DMR significantly reduced the yield per vine in comparison with the control.

Table 1. Identification of phenolic compounds in grape skin and wine of 'Istrian Malvasia' variety in negative ionization with HPLC/MS and MS²

Tentative identification	[M – H] [–] (m/z)	MS ² (m/z)	Analysed in	
			Skin	Wine
<i>Hydroxybenzoic acids</i>				
Protocatechuic acid	153	109		x
<i>Hydroxycinnamic acids</i>				
cis-Caftaric acid	311	179		x
trans-Caftaric acid	311	179	x	x
Ferulic acid derivative 1	355	193		x
Ferulic acid derivative 2	355	193		x
trans-Fertaric acid	325	193		x
Coutaric acid	295	163		x
p-Coumaric acid hexoside 1	325	163		x
p-Coumaric acid hexoside 2	325	163	x	x
<i>Flavanols</i>				
Catechin	289	245	x	x
Epicatechin	289	245		x
Procyanidin dimer 1	577	425, 407, 289	x	
Procyanidin trimer 1	865	577, 425, 407, 289	x	
Procyanidin trimer 2	865	577, 425, 407, 289	x	
Procyanidin tetramer	1153	865, 577, 407, 425, 289	x	
<i>Flavones</i>				
Apigenin pentoside	401	269		x
Apigenin hexoside	431	269	x	
<i>Flavonols</i>				
Quercetin-3-rutinoside	609	301	x	
Quercetin-3-galactoside	463	301	x	
Quercetin-3-xyloside	433	301	x	
Quercetin-3-glucoside	463	301	x	
Quercetin-3-glucuronide	477	301	x	
Kaempferol-3-rutinoside	593	285	x	
Kaempferol-3-galactoside	447	285	x	
Kaempferol-3-glucoside	447	285	x	
Kaempferol-3-glucuronide	461	285	x	
Kaempferol-3-pentoside	417	285	x	
Isorhamnetin-3-glucoside	477	315	x	
Dihydrokaempferol-3-glucoside	449	287		x
Laritracin-3-xyloside	509	463		x
<i>Stilbenes</i>				
Resveratrol-3-glucoside	389	227		x

Grape and wine composition

The most significant impacts of the studied canopy practices were observed in 2014 (Table 3). Only LR1 significantly increased the titratable acidity of the must, which amounted to 7.0 and 7.1 g L^{–1} in 2013 and 2014 respectively compared with the control (6.2 and 6.5 g L^{–1}), while DMR significantly decreased the berry weight. Unexpectedly, DMR did not affect the soluble solids, but in 2014 it significantly decreased the pH, which reached 3.22 in comparison with the control (3.33). According to the weight of 100 berries, the largest berries were obtained with the control (247.1 and 346.8 g), whereas all studied canopy practices had an impact on berry weight, especially DMR, which was expected in view of the on-vine water loss and berry withering. On the other hand, LR1 and LR2 had opposite effects on berry weight in consecutive vintages. Significantly marked differences were observed in 2014, where the contents of glucose and fructose increased with LR1 and LR2 but with DMR remained similar to the control. Sucrose had been found

in trace amounts and therefore was not presented. On the other hand, DMR resulted in the significantly highest contents of citric acid and tartaric acid.

The grape composition undoubtedly affected the presence and abundance of chemical compounds in the wine that was produced and analysed in each year of the study (Table 3). However, greater impacts of the studied canopy practices were observed on the wine. Taking into account both vintages, LR1 was the most effective, especially in the significant reduction of total and sugar-free extracts. Moreover, LR1 showed a similar influence on alcohol content in 2013, which was lowest among all treatments (13.6 vol%). On the other hand, LR1 caused the highest content of alcohol (13.3 vol%) among treatments in 2014 (Table 3). Alcohol content increased significantly under DMR in 2013 compared with the control, but this pattern was not observed in 2014. The produced wines were dry, i.e. containing less than 9 g L^{–1} of residual sugar in accordance with the

Table 2. Growth potential and yield characteristics of 'Istrian Malvasia' variety measured under different canopy practices in two consecutive years

Treatment	Buds per vine			Shoots			Number of clusters per vine	Bunch weight (g)	Yield per vine (kg)
	All	Unfertile	Fertile	All	Unfertile	Fertile			
2013									
C	12.9 ± 0.8	1.9 ± 0.3	11.1 ± 0.7	13.3 ± 1.0	1.7 ± 0.3	11.5 ± 0.9	20.6 ± 2.1	172.3 ± 4.7b	3.5 ± 0.1b
LR1	16.4 ± 1.3	3.5 ± 0.5	12.9 ± 1.1	14.3 ± 1.0	2.2 ± 0.3	12.1 ± 1.0	20.1 ± 1.7	187.1 ± 5.2b	3.7 ± 0.3c
LR2	14.5 ± 0.7	3.5 ± 0.3	10.9 ± 0.5	12.5 ± 0.7	1.4 ± 0.2	11.1 ± 0.7	20.6 ± 1.4	184.7 ± 4.2b	3.8 ± 0.2c
DMR	14.8 ± 0.9	2.5 ± 0.4	12.3 ± 0.8	14.2 ± 0.9	1.5 ± 0.4	12.7 ± 0.9	22.3 ± 1.5	140.1 ± 3.2a	3.1 ± 0.2a
2014									
C	11.0 ± 0.6	1.6 ± 0.4	9.4 ± 0.5	11.2 ± 0.4	2.0 ± 0.4	8.9 ± 0.4	11.2 ± 0.7	287.9 ± 5.2	3.2 ± 0.1b
LR1	10.8 ± 0.8	1.8 ± 0.3	8.6 ± 0.5	11.1 ± 0.4	1.4 ± 0.2	9.3 ± 0.5	14.1 ± 0.7	294.3 ± 3.6	4.1 ± 0.2c
LR2	10.9 ± 0.4	2.6 ± 0.3	8.3 ± 0.4	11.2 ± 0.5	1.9 ± 0.2	8.8 ± 0.4	10.7 ± 0.8	297.8 ± 4.6	3.2 ± 0.2b
DMR	11.3 ± 0.4	2.4 ± 0.3	8.9 ± 0.4	9.7 ± 0.4	1.7 ± 0.3	7.8 ± 0.4	10.1 ± 0.4	287.7 ± 9.2	2.9 ± 0.1a

Treatments: C, control (usual annual practice); LR1, leaf removal in cluster zone at berry set; LR2, leaf removal above second pair of wires at véraison; DMR, Double Maturation Raisonnée. Results are presented as mean ± standard error. Different letters in a column denote statistically significant differences (LSD test, $P < 0.05$) among treatments in each individual year.

Table 3. Characteristics of 'Istrian Malvasia' grape and wine composition measured under different canopy practices in two consecutive years

Parameter	2013				2014				Potential impact		
	C	LR1	LR2	DMR	C	LR1	LR2	DMR	LR1	LR2	DMR
Grape											
Soluble solids (°Brix)	23.3 ± 0.8	24.3 ± 0.9	24.1 ± 0.6	23.5 ± 0.8	21.9 ± 0.3a	23.5 ± 0.4b	23.6 ± 0.5b	21.5 ± 0.3a	↑*	↑*	↓*
Titrateable acidity (g L ⁻¹)	6.2 ± 0.2a	7.0 ± 0.3b	6.5 ± 0.3a	6.3 ± 0.1ab	6.5 ± 0.1a	7.1 ± 0.1b	6.9 ± 0.1b	6.5 ± 0.1a	↑**	↑*	
pH	3.79 ± 0.08	3.52 ± 0.08	3.75 ± 0.09	3.76 ± 0.05	3.33 ± 0.1b	3.34 ± 0.01b	3.31 ± 0.02b	3.22 ± 0.01a			↓*
Weight of 100 berries (g)	247.1 ± 7.0b	221.6 ± 10.6a	225.5 ± 5.6a	230.4 ± 8.0a	346.8 ± 11.0b	311.7 ± 6.3b	331.4 ± 12.9b	262.8 ± 21.2a			↓**
Glucose (g L ⁻¹)	119.7 ± 4.9	114.7 ± 5.1	117.7 ± 5.8	116.8 ± 3.6	101.5 ± 3.1a	113.2 ± 3.4b	112.7 ± 2.4b	103.7 ± 2.1a	↑*	↑*	
Fructose (g L ⁻¹)	103.6 ± 3.7	98.8 ± 4.6	102.5 ± 4.8	101.5 ± 2.8	95.4 ± 3.2a	108.4 ± 3.2b	106.0 ± 2.2b	97.9 ± 1.5a	↑*	↑*	
Citric acid (g L ⁻¹)	0.49 ± 0.01	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.75 ± 0.01a	0.80 ± 0.01a	0.81 ± 0.03a	0.88 ± 0.01b			↑*
Tartaric acid (g L ⁻¹)	4.5 ± 0.2	5.2 ± 0.5	4.8 ± 0.2	5.0 ± 0.3	4.6 ± 0.3a	4.2 ± 0.2a	4.1 ± 0.1a	5.4 ± 0.1b			↑*
Malic acid (g L ⁻¹)	1.9 ± 0.3a	3.7 ± 1.4b	3.5 ± 1.9b	1.8 ± 0.4a	5.3 ± 0.3ab	4.8 ± 0.3a	6.0 ± 0.2b	6.2 ± 0.3b		↑**	
Sugar/acid ratio	33.1 ± 2.9c	24.9 ± 2.9a	29.2 ± 4.7b	29.9 ± 1.0b	18.4 ± 0.7b	22.5 ± 0.7c	20.1 ± 0.7b	16.3 ± 0.7a	↓**↑*	↓**	↓**
Wine											
Relative density	0.9908 ± 0.1	0.9906 ± 0.1	0.9906 ± 0.1	0.9907 ± 0.1	0.9927 ± 0.1b	0.9917 ± 0.1a	0.9936 ± 0.1c	0.9932 ± 0.1c	↓*	↑*	↑*
Alcohol (vol%)	13.8 ± 0.1a	13.6 ± 0.2a	13.9 ± 0.3a	14.0 ± 0.1b	13.1 ± 0.2c	13.3 ± 0.1d	12.5 ± 0.1a	12.9 ± 0.1b	↑*↓*	↑*↓*	↑*↓*
Total extract (g L ⁻¹)	22.0 ± 0.4b	20.4 ± 0.2a	21.6 ± 0.1b	21.7 ± 0.1b	24.4 ± 0.3b	22.6 ± 0.1a	24.8 ± 0.2b	24.9 ± 0.2b	↓**		
Sugar-free extract (g L ⁻¹)	21.8 ± 0.2b	20.0 ± 0.1a	21.2 ± 0.2b	20.9 ± 0.2b	24.3 ± 0.2b	22.6 ± 0.1a	24.6 ± 0.2b	24.6 ± 0.2b	↓**		
Residual sugar (g L ⁻¹)	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1			
Total acidity (g L ⁻¹)	5.7 ± 0.2a	6.5 ± 0.2b	5.4 ± 0.1a	5.8 ± 0.2a	7.9 ± 0.3b	7.1 ± 0.1a	8.4 ± 0.1c	8.5 ± 0.2c	↓*	↑*	↑*
Volatile acidity (g L ⁻¹)	0.24 ± 0.03	0.22 ± 0.02	0.22 ± 0.03	0.21 ± 0.02	0.42 ± 0.02b	0.34 ± 0.01a	0.46 ± 0.01c	0.49 ± 0.01c	↓*	↑*	↑*
pH	3.34 ± 0.02b	3.21 ± 0.04a	3.38 ± 0.02c	3.34 ± 0.01b	3.41 ± 0.03b	3.37 ± 0.01a	3.38 ± 0.01b	3.31 ± 0.01a	↓*	↑*	↓*
Malic acid (g L ⁻¹)	1.2 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.8 ± 0.1b	2.3 ± 0.1a	2.9 ± 0.1b	3.0 ± 0.1b	↓*		
Citric acid (g L ⁻¹)	0.21 ± 0.02	0.19 ± 0.02	0.22 ± 0.01	0.21 ± 0.01	0.19 ± 0.1	0.19 ± 0.1	0.20 ± 0.2	0.21 ± 0.1			
Lactic acid (g L ⁻¹)	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.01	0.30 ± 0.01a	0.40 ± 0.01b	0.26 ± 0.03a	0.30 ± 0.01a	↑*		
Tartaric acid (g L ⁻¹)	2.5 ± 0.1a	2.9 ± 0.1b	2.4 ± 0.1a	2.4 ± 0.1a	2.5 ± 0.1a	2.7 ± 0.1b	2.6 ± 0.1ab	2.7 ± 0.1b	↑**		

Treatments: C, control (usual annual practice); LR1, leaf removal in cluster zone at berry set; LR2, leaf removal above second pair of wires at véraison; DMR, Double Maturation Raisonnée. Results are presented as mean ± standard error. Different letters in a row denote statistically significant differences (LSD test, $P < 0.05$) among treatments in each individual year. Potential impact: ↑* or ↓*, potential increase or decrease in LR1, LR2 and DMR compared with control only in one year; ↑** or ↓**, potential increase or decrease in LR1, LR2 and DMR compared with control in both years.

Ministry of Agriculture, Food and Nutrition.²⁶ Wines from LR2 and DMR augmented the content of total acidity, but only in 2014. On the other hand, LR1 showed a significantly lower pH, which amounted to 3.21 in 2013 and 3.37 in 2014. Individual organic acids in the wine seemed not to be differently affected by canopy practices, especially the contents of tartaric acid, malic

acid and lactic acid. The average content of tartaric acid, irrespective of the treatment, ranged from 2.4 to 2.9 g L⁻¹ and increased significantly with LR1 in both years. Moreover, the LR1-treated wine revealed a significant decline in malic acid content and a subsequent increase in lactic acid content as observed in 2014.



Phenolic composition of grape skin

In the grape skin of 'Istrian Malvasia', 19 different phenolic compounds were identified and quantified; furthermore, the proportion of each phenolic compound according to the total amount of the corresponding group was also calculated (Table 4). In general, treatments had different impacts not only on the contents but also on the proportions of individual phenolic compounds within the berry skin. Different impacts of the individual treatments were especially observed when comparing the studied years, which might be ascribed to certain environmental influences. The content of *p*-coumaric acid hexoside 2, the most abundant hydroxycinnamic acid (HCA), increased with LR1, LR2 and DMR, but only in 2014, by an average of 15% in comparison with the control. Only LR1 increased the content of total hydroxycinnamic acids (THCA) in 2013. However, in 2014 the content of THCA increased compared with the control, also as a consequence of LR2 and DMR treatments. LR1 influenced the quantified flavanols most, especially procyanidins, followed by DMR and LR2. DMR showed a significant influence on procyanidin trimer 2, the second most abundant flavanol, in 2013, where the content increased according to the average percentage of total flavanols of 2.7%, while in 2014 no difference in content compared with the control was noted. The content of catechin in berry skin was also significantly increased with DMR in 2013 and with LR1 in 2014, which was also observed in the proportion regarding total flavanols. Regarding the latter, we observed that total flavanols responded differently in the studied vintages; an impact of the applied treatments was not confirmed. The most abundant flavanol was quercetin-3-glucoside; its content, irrespective of the practice and vintage, ranged from 77.7 to 324.2 mg kg⁻¹. Higher contents of this glucoside were observed in the first year, where the proportion increased to an average of 3.7% in all three treatments. In addition, the second most abundant flavanol (irrespective of the practice) was quercetin-3-galactoside (18.7–69.3 mg kg⁻¹), and only DMR seemed to have a tendency to increase the content in each year. LR2 and DMR augmented the proportion of kaempferol-3-galactoside, regarding total flavanols, but only DMR showed a steady increase in its content. Regarding the content of total flavanols, a significant increase was observed with LR2 and DMR in 2013, while LR2 showed an opposite impact in 2014. Therefore only with DMR might a higher total flavanol content be expected year by year.

Phenolic composition of wine

Fifteen different phenolic compounds were detected and analysed in the 'Istrian Malvasia' wines of vintages 2013 and 2014 (Tables 1 and 5). The most abundant HCA were *cis*- and *trans*-caftaric acid and *p*-coumaric acid hexoside 1, whose average reached 37 and 21% in total, regarding the THCA, respective of a treatment. The proportions of HCA were mostly affected by LR1 and DMR. In addition, DMR significantly increased the contents of protocatechuic acid (hydroxybenzoic acid), which amounted to 4.53 and 2.30 mg L⁻¹ with DMR, and *cis*-caftaric acid (0.72 and 0.45 mg L⁻¹ with DMR), respective of a year. However, only with DMR was the significant increase in *cis*-caftaric acid content and proportion (THCA) observed in both years. The results showed that early leaf removal (LR1) had a significant impact on the content and proportion of *trans*-feraric acid, regarding THCA, which reached 7.7 and 14.2% compared with the control (6.8 and 9.9%), respective of a year. In the context of THCA, we also observed that only LR1 increased the content in the same year (vintage). Among flavanols, epicatechin was

the most abundant regarding content and proportion, being significantly higher with DMR and LR1 in both vintages. LR1 significantly increased the catechin content as well. Leaf removal increased the content of total flavanols in the first year, although the differences were not significant; only with DMR in vintage 2013 was a significantly higher content observed. However, apigenin pentoside accounted for the highest content from the group of flavonols, followed by dihydrokaempferol-3-glucoside and laricitrin-3-xyloside. Only laricitrin-3-xyloside in all three treatments applied in the experiment and in comparison with the control significantly increased its content in both years of the study, ranging from 0.12 to 0.31 mg L⁻¹. Moreover, also its proportions regarding total flavonols increased by treatments and reached an average of 11 and 31%. Resveratrol-3-glucoside was the only stilbenoid identified in the 'Istrian Malvasia' wines, the content of which did not seem to be affected by LR1, LR2 or DMR in comparison with the control. A significant increase in the total analysed phenolic content (TAPC) of wine was only observed in DMR-treated samples of both vintages.

DISCUSSION

Growth and yield components

It is worth mention that growth and yield depend on the climatic conditions during the growing season, of which the most important are certainly air temperature, precipitation and the amount of solar insolation, as well as vineyard practices.³⁰ However, in 2013 a higher number of buds was left on the vines (Table 2), which consequently resulted in a higher number of shoots and clusters. In addition, a significantly lower bunch weight with DMR in 2013, compared with other treatments, according to Bonghi *et al.*¹⁹ could be a result of higher water evaporation from berries that occurred because of an interrupted connection between the plant and the remaining cane. However, in general, a lower bunch weight in 2013 might also be attributed to higher temperatures in July (average 24 °C) and especially to precipitation below the long-term average in July (only 5 mm) and August (only 50 mm) of 2013 (Fig. 1), which could accelerate berry dehydration. Consequently, a reduction in weight could be observed, especially regarding the berries that had undergone DMR treatment. Furthermore, Tardaguila *et al.*¹ reported a 27% decrease in 'Graciano' yield per shoot and a 31% decrease in 'Carignan' compared with the control, averaging all defoliation treatments in their study. Defoliation was performed at pre-bloom stage (E-L 19), when the first eight leaves were manually removed, and at fruit set (E-L 27), all leaves around first 60 cm of foliage on the shoots in the fruiting zone were mechanically removed. However, such decrease in yield per shoot was not observed in our study. Furthermore, Bubola and Persuric¹¹ measured higher yields after bloom leaf removal (3.51 kg per vine) compared with the control (3.22 kg per vine) in 'Istrian Malvasia', which is in accordance with our results. Nevertheless, rather than on the defoliation degree – the intensity of leaf removal, it depends on its exact moment of implementation; Tardaguila *et al.*¹ have shown that defoliation at pre-bloom reduces the yield through the reduced carbohydrate supply. On the other hand, leaf removal applied at fruit set or at véraison (BBCH 81) in our study showed an increase in yield in comparison with the control, which is in accordance with Tardaguila *et al.*¹ and Bavaresco *et al.*³¹ However, LR2 was applied at the time of véraison, when the berries are already well formed, which according to Coombe and McCarthy¹² indicates that after entering véraison the changes could be better observed in terms of the chemical composition of a single berry rather than its weight. Anyway, the study

Table 4. Phenolic compounds in berry skin (mg kg^{-1}) of 'Istrian Malvasia' measured under different canopy practices in two consecutive years

Phenolic compound	2013						2014						Potential impact		
	C	%	LR1	%	LR2	%	C	%	LR1	%	LR2	%	DMR	%	DMR
<i>p</i> -Cou acid hex 2	0.34 ± 0.07a	65.4	0.82 ± 0.17b	78.7	0.24 ± 0.04a	61.6	0.23 ± 0.03a	65.8	1.1 ± 0.1a	68.3	3.1 ± 0.4b	86.7	2.8 ± 0.4b	81.8	3.5 ± 0.5b
<i>trans</i> -Caf acid	0.17 ± 0.02a	34.6	0.24 ± 0.06b	21.3	0.13 ± 0.01a	38.4	0.11 ± 0.01a	34.2	0.62 ± 0.12	31.7	0.49 ± 0.09	13.3	0.66 ± 0.13	18.2	0.70 ± 0.14
Total HCA	0.50 ± 0.09a	0.1	1.1 ± 0.2b	0.2	0.38 ± 0.06a	0.1	0.35 ± 0.02a	0.1	1.9 ± 0.1a	0.6	3.5 ± 0.5b	1.1	4.1 ± 0.5b	1.2	4.1 ± 0.5b
Catechin	8.5 ± 0.8a	12.1	8.3 ± 0.8a	9.8	8.1 ± 0.3a	13.0	11.8 ± 1.7b	22.4	5.4 ± 0.5a	8.5	7.8 ± 1.0ab	14.7	3.6 ± 0.5a	6.2	4.6 ± 0.6a
Procy dimer	45.1 ± 5.8b	63.4	49.3 ± 4.8b	58.1	44.7 ± 10.5b	59.3	24.7 ± 2.7a	46.9	11.4 ± 0.7b	27.4	12.3 ± 1.8b	12.5	8.9 ± 1.5ab	15.3	6.6 ± 1.2a
Procy trimer 1	5.1 ± 1.5a	8.8	12.4 ± 2.5b	9.4	7.2 ± 1.7a	9.1	3.6 ± 0.7a	16.0	18.2 ± 2.3ab	17.9	14.3 ± 2.9a	26.5	18.04 ± 3.1a	29.6	22.6 ± 2.3b
Procy trimer 2	5.7 ± 1.7a	7.0	8.2 ± 1.5b	13.9	6.3 ± 1.7a	11.1	8.4 ± 1.2b	7.0	14.6 ± 1.8b	22.9	12.5 ± 1.4a	23.6	12.3 ± 1.7a	21.0	14.6 ± 0.8b
Procy tetramer	5.7 ± 0.9ab	8.6	8.3 ± 2.4b	8.8	4.8 ± 0.5ab	7.5	4.5 ± 0.6a	7.8	15.7 ± 3.3	23.3	12.4 ± 2.4	22.7	16.8 ± 3.5	28.0	17.9 ± 3.6
Total flavanols	70.3 ± 5.3b	17.6	86.6 ± 9.9b	17.5	71.1 ± 11.8b	11.7	52.7 ± 2.2a	10.0	65.4 ± 3.5b	18.9	53.7 ± 3.2a	16.4	59.8 ± 4.6ab	20.2	72.01 ± 5.2b
Apigenin hex	0.27 ± 0.03b	0.1	0.43 ± 0.08c	0.1	0.17 ± 0.06a	0.1	0.19 ± 0.03a	0.1	0.42 ± 0.07	0.2	0.56 ± 0.07	0.2	0.55 ± 0.09	0.2	0.64 ± 0.11
Iso-3-glu	3.8 ± 0.7ab	1.2	3.2 ± 0.3a	1.0	4.5 ± 0.8ab	1.0	5.9 ± 0.9b	1.0	4.4 ± 1.9a	1.6	7.8 ± 1.2ab	3.1	9.2 ± 2.6ab	3.8	11.9 ± 1.8b
Kaemp-3-gal	12.7 ± 0.6a	3.9	53.9 ± 8.5c	7.7	34.8 ± 6.3b	7.4	32.7 ± 5.5b	7.4	32.7 ± 5.3ab	11.4	25.2 ± 8.8a	8.2	28.6 ± 6.2a	12.5	41.1 ± 5.1b
Kaemp-3-glu	62.2 ± 13.9b	17.9	23.4 ± 8.7a	13.1	55.4 ± 13.6b	12.3	59.8 ± 7.5b	12.4	13.8 ± 2.7a	5.0	30.2 ± 7.5b	11.6	29.4 ± 1.8b	12.6	30.5 ± 1.8b
Kaemp-3-gluc	1.1 ± 0.1ab	0.3	1.7 ± 0.3b	0.4	0.7 ± 0.2a	0.3	0.8 ± 0.1a	0.3	0.69 ± 0.18a	0.2	0.98 ± 0.17ab	0.4	0.91 ± 0.13ab	0.4	1.2 ± 0.2b
Kaemp-3-pent	0.58 ± 0.06ab	0.2	0.90 ± 0.17b	0.2	0.40 ± 0.11a	0.2	0.48 ± 0.02a	0.1	0.87 ± 0.14	0.3	1.2 ± 0.1	0.5	1.2 ± 0.1	0.5	1.3 ± 0.2
Kaemp-3-rut	0.30 ± 0.05a	0.1	0.47 ± 0.04b	0.1	0.46 ± 0.06c	0.1	0.65 ± 0.04b	0.1	0.92 ± 0.13b	0.3	1.04 ± 0.1b	0.4	0.85 ± 0.13ab	0.4	0.57 ± 0.10a
Q-3-gal	52.3 ± 10.5	16.7	51.1 ± 10.4	14.4	69.3 ± 9.1	14.0	66.1 ± 5.7	14.0	21.3 ± 2.5a	7.6	18.7 ± 4.2a	6.9	26.3 ± 3.8a	11.3	37.9 ± 2.8b
Q-3-glu	178.6 ± 41.5a	50.2	230.6 ± 32.5ab	52.3	324.2 ± 34.2b	54.5	271.6 ± 37.9ab	54.7	158.1 ± 10.02b	56.2	146.1 ± 25.7b	51.0	77.7 ± 14.7a	32.4	91.4 ± 13.1a
Q-3-gluc	22.3 ± 3.8a	6.7	43.4 ± 8.5b	8.2	35.8 ± 7.8b	7.8	31.7 ± 6.5b	7.6	35.4 ± 3.4ab	12.5	36.1 ± 3.1ab	13.6	40.5 ± 1.1b	17.5	26.5 ± 4.6a
Q-3-rut	6.7 ± 0.6a	2.1	6.6 ± 1.1a	1.9	8.5 ± 1.1b	1.8	9.9 ± 0.9b	1.8	12.7 ± 1.6a	4.5	11.7 ± 3.5a	4.0	18.5 ± 2.5b	8.1	27.1 ± 1.5b
Q-3-xyl	1.9 ± 0.5	0.6	3.1 ± 1.5	0.6	1.8 ± 0.7	0.5	2.3 ± 0.4	0.5	0.43 ± 0.12a	0.1	0.70 ± 0.04ab	0.3	0.95 ± 0.05ab	0.4	1.2 ± 0.1b
Total flavonols	342 ± 40a	82.3	418 ± 56ab	82.3	535 ± 22b	88.2	482 ± 37b	89.9	281 ± 16b	80.5	279 ± 31ab	82.5	234 ± 15a	78.6	271 ± 15ab
TAPC	413 ± 41a		507 ± 61b		607 ± 23c		535 ± 37b		349 ± 16b		337 ± 33b		298 ± 18a		348 ± 19b

Treatments: C, control (usual annual practice); LR1, leaf removal in cluster zone at berry set; LR2, leaf removal above second pair of wires at veraison; DMR, Double Maturation Raisonnée. Compound abbreviations: cou, coumaric; hex, hexoside; caf, caffeic; HCA, hydroxycinnamic acids; procy, procyanidin; iso, isorhamnetin; glu, glucoside; kaemp, kaempferol; gal, galactoside; gluc, glucuronide; pent, pentoside; rut, rutinoside; Q, quercetin; xyl, xyloside; TAPC, total analysed phenolic content (mg kg^{-1}); %, percentage of each phenolic compound according to total value of associated phenolic group within individual treatment. Results are presented as mean ± standard error. Different letters in a row denote statistically significant differences (LSD test, $P < 0.05$) among treatments in each individual year. Potential impact: † or †*, potential increase or decrease in LR1, LR2 and DMR compared with control only in one year; †** or †**, potential increase or decrease in LR1, LR2 and DMR compared with control in both years.

Table 5. Phenolic compounds in Malvasia wine (mg L^{-1}) measured under different canopy practices in two consecutive years

Phenolic compound	2013										2014										Potential impact		
	C	%	LR1	%	LR2	%	DMR	%	C	%	LR1	%	LR2	%	DMR	%							
Pro acid (HBA)	4.18 ± 0.06b	4.1	3.53 ± 0.11a	3.9	3.92 ± 0.10b	3.9	4.53 ± 0.05b	4.0	1.89 ± 0.03a	1.9	1.90 ± 0.07a	1.9	1.81 ± 0.04a	1.8	2.30 ± 0.08b	2.3	↑**						
cis-Caffaric acid	0.52 ± 0.05a	12.7	0.74 ± 0.06b	13.5	0.60 ± 0.07ab	14.6	0.72 ± 0.03b	16.4	0.16 ± 0.034a	6.6	0.19 ± 0.04a	6.1	0.07 ± 0.02a	2.9	0.45 ± 0.10b	17.0	↑**						
Coumaric acid	0.19 ± 0.14a	4.4	0.08 ± 0.01a	1.5	0.17 ± 0.10a	4.3	0.54 ± 0.01b	12.2	0.20 ± 0.04ab	8.4	0.28 ± 0.01b	8.7	0.27 ± 0.01b	10.4	0.13 ± 0.07a	4.9	↑*	↑*					
Ferulic acid der 1	0.02 ± 0.02a	1.5	0.06 ± 0.02a	1.0	0.02 ± 0.01a	0.7	0.10 ± 0.04b	2.4	0.09 ± 0.05b	3.3	0.07 ± 0.01ab	2.1	0.05 ± 0.01a	2.1	0.08 ± 0.01b	3.0	↓*	↑*					
Ferulic acid der 2	0.19 ± 0.03	4.6	0.20 ± 0.04	3.7	0.19 ± 0.03	4.7	0.22 ± 0.03	5.0	0.10 ± 0.01	4.1	0.11 ± 0.01	3.8	0.11 ± 0.01	4.3	0.12 ± 0.01	4.9							
p-Cou acid hex 1	0.31 ± 0.02	7.7	0.34 ± 0.03	6.1	0.31 ± 0.02	7.5	0.36 ± 0.01	8.3	0.20 ± 0.04b	9.2	0.20 ± 0.03b	6.3	0.12 ± 0.01a	6.9	0.17 ± 0.01b	6.2	↓*						
p-Cou acid hex 2	0.85 ± 0.02a	20.7	0.85 ± 0.02a	15.6	0.89 ± 0.05a	21.6	1.05 ± 0.02b	23.8	0.62 ± 0.02c	26.1	0.59 ± 0.02bc	18.6	0.53 ± 0.02ab	20.9	0.49 ± 0.02a	18.8	↓*	↑*	↑*				
trans-Ferulic acid	0.28 ± 0.03a	6.8	0.42 ± 0.03b	7.7	0.29 ± 0.04a	7.0	0.32 ± 0.02ab	7.3	0.23 ± 0.01a	9.9	0.45 ± 0.02c	14.2	0.34 ± 0.01a	13.3	0.47 ± 0.03b	17.9	↑**						
trans-Caffaric acid	1.70 ± 0.06b	41.6	2.78 ± 0.12c	50.9	1.63 ± 0.32ab	39.6	1.09 ± 0.07a	24.8	0.78 ± 0.03a	33.1	1.28 ± 0.09b	40.1	1.00 ± 0.08ab	39.1	0.71 ± 0.11a	27.3	↑**	↓**					
Total HCA	4.1 ± 0.2a	21.0	5.5 ± 0.2b	21.3	4.1 ± 0.2a	21.0	4.4 ± 0.1a	20.2	2.3 ± 0.1a	38.6	3.2 ± 0.2b	36.8	2.5 ± 0.1a	43.1	2.6 ± 0.1a	7.8	↑**						
Catechin	0.21 ± 0.04a	13.5	1.62 ± 0.14b	45.2	0.24 ± 0.04a	11.0	0.27 ± 0.02a	7.0	0.14 ± 0.01ab	22.0	0.19 ± 0.03b	8.7	0.10 ± 0.02a	17.1	0.16 ± 0.02ab	9.1	↑**						
Epicatechin	1.29 ± 0.15a	86.5	1.99 ± 0.25b	54.8	2.16 ± 0.71ab	89.0	3.59 ± 0.14c	93.0	1.08 ± 0.33a	78.0	1.94 ± 0.13b	91.3	0.79 ± 0.33a	82.9	1.95 ± 0.15b	90.9	↑**						
Total flavanols	1.50 ± 0.19a	7.6	3.61 ± 0.39bc	10.5	2.40 ± 0.73ab	10.6	3.86 ± 0.16c	11.6	1.22 ± 0.33a	18.1	2.13 ± 0.35b	26.6	0.89 ± 0.34a	13.8	2.10 ± 0.52b	33.6	↑**						
Apigenin pentoside	0.68 ± 0.08a	68.4	0.80 ± 0.07ab	67.2	0.74 ± 0.09a	65.5	0.90 ± 0.02b	69.1	0.44 ± 0.13	51.3	0.46 ± 0.09	47.5	0.32 ± 0.03	40.7	0.42 ± 0.05	45.3	↑*						
Dihydrokaemp-3-glu	0.24 ± 0.04	24.4	0.27 ± 0.04	23.1	0.24 ± 0.02	22.0	0.20 ± 0.01	15.1	0.19 ± 0.01ab	26.7	0.19 ± 0.05ab	18.4	0.17 ± 0.04a	21.1	0.21 ± 0.04b	22.4		↑**	↑**				
Laricitrin-3-xyloside	0.07 ± 0.01a	7.2	0.12 ± 0.02b	9.7	0.14 ± 0.02b	12.6	0.20 ± 0.01c	15.8	0.15 ± 0.02a	22.0	0.31 ± 0.03b	34.1	0.30 ± 0.03b	38.2	0.29 ± 0.01b	32.4	↑**	↑**	↑**				
Total flavonols	1.01 ± 0.09a	49.9	1.20 ± 0.02ab	50.0	1.14 ± 0.11ab	50.0	1.31 ± 0.04b	50.0	0.79 ± 0.14a	12.3	0.97 ± 0.06b	12.2	0.80 ± 0.05a	13.3	0.94 ± 0.07b	17.2	↑**	↑**	↑**				
Res-3-glu (STIL)	0.01 ± 0.004	0.05	0.01 ± 0.003	0.05	0.01 ± 0.003	0.1	0.01 ± 0.006	0.1	0.01 ± 0.004	0.2	0.01 ± 0.002	0.2	0.01 ± 0.002	0.2	0.01 ± 0.005	0.2							
TAPC	10.8 ± 0.4a		13.8 ± 0.7b		11.6 ± 0.9a		14.1 ± 0.3b		6.2 ± 0.7a		8.2 ± 0.7b		6.1 ± 0.5a		7.9 ± 0.6b		↑**						
Treatments: C, control (usual annual practice); LR1, leaf removal above cluster zone at berry set; LR2, leaf removal above second pair of wires at véraison; DMR, Double Maturation Raisonnée. Compound abbreviations: pro, protocatechuic; HBA, hydroxybenzoic acids; der, derivative; cou, coumaric; hex, hexoside; HCA, hydroxycinnamic acids; dihydrokaemp, dihydrokaempferol; glu, glucose; res, resveratrol; STIL, stilbenoids; TAPC, total analysed phenolic content (mg kg ⁻¹); %, percentage of each phenolic compound according to total value of associated phenolic group within individual treatment. Results are presented as mean ± standard error. Different letters in a row denote statistically significant differences (LSD test, P < 0.05) among treatments in each individual year. Potential impact: ↑* or ↓*, potential increase or decrease in one year; ↑** or ↓**, potential increase or decrease in two years.																							

Treatments: C, control (usual annual practice); LR1, leaf removal in cluster zone at berry set; LR2, leaf removal above second pair of wires at véraison; DMR, Double Maturation Raisonnée. Compound abbreviations: pro, protocatechic; HBA, hydroxybenzoic acid; der, derivative; cou, coumaric; hex, hexoside; HCA, hydroxycinnamic acid; dihydrokaemp, dihydrokaempferol; glu, glucoside; res, resveratrol; STIL, stilbenoids; TAPC, total analysed phenolic content (mg kg^{-1}); %, percentage of each phenolic compound according to total value of associated phenolic group within individual treatment. Results are presented as mean ± standard error. Different letters in a row denote statistically significant differences (LSD test, $P < 0.05$) among treatments in each individual year. Potential impact: ↑* or ↑, potential increase or decrease in LR1, LR2 and DMR compared with control only in one year; ↑** or ↓**, potential increase or decrease in LR1, LR2 and DMR compared with control in both years.

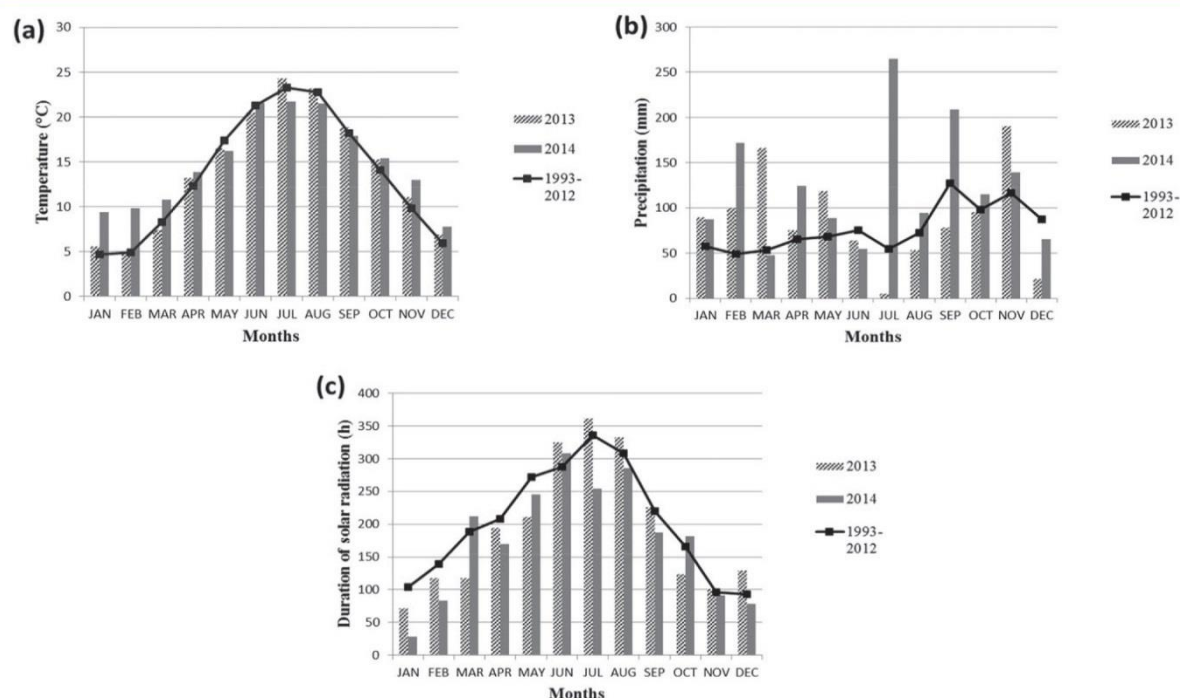


Figure 1. (a) Average monthly temperature, (b) average monthly precipitation and (c) average duration of solar radiation at weather station Portorož for years 2013 and 2014 and long-term average between 1993 and 2012.

showed that only DMR has the potential to decrease the final yield per vine, which ranged from 2.9 to 3.1 kg per vine, and at 4000 vines ha^{-1} can still represent a quite abundant quantity of 11 600 and 12 400 kg ha^{-1} respectively.

Grape composition

The soluble solids content, titratable acidity and pH of grape berries could serve as a rough preview on further wine quality and composition.² Early leaf removal may advance the sugar accumulation in berries,⁴ since lower old leaves are less photosynthetically active and often receive less light than upper young leaves. On this account, an increase in soluble solids content could be expected,⁶ as clearly shown in our study as well. Additionally in our experiment, the increase in soluble solids in both years despite the removal of younger leaves on shoots (LR2) could be related to a certain photosynthetic activity of the lower leaves, including lateral shoots positioned under the second pair of wires.³³ Moreover, Poni *et al.*¹⁰ reported that after removing the upper leaves on the shoots a delay in ripening occurs, i.e. also a delay in augmentation of soluble solids, which could be lesser compared with non-defoliated vines. Regarding the latter and already mentioned above, a cause for this phenomenon is in the decreased photosynthetic activity of bunch zone leaves.³³ The reason for the increased titratable acidity with LR1 might be assigned to the fact that titratable acids emerge in berries during the time of berry growth³⁴ and to the lower average temperatures and higher precipitation at the time of berry set, which was especially observed in 2014 (Fig. 1). In contrast, bunches exposed to the sunlight could be less abundant in titratable acidity, as malic acid degrades at higher temperatures.⁵ On the other hand, Bubola *et al.*⁹ reported that the

exposure of grapes to sunlight can lead to increased water transpiration from berries as a consequence of which an increased synthesis of acids occurs, which has also been observed in our study with LR1 and even LR2 compared with the control. Conversely to an increase in acidity in berry must, a decline in pH should be obtained.¹⁵ As reported by Bubola and Persurić¹¹ after an early defoliation applied before bloom, immediately after bloom and at the beginning of bunch closure (E-L 18, 27 and 32 respectively), the remaining leaves on the vine provide a sufficient assimilate supply during berry growth and ripening, on account of which the 100-berry weight loss is negligible. The composition and contents of primary metabolites (sugars, organic acids) in berries influence further composition and especially the contents of organic acids in wines.^{34,35} Poni *et al.*⁵ claimed that leaf removal applied especially at an earlier stage (BBCH 55–57) causes a higher translocation of sugars into the clusters, which might have occurred in our experiment, whereas glucose and fructose contents significantly increased only with both leaf removals in 2014, while soluble solids increased in both years. The reason for the negative response of glucose and fructose in the first year might be assigned to the low rainfall and dry conditions during the summer months of 2013. On the other hand, in the process of DMR, water evaporates through the berry skin, since the connection between the vine and shoot is interrupted, consequently sugars and acids concentrate in the pulp. The effect is very similar to drought conditions or berry shrinkage, which causes sugar accumulation in berries.^{17,19,33} In addition, a 2-week-long DMR on an 'Italian Riesling' increased sugars and lowered organic acid contents in three vintages as reported by Májer *et al.*³⁶ An extensive leaf removal, especially applied in bunch zones, causes a sudden increase in light and temperature around the clusters, which according to Bubola *et al.*⁸ leads to

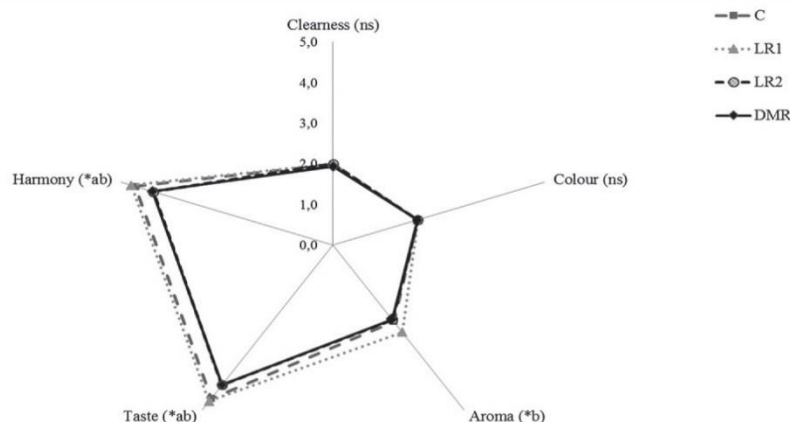


Figure 2. Sensory evaluation of clearness, colour, aroma, taste and harmony (overall impression) of Malvasia C, LR1, LR2 and DMR experimental wines. For each category, a score is given (sum of maximum scores is 20). The results of the sensory wine evaluation are given as means of each sensory attribute: 'ns' denotes statistically significant differences (LSD test, $P < 0.05$) of each attribute between treatments; 'ns' denotes no significant differences; 'a' denotes statistically significant differences (LSD test, $P < 0.05$) between LR1 and DMR treatments; 'b' denotes statistically significant differences (LSD test, $P < 0.05$) between LR1 and DMR treatments.

a higher concentration of tartaric acid in grape juice and wine. However, in our experiment, a contrary effect was noted, since the contents of citric acid and tartaric acid remained unchanged by the leaf removals but increased with DMR, which might be attributed to varietal characteristics.

Table 6. Results of sensory evaluation of wines under different canopy practices

Treatment	C	LR1	LR2	DMR
Total points	16.0	16.5	15.0	15.0

Wine composition

Wines produced from 'Istrian Malvasia' are usually known as dry (residual sugar $< 9 \text{ g L}^{-1}$), full-bodied, slightly bitter, reaching higher alcohol contents of 13–14 vol%, which nevertheless is not always desired.⁶ However, the chemical composition of wine is firstly conditioned by the grape composition, followed by the macro- and micro-location, and secondly by the techniques and winemaking conditions.³⁵ Kozina *et al.*,³⁵ in their study on 'Sauvignon Blanc' and 'Riesling' wines produced under basal leaf removal at véraison and compared with the control, showed a decrease in total acidity caused by enhanced malic acid degradation and an increase in volatile acidity partly ascribed to the volatile compounds derived from grape and not synthesized during fermentation. Moreover, LR1 increased the tartaric acid content, which according to Tardaguila *et al.*¹ could be a consequence of the changes between tartaric acid catabolism and early (before pea-size stage) leaf removal. However, LR2 and DMR increased the malic acid amount in the wine, leading to a higher freshness or astringency of the wine;³⁴ on the other hand, a great abundance of malic acid can also result in a negative response in terms of organoleptic characteristics.³⁷ Usually, a higher content of soluble solids in berries leads to a higher alcohol content of the wine, but according to our results it depends on fermentation conditions, nutrient supply and yeast activity.³⁴ However, panellists found the wines from DMR and LR2 less tasteful and less harmonious/balanced in comparison with LR1 and control wines. On the other hand, according to the results and sensory evaluation, lighter, less-bodied but more preferable wine could be produced from LR1 grapes (Fig. 2 and Table 6).

Phenolic composition of berry skin and wine

Vineyard practices certainly affect the synthesis and contents of phenolic compounds in grapes and wines,^{1,5,13} but factors such

as variety, plant diseases, grape maturity and seasonal climatic conditions must also be considered.^{38–40} The different precipitation rates and the possible resulting plant stress might have influenced the phenolics in the berry skins, especially the contents of individual and total HCA, irrespective of treatment, especially taking into consideration the water supply at the time of berry growth, i.e. extremely low precipitation (5 mm on average) recorded in July 2013 and extremely high rainfall (270 mm) in July 2014 (Fig. 1(b)). However, the removal of lower leaves early in the season (around berry set) could result in a reduction of cluster compactness,⁴¹ which leads to an improved cluster microclimate and consequently to the promotion of berry skin growth.¹ Regarding the latter, the result is the increased phenolic content of berries,¹ which was also observed in our study, especially in the contents of HCA. Another reason for the increased phenolic concentration in sun-exposed grapes from bunch zone-defoliated vines could be the higher temperatures of the berries ($> 30^\circ\text{C}$, overheated berries) at the time of véraison.^{33,41} However, average monthly air temperatures within our study did not exceed 25°C . Moreover, it is likely to ascribe the increase in berry skin flavonols to increased cluster exposure to sunlight, as the synthesis of flavonols is inhibited in shaded clusters.^{14,37,42} Suklje *et al.*⁶ reported that the leaf area/yield ratio has an influence on the concentration of phenolics as well, while Poni *et al.*³³ reported that leaf removal above the bunch zone, despite being applied post-véraison, did not significantly influence the phenolic ripening and phenolic substances in 'Sangiovese'. Moreover, LR2 in our experiment seemed to have no greater tendency to increase individual phenolics, which is similar to the above mentioned reports and might be associated with the fact that removing the leaves above the fruiting zone can hardly influence the cluster microclimate.³³ Furthermore, upper leaves are also more photosynthetically active and thus more

prone to change berry sugar and acidity contents and pH than phenolic composition.^{1,33} DMR had a great impact on the phenolic composition of the grape skin of 'Istrian Malvasia', which was especially observed in the contents of individual flavonols. Changes in water potential and higher berry transpiration after the cut in DMR induce stress conditions to the grape, similar to drought, and as a response to these conditions an increase in berry skin and wine phenolics is usually expected,^{17,18} which has also been confirmed in our study. On the other hand, Corso *et al.*¹⁸ reported that xylem backflow occurs and phloem functionality is reduced in berries exposed to late harvest, similarly to berry shrinkage,¹² but the result is often a decrease in phenolic content. However, an increase in some individual and total phenolics, observed only in one year, could also be attributed to higher or lower monthly precipitation (Fig. 1(b)), which influences water transpiration from the berries. The results showed that DMR had the greatest influence on the phenolic content of 'Istrian Malvasia' wine, which is undoubtedly connected with the fact that the major changes in phenolic composition occurred in the berries under DMR. Related to that, several authors^{16,18,19} reported that DMR causes a faster phenolic ripening in relation to skin texture,²¹ which could lead to earlier phenolic maturity and to better phenolic extraction from skin to wine. Tardaguila *et al.*¹ reported on the relationship between grape and wine phenolic composition under early leaf removal, where the same impact was observed in grape and wine. However, such effect in our study was observed only under DMR. According to Bubola *et al.*,⁹ HCA contribute to wine astringency and represent the most abundant phenolic compounds in white wines, which is not completely in accordance with our results. DMR affected HCA, because of which the obtained wine was more complex as the HCA were involved in the formation of ethyl esters during fermentation.³⁸ Moreover, a different phenolic composition from skin to wine according to Conde *et al.*³⁴ might be attributed to the fact that phenolic compounds are also formed and transformed during the vinification process. Taking into account the changes in grape and wine chemical composition, the applied treatments seemed to have influenced them, especially in terms of the sensory evaluation, mostly regarding the taste and harmony (overall impression), a less intensive aroma and colour characteristics.

CONCLUSIONS

The present study shows that different canopy managements had different impacts on the yield and chemical composition of 'Istrian Malvasia' grapes and wines. Specifically, early leaf removal of four or five leaves per shoot under the first pair of wires augmented the yield per vine compared with the control and had an influence on most of the general grape and wine parameters, while less on the detected secondary metabolites of berries, from which a lighter, less-bodied wine with moderate alcohol content is expected. On the other hand, removal of leaves above the second pair of wires at véraison increased the malic acid content of grapes, the total and volatile acidity of wines and most of the flavonol contents in berry skins but had almost no influence on wine phenolics. DMR application has proven that grape dehydration and the stress condition of the remaining cane lead to an increased synthesis of phenolic compounds in berry skins to the point of harvest as well as consequently in the wine. Hence a richer taste and full body could be expected from a DMR-treated wine. This study demonstrates the potential of applied canopy managements to produce wines differing in style. However, regarding the listed features of individual canopy managements, environmental conditions must also

be taken into account and the plant stress affecting vine growth should be further studied.

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2.1.3 Vpliv delnega sušenja na kemijsko sestavo grozdja in vina belih sort žlahtne vinske trte (*Vitis vinifera* L.)

Reščič J., Mikulič-Petkovšek M., Rusjan D. 2016. The impact of partial dehydration on grape and wine chemical composition of white grapevine (*Vitis vinifera* L.) varieties. European Journal of Horticultural Sciences, 81, 6: 310-320

S to raziskavo smo želeli ugotoviti vpliv dvojnega namenskega zorenja (DMR - Double Maturation Raisonnée) grozdja na kemijsko sestavo grozdja in vina dveh zelo rodni belih sort 'Rebula' in 'Vitovska grganja' v dveh zaporednih letnikih. Merili smo parametre dozorevanja ter določali vsebnost fenolnih spojin v jagodni kožici in vinu. Pod vplivom DMR se je v jagodah povečala vsebnost topne suhe snovi, vsebnost titracijskih kislin, posameznih sladkorjev in organskih kislin, v primerjavi z grozdom v kontroli. Vina iz grozdja, ki je bilo podvrženo DMR, so imela večjo vsebnost alkohola ter skupnega ekstrakta, kar pripomore k večji kakovosti. Zanimiv odziv na DMR se je pokazal pri fenolnih spojinah. Vsebnost procianidin-dimera se je v jagodni kožici sorte 'Rebula' v povprečju povečala za 22 mg/kg. Podobno, v jagodni kožici sorte 'Vitovska grganja' se je povečala vsebnost kvercetin-3-glukozida v povprečju za 69,5 mg/kg v primerjavi s kontrolo. Ugotovili smo, da je DMR različno, vendar ne vedno značilno spremenil in vplival na sestavo grozdja in vina in s tem prispeval k večji kakovosti vina. Rezultati kažejo, da bi se v prakso lahko vpeljalo DMR, vendar pri sortah, ki dajejo bujen in velik pridelek, z namenom pridelave polnih vin.

Original article



The impact of partial dehydration on grape and wine chemical composition of white grapevine (*Vitis vinifera* L.) varieties

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Summary

The aim of this study was to investigate the impact of Double Maturation Raisonnée (DMR) partial dehydration technique on chemical composition of two white and high-yielding grapevine varieties: 'Rebula' and 'Vitovska grganja' in two consecutive years. DMR technique was applied, where a cut of one-year old cane two to three weeks before harvest was done and grape remained on the vine till suitable chemical composition was reached. Grape and wine characteristics were monitored along with primary metabolites and phenolic profiles of berry skin and wine. DMR decreased the weight of 100 berries and caused an increase in soluble solids, titratable acidity, individual sugars and organic acids in comparison with the control berries. The wines produced from DMR grapes had higher alcohol content and total extracts. An interesting impact of DMR was observed on phenolic compounds. Procyanidin dimer 1 averagely increased for 22.0 mg kg⁻¹ in 'Rebula' DMR berry skin. Similarly, 69.5 mg kg⁻¹ more quercetin-3-glucoside was determined in 'Vitovska grganja' DMR berry skin compared to the control. In our study, DMR influenced and modified differently but significantly the grape and wine composition, especially the phenolic profiles of two white varieties and contributed to higher wine quality. The results propose the implementation of DMR partial dehydration technique for high-yielding white grapevine varieties in order to produce richer, full-bodied wines.

Keywords

canopy management, phenolic maturation, partial dehydration, quality, 'Rebula', 'Vitovska grganja'

Significance of this study

What is already known on this subject?

- Vineyard canopy practices and dehydration techniques are one of the main tools to manage and improve grape and wine quality for the production of different wine styles.

What are the new findings?

- This study clearly demonstrated that DMR partial dehydration in the vineyard increased soluble solids and sugars, alcohol content and most of individual phenolic compounds determined in local, white 'Rebula' and 'Vitovska grganja' (*Vitis vinifera* L.) grapes and wine.

What is the expected impact on horticulture?

- The DMR partial dehydration technique could be of much importance for winegrowers and winemakers, who struggle with difficulties in production of desired wine styles, demanded by the consumers, especially dealing with high-yielding varieties and high production costs.

Introduction

Grape and wine quality is generally affected by the variety (Landraut et al., 2001; Prajitna et al., 2007), environmental conditions (Jackson and Lombard, 1993; Jones and Davis, 2000) and plant status (Rusjan et al., 2012). Vigorous and high-yielding varieties often produce copious grapes, resulting in an inadequate berry composition and inferior wine quality. Also, winegrowers and winemakers are struggling to place their wines on the market, dealing also with high production costs. Canopy practices (shoot thinning and hedging, leaf removal, bunch and berry thinning) have a significant impact on grape and wine characteristics (Gil et al., 2013; Šuklje et al., 2013).

Partial grape dehydration is a common practice for the production of specific wine types rich in sugars (sweet

wines), aroma and phenolic compounds (Panceri et al., 2013), but it can also be utilized for dry wines (Rolle et al., 2009). Grape dehydration is usually performed as an off-vine postharvest drying method (Mencarelli et al., 2010; Panceri et al., 2013). However, on-vine drying, where grapes are left on the vine until harvest, is frequent in ice wine production (Rolle et al., 2009).

To improve chemical composition of grapes and produce more complex, full-bodied wines with higher alcohol content, a technique called Double Maturation Raisonnée (DMR) has been developed and introduced by Cargnello (Cargnello, 2000) and Peršurić (Peršurić et al., 2000). DMR proposes to remove one-year-old canes two to three weeks before harvest and leave the clusters to partially dehydrate on the vine till reaching suitable chemical composition for a specific wine style (Cargnello et al., 2005). Cane wounding results in increased water loss due to high summer temperatures, which cause faster berry transpiration and shriveling (Rolle et al., 2011; Corso et al., 2013). Consequently, a rapid increase in total soluble solids, titratable acidity and phenolic maturity has been recorded in pulp and skin of berries, subjected to berry dehydration (Rolle et al., 2009). However, Corso et al. (2013) reported a decrease in individual organic acids in DMR berries of the 'Raboso Piave' variety. Biochemical changes are subsequently reflected in earlier phenolic maturity, leading to higher contents of phenolic compounds

in berry skin (Conde et al., 2007; Corso et al., 2013). Moreover, the loss of skin compactness in relation to skin thickness causes an easier release of phenolic compounds into the must, which improves chemical composition and sensorial characteristics of the wine (Conde et al., 2007; Rolle et al., 2009). Also, it is well known that wine composition and quality not only depends on enological practices but also on ripening status of grape (Canals et al., 2005; del Llaudy et al., 2008). Therefore insufficiently ripened grape and/or insufficient phenolic maturity is usually reflected in wine that is more bitter, astringent and poor in colour (Castillo-Muñoz et al., 2007; Kontoudakis et al., 2011).

Up to now, studies have focused on the effect of DMR on berry and wine characteristics, wine sensorial changes and total phenolic composition (János et al., 2007; Corso et al., 2013). Nevertheless, information on the impact of DMR on individual phenolic profiles already exist (Corso et al., 2013), but information particularly on the white *Vitis vinifera* L. varieties are still scarce.

'Rebula' and 'Vitovska grganja' (*Vitis vinifera* L.) are white grapevine varieties, mostly cultivated in western Slovenia and northeastern Italy. 'Rebula' is also frequently planted in Kefalonia, an Ionian island in Greece (De Lorenzis et al., 2013). Both are local (probably indigenous), but high-yielding (5.1–6.3 kg vine⁻¹) varieties, with an average bunch weight of 202–265 g for 'Rebula' and 270–370 g for 'Vitovska grganja'. The berries are characterized by lower soluble solids content (18.9 to 20.0° Brix) and higher acidity (6.9 to 7.9 g L⁻¹). Wines, produced from both varieties are considered fresh and dry, with a distinct acidity, bitterness and low or moderate alcohol content (Koruza et al., 2012). Therefore, winegrowers strive to overcome the difficulties in the production of these varieties and subsequent placement of the wines on the market. Nevertheless, DMR might reduce the costs through an improved quality of the final product, which would consequently achieve better prices.

The aims of the study were: (i) to investigate the impact of DMR technique on grape and wine composition and phenolic profiles of two white grapevine varieties ('Rebula' and 'Vitovska grganja') and (ii) to determine the potential of DMR as a common technique in the production of full-bodied wines with higher extract content.

Materials and methods

Experimental vineyard

The study was conducted on 'Rebula' and 'Vitovska grganja' (*Vitis vinifera* L.) grapevine varieties in two commercial vineyards; 'Rebula' in Goriška brda and 'Vitovska grganja' in Kras winegrowing district of the Primorska winegrowing region in Slovenia. The vines were planted at the distance of 2.5 × 0.8 m, grafted on 5BB rootstocks and trained to a vertical shoot-positioned double guyot on two pairs of wires. The grape production in both vineyards follows the cultivation practices regulated by integrated pest management. The vineyards are not equipped with an irrigation system, the rows are N-S orientated and in-row tillage is practiced. Climatic conditions in Goriška brda are sub-Mediterranean with an average annual temperature of 11.8°C and average precipitation of 1,456 mm per year. The climate in Kras is sub-Mediterranean with distinct effects of continental climate with an average annual temperature of 10.5°C and average precipitation of 1,420 mm per year (Slovenian Environment Agency, 2016).

Experimental design and winemaking

The experiment was performed in 2012 and 2013 as a randomized three-block design. Each block was organized as a partial vineyard row and consisted of two treatments; (i) control (C) – common annual practice and (ii) Double Maturation Raisonnée (DMR) – one-year-old canes with shoots and clusters were cut off manually when soluble solids reached approximately 18.0° Brix ('Rebula') and 17.5° Brix ('Vitovska grganja'). Three to four shoots were left on the head of each trunk for further growth. Each treatment per block included 20 consecutive vines in the same row. Grapes were sampled (harvested) when the content of soluble solids of DMR treatment reached approximately 22° Brix; on 1st October ('Rebula') and on 2nd October ('Vitovska grganja') of 2012 and 2013. Clusters were collected at harvest separately for each treatment and replicate (approximately 0.5 kg per replicate), transported to the laboratory and stored at -20°C until biochemical analysis. After the sampling, all the grapes from individual treatments were manually harvested for vinification, which was performed under equal conditions and replicated three times per treatment. Approximately 100 kg of grapes were used for each individual vinification. Grapes were de-stemmed, crushed and pressed and 50 mL 100 L⁻¹ of 5 to 6% aqueous solution of sulphur dioxide (H₂SO₃) was added to prevent oxidation. The must was cooled and left on low temperature (10°C) during the night (approximately 15 hours). Must (without the sediment) was decanted into 50-L stainless steel experimental tanks (Mühlfeller Tankbau GmbH, Ehrenhausen, Germany) separately for each treatment (*n*=3) and fermented for 14 days at controlled temperature (17°C) until residual sugar levels were below 5 g L⁻¹, according to the limits for dry wine (Ministry of Agriculture, Forestry and Nutrition of the Republic of Slovenia, 2004). The must was inoculated with 30 g 100 L⁻¹ of rehydrated yeast *Saccharomyces cerevisiae* Anchor VIN 13 (Anchor Yeast, Industria, South Africa) and a yeast nutrient OptiMUM WHITE (Lallemand, Milwaukee, USA) were added into each tank – 30 g 100 L⁻¹ at the start of fermentation and 30 g 100 L⁻¹ seven days later, according to the instructions of the producers. After the completion of fermentation process, the wine was moved to 30-L stainless steel tanks (Mühlfeller Tankbau GmbH, Ehrenhausen, Germany) and 50 mg L⁻¹ of potassium metabisulfite (K₂S₂O₅) was added into each tank.

Berry characteristics and wine analysis

Measurements of berry characteristics were conducted immediately after sampling. Chemical analyses were carried out during the following months in the laboratory. Soluble solids content of grapes was assessed with a digital refractometer (ATAGO PAL87S) and expressed in °Brix scale degree. The titration method by Ough and Amerine (1988) was used to determine titratable acidity of must. The required volume of 0.1 M NaOH (pH 7.0) was recorded for further calculation of titratable acidity, which was expressed in g L⁻¹. Before the titration, initial pH value of grape must and wine was recorded. A sample of wine was taken from each tank for chemical analyses on WineScan FT120 (Foss, Denmark), which were carried out one month after sulphuring.

Individual sugars and organic acids

The extraction of individual sugars and organic acids followed a protocol described by Rusjan et al. (2008). Twenty grape berries per sample were manually crushed, pressed and filtered through an extraction bag (BIOREBA, Switzerland), to obtain 1 mL of grape juice, which was topped with

9 mL of double distilled water; and left at room temperature for 30 min. Sample was then centrifuged (Eppendorf Centrifuge 5810R) for 7 min at 10,000 rpm and 4°C. The supernatant was filtered through a 0.45 µm cellulose ester filter (Macherey-Nagel, Düren, Germany) and transferred into a vial. 20 µL of the sample was used for the analysis on high-performance liquid chromatograph (HPLC Accela Systems) of Thermo Scientific with a Rezex - RCM – monosaccharide Ca+2 (300 mm × 7.8 mm) separation column from Phenomenex for sugars and a Rezex ROA for organic acids (Phenomenex), both operating at 65°C. Further procedure was carried out as described by Mikulič-Petkovšek et al. (2007). Retention times of sugars and organic acids were used for their identification and their levels were calculated from standard curves of the corresponding external standards and expressed in g L⁻¹.

Individual phenolic compounds – HPLC analysis

Extraction of individual hydroxycinnamic acids, hydroxy-benzoic acids, flavanols, flavonols and stilbenoids from berry skin was performed according to the method of Mikulič-Petkovšek et al. (2012). Skin was removed from frozen berries and skin samples were ground to a fine powder using liquid nitrogen. Approximately 0.5 g of berry skin sample (exact weight was recorded) was placed into a centrifuge tube and topped with 10 mL of methanol containing 3% (v/v) formic acid and 1% (w/v) butylated hydroxyl-toluene (BHT). BHT was added to the samples to prevent oxidation. The extraction was performed in a cooled ultrasonic bath for 1 h and the extracts were centrifuged (Eppendorf Centrifuge 5810R) at 10,000 rpm and 4°C for 7 min. Supernatant was filtered through an injection filter (0.20 µm Chromafil A-20/25, Macherey-Nagel) into a vial. Further procedure was carried out with a HPLC-MS system (Thermo Finnigan Surveyor with quaternary pump, San Jose, USA) according to the methods

described by Wang et al. (2002) and Mikulič-Petkovšek et al. (2012). The contents of individual phenolic compounds were quantified from peak areas with the help of corresponding standards and expressed in mg kg⁻¹ FW and mg L⁻¹ of grape and wine, respectively. For compounds lacking standards the quantification was carried out using similar compounds as standards. The content of total hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols and stilbenoids was calculated as the sum of all identified and quantified individual phenolics of the corresponding phenolic group.

Total phenolic content (TPC)

The extraction of total phenolic compounds was carried out according to the same procedure as for the extraction of individual phenolic compounds (Mikulič-Petkovšek et al., 2012). BHT was not used in the extract solution. Berries were peeled and 0.5 g of skins was ground to a fine powder with liquid nitrogen. Skin samples were placed into a centrifuge tube and topped with 10 mL of methanol. After 1 h of cool-bathing, the extracts were centrifuged (Eppendorf Centrifuge 5810R) and filtered into vials. Total phenolic content was analyzed with a Folin-Ciocalteu reagent method described by Singleton et al. (1999). The absorbance was measured in three replications on a spectrophotometer (Perkin-Elmer, UV/visible Lambda Bio 20) at 765 nm. Total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) in mg kg⁻¹ FW of berry skin and in mg L⁻¹ of wine.

Chemicals

For the quantification of sugars, organic acids and phenolic compounds the following standards were used: glucose, fructose, tartaric acid, *p*-coumaric acid, caffeic acid, procyanidin B1, catechin, epicatechin, vanillic acid, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside and myricetin-3-*O*-rhamnoside from

TABLE 1. General characteristics of 'Rebula' berries at harvest and 'Rebula' wine of two consecutive vintages.

	2012		2013	
	C	DMR	C	DMR
Berries				
Weight of 100 berries (g)	240±12 ^b	148±2 ^a	253±14	249±9
Total soluble solids (°Brix)	21.2±0.3 ^a	22.3±0.3 ^b	19.1±0.3 ^a	20.9±0.3 ^b
Titrateable acidity (g L ⁻¹)	2.8±0.02 ^a	4.4±0.06 ^b	4.2±0.1 ^a	4.5±0.1 ^b
pH	4.0±0.02 ^b	3.8±0.01 ^a	3.5±0.02	3.4±0.03
Glucose (g L ⁻¹)	122±1 ^a	152±2 ^b	100±1 ^a	131±3 ^b
Fructose (g L ⁻¹)	109±1 ^a	137±1 ^b	96.3±0.9 ^a	125±3 ^b
Tartaric acid (g L ⁻¹)	2.8±0.1 ^a	4.9±0.4 ^b	2.2±0.3 ^a	2.5±0.2 ^b
Malic acid (g L ⁻¹)	3.9±0.2 ^a	5.2±0.2 ^b	5.3±0.7 ^b	4.7±0.6 ^a
Citric acid (g L ⁻¹)	0.03±0.002 ^a	0.04±0.002 ^b	0.05±0.006 ^a	0.06±0.007 ^b
Wine				
Alcohol (% vol.)	12.5±0.3 ^a	13.1±0.2 ^b	11.3±0.1 ^a	12.3±0.3 ^b
Total acidity (g L ⁻¹)	3.8±0.2 ^a	4.8±0.4 ^b	5.1±1.2	5.9±0.4
pH	3.81±0.3	3.71±0.1	3.41±0.2	3.34±0.3
Total extract (g L ⁻¹)	20.8±1.2 ^a	25.3±1.8 ^b	18.7±0.4	21.1±0.8
Residual sugar (g L ⁻¹)	1.2±0.1	1.5±0.2	1.3±0.7	2.1±0.3
Tartaric acid (g L ⁻¹)	1.9±0.2	1.8±0.1	1.4±0.3	2.2±0.4
Malic acid (g L ⁻¹)	0.3±0.0 ^a	2.7±0.3 ^b	0.8±0.3 ^a	3.1±0.7 ^b
Lactic acid (g L ⁻¹)	1.2±0.1 ^b	0.2±0.0 ^a	1.7±0.6 ^b	0.4±0.1 ^a

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean ± SE. C: control (common annual practice); DMR: Double Maturation Raisonnée.

Fluka Chemie GmbH (Buchs, Switzerland); citric acid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-xyloside and naringenin from Sigma-Aldrich Chemical (St. Louis, MO, USA); malic acid and gallic acid from Merck KGaA (Darmstadt, Germany) and isorhamnetin-3-*O*-glucoside from Extrasynthèse (Lyon, France). 1% (w/v) butylated hydroxyl-toluene (BHT) from Sigma-Aldrich GmbH was used to prevent the oxidation of individual phenolics. To determine total phenolic content, Folin-Ciocalteu reagent from Merck KGaA (Darmstadt, Germany) was used. Methanol was from Sigma-Aldrich GmbH. The chemicals used for the mobile phases were acetonitrile HPLC-MS grade and formic acid from Sigma-Aldrich GmbH (St. Louis, MO, USA). Water was purified and double distilled with a Milli-Q-system (Millipore, Bedford, MA, USA).

Statistical analysis

Statistical analysis was carried out using a Statgraphics Centurion XV program (Statpoint Technologies Inc., Warrenton, Virginia, USA). Differences between treatments were assessed with one-way analysis of variance (ANOVA). Significant means were determined using Fisher's least significant difference (LSD) multiple range test at 95% confidence level ($P \leq 0.05$) and significant differences are denoted by different letters.

Results

Grape characteristics

DMR significantly altered most of 'Rebula' and 'Vitovska grganja' grape characteristics in both experimental years (Tables 1 and 2). 'Rebula' grapes were subjected to partial dehydration for 19 and 20 days and 'Vitovska grganja' grapes for 14 and 15 days, depending on the vintage. The single berry weight loss from the day when the cane cut was done till harvest of DMR berries/day amounted 2.5 g ('Rebula') and

0.8 g ('Vitovska grganja'). Correspondingly, DMR significantly decreased the weight of 100 berries in 'Vitovska grganja' (from 6.2 to 10.2 g, depending on the year). However, a significant decrease in the weight of 100 DMR 'Rebula' berries has only been recorded in the first experimental year. As expected, DMR significantly increased soluble solids content in both varieties and both years. DMR 'Rebula' berries were characterized by 1.45 higher °Brix and DMR 'Vitovska grganja' berries by 2.65 °Brix in comparison with the control. Moreover, titratable acidity of DMR berries was significantly increased compared to the control: for 0.95 g L⁻¹ in 'Rebula' and for 0.45 g L⁻¹ in 'Vitovska grganja', irrespective of the vintage. Conversely, pH value of DMR berries was lower compared to the control berries, but the differences were only significant in the first year in both analyzed varieties (Tables 1 and 2). A detailed analysis of individual sugars revealed a consistent impact of DMR on berry sugar composition. Glucose and fructose were the main individual sugars in 'Rebula' and 'Vitovska grganja' berries and their contents increased for an average of 27 g L⁻¹ and 22.9 g L⁻¹ in DMR berries. The most abundant organic acid in 'Rebula' berries was malic acid, but in 'Vitovska grganja' tartaric acid (3.8 to 4.7 g L⁻¹) represented the main share of organic acids. As expected, DMR generally increased the contents of tartaric, malic and citric acids in both varieties and the effect was particularly strong in 2012.

Wine composition

Irrespective of the year, DMR significantly increased the alcohol content in 'Rebula' (12.7% vol.) and 'Vitovska grganja' (12.9% vol.) wines (Tables 1 and 2). As expected, total acidity of wines was slightly increased in DMR treatment, although the significance has only been determined for 'Rebula' wine in 2012. In accordance with an increase in total acidity, a subsequent decrease in pH value has been observed

TABLE 2. General characteristics of 'Vitovska grganja' berries at harvest and 'Vitovska grganja' wine of two consecutive vintages.

	2012		2013	
	C	DMR	C	DMR
Berries				
Weight of 100 berries (g)	167±3 ^b	150±2 ^a	193±4.2 ^b	181 ± 4.2 ^a
Total soluble solids (°Brix)	17.7±0.2 ^a	21.8±0.5 ^b	20.8±0.2 ^a	22.0 ± 0.1 ^b
Titratable acidity (g L ⁻¹)	4.0±0.2 ^a	4.5±0.1 ^b	3.8±0.04 ^a	4.1 ± 0.06 ^b
pH	3.6±0.04 ^b	3.4±0.01 ^a	3.5±0.05	3.5 ± 0.04
Glucose (g L ⁻¹)	116±1 ^a	150±3 ^b	109±9 ^a	120 ± 12 ^b
Fructose (g L ⁻¹)	112±1 ^a	138±2 ^b	102±8 ^a	111 ± 12 ^b
Tartaric acid (g L ⁻¹)	4.5±0.2 ^a	4.7±0.2 ^b	3.8±0.2	3.9 ± 0.1
Malic acid (g L ⁻¹)	1.9±0.07 ^a	2.2±0.04 ^b	1.6±0.1 ^a	2.3 ± 0.1 ^b
Citric acid (g L ⁻¹)	0.1±0.02 ^a	0.2±0.01 ^b	0.01±0.002	0.01 ± 0.005
Wine				
Alcohol (% vol.)	10.4±0.1 ^a	12.9±0.4 ^b	12.3±0.2 ^a	13.0±0.2 ^b
Total acidity (g L ⁻¹)	5.6±0.2	6.0±0.3	5.2±1.2	5.5±0.3
pH	3.51±0.2	3.42±0.1	3.48±0.3	3.52±0.1
Total extract (g L ⁻¹)	18.1±1.4 ^a	22.3±1.4 ^b	19.8±0.4 ^a	24.1±1.4 ^b
Residual sugar (g L ⁻¹)	1.2±0.0 ^a	2.5±0.1 ^b	1.7±0.4	2.3±0.7
Tartaric acid (g L ⁻¹)	3.2±0.2 ^b	2.1±0.1 ^a	2.2±0.3	1.8±0.4
Malic acid (g L ⁻¹)	0.6±0.1 ^a	3.2±0.4 ^b	0.4±0.3 ^a	3.1±0.2 ^b
Lactic acid (g L ⁻¹)	1.7±0.0 ^b	0.6±0.0 ^a	1.8±0.3 ^b	0.2±0.2 ^a

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean±SE. C: control (common annual practice); DMR: Double Maturation Raisonnée.

in 'Rebula' wines prepared from DMR grapes in 2012. Total extracts were significantly increased in 'Vitovska grganja' DMR wine of both vintages and in 'Rebula' wine of 2012 vintage. Interestingly, DMR slightly (but not significantly) increased the contents of residual sugars, which ranged from 1.5 to 2.5 g L⁻¹ irrespective of the variety and vintage. The content of tartaric and lactic acids in 'Rebula' and 'Vitovska grganja' wines was not affected by the treatment. Contrary, significantly higher levels of malic acid have been recorded in 'Rebula' (from 3.8 to 9.0 fold higher) and 'Vitovska grganja' (from 5.3 to 7.7 fold higher) wines compared to the control in both vintages.

Phenolic composition of berry skin

Twenty-one and nineteen different phenolic compounds have been identified and quantified in berry skins of 'Rebula' and 'Vitovska grganja' varieties, respectively (Tables 3 and 4). The results showed a significant impact of DMR on the accumulation of individual phenolic compounds (but not on

the proportions), which was subsequently reflected in total phenolic content (TPC). Irrespective of the vintage, flavanols were the most abundant group in 'Rebula' berry skin in terms of the total content of each phenolic group. On the other hand, 'Vitovska grganja' berry skin contained highest levels of total flavanols. The content of *p*-Coumaric acid hexoside was significantly increased by DMR treatment compared to the control, irrespective of the variety and year. Coumaric acid was the main HCA in 'Rebula' berry skin (comprising from 51.1 to 55.2% HCA) and *p*-Coumaric acid hexoside was the most abundant HCA in 'Vitovska grganja' (representing from 69.9 to 77.1% HCA). A most significant impact of DMR was exhibited on the levels of individual flavanols, especially procyanidins in 'Vitovska grganja'. However, their share in terms of total flavanols remained unaffected by the treatment. Procyanidin dimer 1 was the most abundant flavanol in 'Rebula' berry skin (detected in levels from 63.5 to 143 mg kg⁻¹) and procyanidin trimer 4 prevailed in 'Vitovska grganja' (from 33.1 to 72.1 mg kg⁻¹). The impact of DMR on individual fla-

TABLE 3. Phenolic composition of 'Rebula' berry skin (mg kg⁻¹ ± SE) and their percentage (%) according to the corresponding phenolic group in two consecutive vintages.

	2012				2013				PI
	C	%	DMR	%	C	%	DMR	%	
<i>p</i> -Coumaric acid hexoside	1.4±0.1 ^a	47.9	1.8±0.1 ^b	44.8	1.5±0.1 ^a	46.0	2.2±0.1 ^b	48.9	↑**
Coumaric acid	1.5±0.1 ^a	52.1	2.7±0.2 ^b	55.2	1.7±0.1 ^a	54.0	2.1±0.3 ^b	51.1	↑**
Total hydroxycinnamic acids	2.8±0.1^a	100	4.6±0.2^b	100	3.9±0.2	100	4.2±0.3	100	↑*
Catechin	47.3±2.8 ^b	12.0	28.1±4.2 ^a	9.9	34.9±3.6 ^a	6.3	45.1±3.7 ^b	6.3	↓↑*
Procyanidin dimer 1	63.5±3.5 ^a	19.2	78.8±10.3 ^b	18.4	115±9 ^a	20.7	143±3 ^b	20.5	↑**
Procyanidin dimer 2	59.8±4.8 ^a	18.0	89.3±3.2 ^b	18.9	108±2 ^a	19.6	136±4 ^b	19.4	↑**
Procyanidin trimer 1	44.5±1.7 ^a	13.4	60.9±1.4 ^b	13.6	60.6±3.9 ^a	10.8	76.4±3.2 ^b	10.8	↑**
Procyanidin trimer 2	9.1±0.6 ^b	2.7	8.3±0.6 ^a	2.3	35.2±3.7	6.3	38.6±2.6	5.9	↓*
Procyanidin trimer 3	22.9±1.1 ^a	6.9	31.4±2.1 ^b	7.0	25.9±2.5 ^a	4.6	31.9±2.3 ^b	4.6	↑**
Procyanidin trimer 4	32.1±0.8 ^a	9.7	53.7±5.3 ^b	10.7	101±3 ^a	18.3	129±5 ^b	18.3	↑**
Procyanidin tetramer 1	43.7±3.2 ^a	13.1	58.9±4.4 ^b	13.2	43.3±3.3 ^a	7.8	75.1±3.4 ^b	9.0	↑**
Procyanidin tetramer 2	16.1±0.6 ^a	4.9	31.3±2.6 ^b	5.8	30.3±1.9	5.5	35.2±2.1	5.2	↑*
Total flavanols	331±9^a	100	462±17^b	100	580±18^a	100	708±9^b	100	↑**
Apigenin glycoside	1.7±0.1 ^a	0.7	3.1±0.3 ^b	0.8	3.3±0.6 ^a	0.9	5.8±0.6 ^b	1.0	↑**
Dihydroquercetin-rhamnoside	2.2±0.2 ^a	0.8	3.5±0.6 ^b	0.9	3.4±0.3 ^a	1.0	6.1±0.7 ^b	1.1	↑**
Isorhamnetin-3-glucoside	6.0±0.2 ^a	2.3	10.3±1.1 ^b	2.7	11.6±0.4 ^a	3.3	15.8±0.5 ^b	2.9	↑**
Kaempferol-3-glucoside	60.9±4.1 ^a	23.5	97.7±6.6 ^b	25.3	83.9±4.6 ^a	24.3	102.9±6.2 ^b	18.8	↑**
Kaempferol-3-glucuronide	1.7±0.1 ^a	0.7	3.2±0.2 ^b	0.8	3.5±0.3 ^a	1.0	6.4±0.5 ^b	1.2	↑**
Kaempferol-3-rutinoside	1.2±0.2 ^a	0.5	2.3±0.1 ^b	0.6	2.5±0.5 ^b	0.7	2.1±0.2 ^a	0.4	↓↑*
Quercetin-3-glucoside	146±4 ^a	56.6	200±4 ^b	52.1	202±16 ^a	57.1	350±33 ^b	62.4	↑**
Quercetin-3-glucuronide	30.3±3.7 ^a	11.7	50.9±2.6 ^b	13.2	17.6±2.1 ^a	4.9	46.4±4.2 ^b	8.3	↑**
Quercetin-3-rutinoside	5.5±0.9 ^a	2.1	10.9±0.4 ^b	2.8	22.9±4.4 ^b	5.2	18.7±3.1 ^a	2.8	↓↑*
Quercetin-3-xyloside	2.9±0.4	1.1	2.5±0.4	0.6	1.6±0.1 ^a	0.5	2.7±0.1 ^b	0.5	↑*
Total flavonols	258±4^a	100	384±7^b	100	353±21^a	100	557±31^b	100	↑*
TPC	667±76^a		860±70^b		854±71		1112±125		↑*

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean ± SE and as proportion (%) of each phenolic compound according to total value of the corresponding phenolic group within individual treatment. C – control (common annual practice); DMR – Double Maturation Raisonnée. TPC – total phenolic content (mg GAE kg⁻¹). PI – potential impact: ↑* or ↓* – potential increase or decrease in DMR berries compared to control in a single year (vintage). ↑** or ↓** – potential increase or decrease in DMR berries compared to control in both years (vintages). ↑↓* – potential increase and decrease in DMR berries compared to control in a single year. ↓↑* – potential decrease and increase in DMR berries compared to control in a single year.

vonols was more prominent in 'Rebula' berry skin, compared to 'Vitovska grganja' (Tables 3 and 4). Quercetin-3-glucoside was the major flavonol in 'Rebula' (comprising 52.7 to 62.5% total flavonols) and 'Vitovska grganja' (51.7 to 62.4%) berry skin. Quercetin-3-rutinoside was the most affected flavonol by DMR treatment as twofold levels have been recorded in 'Rebula' berry skin in 2012 (5.4 mg L⁻¹). DMR berries were namely characterized by significantly higher levels of total phenolics compared to berries from the control vines (Tables 3 and 4). Regarding the latter, only TPC of 'Rebula' berry skin in 2013 resulted lower than the sum of all phenolic groups presented (Table 3).

Phenolic composition of wine

DMR generally increased the levels of most phenolics in 'Rebula' and 'Vitovska grganja' wines in 2012 and 2013 vintages (Table 5 and 6) compared to control wines. Flavanols were the most abundant phenolic group in 'Rebula' (29.3 to 44.3 mg L⁻¹) and 'Vitovska grganja' (70.2 to 136 mg L⁻¹) wines,

followed by hydroxycinnamic acids, hydroxybenzoic acids, flavonols and stilbenoids. Protocatechuic acid was the most abundant hydroxybenzoic acid in 'Rebula' wine (3.1 to 6.4 mg L⁻¹) and a significant increase of its content has been detected in wine from 2012 DMR treatment. Moreover, DMR significantly increased the levels of protocatechuic acid in 'Vitovska grganja' wine in both years compared to the control. From the hydroxycinnamic acids (HCA), caftaric acid was the most abundant in 'Rebula' wine (4.5 to 10.6 mg L⁻¹); however, DMR increased its levels only in the first year. Similarly, 'Vitovska grganja' wine was also characterized by highest levels of caftaric acid (5.6 to 15.4 mg L⁻¹), which represented from 41.2 to 57.8% total HCA. The second most abundant HCA in 'Rebula' wine was *p*-Coumaric acid derivative 1 (8.4 to 9.6 mg L⁻¹). DMR treatment increased its contents but decreased the proportions in terms of total HCA only in 2013. On the other hand, DMR did not increase the share of selected HCA in 'Vitovska grganja' wine, with the exception of caftaric acid (9.4% increase) and *p*-Coumaric acid derivative 2 (4.5% in-

TABLE 4. Phenolic composition of 'Vitovska grganja' berry skin (mg kg⁻¹ ± SE) and their percentage (%) according to the corresponding phenolic group in two consecutive vintages.

	2012				2013				PI
	C	%	DMR	%	C	%	DMR	%	
<i>p</i> -Coumaric acid hexoside	1.1±0.1 ^a	77.1	1.4±0.7 ^b	77.1	0.4±0.01 ^a	69.9	0.6±0.03 ^b	71.1	↑**
Caftaric acid	0.3±0.03	22.9	0.4±0.02	22.9	0.2±0.01	30.1	0.3±0.02	28.9	
Total hydroxycinnamic acids	1.4±0.1^a	100	1.8±0.1^b	100	0.6±0.02^a	100	0.9±0.05^b	100	↑**
Catechin	5.9±0.5 ^a	4.9	7.5±1.2 ^b	5.2	6.1±0.3 ^a	3.5	7.6±0.5 ^b	3.5	↑**
Epicatechin	3.3±0.3 ^a	2.8	4.2±0.2 ^b	2.9	1.2±0.1 ^a	0.7	1.8±0.2 ^b	0.8	↑**
Procyanidin dimer	27.3±2.6 ^a	22.6	36.3±3.1 ^b	25.7	34.8±2.4 ^a	20.1	37.6±4.0 ^b	17.4	↑**
Procyanidin tetramer 1	17.2±2.1	14.2	16.9±1.1	11.9	26.5±1.3 ^a	15.3	28.4±2.6 ^b	13.2	↑*
Procyanidin tetramer 2	6.2±0.4 ^a	5.1	7.1±0.4 ^b	4.9	11.4±1.0 ^a	6.6	15.2±0.9 ^b	7.0	↑**
Procyanidin trimer 1	12.9±0.3 ^a	10.8	16.2±0.7 ^b	11.5	5.5±0.2 ^a	3.2	8.3±0.4 ^b	3.8	↑**
Procyanidin trimer 2	7.5±0.5 ^a	6.2	10.7±0.7 ^b	7.5	20.5±1.6 ^a	11.8	29.6±3.4 ^b	13.7	↑**
Procyanidin trimer 3	7.2±0.5 ^a	6.0	9.2±0.7 ^b	6.5	10.3±0.6 ^a	6.0	15.4±0.7 ^b	7.2	↑**
Procyanidin trimer 4	33.1±1.7	27.4	33.6±1.7	23.7	56.6±1.6 ^a	32.8	72.1±4.8 ^b	33.3	↑*
Total flavanols	120±5^a	100	141±6^b	100	173±4^a	100	215±6^b	100	↑**
Isorhamnetin-3-glucoside	3.4±0.4 ^a	0.7	4.8±0.7 ^b	0.7	7.7±1.0 ^a	1.2	10.8±0.4 ^b	1.6	↑**
Kaempferol-3-glucoside	103.5±7.5 ^a	21.6	108±2 ^b	20.1	115±13 ^b	18.0	83.4±13.7 ^a	11.8	↓↑*
Kaempferol-3-glucuronide	7.2±0.8 ^b	1.1	5.8±0.7 ^a	1.1	7.5±0.5	1.2	10.4±0.8	1.5	↓*
Kaempferol-3-rutinoside	2.9±0.4 ^a	0.5	3.9±0.8 ^b	0.4	2.2±0.1 ^a	0.3	3.2±0.1 ^b	0.5	↑**
Quercetin-3-glucoside	248±18 ^a	51.7	321±22 ^b	58.7	365±28 ^a	56.8	431±35 ^b	62.5	↑**
Quercetin-3-glucuronide	98.9±12.1	18.4	103±12	14.8	115±10 ^a	18.1	119±12 ^b	17.1	↑*
Quercetin-3-rutinoside	27.3±2.6 ^b	4.8	21.5±4.1 ^a	3.3	27.1±1.4 ^a	4.3	33.1±1.6 ^b	4.8	↓↑*
Quercetin-3-xyloside	2.2±0.3 ^a	0.4	3.1±0.6 ^b	0.3	0.9±0.1 ^a	0.1	1.5±0.2 ^b	0.2	↑**
Total flavonols	493±36^a	100	571±33^b	100	642±27	100	693±56	100	↑*
TPC	724±70^a		901±94^b		744±59^a		952±93^b		↑**

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean ± SE and as proportion (%) of each phenolic compound according to total value of the corresponding phenolic group within individual treatment. C – control (common annual practice); DMR – Double Maturation Raisonnée. TPC – total phenolic content (mg GAE kg⁻¹). PI – potential impact: ↑* or ↓* – potential increase or decrease in DMR berries compared to control in a single year (vintage). ↑** or ↓** – potential increase or decrease in DMR berries compared to control in both years (vintages). ↑↓* – potential increase and decrease in DMR berries compared to control in a single year. ↓↑* – potential decrease and increase in DMR berries compared to control in a single year.

crease) in 2013. Moreover, DMR increased the proportions of procyanidin dimer, procyanidin trimer 2 and procyanidin trimer 3 in 'Rebula' wine, irrespective of the year. Contrary, the shares of catechin, epicatechin and procyanidin dimer 1 were most affected by DMR in 'Vitovska grganja' wine. Additionally, DMR increased the contents of two and four flavanol compounds, depending on the variety. The most abundant flavonols were laricitrin-3-xyloside ('Rebula' wine) and kaempferol-diglucoside ('Vitovska grganja' wine). From the group of stilbenoids, *cis*- and *trans*-Resveratrol-3-glucoside were identified and quantified; the latter was the main stilbenoid in both varieties. DMR positively affected resveratrol

levels in analyzed wines of both vintages, with the exception of *cis*-resveratrol-3-glucoside in 2012 'Rebula' wine. Lastly, higher levels of total phenolic compounds (TPC) have been observed in wines prepared from DMR grapes compared to the control.

Discussion

Vineyard practices are one of the most important factors affecting grape composition and ripening, which additionally depend on the variety and climatic conditions during the season (Koundouras et al., 2006; Mazza et al., 1999). DMR increases water transpiration through the berry skin, which is

TABLE 5. Phenolic composition of 'Rebula' wine (mg L⁻¹ ± SE) and their percentage (%) according to the corresponding phenolic group in two consecutive vintages.

	2012				2013				PI
	C	%	DMR	%	C	%	DMR	%	
Gallic acid	4.3±0.1 ^a	36.5	6.2±0.2 ^b	35.3	1.5±0.02 ^a	21.6	1.6±0.02 ^b	21.4	↑**
Protocatechuic acid	3.1±0.5 ^a	25.9	6.4±0.2 ^b	29.8	4.6±0.06	61.1	4.5±0.03	60.2	↑*
Syringic acid ethyl gallate	4.5±0.6 ^a	37.6	5.8±0.7 ^b	34.9	1.2±0.1 ^a	17.2	1.5±0.2 ^b	18.4	↑**
Total hydroxybenzoic acids	7.4±0.6^a	100	12.6±0.4^b	100	4.4±0.06	100	4.5±0.03	100	↑*
Caffeic acid	2.4±0.1 ^a	9.7	3.5±0.5 ^b	9.8	2.8±0.3 ^b	11.1	2.3±0.2 ^a	11.8	↑↓*
Caftaric acid	4.5±0.2 ^a	18.5	10.6±0.3 ^b	23.6	4.6±0.6	22.3	4.7±0.1	21.8	↑*
<i>cis</i> -Coumaric acid	1.2±0.04 ^a	4.8	2.2±0.08 ^b	5.3	0.3±0.04 ^a	1.4	0.4±0.05 ^b	1.5	↑**
<i>p</i> -Coumaric acid derivative 1	9.6±0.1 ^b	39.1	8.6±0.1 ^a	32.5	8.4±0.1 ^a	40.7	9.1±0.8 ^b	40.6	↑↓*
<i>p</i> -Coumaric acid derivative 2	1.8±0.1 ^a	7.6	2.4±0.1 ^b	7.2	1.9±0.05	9.4	1.7±0.08	8.6	↑*
<i>p</i> -Coumaric acid derivative 3	2.7±0.1 ^a	10.9	4.1±0.1 ^b	11.2	2.0±0.1 ^a	9.9	2.3±0.1 ^b	10.0	↑**
<i>p</i> -Coumaric acid derivative 4	0.1±0.02 ^a	0.5	0.3±0.04 ^b	0.6	0.1±0.01	0.3	0.1±0.01	0.3	↑*
<i>p</i> -Coumaric acid derivative 5	0.4±0.02 ^a	1.5	0.6±0.06 ^b	1.6	0.4±0.04 ^a	2.0	0.5±0.01 ^b	2.1	↑**
<i>trans</i> -Coumaric acid	1.1±0.04 ^a	4.5	2.0±0.08 ^b	5.1	0.5±0.06 ^a	2.2	0.6±0.05 ^b	2.4	↑**
<i>trans</i> -Fertaric acid	0.7±0.02 ^a	3.0	1.2±0.04 ^b	3.2	0.1±0.05	0.6	0.2±0.01	0.8	↑*
Total hydroxycinnamic acids	24.6±0.5^a	100	35.6±1.3^b	100	20.6±1.2^a	100	22.4±1.2^b	100	↑**
Catechin	3.2±0.6 ^a	4.5	4.5±0.9 ^b	4.0	5.0±1.1 ^a	7.7	7.1±0.8 ^b	7.8	↑**
Epicatechin	0.7±0.03 ^a	1.0	1.3±0.1 ^b	1.0	1.1±0.3	1.7	1.1±0.2	1.5	↑*
Naringenin	1.8±0.1 ^a	2.6	3.4±0.2 ^b	2.6	2.8±0.1	4.4	2.7±0.1	3.9	↑*
Procyanidin dimer	8.8±1.8 ^a	12.3	24.6±5.7 ^b	14.5	6.5±0.9 ^a	10.0	11.5±1.1 ^b	11.3	↑**
Procyanidin tetramer	8.1±0.4 ^a	11.6	14.1±1.4 ^b	11.0	1.6±0.1 ^a	2.5	2.2±0.2 ^b	2.5	↑**
Procyanidin trimer 1	21.8±0.3 ^a	31.2	27.8±0.5 ^b	26.5	25.8±0.4 ^a	39.9	35.4±0.4 ^b	40.0	↑**
Procyanidin trimer 2	4.7±0.1 ^a	6.7	12.4±1.7 ^b	7.7	6.8±0.6 ^a	10.4	10.2±0.6 ^b	10.8	↑**
Procyanidin trimer 3	21.2±1.1 ^a	30.2	48.8±2.5 ^b	32.7	14.8±0.9 ^a	22.8	17.6±0.5 ^b	21.6	↑*
Total flavanols	70.2±3.8^a	100	136±12^b	100	64.9±3.3^a	100	88.6±2.2^b	100	↑**
Laricitrin-3-pentoside	0.2±0.02 ^a	42.1	0.3±0.01 ^b	24.9	3.3±0.1 ^a	66.1	5.1±0.1 ^b	62.8	↑**
Laricitrin-3-xyloside	0.3±0.02 ^b	30.0	0.2±0.02 ^a	20.3	0.5±0.1 ^a	17.4	0.6±0.03 ^b	17.4	↑↓*
Quercetin-3-glucoside	0.4±0.01 ^b	9.9	0.2±0.12 ^a	10.0	0.03±0.01	1.6	0.03±0.01	1.3	↓*
Dihydroquercetin-3-rhamnoside	0.02±0.01 ^a	3.6	0.06±0.01 ^b	4.7	0.1±0.01	4.8	0.1±0.02	4.5	↑*
Quercetin-3-glucuronide	0.1±0.04 ^a	14.5	0.6±0.2 ^b	40.1	0.2±0.1 ^a	10.1	0.6±0.1 ^b	14.0	↑**
Total flavonols	0.5±0.1^a	100	1.4±0.4^b	100	4.5±0.4^a	100	7.3 ± 0.1^b	100	↑**
<i>cis</i> -Resveratrol-3-glucoside	0.1±0.01	20.7	0.1±0.02	24.0	0.2±0.06 ^a	23.2	0.5±0.04 ^b	24.5	↑*
<i>trans</i> -Resveratrol-3-glucoside	0.2±0.02 ^a	79.3	0.3±0.03 ^b	76.0	0.9±0.1 ^a	76.8	1.3±0.1 ^b	75.5	↑*
Total stilbenoids	0.2±0.03^a	100	0.4±0.04^b	100	1.0±0.2^a	100	1.8±0.1^b	100	↑*
TPC	252±8^a		382±38^b		188±13^a		218±10^b		↑**

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean±SE and as proportion (%) of each phenolic compound according to total value of the corresponding phenolic group within individual treatment. C – control (common annual practice); DMR – Double Maturation Raisonnée. TPC – total phenolic content (mg GAE kg⁻¹). PI – potential impact: ↑* or ↓* – potential increase or decrease in DMR berries compared to control in a single year (vintage). ↑** or ↓** – potential increase or decrease in DMR berries compared to control in both years (vintages). ↑↓* – potential increase and decrease in DMR berries compared to control in a single year. ↓↑* – potential decrease and increase in DMR berries compared to control in a single year.

reflected in a significantly lower weight of 100 berries compared to control berries (Cargnello et al., 2005). Moreover, transpiration increases the concentration of soluble solids, individual sugars (glucose, fructose) and organic acids in grapes produced on DMR treated vines (Peršurić et al., 2000). Therefore, superior grape chemical characteristics lead to improved wine composition since the alcohol content greatly depends on the amount of soluble solids (Prajitna et al., 2007). In addition, alcohol content influences the body of the wine, making wines with higher alcohol levels more complex (Garrido and Borges, 2013). Although the differences were not significant, 'Rebula' DMR berries were characterized by increased contents of tartaric acid (Table 1), which is similar to the reports by Corso et al. (2013), who determined higher acidity of DMR berries compared to the control due to slow malic and tartaric acid catabolism. Moreover, organic acids in wine strongly affect the palatability, taste and freshness of the wines and change their stability during aging (Bauer and Dicks, 2004). Since tartaric and malic acids emerge into the wine from berries during the fermentation process, their content is mainly pre-determined (Conde et al., 2007). Our

results indicate that DMR even reduced the content of lactic acid in wine (Tables 1 and 2), which suggests that the spontaneous malolactic fermentation did not occur, since higher contents of lactic acid are usually the result of malolactic fermentation, and which (if it occurs spontaneously) potentially leads to wine spoilage (Bauer and Dicks, 2004). Related to that, increased alcohol content in DMR wine (Tables 1 and 2) probably inhibited the growth and metabolism of lactic acid bacteria, the main promotor of malolactic fermentation (Bauer and Dicks, 2004). In our study and compared to the reports by Bauer and Dicks (2004), a significant decrease in pH of berry must was not observed (Tables 1 and 2), likely due to the initially low acidity of the samples (Koruza et al., 2012). Therefore, a direct impact of DMR on the pH value of the must is difficult to confirm as it depends on absolute changes in the acidity. In accordance to our results (Tables 1 and 2), Panceri et al. (2013) monitored the impact of 'Merlot' and 'Cabernet Sauvignon' off-vine drying and detected an increase in pH value with a concomitant decrease in must acidity. Therefore, this phenomenon has to be taken into account as it directly impacts the pH value of the produced wine.

TABLE 6. Phenolic composition of 'Vitovska grganja' wine (mg L⁻¹ ± SE) and their percentage (%) according to the corresponding phenolic group in two consecutive vintages.

	2012				2013				PI
	C	%	DMR	%	C	%	DMR	%	
Protocatechuic acid	2.9±0.1 ^a	100	3.6±0.1 ^b	100	2.4±0.02 ^a	100	3.3±0.02 ^b	100	↑**
Total hydroxybenzoic acids	2.9±0.1 ^a	100	3.6±0.1 ^b	100	2.4±0.02 ^a	100	3.3±0.02 ^b	100	↑**
Caftaric acid	9.8±0.8 ^a	41.2	15.4±1.6 ^b	41.2	5.6±0.8 ^a	48.4	8.5±0.4 ^b	57.8	↑**
cis-Coutaric acid	2.2±0.2 ^a	9.4	3.5±0.3 ^b	9.3	2.1±0.2	18.3	2.2±0.4	15.1	↑*
p-Coumaric acid derivative 1	0.5±0.02 ^a	2.3	0.8±0.02 ^b	2.4	0.2±0.004 ^a	2.1	0.3±0.005 ^b	1.8	↑**
p-Coumaric acid derivative 2	8.7±0.3 ^a	36.9	13.2±0.8 ^b	35.8	1.5±0.2 ^a	13.2	1.9±0.5 ^b	17.7	↑**
trans-Coutaric acid	1.9±0.1 ^a	7.9	3.5±0.3 ^b	9.2	1.3±0.01	11.6	1.3±0.02	9.0	↑*
trans-Fertaric acid	0.6±0.1 ^a	2.3	0.8±0.1 ^b	2.2	0.7±0.1 ^b	6.4	0.5±0.1 ^a	3.7	↑↓*
Total hydroxycinnamic acids	23.7±1.1 ^a	100	37.2±2.8 ^b	100	11.5±0.7 ^a	100	14.7±1.3 ^b	100	↑**
Catechin	2.8±0.1 ^a	9.6	5.0±0.1 ^b	11.4	5.5±0.5 ^a	15.8	7.2±0.4 ^b	16.7	↑**
Epicatechin	0.9±0.1 ^a	2.3	1.2±0.1 ^b	2.4	1.1±0.1	3.1	1.2±0.2	2.7	↑*
Procyanidin dimer 1	4.1±0.2 ^a	11.1	5.8±0.3 ^b	11.5	6.2±0.4 ^a	17.5	6.8±0.1 ^b	15.9	↑**
Procyanidin dimer 2	2.6±0.2 ^a	6.9	2.9±0.6 ^b	5.7	2.1±0.1 ^a	5.9	2.4±0.3 ^b	5.6	↑**
Procyanidin dimer 3	18.9±1.5 ^a	50.9	29.3±2.2 ^b	57.7	20.3±1.1 ^a	57.7	25.4±1.9 ^b	59.1	↑**
Total flavanols	29.3±1.9 ^a	100	44.3±2.8 ^b	100	35.1±0.9 ^a	100	42.9±1.7 ^b	100	↑*
Quercetin dihexoside	1.0±0.1 ^a	29.2	1.5±0.2 ^b	21.5	1.0±0.1 ^a	35.1	1.5±0.2 ^b	35.3	↑**
Kaempferol diglucoside	1.9±1.0 ^b	25.0	1.4±0.1 ^a	10.6	1.9±1.0 ^b	45.6	1.4±0.1 ^a	41.9	↓**
Quercetin hexose glucuronide	0.4±0.1 ^a	10.7	0.8±0.1 ^b	11.9	0.4±0.1 ^a	13.1	0.8±0.1 ^b	14.9	↑**
Eriodictyol hexoside	0.1±0.04 ^a	30.9	0.2±0.03 ^b	49.2	0.1±0.04 ^a	2.8	0.2±0.03 ^b	3.6	↑**
Eriodictyol dihexoside	0.01±0.003	2.2	0.02±0.002	3.4	0.01±0.003	0.2	0.02±0.002	0.2	
Syringetin-3-glucoside	0.1±0.04 ^a	2.0	0.3±0.04 ^b	3.4	0.1±0.04 ^a	3.3	0.3±0.04 ^b	4.1	↑**
Total flavonols	1.9±0.4 ^a	100	3.3±0.2 ^b	100	3.6±1.0 ^a	100	4.3±0.4 ^b	100	↑**
cis-Resveratrol-3-glucoside	0.5±0.2 ^a	51.6	1.4±0.2 ^b	71.0	0.4±0.2 ^a	69.3	1.4±0.2 ^b	73.4	↑**
trans-Resveratrol-3-glucoside	0.2±0.5 ^a	48.4	0.3±0.1 ^b	29.0	0.2±0.1 ^a	30.7	0.3±0.1 ^b	26.6	↑**
Total stilbenoids	0.2±0.1 ^a	100	0.4±0.1 ^b	100	0.6±0.3 ^a	100	1.6±0.3 ^b	100	↑**
TPC	249± ^b		254± ^c		179± ^a		187± ^b		↑*

Different letters in columns at apex denote statistically significant differences (LSD test, $P < 0.05$) between treatments in a single year and the results are presented as mean±SE and as proportion (%) of each phenolic compound according to total value of the corresponding phenolic group within individual treatment. C – control (common annual practice); DMR – Double Maturation Raisonnée. TPC – total phenolic content (mg GAE kg⁻¹). PI – potential impact: ↑* or ↓* – potential increase or decrease in DMR berries compared to control in a single year (vintage). ↑** or ↓** – potential increase or decrease in DMR berries compared to control in both years (vintages). ↑↓* – potential increase and decrease in DMR berries compared to control in a single year. ↓↑* – potential decrease and increase in DMR berries compared to control in a single year.

Increased acidity is additionally reflected in lower pH value (Conde et al., 2007), which may be favorable in wine production as higher pH of must encourages the development of adverse bacteria and spontaneous malolactic fermentation of the wine (Bauer and Dicks, 2004).

Phenolics predominately accumulate in berry skin during ripening and their content at harvest largely depends on the variety, environmental conditions and ampelotechnic measures in the vineyards (Rolle et al., 2009; Fanzone et al., 2011). DMR is a delicate practice as only a proportion of canes on each vine may be cut in order to promote berry ripening and on-vine dehydration (Carbonneau and Murisier, 2009). Generally, the content of most phenolic compounds increased in berries, subjected to DMR in our study, compared to the control and irrespective of the variety and year (Tables 3 and 4). This can be ascribed to stress-like conditions of all grapes on DMR treated vines (Cargnello et al., 2005). The increase in contents of hydroxycinnamic acids in our study (Tables 3 and 4) is in accordance with Bonghi et al. (2012), suggesting the changes in phenylpropanoid metabolic pathway. Furthermore, stress conditions and dehydration provoke changes in water potential, leading to modified expression of enzymes and precursors in the phenylpropanoid pathway (Bonghi et al., 2012; Corso et al., 2013). Particularly *p*-Coumaric acid is involved in lignification, which is crucial for diminished water evaporation from berry skin. Increased flavonol contents in DMR berries (Tables 3 and 4) correspond to the findings of Deluc et al. (2009) and Bonghi et al. (2012), who explained their accumulation as a response to provoked stress conditions, which launch the activation of lignin pathway and increase gene expression in berry skin as a mechanism against stress. However, the increase of flavonols in DMR berries (Tables 3 and 4) can additionally be linked to their absorbance in UV-A and UV-B solar spectrum (Castillo-Muñoz et al., 2007). As DMR causes leaf wilting, the berries intercept more light. Moreover, Panceri et al. (2013) reported that dehydrated 'Cabernet Sauvignon' berries are abundant in flavonol compounds and contain particularly high levels of kaempferol and quercetin glycosides. A significant increase of the majority of individual flavanols in our study (Tables 3 and 4) could be ascribed to the concentration effect caused by water loss promoted by DMR (Panceri et al., 2013). Contrary, Rolle et al. (2009) and Bonghi et al. (2012) demonstrated that grape dehydration results in progressive oxidation and increased polyphenoloxidase activity, which consequently lead to the loss of flavanols and other phenolics. As enzyme activity was not monitored in the present study this pattern cannot be confirmed. However, it has to be taken into account that Rolle et al. (2009) and Bonghi et al. (2012) assessed postharvest dehydration when phenolic ripeness was at (or even past) its peak (Río Segade et al., 2008). Several authors (Río Segade et al., 2008; Hernández-Hierro et al., 2014) have indeed confirmed that phenolic compounds tend to degrade slowly after reaching their peak. This was not observed in our study (Tables 3 and 4). It is only worth to mention that the difference between TPC and the sum of phenolic groups determined in year 2013 (Table 3) may not be ascribed to DMR effect, but rather to the detection and quantification procedure. However, the results of phenolic turnover are in accordance with the reports of Corso et al. (2013) who measured a 25% increase of total polyphenols in DMR 'Raboso Piave' berries compared to the control.

DMR significantly impacted phenolic composition and contents of 'Rebula' (Table 5) and 'Vitovska grganja' wines (Table 6). Although hydroxybenzoic acids were only detected

in the wine, higher levels of protocatechuic acid in DMR wines (Tables 5 and 6) suggest a significant impact of this measure on its composition. The latter may be ascribed to increased skin/pulp ratio of DMR berries linked to their dehydration. It has namely been reported that hydroxycinnamic acids are mostly present in berry skin (Cargnello et al., 2005). Wine composition also depends on the variety and therefore, 'Vitovska grganja' wine only contained protocatechuic acid (Table 6) as opposed to 'Rebula' wine, which was additionally characterized by gallic acid and syringic acid ethyl gallate (Table 5). Flavonols generally represent a minor phenolic group in white wines (Monagas et al., 2005). According to Garrido and Borges (2013), laricitrin and syringetin-glycosides are not present in white wines since the flavonoid 3',5'-hydroxylase enzyme is not expressed in white grapevine varieties. However, these compounds have been detected in 'Rebula' and 'Vitovska grganja' wines in the present study but in very low levels (from 0.1 to 0.2 mg L⁻¹) (Tables 5 and 6), which is in accord to the report of Jeffery et al. (2008). Stilbenoids are a class of health-beneficial compounds found in wine, which tend to inhibit the onset and progression of cardiovascular diseases (Romero-Pérez et al., 1999, 2001). Interestingly, these compounds were only detected in analyzed wines (Tables 5 and 6) and not in berry skin. Skin generally contains very low amounts of resveratrol, which are below limits of detection on HPLC (Garrido and Borges, 2013). In contrast, Romero-Pérez et al. (2001) reported *cis*- and *trans*-resveratrol in berry skin. Furthermore, authors also claim that stress conditions increase the contents of resveratrol compounds in berry skin. This is the potential reason for augmented resveratrol contents in DMR wines (Tables 5 and 6). Moreover, several authors (Romero-Pérez et al., 2001; Garrido and Borges, 2013) linked the increase of resveratrol levels to fungal infections such as *Botrytis cinerea*. As DMR improves grape microclimate by leaf wilting it reduces fungal activity and their proliferation. The results suggest that DMR particularly affects the content of hydroxycinnamic acids and flavanols in wine (Tables 5 and 6). These are the main phenolic groups present in white wines and contribute to color, bitterness, antioxidant activity and possess beneficial impacts for our health (Monagas et al., 2005; Tourtoglou et al., 2014). Individual phenolic contents of 'Rebula' (Table 5) and 'Vitovska grganja' (Table 6) wines are in accordance with data reported for white wines by Makris et al. (2006) and Ružić et al. (2011). The differences in phenolic composition of wine in our study, can be according to Monagas et al. (2005) and Conde et al. (2007) attributed to diverse levels of phenolics in grape berries, which are specific for the variety and affected by vineyard conditions. Moreover, DMR may provoke faster phenolic ripening, leading to higher levels of phenolic compounds in berries (Conde et al., 2007). Correspondingly, Río Segade et al. (2008) and Hernández-Hierro et al. (2014) have set an experiment on assessing the phenolic ripeness and extraction of phenolic compounds (mostly anthocyanins) of DMR and control grapes into the must. It has been demonstrated that even short-term berry dehydration (such as DMR) modifies structural, mechanical and chemical properties of grape berry skin (Rolle et al., 2009), which might contribute to an easier release of phenolics into the must during grape processing. Furthermore, phenolic compounds are structurally modified and synthesized during the wine extraction and fermentation processes (Conde et al., 2007), which explains the incidence of wine-specific phenolic compounds.

The present study demonstrated the impact of DMR on grape and wine composition of 'Rebula' and 'Vitovska grganja'

ja' grapevine varieties. The significance of this practice has been observed on general grape composition (decreased weight of 100 berries and increased soluble solids as well as titratable acidity) caused by enhanced water transpiration of the berries on cut canes. DMR significantly affected the levels of individual sugars and organic acids in berries and alcohol and total extract contents in wine. DMR also altered the composition and contents of secondary metabolites in grape berries and wine. Hydroxycinnamic acids, flavanols and flavonols were significantly increased in DMR berries as a result of partial dehydration and potential modifications of berry metabolism. The changes in wine composition can mainly be ascribed to DMR as increased levels of hydroxybenzoic, hydroxycinnamic acids, flavanols and stilbenoids have been detected in DMR wine. The group of flavonols was less affected by DMR treatment. Further studies should be conducted to evaluate the impact of partial grape dehydration on phenolic ripening and plant stress as well as on the costs of DMR practicability in a production vineyard. The results promote the application of DMR technique to high-yielding varieties in order to improve their quality and produce dry wines with distinct styles.

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2.1.4 Vpliv tehnik delnega sušenja na sestavo metabolitov grozdja in vina sorte 'Refošk'

Mikulič-Petkovšek M., Jug T., Reščič J., Rusjan D. 2017. Effects of partial dehydration techniques on the metabolite composition in 'Refošk' grape berries and wine. Turkish Journal of Agriculture and Forestry, 41: 10-22

V poskusu smo ugotavljali vplive dvojnega namenskega zorenja grozdja (DMR) ter delnega sušenja grozdja v komori na kemijsko sestavo grozdja in vina sorte 'Refošk' (*Vitis vinifera* L.). Delno sušenje grozdja je že precej raziskan in uporabljen ukrep pri pridelavi sladkih vin, vendar manj za pridelavo suhih vin. Grozdje pod vplivom DMR je doseglo tehnološko zrelost 14 dni po rezi rozge, grozdje za delno sušenje v komori pa 22 dni po trgatvi. Grozdje sušeno v komori je doseglo večjo izgubo mase posameznih jagod (14 %) kot grozdje pod vplivom DMR (8 %). Ugotovili smo, da sta obe obravnavanji značilno povečali vsebnost titracijskih in skupnih kislin v grozdju in vinu, medtem ko je izrazito nizek pH doseglo grozdje iz DMR. Ukrep DMR je vplival na večjo vsebnost fenolnih spojin v grozdju in vinu, v primerjavi s kontrolo in delnim sušenjem v komori. Določili smo 1,6-krat večjo vsebnost skupnih flavonolov ter 1,7-krat večjo vsebnost skupnih antocianov v jagodni kožici iz DMR, v primerjavi z grozdem iz delnega sušenja. Večjo vsebnost skupnih hidroksicimetnih kislin in flavanolov, a zmanjšanje vsebnosti antocianov pa smo zabeležili v vinu iz grozdja, delno sušenega v komori. Raziskava je pokazala različen odziv metabolitov v grozdju in vinu na DMR in delno sušenje v komori, kar je povezano z različnim načinom dehidracije grozdnih jagod. Kljub temu pa bi se ukrep DMR grozdja lahko priporočal v praksi, za izboljšanje kemijske sestave grozdja in vina, tudi pri pridelavi suhih vin.



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Effects of partial dehydration techniques on the metabolite composition in 'Refošk' grape berries and wine*

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Abstract: The aim of the study was to investigate the impact of the on-vine double maturation raisonnée (DMR) and off-vine berry partial dehydration in the chamber (PDC) on the chemical characteristics of the grape and wine grapevine cultivar 'Refošk'. A liquid chromatography-mass spectrometry (LC-MS) system was used for the identification and quantification of phenolics and the HPLC for sugars and organic acids. Berries subjected to DMR partial dehydration reached technological maturity (18.5 °Brix) within 14 days of cane cutting and those under PDC in 22 days upon harvest. The greatest decline in berry weight was recorded in the PDC treatment (14%), followed by the DMR technique (approx. 8%). Both dehydrations significantly increased titratable and total acidity in berry and wine, while the pH level was significantly lower in DMR treated berries. Compared to PDC, DMR significantly increased the content of total flavonols and anthocyanins in berries and wine, as well as the total flavanols in berries and stilbenoids in wine. DMR treated berries contained 1.7-fold higher content of total anthocyanins, up to 2-fold higher content of total flavonols, and 1.6-fold higher total phenolic content in the skin compared to those from PDC treatment. Moreover, it increased the content of total anthocyanins, stilbenoids, and flavonol glycosides in wine. On the other hand, PDC treatment showed greater impact on phenolics in wine, especially with an increase of total hydroxycinnamic acids, flavanols, and total phenolics but with a decrease in anthocyanins contents. The study suggests that berries subjected to DMR respond differently to partial dehydration than those subjected to PDC, which was evident from the accumulation of secondary metabolites. Undoubtedly, DMR showed interesting results regarding berry and wine composition and therefore it could be introduced in the vineyard for production of dry wine as well.

Key words: Dehydration, anthocyanins, organic acids, phenolics, 'Refošk', sugars

1. Introduction

Grape quality is reflected in a balanced composition of primary and secondary metabolites, which constitute different parts of the fresh berries during their development and ripening. The accumulation of these metabolites within the berries is affected by genetic characteristics, cultivation practices, and climate conditions (Jackson and Lombard, 1993).

The phenolic compounds in the skins of red berry grapevine varieties are mostly anthocyanins, hydroxycinnamic acids, stilbenoids, flavanols, and flavonols, which are also the building blocks of the grapes' and wines' sensorial properties (Jackson and Lombard, 1993; Gonzalez-Alvarez et al., 2013). In red grapes, anthocyanins are responsible for the red color of the skins and consequently define the color and quality of the must and wine (Liang et al., 2008). Other polyphenols, such as flavonols and flavanols, mostly contribute to wine flavor and astringency (Li et al., 2009). Phenolic compounds are

biosynthesized through the phenylpropanoid and flavonoid pathways and originate from the amino acid precursor, phenylalanine. These pathways are among the most studied and thoroughly described secondary metabolic pathways in plants (Yang et al., 2012). Polyphenolic compounds play an important role in the sensorial quality of grapes and wines. Their transformation during the vinification process directly or indirectly influences the quality of the wine: its structure and sensorial properties (Cheynier, 2005; Noguerol-Pato et al., 2013).

In order to change the composition of fresh berries and improve the quality of the wines produced from these, different canopy techniques, especially training systems, bunch thinning, defoliation, dehydration etc., have been introduced in viticulture (Kyrleou et al., 2015; Liu et al., 2015). Grape dehydration, used for the production of late harvest, botrytized, and ice wines, represents a measure applied for the production of sweeter wines in general, in which the contents of soluble solids, phenolics, and aroma

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compounds are usually altered in respect to the dry wine (Figueiredo-Gonzalez et al., 2013).

Mencarelli and Tonutti (2013) have summarized common on- and off-vine grape (partial dehydration in chamber (PDC)) refinement techniques, usually used for dehydration and their significant impacts on grape composition. Sun-drying is the oldest used technique for off-vine grape dehydration, which increases the content of soluble solids and phenolics in grape berries, especially hydroxycinnamic acids (caftaric, coumaric, and ferulic acid), flavanols (catechin, epicatechin, and procyanidins) and flavonols (kaempferol and quercetin glycosides) (Mencarelli and Tonutti, 2013).

The double maturation raisonnée (DMR) or double reasoned maturation first reported by Cargnello et al. (1996) and later by Carbonneau and Murisier (2009) was introduced as an on-vine berry partial dehydration technique for the production of dry wines. The same authors claim that DMR accelerates wine aging especially regarding phenolics synthesis. Double reasoned maturation proposes the cutting (wounding) of bearing canes during ripening when the berries have reached the determined composition of soluble solids. After the cutting, the berries were left for partial dehydration on vines until technological ripeness is reached. Until today, studies of the DMR technique have demonstrated the method's capability of altering soluble solids, organic acids, and phenolic compounds in berries and wines (Bonghi et al., 2012; Corso et al., 2013). DMR is still considered a less-known viticultural technique although it might replace expensive grape partial dehydration in chambers.

'Refošk' (*Vitis vinifera* L.) is a high-yielding red grapevine cultivar traditionally cultivated in the neighboring wine regions of three countries, namely Friuli-Venezia Giulia (Italy), Primorska (Slovenia), and Hrvatska Istra (Croatia), where denominations 'Teran' and 'Terrano d'Istria' are also used for the cultivar 'Refošk'. Generally, 'Refošk' produces soft berries with a low individual berry weight (about 3 g) according to OIV descriptor 503 (OIV descriptors, 2001), colorless flesh with a moderate must yield (up to 75%), and a thin blackish-blue berry skin (average thickness up to 150 µm), which is rather susceptible to sun burns. Furthermore, high contents of phenolics are accumulated in the skins of this cultivar, especially anthocyanins and stilbenoids. Therefore, the wines produced from 'Refošk' are often promoted as abundant in resveratrol, which is supposed to have a positive impact on human health (de la Lastra and Villegas, 2007).

The aim of the present study was to investigate the effect of these two different partial dehydrations techniques, especially the DMR method, on the berry maturation of the high-yielding grapevine cultivar 'Refošk'. Since such a study has never been performed to date, the experiment

was conducted with a view to determine (i) the detailed phenolic fingerprint of the 'Refošk' berry skin and wine, (ii) the dynamics of phenol synthesis during berry ripening, and (iii) the specific responses of the primary and secondary metabolites in the berry skins to different techniques of partial dehydration, aimed to improve grape and wine quality.

2. Material and methods

2.1. Experiment

The experiment was carried out in 2013 on 12-year-old 'Refošk' grapevines (*Vitis vinifera* L.), flourishing in terra rossa soil (the "red earth"; a type of red clay soil formed by the weathering of limestone), located in a nonirrigated vineyard in the sub-Mediterranean part of Slovenia, the winegrowing district (45°08'N; 13°75'E). The vines were grafted on SO4 rootstocks, trained on a double guyot, and planted on a distance of 1.0 m × 2.3 m. Three treatments were established: (i) C, control (common harvest practice in which the grapes were harvested when soluble solids reached around 18.5 °Brix; grapes of the control treatment reached technological maturity on 10 October); (ii) DMR, double maturation raisonnée (bearing shoots were cut when soluble solids reached 17.0 °Brix; bunches were left on the vines for partial dehydration until soluble solids reached the same content as in the control and PDC, around 18.5 °Brix, then the DMR grapes were harvested); and (iii) PDC, partial dehydration in chamber (the bunches were harvested when soluble solids reached 17.0 °Brix and placed in a drying chamber with air circulation until soluble solids reached the exact content as in the control and DMR, around 18.5 °Brix). The experiment was conducted in five blocks, each randomly built up of three treatments. Each treatment was composed of ten consecutive vines per block. Irrespective of the treatment, the berries reached 17.0 °Brix on 23 September (1st sampling), when the grapes for the PDC treatment were harvested, placed in perforate cases, transported to the drying chamber, and partially dehydrated with air circulation in darkness. Ambient air temperature was controlled using a thermostat in the range of 22–23 °C while outdoor air was pumped in by ventilators, retaining a relative humidity in the chamber of around 60%. On the same day (23 September) DMR was implemented, the 1-year old canes were cut, whereupon bunches were left on the vines for partial dehydration. The ripening of the control and DMR grapes had been exposed to the same environmental conditions (Figure 1). The second sampling in all treatments was performed when the berries reached technological maturity commonly defined for the 'Refošk' cultivar, meaning the content of the soluble solids reaching around 18.5 °Brix. The berries of the control treatment reached technological maturity on 10 October, the DMR grapes on 7 October (14 days

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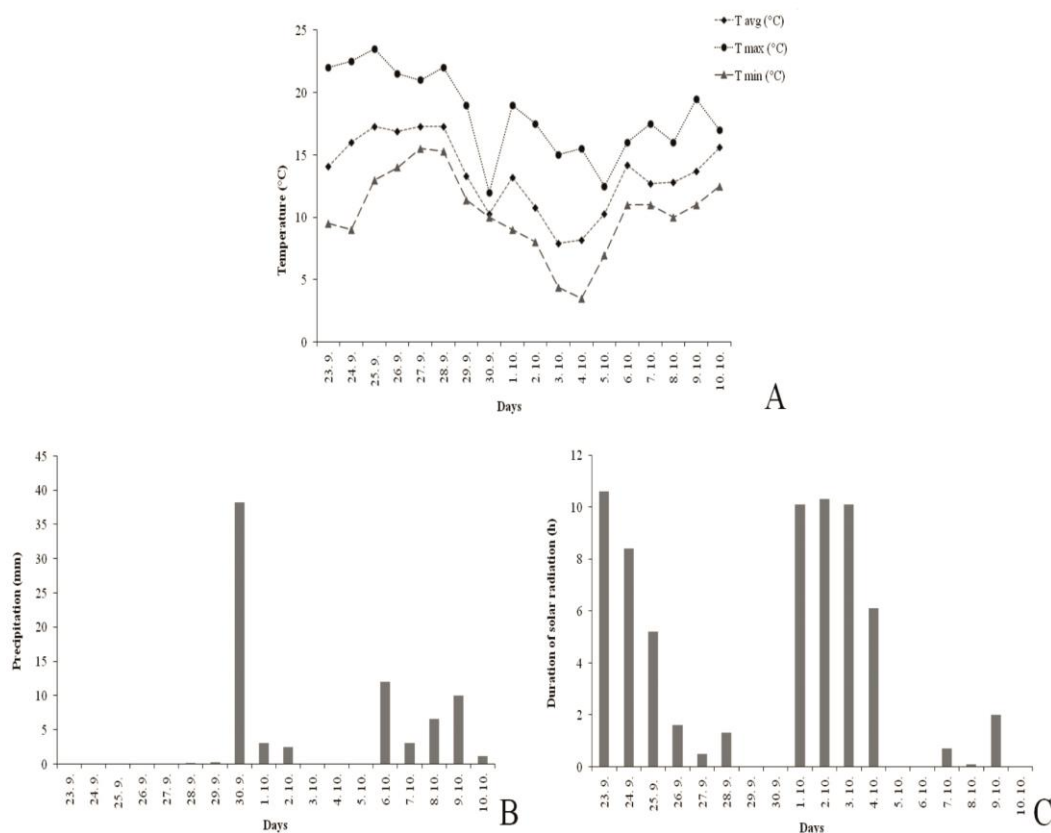


Figure 1. Meteorological data of the weather station Godnje during the experimental period from 23 September to 10 October 2013 (A): Average, maximal and minimal daily temperature (°C). (B): Average daily precipitation (mm). (C): Average daily duration of solar radiation (h).

after cane cutting, partial dehydration), and the PDC grapes on 15 October (22 days after harvest and partial dehydration in the drying chamber). The berries, sampled in five replications per treatment, were stored in PVC bags at -80°C until further analyses. Furthermore, a respective number of berry skins per treatment were also dried at 105°C for 72 h to determine water loss and dry weight content (Skupien, 2006).

The harvested grapes, from each treatment, were divided into four equal parts and then vinified under the same conditions. The grapes were first stemmed; then berries were crushed and the maceration lasted for 10 days. At the beginning of the maceration, 20 g h L^{-1} of rehydrated yeast *Saccharomyces cerevisiae* Mycoferm ROUGE was added to each vessel, according to the producer instruction (INRA, Bordeaux, France). During the maceration, punching down the cap of grape marc was manually done twice per day, and at the end of

maceration they were pressed separately per treatment and replicate. Obtained semifermented must was decanted into individual 30-L tanks, where the wine fermentation continued for the next 14 days. After that, the wine was decanted and 25 mL h L^{-1} of 5% sulfuric acid was added to the individual tank to prevent wine oxidation. Wine samples were taken from each tank ($n = 4$ per treatment) for further analysis.

2.2. Berry characteristics and wine analysis

Firstly, the weight of 100 berries was recorded in five replicates per sample. The content of soluble solids was assessed with a digital refractometer (ATAGO PAL87S) and expressed in °Brix. The initial pH value of the grape juice was recorded before measuring titratable acidity according to the method by Ough and Amerine (1988). A solution of 0.1 M NaOH was added to the sample with a semiautomatic titrator until a pH value of 7.0 was reached, while the required volume was recorded to calculate the

titratable acidity, which was expressed in grams of tartaric acid per liter. To determine alcohol content in the wine, pH, total acidity, total extract, lactic, malic, and tartaric content, a sample of wine (50 mL) was picked from each tank. Chemical analyses were carried out using a WineScan FT120 (Foss, Denmark).

2.3. Chemicals

For the quantification of sugars, organic acids, and phenolic compounds the following standards were used: glucose, fructose, tartaric acid, *p*-coumaric acid, caffeic acid, procyanidin B1, catechin, epicatechin, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, and myricetin-3-*O*-rhamnoside from Fluka Chemie GmbH (Buchs, Switzerland); citric acid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-xyloside, peonidin-3-*O*-glucoside, resveratrol, caftaric acid, and cyanidin-3-*O*-glucoside from Sigma-Aldrich (St. Louis, MO, USA); malic acid and gallic acid from Merck KGaA (Darmstadt, Germany); and isorhamnetin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside from Extrasynthese (Genay Cedex, France). Moreover, 1% (w/v) butylated hydroxytoluene (BHT) from Sigma-Aldrich GmbH was used to prevent the oxidation of individual phenolics. To determine total phenolic content, Folin-Ciocalteu reagent from Merck KGaA (Darmstadt, Germany) was used. Methanol was from Sigma-Aldrich GmbH. The chemicals used for the mobile phases were acetonitrile HPLC-MS grade and formic acid from Sigma-Aldrich GmbH (St. Louis, MO, USA). Water was purified and twice distilled with a Milli-Q-system (Millipore, Bedford, MA, USA).

2.4. Extraction and determination of sugars and organic acids

The extraction of sugars and organic acids followed the method reported by Rusjan et al. (2008). For each replication per treatment, 20 berries were crushed and 1 mL of obtained grape juice was diluted with double distilled water (1:10, v/v). Diluted grape juice was centrifuged and filtrated. The separation of sugars and organic acids was done according to the HPLC method described by Mikulic-Petkovsek et al. (2012a). The contents of all analyzed sugars and organic acids were expressed in g L⁻¹ and summed up and presented as total sugar and acid contents in g L⁻¹, respectively.

2.5. Extraction of phenolic compounds

The extraction of phenolic compounds from the dry berry skins was performed as described by Mikulic-Petkovsek et al. (2012b, 2015). Berry skins (0.1 g) were extracted with 5 mL of methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-di-tert-butyl-4-methylphenol (BHT). Extraction was performed in five repetitions per treatment.

2.6. Determination of individual phenolic compounds using HPLC-DAD-MSⁿ analysis

Phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA, USA) with a diode array detector (DAD) at 280 nm (flavanols, hydroxycinnamic acid derivatives), 350 nm (flavonols), and 530 nm (anthocyanins) according to the chromatographic conditions as it was previously described in the study by Mikulic-Petkovsek et al. (2012b, 2015). All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative and positive (for anthocyanins) ion mode. Contents of phenolics were expressed in mg 100 g⁻¹ dry weight (DW) of berry skin and mg L⁻¹ of wine. For phenolics missing standards, quantification was carried out using similar compounds as standards.

2.7. Determination of total phenolic content

The same extraction procedure of berry skins used for individual phenolics, but without BHT, were used to prepare extracts for total phenolics content analysis. The total phenolic content (TPC) of the extracts was assessed by the Folin-Ciocalteu phenol reagent protocol (Singleton et al., 1999). Total phenolic content was expressed as gallic acid equivalents (GAE) in mg 100 g⁻¹ dry weight (DW) of berry skin and in mg L⁻¹ of wine.

2.8. Statistical analysis

The data were analyzed with the Statgraphics Plus 4.0 program (Manugistics, Inc.) using one-way analysis of variance (ANOVA) and some data of the produced wine were analyzed by principal component analysis (PCA). The significance of the treatment on the content of primary and secondary compounds was analyzed separately for each sampling, using the LSD multiple range test with a significance level of $P \leq 0.05$. Means and standard errors are presented (mean $\pm$ SE) and statistical differences among treatments are denoted by different letters.

3. Results

3.1. Berry and wine characteristics, and sugar and organic acid contents

The meteorological data depict increased solar radiation on most days during the experimental period (as high as 10 h of sunny weather per day) and only 4 days of cloudy weather (Figure 1). Additionally, intense daily temperature oscillations, which potentially caused changes in the berry metabolism, were recorded.

All berries were characterized by comparable soluble solids contents, approx. 18.5 °Brix, which is common for the technologically mature berries of the high-yielding grapevine cultivar 'Refošk'. Single berry weight, one of the main indicative factors of grape ripeness, increased 11%

from the first sampling until harvest in control plants. However, berry weight decreased by 8% in DMR and by 13% in PDC treatment (Table 1). In a detailed study on individual sugars' accumulation, no significant impact of partial dehydrations was recorded (Table 1). Glucose (from 86.8 to 88.1 g L⁻¹) and fructose (from 83.3 to 84.9 g L⁻¹) represented the main sugars in the examined grape berries (approx. 98% of the total sugars). Sucrose was only detected in small amounts (from 2.7 to 5.3 g L⁻¹) (Table 1). The highest rate of sugar increase was measured in DMR treated berries (0.93 g L⁻¹ per day), followed by PDC (0.64 g L⁻¹ per day) and control berries (0.62 g L⁻¹ per day).

The titratable acidity of DMR and PDC treated berries increased by 12% and 6% by the second sampling, respectively; however, an average decrease of 0.08 g L⁻¹ per day was observed in the control, with an average increase of 0.14 g L⁻¹ per day in DMR and 0.10 g L⁻¹ per day in PDC

treatments. The most abundant organic acid in 'Refošk' grapes was malic acid. In the technologically mature DMR and PDC berries a significantly higher content of malic acid was measured in comparison to the control. The same response to partial dehydration was observed in terms of tartaric acid; its content was 1.2- to 1.4-fold higher in DMR and PDC compared to the control, respectively. Furthermore, the content of tartaric acid decreased by 18% and malic acid by 5% from the first to the second sampling. Fumaric, shikimic, and citric acids were only detected in traces (less than 0.5 g L⁻¹). However, the partial dehydration of the berries resulted in a significantly higher content of total organic acids in DMR (27% increase) and PDC (30% increase) berries compared to the control. When it comes to the pH value of the analyzed berries, the most interesting response was observed since the significantly lowest pH level was measured in DMR in respect to the

Table 1. Grapevine cultivar 'Refošk' (*Vitis vinifera* L.) characteristics treated with different dehydration techniques.

Grape characteristics	1st sampling [§]	2nd sampling		
	23 Sep.	10 Oct.	7 Oct.	15 Oct.
		Control	DMR	PDC
Weight of 100 berries (g)	205.7 ± 7.5	227.1 ± 5.5 c*	190.5 ± 2.0 b	178.4 ± 6.5 a
Soluble solids (°Brix)	17.6 ± 0.4	18.4 ± 0.2 a	18.8 ± 0.2 a	18.6 ± 0.2 a
Titratable acidity (g L ⁻¹)	11.6 ± 0.5	10.1 ± 0.1 a	13.1 ± 0.4 b	12.3 ± 0.2 b
pH level	2.52 ± 0.06	2.96 ± 0.05 b	2.54 ± 0.04 a	2.74 ± 0.03 b
Fructose (g L ⁻¹)	79.3 ± 1.9	84.9 ± 0.7 a	83.3 ± 1.0 a	84.9 ± 1.9 a
Glucose (g L ⁻¹)	81.7 ± 1.1	86.9 ± 0.7 a	88.1 ± 0.6 a	87.8 ± 1.5 a
Sucrose (g L ⁻¹)	2.69 ± 0.51	2.75 ± 0.86 a	5.32 ± 0.18 b	5.22 ± 0.19 b
Total sugars (g L ⁻¹)	163.8 ± 2.08	174.5 ± 1.7 a	176.7 ± 0.8 a	177.9 ± 2.7 a
Tartaric acid (g L ⁻¹)	4.91 ± 0.40	4.04 ± 0.14 a	5.13 ± 0.32 b	5.46 ± 0.46 b
Malic acid (g L ⁻¹)	7.87 ± 0.54	7.48 ± 0.12 a	9.56 ± 0.73 b	9.61 ± 0.46 b
Total acidity (g L ⁻¹)	13.2 ± 0.9	11.9 ± 0.1a	15.2 ± 0.9 b	15.5 ± 0.9 b
WINES				
Alcohol (vol %)		10.3 ± 0.03a	10.5 ± 0.04a	10.4 ± 0.02a
pH		3.03 ± 0.01a	3.00 ± 0.05a	3.13 ± 0.07a
Lactic acid		0.075 ± 0.03a	-	0.66 ± 0.02b
Malic acid		5.25 ± 0.03a	6.23 ± 0.08c	5.52 ± 0.08b
Tartaric acid		4.93 ± 0.04b	5.40 ± 0.05c	3.70 ± 0.09a
Total acidity		10.65 ± 0.09a	11.70 ± 0.5b	12.47 ± 0.3b
Total extract		27.35 ± 0.06a	28.37 ± 0.08b	30.02 ± 0.18c

*Different letters in rows of the 2nd sampling of berry skin or among different types of wine denote significant differences (LSD test, P < 0.05) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.

control and PDC treatment. The data suggest that the pH level of partially dehydrated grape berries subjected to DMR remains stable.

Wine composition undoubtedly reflects the contents of primary and secondary metabolites in grape berries. Regarding the soluble solids content in berries, insignificant differences in alcohol contents of wines among treatments were expected and they ranged between 10.3 and 10.5 vol % (Table 1). Significantly lower pH in grape from DMR treatment did not influence the pH in wine. The measured contents of organic acids in wines reflected the grape total acidity, where a wine from PDC berries had the significantly highest content and total acidity (for 17%), followed by DMR (for 9.5%) compared to the control wine. The total acidity of the produced wines could be primarily attributed to the content of malic and tartaric acid. The content of malic acid prevailed, especially in DMR wine. According to the measured content of lactic acid, we can observe that the spontaneous malolactic fermentation did

not expire in the produced wine. Partial dehydration of berries also resulted in significantly higher content of total extract in wines; the significantly highest was measured in PDC treatment, followed by DMR, with the lowest in the control wine (Table 1).

3.2. Phenolic compounds in berry skins

Anthocyanins were the most abundant phenolic compounds in berry skins (approx. 93% of the total analyzed phenolic compounds in 'Refošk'), followed by flavonols, flavanols, and stilbenoids (Table 2; Figure 2). Irrespective of treatment, malvidin glycosides represented 48%–52%, delphinidin glycosides 17%–18%, petunidin glycosides 14%–18%, peonidin glycosides 10%–12%, and cyanidin glycosides 3%–5% of the total analyzed anthocyanins (TAA) in berry skins. Malvidin-3-glucoside was the major anthocyanin from the group of malvidin derivatives, determined in the analyzed berry skins, followed by malvidin-3-acetylglucoside and malvidin-3-coumaroylglucoside.

Table 2. Anthocyanins (mg 100 g⁻¹ DW) in berry skin of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) treated with different dehydration techniques.

Phenolics	1st sampling [§]		2nd sampling					
	23 Sep.		10 Oct.		7 Oct.		15 Oct.	
		% [‡]	Control	%	DMR	%	PDC	%
Cyanidin-3-glucoside	158.2 ± 17.8	2.3	158.9 ± 14.1 b*	2.8	99.0 ± 8.3 a	1.3	109.9 ± 9.9 a	2.4
Cyanidin-3-acetylglucoside	52.7 ± 5.2	0.7	45.01 ± 4.51 ab	0.8	51.5 ± 4.5 b	0.7	36.7 ± 3.3 a	0.7
Delphinidin-3-glucoside	858.1 ± 62.2	12.2	739.8 ± 34.0 b	13.1	875.3 ± 38.5 c	11.3	453.2 ± 25.5 a	10.0
Delphinidin-3-acetylglucoside	201.2 ± 20.3	2.7	114.7 ± 7.3 a	2.0	203.8 ± 16.3 b	2.6	116.5 ± 11.4 a	2.5
Delphinidin-3-coumaroylglucoside	201.1 ± 20.1	2.8	176.9 ± 16.4 a	3.1	242.1 ± 19.3 b	3.1	148.1 ± 13.0 a	3.2
Malvidin-3-glucoside	2328 ± 247	35.4	2065 ± 92 a	36.5	2778 ± 269 b	35.1	1661.2 ± 93.4 a	37.3
Malvidin-3-acetylglucoside	9.59 ± 0.93	0.1	6.91 ± 0.84 a	0.1	11.80 ± 1.00 b	0.2	8.44 ± 0.94 ab	0.2
Malvidin-3-coumaroylglucoside	531.9 ± 42.0	7.9	376.1 ± 36.3 a	6.6	678.3 ± 45.9 b	8.7	408.7 ± 42.5 a	8.7
Petunidin-3-glucoside	738.5 ± 62.1	10.9	683.0 ± 66.7 ab	11.9	875.4 ± 43.2 b	11.3	582.2 ± 66.4 a	12.6
Petunidin-3-acetylglucoside	142.9 ± 15.7	2.0	108.2 ± 12.9 a	1.9	162.1 ± 8.3 b	2.1	80.0 ± 7.3 a	1.8
Petunidin-3-coumaroylglucoside	175.7 ± 12.5	2.5	146.4 ± 14.3 a	2.6	222.0 ± 21.0 b	2.8	117.2 ± 21.6 a	2.6
Peonidin-3-glucoside	630.5 ± 72.5	9.4	529.1 ± 44.2 a	9.2	755.2 ± 82.1 b	9.5	355.4 ± 32.7 a	7.8
Peonidin-3-acetylglucoside	83.4 ± 9.2	1.2	58.9 ± 5.7 a	1.0	97.8 ± 10.2 b	1.2	50.3 ± 6.4 a	1.1
Peonidin-3-coumaroylglucoside	122.93 ± 13.2	1.9	93.3 ± 9.7 a	1.6	145.3 ± 16.5 b	1.8	67.8 ± 6.3 a	1.5
Total anthocyanins	6734 ± 528	86.2	5682 ± 358 a	90.2	7869 ± 600 b	89.4	4533 ± 294 a	88.6

*Different letters in rows of the 2nd sampling denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.

‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

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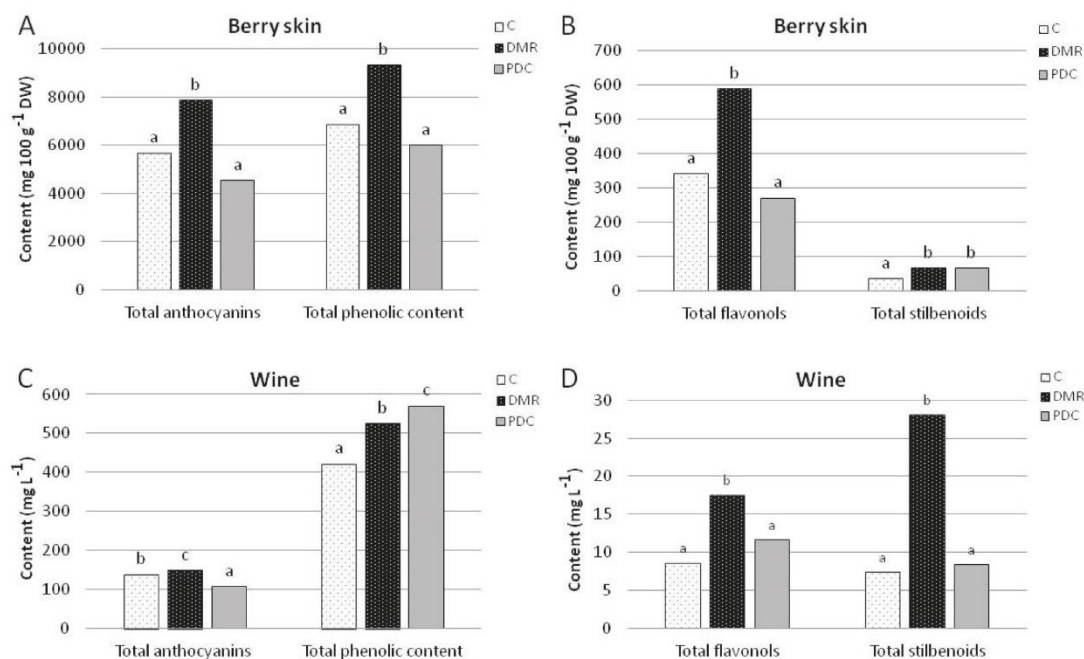


Figure 2. Content of phenolics from different phenolic groups in berry skins of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) (mg 100 g⁻¹ DW) (A, B) and refošk wine (mg L⁻¹) (C, D) treated with different dehydration techniques.

The malvidin aglycones prevailed in wine where 15 anthocyanins were identified and quantified (Table 3). However, malvidin-3-glucoside with cyanidin-3-glucoside and petunidin-3-glucoside represented 50%–60% of TAA in refošk wine. Moreover, several anthocyanins, which were not present in berry skin, have been identified in wine. Total anthocyanin content of the refošk wine ranged from 106.8 to 148.7 mg L⁻¹.

DMR treatment significantly increased the total anthocyanin content in the skins compared to the control and PDC treatment, reaching 38% higher levels in comparison to the control and 73% higher than PDC berries (Table 2; Figure 2). Based on the anthocyanin content in the berry skins we can assume that 'Refošk' grapes reached their optimal phenolic maturity on the day of the first sampling but in order to increase the berries' sugar content the bunches were left on the vines for an additional 17 days for the control treatment, 14 days for DMR, and 22 days for PDC treated berries.

A 50% increase in delphinidin-, malvidin-, petunidin-, and peonidin-3-acetylglucoside, and malvidin- and petunidin-3-coumaroylglucoside content was measured in DMR berries compared to the control treatment at the second sampling. In contrast, the content of cyanidin-3-glucoside significantly decreased in DMR berries (Table 2). Interestingly, the content of individual and total

anthocyanin was not significantly different between the control and PDC treatment, except in terms of cyanidin- and delphinidin-3-glucoside contents.

Significant differences in the contents of individual anthocyanins were also observed among wines of different treatments (Table 3). Wine prepared from DMR berries contained significantly higher anthocyanins contents when compared to the wine of other treatments. DMR wine contained 7% higher content of TAA in comparison to the control wine. Moreover, PDC wine was characterized by 23% lower content of TAA compared to the wine prepared from control berries (Figure 2).

The highest share of total analyzed flavonols (TAF) in the berry skins of 'Refošk' was represented by myricetin glycosides (45%), followed by quercetin glycosides (35%), syringetin glycosides (10%), kaempferol glycosides (5%), and isorhamnetin glycosides (5%) (Table 4). The major flavonols were myricetin-3-glucoside and quercetin-3-glucoside, combined representing 3/4 of TAF. The content of all individual flavonols decreased in range from 36% to 69% from the 1st to the 2nd sampling in the control berries (Table 4). 'Refošk' berry skin in the control treatment contained 340.3 mg 100 g⁻¹ DW TAF at harvest. In comparison to that, approximately 1.7-fold higher levels were measured in the berry skins of the DMR treatment and 13% lower TAF levels in PDC treatment (Figure 2).

Table 3. Anthocyanins (mg L⁻¹) in refošk wine prepared from grapes of different dehydration techniques.

Phenolics	Control	% [‡]	DMR	%	PDC	%
Cyanidin-3-glucoside	34.02 ± 1.32b*	24.6	25.74 ± 1.62a	17.3	25.83 ± 1.43a	24.2
Malvinidin-3-acetylglucoside	2.25 ± 0.07a	1.6	2.69 ± 0.17b	1.8	1.93 ± 0.11a	1.8
Malvinidin-3,6- <i>p</i> -coumaroyl-glucoside	8.92 ± 0.32b	6.5	11.82 ± 0.17c	8.0	7.62 ± 0.12a	7.2
Malvinidin-3-glucose-ethyl(epi)catechin 1	1.08 ± 0.17a	0.8	3.07 ± 0.22b	2.1	0.96 ± 0.11a	0.9
Malvinidin-3-glucose-ethyl(epi)catechin 2	11.94 ± 0.41b	8.7	16.12 ± 0.48c	10.9	8.11 ± 0.67a	7.6
Malvinidin-3-glucoside	18.62 ± 1.19a	13.5	25.98 ± 1.80b	17.5	15.99 ± 1.28a	15.0
Peonidin-3-acetylglucoside	3.29 ± 0.09b	2.4	3.78 ± 0.12c	2.5	2.31 ± 0.12a	2.2
Peonidin-3,6- <i>p</i> -coumaroyl-glucoside	2.07 ± 0.07a	1.5	2.41 ± 0.18ab	1.6	2.86 ± 0.18b	2.7
Peonidin-3-glucose-ethyl(epi)catechin	0.87 ± 0.26ab	0.6	1.48 ± 0.21b	1.0	0.35 ± 0.13a	0.3
Peonidin-3-glucoside	13.65 ± 0.56b	9.9	18.36 ± 1.15c	12.1	10.78 ± 0.08a	10.1
Petunidin-3-acetylglucoside	5.60 ± 0.12b	4.1	5.03 ± 0.10a	3.4	5.23 ± 0.16ab	4.9
Petunidin-3-glucoside	30.58 ± 1.30c	22.2	26.36 ± 0.77b	17.7	20.73 ± 0.37a	19.4
Pyranol-malvidin-3-glucoside (vitisin B)	2.51 ± 0.31b	1.8	2.46 ± 0.06b	1.7	1.33 ± 0.17a	1.2
Pyranol-malvidin-3- <i>p</i> -coumaroylglucoside	1.08 ± 0.15a	0.8	2.11 ± 0.29b	1.4	1.68 ± 0.13b	1.6
Coumaroylvitisin A	1.47 ± 0.037b	1.1	1.58 ± 0.11b	1.1	1.07 ± 0.04a	1.0
Total anthocyanins	137.98 ± 1.25b	32.8	148.70 ± 4.17c	56.8	106.82 ± 2.40a	23.1

*Different letters in rows denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

[‡] % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

In contrast to the berry skin, the contents of TAF in wine were relatively low, ranging from 8.65 to 17.48 mg L⁻¹ (Table 5). Wine produced from DMR berries contained 2-fold higher TAF than the control wine. However, no significant differences in TAF content were determined between wines produced from PDC and control berries (Figure 2).

From the group of hydroxycinnamic acids (HCA) only *p*-coumaric acid hexose was detected in the berry skins of 'Refošk' (Table 4). Grape skin contained from 0.72 to 1.48 mg 100 g⁻¹ DW of *p*-coumaric acid hexose and its content generally decreased from the first to the second sampling. However, the lowest content of *p*-coumaric acid hexose was measured in PDC treated berries.

However, seven hydroxycinnamic acid derivatives were identified in refošk wine (Table 5). Irrespective of the treatment, the most abundant was caftaric acid, which ranged from 11.43 to 20.68 mg L⁻¹. The remaining hydroxycinnamic acid derivatives were detected in contents below 4.13 mg L⁻¹. Wine prepared from DMR berries contained significantly lower contents of *p*-coumaric acid derivative 1 and 2, *trans*-ferric acid, and caftaric acid compared to the control and PDC wine. On the other hand, wines prepared from PDC berries were characterized by 1.1–1.8 higher contents of

hydroxycinnamic acid derivatives than the control.

Procyanidin dimer was the only phenol from the group of flavanols identified in berry skin. Its content ranged from 215.7 to 244.6 mg 100 g⁻¹ DW on the second sampling (Table 4). A significantly higher procyanidin dimer content was quantified in DMR berries compared to the control. On the other hand, PDC treatment did not impact the content of the identified flavanol. Six flavanols were identified in wine refošk and the most abundant were catechin and procyanidin trimer (Table 5). The results suggested that DMR significantly decreased the total flavanol content and most individual flavanols in wines, which cannot be affirmed for epicatechin. On the other hand, a significant increase in total flavanols was observed in wine produced from PDC berries, especially of epicatechin, procyanidin tetramer, and trimer.

Two resveratrol hexosides were identified and quantified from the group of stilbenoids in the berry skins, which were termed resveratrol hexosides 1 and 2, according to their retention (elution) time characteristics. The fragmentation patterns suggest these compounds to be *cis*- and *trans*-resveratrol-3-glucosides previously reported in berry skins by Chiva-Blanch et al. (2011). The contents of both identified stilbenoids decreased during grape maturation (Table 4), which is in accordance with

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Table 4. Phenolic compounds (mg 100 g⁻¹ DW) in berry skins of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) treated with different dehydration techniques.

Phenolics	1st sampling [§]				2nd sampling			
	23 Sep.		10 Oct.		7 Oct.		15 Oct.	
		% [‡]	control	%	DMR	%	PDC	%
Procyanidin dimer	300.0 ± 21.8	4.4	244.6 ± 17.4 a*	3.6	288.0 ± 20.7 b	3.1	215.7 ± 15.4 a	3.6
<i>p</i> -coumaric acid derivative	1.48 ± 0.16	0.02	1.30 ± 0.14 b	0.02	1.08 ± 0.17 ab	0.01	0.72 ± 0.08 a	0.01
Myricetin-3-galactoside	30.7 ± 2.8	4.3	13.9 ± 1.4 a	4.1	25.0 ± 2.4 b	4.3	17.6 ± 1.3 ab	5.9
Myricetin-3-glucoside	247.8 ± 23.5	36.0	144.8 ± 14.4 a	42.5	210.9 ± 17.8 b	36.1	117.8 ± 6.8 a	39.7
Myricetin-3-glucuronide	5.85 ± 0.52	0.9	3.42 ± 0.33 a	1.0	6.21 ± 0.67 b	1.1	4.16 ± 0.52 ab	1.4
Quercetin-3-galactoside	27.5 ± 2.9	3.9	8.50 ± 0.95 a	2.4	15.7 ± 1.8 b	2.7	11.9 ± 1.8 ab	4.0
Quercetin-3-glucoside	182.9 ± 17.6	25.3	73.7 ± 5.5 a	22.1	106.7 ± 12.2 b	18.5	57.6 ± 4.1 a	19.8
Quercetin-3-glucuronide	56.1 ± 4.2	9.5	25.9 ± 2.7 a	7.4	74.8 ± 8.1 b	12.5	22.4 ± 3.6 a	7.5
Quercetin-3-rutinoside	9.30 ± 0.87	1.4	5.43 ± 0.53 a	1.6	9.41 ± 1.21 b	1.6	5.42 ± 0.93 a	1.8
Kaempferol-3-galactoside	4.16 ± 0.35	0.7	1.91 ± 0.24 a	0.6	6.15 ± 0.93 b	1.0	4.53 ± 0.72 b	1.5
Kaempferol-3-glucoside	8.03 ± 0.95	1.2	3.17 ± 0.43 a	0.9	7.52 ± 0.85 b	1.3	4.61 ± 0.54 a	1.5
Kaempferol-3-coumaroylglucoside	17.4 ± 1.8	2.6	11.0 ± 1.3 a	3.2	19.0 ± 1.7 b	3.2	12.0 ± 1.2 a	4.0
Isorhamnetin-3-glucoside	6.82 ± 0.72	1.0	3.72 ± 0.46 a	1.1	6.87 ± 5.07 b	1.2	3.57 ± 0.43 a	1.2
Isorhamnetin-3-rutinoside	24.5 ± 1.4	3.8	14.3 ± 1.4 a	4.2	26.6 ± 2.8 b	4.5	10.2 ± 1.2 a	1.4
Syringetin-3-glucoside	45.4 ± 4.2	6.7	17.9 ± 2.4 a	5.2	38.6 ± 3.9 b	6.4	14.7 ± 3.1 a	5.0
Syringetin-3-rutinoside	19.0 ± 1.7	2.8	12.3 ± 1.6 a	12.3	32.9 ± 4.5 b	5.7	10.4 ± 1.3 a	3.4
Total flavonols	685.7 ± 71.5	8.4	340.3 ± 26.7 a	5.4	586.6 ± 47.9 b	6.6	296.9 ± 16.0 a	5.8
Resveratrol-hexoside 1	38.4 ± 2.8	63.3	20.9 ± 2.6 a	60.8	35.7 ± 4.6 b	56.0	47.4 ± 3.8 c	74.7
Resveratrol-hexoside 2	19.6 ± 1.7	36.7	13.7 ± 1.8 a	39.2	28.1 ± 3.2 b	44.0	17.4 ± 1.9 ab	25.3
Total stilbenoids	58.1 ± 5.9	0.7	34.7 ± 4.4 a	0.5	63.9 ± 7.8 b	0.7	65.0 ± 7.4 b	1.3

* Different letters in rows of the 2nd sampling denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.

‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

the reports by Moreno et al. (2008). The results suggested that the DMR and PDC treatments significantly increased the content of resveratrol hexosides approximately 1.3 to 2.3 times in comparison to the control. DMR berries contained particularly high levels of resveratrol-hexoside 1. On the other hand, PDC berries were characterized by high contents of resveratrol-hexoside 2. However, the wine produced from DMR berries contained significantly higher content of total stilbenoids compared to the wine prepared from control and PDC berries (3.8- and 3.3-fold, respectively) (Figure 2).

3.3. Total phenolic content in berry skin and wine

The total phenolic content (TPC) is in close connection to the levels of analyzed individual phenolics and thus

higher levels of each phenolic compound result in a higher TPC in plant tissues (Mikulic-Petkovsek et al., 2013). In general, the berry skins of DMR treated grapes contained significantly higher levels of total phenolics (9334 mg GAE 100 g⁻¹ DW) compared to PDC and control treatment (Table 4). Moreover, PDC berry skins contained approx. 13% less total phenolics compared to the control berries, but the difference between them was insignificant. The wine made from PDC/DMR berries was characterized by 1.35- and 1.24-fold higher total phenolic content in comparison with the control wine, respectively (Figure 2).

3.4. Principal component analysis

Principal component analysis (Figure 3) performed on total acidity, total extract, total hydroxycinnamic acids,

Table 5. Phenolic compounds (mg L⁻¹) in refošk wine prepared from grapes of different dehydration techniques.

Phenolics	Control	% [‡]	DMR	%	PDC	%
<i>p</i> -coumaric acid derivative 1	2.78 ± 0.14b*	10.5	1.09 ± 0.07a	5.7	3.23 ± 0.10c	8.9
<i>p</i> -coumaric acid derivative 2	2.64 ± 0.12b	9.9	1.44 ± 0.02a	7.6	2.59 ± 0.04b	7.1
<i>p</i> -coumaric acid derivative 3	0.087 ± 0.002a	0.3	0.122 ± 0.002b	0.6	0.152 ± 0.001c	0.4
<i>cis</i> -couteric acid	1.25 ± 0.03a	4.7	1.74 ± 0.04b	9.1	2.19 ± 0.02c	6.0
<i>trans</i> -couteric acid	2.24 ± 0.07a	8.4	2.00 ± 0.13a	10.4	3.45 ± 0.06b	9.5
<i>trans</i> -fertaric acid	3.23 ± 0.04b	12.2	1.29 ± 0.02a	6.8	4.13 ± 0.33c	11.4
Caftaric acid	14.36 ± 0.34b	54.0	11.43 ± 0.49a	59.7	20.68 ± 1.05c	56.7
Total hydroxycinnamic acid	26.61 ± 0.62b	6.3	19.14 ± 0.71a	7.3	36.44 ± 0.81c	7.9
Catechin	74.37 ± 1.60b	31.0	29.3 ± 0.04a	6.1	87.14 ± 7.51b	29.1
Epicatechin	0.99 ± 0.02a	0.4	1.18 ± 0.03b	2.4	1.72 ± 0.04c	0.6
Procyanidin dimer 1	50.78 ± 0.23b	21.2	25.35 ± 0.23a	52.3	60.82 ± 1.32c	20.4
Procyanidin dimer 2	13.81 ± 0.29b	5.8	7.25 ± 0.05a	15.0	17.33 ± 1.11c	5.8
Procyanidin trimer	73.20 ± 1.58b	30.5	28.8 ± 0.04a	6.0	93.38 ± 3.56c	31.3
Procyanidin tetramer	26.88 ± 0.79b	11.2	8.87 ± 0.74a	18.3	37.95 ± 3.90c	12.8
Total flavanols	240.05 ± 5.01b	57.0	48.48 ± 0.79a	18.5	498.36 ± 3.09c	64.6
Resveratrol hexoside 1	1.78 ± 0.09a	23.8	7.07 ± 1.07b	24.9	1.96 ± 0.18a	24.0
Resveratrol hexoside 2	3.26 ± 0.21a	43.4	12.35 ± 0.77b	44.0	2.53 ± 0.35a	30.2
Resveratrol hexoside 3	2.45 ± 0.42a	32.8	8.70 ± 0.55b	31.1	3.93 ± 0.78a	45.7
Total stilbenoids	7.49 ± 0.33a	1.9	28.13 ± 2.25b	10.7	8.44 ± 1.29a	1.8
Laricitrin-3-glucoside	5.41 ± 0.35a	62.3	14.58 ± 1.96b	83.0	8.41 ± 1.35a	70.9
Quercetin-3-glucuronide	0.96 ± 0.06b	11.0	0.53 ± 0.03a	3.1	1.16 ± 0.06c	10.2
Syringetin-3-glucoside	2.29 ± 0.01b	26.7	2.39 ± 0.06b	13.9	2.09 ± 0.06a	18.8
Total flavonols	8.65 ± 0.41a	2.1	17.48 ± 2.05b	6.7	11.66 ± 1.35a	2.5

*Different letters in rows denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

[‡] % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

total flavanols, total stilbenoids, total flavanols, total anthocyanins, and total phenolic content of refošk wine showed two principal components; the first explained 61.75% and the second 38.24% of the data variability. The analysis showed (data not shown) that the variables with the highest contributions to the first component were total acidity, total extract, total flavanols, total hydroxycinnamic acids, and total phenolic content. The highest contribution in relation to the second component was obtained by total anthocyanins, total flavonols, and total stilbenoids.

4. Discussion

Grape and wine composition depends on many factors, most importantly environmental conditions and cultivation practices. Weather conditions (temperature, precipitation, and insolation) greatly affect the chemical parameters of berries while they are exposed to them on the vine.

As for the control and DMR treated berry in the present study, metabolisms were altered by their environment. The greatest impact could potentially be ascribed to the intense solar radiation during the experimental period as control and DMR berries were subjected to as much as 10 h of insolation per day. On the other hand, controlled temperature and darkness in the drying chamber triggered a different berry response, which is in accordance with Mencarelli and Tonutti's (2013) findings. Generally, DMR and PDC practices dehydrate the berries, however, under different conditions. DMR berry dehydration takes place on the vine itself and is affected by specific annual weather conditions, which impact the duration of the dehydration and consequently alter the berry composition. On the other hand, PDC takes place in a drying chamber and is operated by air circulation. Generally spoken, berry dehydration increased the soluble solids content in

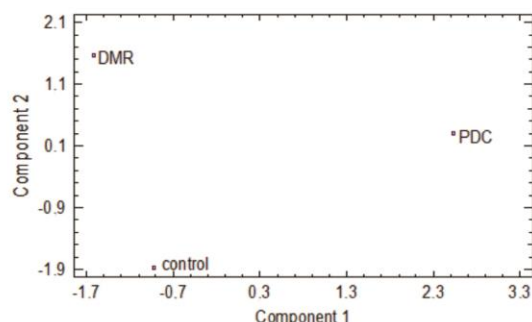


Figure 3. Principal component analysis (PCA) of the results of Refošk wine for total acidity, total extract, total hydroxycinnamic acids, total flavanols, total stilbenoids, total anthocyanins, and total phenolic content according to different dehydration techniques.

grape berries (Corso et al., 2013). Furthermore, a faster concentration or accumulation of soluble solids in DMR grapes despite the slightly slower dehydration with respect to the PDC technique could be attributed to the fact that after cane cutting the leaves on the shoots of the vigorous 'Refošk' cultivar might remained photosynthetically active for a certain period, several days, resulting in an increase in soluble solids in berries. Moreover, periods of high precipitation and lower insolation during the DMR (Figure 1) might have decelerated wilting of leaves and consequently also the photosynthesis. Corso et al. (2013) suggested that increased sugar accumulation in 'Raboso Piave' (*Vitis vinifera* L.) berries under DMR could be associated with xylem backflow and reduced phloem functionality, resulting in berry water loss.

DMR and PDC treatments of 'Refošk' berries increased the total acidity from the 1st to 2nd sampling. Greater importance should be ascribed to the modifications in the pH level due to its impact on wine chemical and microbiological stability. Zoecklein et al. (1999) reported that a grape pH level above 3.60 might negatively impact the wine's sensory attributes. In our study, a higher pH level was only measured in the berries that underwent PDC treatment. There were no differences in pH values among different types of wines. The measured alcohol content in refošk wine was in accordance with the requirements of the Rules on Wine (2000), which was expected according to the aim of the study. In accordance with reports by Cargnello et al. (2006), the PDC and DMR undoubtedly increased the content of total acidity and extract content, which makes wine more pleasant, sweet, and full bodied (Reboredo-Rodriguez et al., 2015).

In terms of the 'Refošk' cultivar slow leaf wilting was observed after cane wounding, suggesting that the leaves remain photosynthetically active for a few days, despite

the xylem flow from the rootstock and trunk to the shoots being interrupted. Zsofi et al. (2014) suggested that mild to moderate water stress results in increased anthocyanin synthesis in the berry skins, which could be the case in our study as well. Wine prepared from DMR berries contained significantly higher individual and total anthocyanin contents compared to other treatments, which was expected according to their contents in the berry skin and which was also clearly demonstrated in principal component analysis (Figure 3).

The content of flavonols in 'Refošk' berry skin from DMR was almost 2-fold the content of that from the control, which was also reflected in significantly higher content of total flavonols in DMR wine. Similarly, Corso et al. (2013) detected that the application of DMR induced the accumulation of quercetin. In grape berries, flavonols play a role as UV protectants and are strongly correlated with the accumulated dose of UV-B radiation (Martinez-Luscher et al., 2014), which has also been observed in our experiment in DMR treated vines and the control treatment. Drying in chamber (PDC) resulted in 13% lower content of total flavonols in berry skin. However, the observed decrease in our study was not severe and was insignificant.

A decrease in hydroxycinnamic acid derivative *p*-coumaric acid hexoside was recorded in berry skin after drying in chamber. Similarly, this trend was observed with sun drying, which resulted in 67% loss of hydroxycinnamic acids (HCAs) in comparison to fresh grapes (Karadeniz et al., 2000), proposing that HCAs are subject to oxidative degradation by different enzymatic pathways. Interestingly, wine prepared from PDC berries contained almost 40% higher content of total HCAs compared to the control wine. Contrary to this, DMR wine was characterized by 27% lower contents of HCAs than the control wine.

DMR berries contained higher contents of procyanidin dimer compared to the control and PDC berries. Contrary to the control and PDC, DMR wine had significantly reduced flavanol concentrations. Also Bonghi et al. (2012) found that the procyanidins B1 and B2 reduction in berry skin of 'Raboso Piave' cultivar is in correlation with the DMR technique. The flavanol profile of wine was more diverse compared to the berry skin. Catechin, procyanidin dimer 1, and procyanidin trimer combined represented 65% (DMR wine) to 85% (control) of total analyzed flavanols in wine. PDC wine resulted in 2-fold higher total flavanol content in comparison with the control wine.

It has been established that the synthesis of resveratrol in grape berries and leaves is stimulated by stress such as fungal infection, injury, light penetration, and UV light exposure (Crupi et al., 2013). The results suggested that DMR and PDC treatment significantly increased the content of resveratrol hexosides from 1.3- to 2.2-fold,

compared to the control berries, especially resveratrol-hexoside 1 (PDC) and resveratrol-hexoside 2 (DMR). Possibly, increased levels of stilbenoids in 'Refošk' DMR berries can be ascribed to prolonged exposure to UV light and water deficit in canes and leaves. DMR wine in our study also contained the highest contents of stilbenoids but no significant differences were confirmed between the control and PDC wine.

TPC in the research was significantly higher in DMR berries in comparison with the control and PDC berries. The reason for this result can be primarily attributed to water deficit. Zsofi et al. (2014) observed that berries undergoing moderate water stress contained higher levels of total phenolics in comparison to well-watered plants. As opposed to DMR berries, PDC berries were dehydrated under a controlled atmosphere of the drying chamber: constant temperature and darkness. Therefore, differences in levels of phenolics between these two treatments could be ascribed to diverse dehydrating conditions. Wine prepared from PDC/DMR berries was characterized by 35%/24% higher TPC compared to the control wine, respectively. This can be ascribed to higher total extract content and lower water content of DMR and PDC berries.

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- The partial dehydrations of berry skin of red grapevine variety with DMR or PDC have undoubtedly different impact on grape and wine composition. PDC and DMR berries increased the content of organic acids, which affected the wine, but pH remained stable only in grapes at DMR. DMR treatment significantly increased the content of anthocyanins, flavonol glycosides, and stilbenoids in berry skin and wine. On the other hand, wine made from dried grapes in chamber (PDC) was characterized by increased contents of hydroxycinnamic acids and flavanols, as well as total phenolics. Significant differences in the levels of phenolic contents between DMR and PDC berries can potentially be ascribed to the different dehydration conditions and water status of the plant upon DMR treatment. In contrast, PDC treatment was conducted under controlled conditions in the drying chamber. It can be concluded that the application of the DMR technique is much simpler, cheaper, and faster compared to partial dehydration in a chamber. Double maturation raisonnée can potentially be utilized for the production of dry 'Refošk' wine.

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

Čeprav so ampelotehnični ukrepi že precej raziskani in njihova uporaba v praksi zelo pogosta, pa kakovost grozdja in vina še vedno precej niha. Poleg tega določeni ampelotehnični ukrepi in njihova intenzivnost ni prilagojena odzivom posamezne sorte. Zato smo v prvem poskusu ugotavljali vpliv redčenja grozdja na rodnost trt ter kemijsko sestavo grozdja in vina rdeče sorte 'Portugalka' (*Vitis vinifera* L.). Redčili smo manj intenzivno oziroma omejeno (nadalje CT1), kjer smo odstranili vsak tretji grozd na mladiki ter vsak drugi grozd na vsaki drugi mladiki (približno 20-30 % odstranjenih grozdov) ter intenzivno (nadalje CT2), kjer smo pustili samo po en grozd na vsaki mladiki (približno 40-50 % odstranjenih grozdov). Zanimal nas je vpliv ukrepa redčenja na rodnost in kakovostne parametre, barvo grozdja in vina, v zadnjem letu poskusa pa smo spremljali še vpliv na sekundarne metabolite.

Število grozdov na trti neposredno vpliva na njegovo kakovost, saj grozdje predstavlja ponor hranil oziroma asimilatov, ki jih rastlina tvori v procesu fotosinteze (Ough in Nagaoka, 1984; Dami in sod., 2006; Bubola in sod., 2011).

Pri obravnavanjih z redčenjem grozdja v primerjavi s kontrolo nismo zabeležili značilnih razlik v rastnem in rodnem potencialu v vseh treh letih poskusa, kar je potrdilo, da med leti in obravnavanji ni bilo razlik v rastnih razmerah. Hkrati pa nam je ta podatek podal informacijo, da redčenje iz prejšnjega leta ni vplivalo na število rodnihih oči in mladik v naslednjem letu, kar sovпада z navedbami Bubola in sod. (2011). Razlike smo zabeležili samo v količini in kakovosti pridelka. Hrček in Korošec-Koruza (1996) v opisih sort poročata, da je 'Portugalka' precej bujna sorta, ki daje velik pridelek, po Shellie (2007) naj bi bila povprečna masa grozdja pri sorti 'Portugalka' okrog 4,5 kg na trto. Pravilnik o vinu z oznako priznanega tradicionalnega poimenovanja - Metliška črnina in Belokranjec določa, da pridelek sorte 'Portugalka' ne sme presegati 11 ton na hektar (Pravilnik o vinu ..., 2008). V našem poskusu je povprečni pričakovan pridelek znašal med 12 in 18 ton na hektar pri kontrolnem obravnavanju. Samo CT2 je značilno zmanjšalo maso pridelka, medtem ko med CT1 in kontrolo ni bilo razlik. Podobne odzive sorte 'Syrah' na redčenje navajajo tudi Gil in sod. (2013).

Bubola in sod. (2011) poročajo o povečanju povprečne mase grozdov sorte 'Merlot' za med 18 in 57 %, čeprav je bil delež odstranjenih grozdov (34 %) podoben kot pri nas. Prav tako Gil in sod. (2013) poročajo o 19 % povečanju mase grozdov pri redčenju, kar kot navajajo, gre ta pojav pripisati dejstvu, da trta porazdeli hranila med manjše število grozdov. Kljub manjši obremenitvi trt s pridelkom v naši raziskavi ni bilo opaziti značilnega povečanja mase jagod oziroma grozdov na obravnavanih trtah, kar Naor in sod. (2002) povezujejo z ugotovitvami, da grozdi po redčenju najbrž niso neposredno povezani z virom hranil in njihovo razporeditvijo. V tretjem letu raziskave smo opazili manjši pridelek na trto pri CT2 obravnavanju, v primerjavi s prejšnjima letnikoma, kar bi lahko pripisali nižji povprečni temperaturi meseca julija ter nadpovprečno veliki količini padavin v istem mesecu ter zelo majhni količini padavin v mesecu avgustu (samo 5 mm).

Vino, pridelano iz sorte 'Portugalka', se običajno trži kot mlado, sortno vino (Nemanič, 2011). Na barvo vina vpliva precej dejavnikov, eden izmed teh je tudi zrelost grozdja oziroma tudi fenolna zrelost, ki je odvisna predvsem od vsebnosti antocianov, ki se nahajajo v kožici jagod ter njihove stabilnosti. Pri rdečih vinskih sortah je to še posebej pomembno, saj so rdeča barvila (antociani) locirana v jagodni kožici (Gómez-Plaza in sod., 2000; Fragoso in sod., 2011).

Manjši pridelek lahko poveča vsebnost antocianov v grozdju (Prajitna in sod., 2007), zato smo v našem poskusu spremljali barvo jagod (CIRG indeks) po metodi, ki jo navajajo Carreño in sod. (1995) in barvo grozdnega soka (mošta) po metodi, kot jo navajata Ough in Amerine (1988).

Barvne lastnosti grozdnega soka oziroma mošta so pomembna informacija za nadaljnje vinifikacijske in kletarske prakse (Fragoso in sod., 2011), še posebno, če je vino namenjeno staranju (Gómez-Plaza in sod., 2000). Intenziteta in ton barve sta neposredno povezana z vsebnostjo antocianov, prva je podana kot vsota absorbanc, izmerjenih pri valovnih dolžinah 420 nm, 520 nm in 620 nm, ton barve pa predstavlja razmerje med absorbancama, izmerjenima pri 420 nm ter 520 nm (Fragoso in sod., 2011).

Redčenje grozdja sorte 'Portugalka' je značilno vplivalo na povečanje indeksa obarvanosti jagod, kar smo potrdili v obeh letih. Ugotovili smo tudi, da je redčenje grozdja pri sorti 'Portugalka' v obeh letih značilno vplivalo na večjo intenziteto barve grozdnega soka. Tudi Ough in Nagaoka (1984) in Gil in sod. (2013) v svojih raziskavah poročajo, da redčenje grozdja vpliva na intenziteto in ton barve grozdnega soka. Podobne rezultate so dobili tudi Bubola in sod. (2011) v raziskavi na sorti 'Merlot', kjer se je intenziteta barve v vinu značilno povečala z redčenjem grozdja. Isti avtorji povečanje intenzitete barve povezujejo z večjo vsebnostjo fenolov, posebej antocianov, in kot navajajo, redčenje lahko neposredno vpliva na vsebnost omenjenih spojin s spodbujenim zorenjem grozdja ali posredno z regulacijo razmerja med viri in ponori hranil. Poleg tega Fragoso in sod. (2011) navajajo še, da imajo večje jagode večje razmerje med kožico in mesom (pulpo) in zato manjšo vsebnost antocianov, v primerjavi z manjšimi jagodami.

Odstranitev dela pridelka pred začetkom zorenja vpliva na procese sinteze in nalaganja asimilatov (sladkorjev), kislin in drugih snovi v jagode (Gil in sod., 2013). CT2 je v primerjavi s CT1 in kontrolo značilno povečalo vsebnost topne suhe snovi v grozdju, kar je bilo pričakovano glede na ugotovitve, ki jih poročajo Bubola in sod. (2011) z redčenjem pri sorti 'Merlot', kjer je bila vsebnost topne suhe snovi značilno večja pri 30 in 60 % redčenju v primerjavi s kontrolo. CT1 ni značilno vplivalo na večjo vsebnost topne suhe snovi v primerjavi s kontrolo. Na drugi strani pa je redčenje v naši raziskavi, ne glede na intenziteto, vplivalo na značilno zmanjšanje vsebnosti titracijskih kislin v grozdnem soku, v povprečju za 0,9 g/l (CT1) ter za 1 g/l (CT2). O podobnih ugotovitvah poročajo tudi Bubola in sod. (2011). V nasprotju z zmanjšanjem vsebnosti titracijskih kislin pa je redčenje vplivalo na višji pH. Zmanjšanje vsebnosti kislin naj bi bilo, kot še navajajo Bubola in sod. (2011), posledica hitrejšega zorenja grozdja pri redčenju. Dami in sod. (2006) poročajo, da je bila pri samo 10 grozdih na trto vsebnost titracijskih kislin največja, čeprav je bilo to ugotovljeno samo v enem letu raziskave, v ostalih štirih letih razlik med obravnavanji v vsebnosti titracijskih kislin ni bilo. Obenem Dami in sod. (2006) še

poročajo, da je bil pH vedno najvišji pri največji intenziteti redčenja, kar je prav tako v skladu z našimi rezultati.

V tretjem letu poskusa (2011) smo se osredotočili na pomembnejše parametre kakovosti vina, pridelanega iz grozdja vseh treh obravnavanj. Pri CT2 smo izmerili večjo vsebnost alkohola kakor tudi vsebnost skupnega ekstrakta in hlapnih kislin ter manjšo vsebnost skupnih kislin v primerjavi s CT1 in kontrolo, podobno navajajo tudi Bubola in sod. (2011) pri sorti 'Merlot'.

Velik pomen za kakovost grozdja in vina imajo tudi fenolne spojine. V poskusu smo s HPLC ter HPLC-MS metodama identificirali 12 različnih antocianov v jagodni kožici sorte 'Portugalka' in ravno toliko tudi v pridelanem vinu. Najbolj zastopan antocian v jagodni kožici in vinu je bil malvidin-3-glukozid, saj je predstavljal kar med 27 in 30 % delež vseh antocianov v kožici ter med 62 in 65 % skupnih antocianov v vinu, s čimer smo potrdili navedbe Kumšta in sod. (2014) o vinu iz sorte 'Portugalka'. Z redčenjem se je povečala vsebnost posameznih antocianov v jagodni kožici, največji delež povečanja ne glede na intenziteto redčenja smo ugotovili pri delfinidin-, cianidin-, petunidin ter peonidin-3-glukozidu, pri delfinidin- ter petunidin-3-(6" acetil) glukozidu in delfinidin-, petunidin- ter peonidin-3-(6-*p*-kumaroil) glukozidu. Povečanje vsebnosti antocianov je poleg že prej omenjenega hitrejšega zorenja in regulacije razmerja hranil med viri in ponori, lahko posledica tudi povečanja mase in volumna posameznih jagod (Bubola in sod., 2011).

Poleg antocianov, nas je zanimal tudi vpliv redčenja na ostale fenolne spojine, ki smo jih identificirali in kvantificirali v grozdju in vinu. Izmed hidroksicimetnih kislin sta se vsebnosti kaftarne kisline ter *p*-kumaroil pentoze značilno povečali pri CT2 tako v jagodni kožici kot v vinu, kar nakazuje k boljši obarvanosti vina.

Flavanoli pomembno vplivajo na okus in telo vina (Fanzone in sod., 2011). V vinu, ki je bilo pridelano iz redčenega grozdja je bila vsebnost nekaterih flavanolov bistveno večja, kot v vinu iz grozdja kontrole. Ugotovili smo, da je CT2 najbolj vplivalo na vsebnost procianidin dimera 2 (značilno povečanje za 33 mg/l) ter epikatehina (značilno povečanje za 34 mg/l). Peña-Neira in sod. (2007) so v raziskavi z redčenjem grozdja na sorti 'Syrah' v grozdnih jagodah identificirali katehin ter procianidin B1, in poročajo, da je bolj intenzivno redčenje povečalo vsebnosti omenjenih fenolov v kožici, v primerjavi z manj intenzivnim, kar je tudi skladno z našo raziskavo.

Na barvo vin, poleg antocianov vplivajo tudi flavanoli. Imajo vlogo kopigmentov ter pripomorejo k stabilizaciji barve. Peña-Neira in sod. (2007) povezujejo flavonole z antioksidativnim delovanjem. Vendar pa smo v raziskavi ugotovili da redčenje grozdja ni imelo bistvenega vpliva na flavonole, kar je povezano z dejstvom, da je sinteza flavanolov odvisna predvsem od osvetlitve (Peña-Neira in sod., 2007). Kljub temu pa je redčenje vplivalo na značilno večjo vsebnost določenih flavanolov v jagodni kožici, manj razlik smo sicer opazili v vinu. Med flavanoli v kožici smo identificirali največ glikozidov kamferola in kvercetina. Podobno, v kožici sorte 'Syrah' so izmed flavanolov najbolj zastopani kvercetin in kamferol glikozidi, poleg teh še glikozidi miricetina (Peña-Neira in sod., 2007). Zastopanost posameznih fenolnih spojin v kožici je predvsem lastnost določene sorte (Montealegre in sod., 2006), manj razlik pa je med rdečimi in belimi

sortami (Castillo-Muñoz in sod., 2007; Castillo-Muñoz in sod., 2010). Glede na vsebnost posameznih flavonolov v jagodni kožici je bilo v naši raziskavi največ kvercetin-3-glukozida, kar so potrdili tudi Peña-Neira in sod. (2007) pri sorti 'Syrah'. Nasprotno pa nekatere raziskave (Montealegre in sod., 2006) poročajo, da kvercetin-3-glukozid ni najbolj zastopan flavonol glede na vsebnost. Kljub temu pa redčenje grozdja ni bistveno spremenilo deleža posameznega flavonola glede na vsoto vseh določenih flavonolov v grozdju in vinu. Vsebnosti za flavonole iz naše raziskave se ujemajo z vsebnostmi, ki jih podajajo Montealegre in sod. (2006).

Prva raziskava (Reščič in sod., 2015) je pokazala, da je ukrep redčenja grozdja upravičen za izboljšanje kakovosti grozdja in vina pri sorti 'Portugalka'. Ker je tradicionalno razlivanje že precej raziskan ukrep, nas je zanimalo, kako na sestavo grozdja in vina vpliva odstranitev listov v predelu grozdov v nekoliko zgodnejšem času, to je kmalu po cvetenju in pa razlivanje nad drugim parom žic v začetku zorenja grozdja. Ob tem smo v poskus vključili še manj poznan ukrep dvojnega namenskega zorenja grozdja (DMR), da bi raziskali še drugo stran potrošnikovih preferenc, torej možnost pridelave kompleksnejših in bolj polnih vin z večjo vsebnostjo alkohola in ekstrakta.

Zgoraj omenjene ukrepe smo izvedli na sorti 'Istrska malvazija', pri kateri so, kot navajata Hrček in Korošec-Koruza (1996), zaradi bujne rasti in obilnega pridelka vina manj zaokrožena in harmonična v ustih, čeprav dosegajo precej velike vsebnosti sladkorjev ter manjše vsebnosti kislin. Vplive zgoraj omenjenih ampelotehničnih ukrepov smo ugotavljali s spremljanjem in merjenjem rastnega in rodnega potenciala, grozdje smo vzorčili v tehnološki zrelosti in v laboratoriju izmerili osnovne kemijske in fizikalne značilnosti. S HPLC in HPLC-MS metodama smo izmerili tudi vsebnost primarnih metabolitov (sladkorjev in organskih kislin) ter sekundarnih metabolitov (fenolnih spojin). Z WineScan FT 120 smo v pridelanem vinu izmerili ključne parametre kakovosti.

Med obravnavanji, znotraj posameznega leta poskusa, nismo dobili značilnih razlik v številu oces ter mladik na trto, kar potrjuje, da so bile trte v enaki kondiciji. Le v drugem letu poskusa, smo ne glede na obravnavanje opazili manjše število rodnihi oces, kar se je kasneje odrazilo v manjšem številu rodnihi mladik ter grozdov, kar prepisujemo slabši diferenciaciji oces v predhodnem letu in je najverjetneje posledica okoljskih, predvsem klimatskih razmer. Več razlik smo opazili v količini pridelka, največji povprečni pridelek je bil izmerjen pri obeh razlivanjih, medtem ko je pričakovano ukrep DMR značilno zmanjšal pridelek v primerjavi s kontrolo. Poleg tega pa je grozdje iz kontrolnega obravnavanja imelo največjo maso 100 jagod v obeh letih, medtem ko je bila masa 100 jagod pri DMR značilno najmanjša samo v drugem letu, v primerjavi s kontrolo in obravnavanema razlivanja.

Zaradi izhlapevanja vode iz grozdnih jagod se v njih koncentrirajo sladkorji, zato bi pričakovali da bo vsebnost topne suhe snovi večja pri DMR, v primerjavi z ostalimi obravnavanji. Vendar se vsebnost topne suhe snovi pri DMR ni značilno razlikovala od vsebnosti pri kontrolnem obravnavanju. Prav takšen odziv smo ugotovili tudi v vsebnosti titracijskih kislin, kar pa je v skladu s poročanji iz raziskave, ki so jo opravili Corso in sod. (2013). Bonghi in sod. (2012) navajajo, da lahko mikroklimatske razmere precej vplivajo na razvoj in zorenje grozdja, zato bi razlog za manjše vsebnosti topne suhe snovi pri

obravnavanju DMR lahko bil v okoljskih oziroma vremenskih razmerah, posebno v letu 2014, ko je bila v času zorenja grozdja količina padavin nadpovprečna.

Poni in sod. (2006) navajajo, da so spodnji listi pri trti starejši in fotosintezno manj učinkoviti, zato ob odstranitvi le-teh postanejo bolj aktivni zgornji listi na mladikah, ki so tudi bolj osvetljeni. Za razlistanje zgornjih, fotosintezno aktivnejših listov smo se odločili na podlagi poročanj Hrček in Koruza (1996), da grozdje sorte 'Istrska Malvazija' pogosto doseže veliko vsebnost sladkorjev, posledično ima vino lahko večjo vsebnost alkohola. A vendar je s strani potrošnikov večje povpraševanje po belih vinih z manjšo vsebnostjo alkohola, zato smo odstranili liste, ki so fotosintezno najbolj aktivni, da bi s tem zmanjšali vsebnost sladkorja v grozdju in posledično alkohola v vinu. V kasnejši raziskavi, Poni in sod. (2013) poročajo, da je odstranitev zgornjih listov na trtah sorte 'Sangiovese' upočasnila zorenje grozdja, kar se je odrazilo v manjši vsebnosti topne suhe snovi v primerjavi s kontrolo. Takšnega odziva pri odstranitvi zgornjih listov v naši raziskavi pa nismo opazili, saj smo največje vsebnosti topne suhe snovi zabeležili pri obeh razlistanjih, posebno je bilo povečanje v primerjavi s kontrolo značilno v drugem letu. Omenjeni pojav pri odstranitvi zgornjih mlajših listov najbrž nakazuje na določen delež fotosintezne aktivnosti spodnjih, starejših listov ter listov zalistnikov, kot poročajo Poni in sod. (2006). Kljub povečanju vsebnosti topne suhe snovi pri obravnavanjih z razlistanjem pa se je povečala tudi vsebnost titracijskih kislin, kar je skladno z rezultati, ki so jih podali Poni in sod. (2013). Rezultati naše raziskave so skladni tudi z rezultati raziskave z odstranitvijo listov v času pred cvetenjem, ki so jo opravili Poni in sod. (2006) na sorti 'Sangiovese', kjer poročajo o značilnem povečanju vsebnosti topne suhe snovi in titracijskih kislin. Kljub povečanju vsebnosti titracijskih kislin v soku grozdnih jagod pa v okviru našega poskusa značilnih sprememb v vrednosti pH nismo zaznali.

Sestava in vsebnost sladkorjev ter organskih kislin je neposredno odvisna od fotosintezne aktivnosti trte in od poteka zorenja grozdja (Liu in sod., 2006; Conde in sod., 2007) ter tudi od mikroklimatskih razmer med rastno dobo trte (Haselgrove in sod., 2000). V prvem letu poskusa ni bilo značilnih razlik v vsebnosti sladkorjev in kislin, kar gre najverjetneje pripisati nadpovprečnim temperaturam v mesecu juliju ter podpovprečni količini padavin v mesecih juliju in avgustu. V letu 2014 pa je bila vsebnost glukoze in fruktoze največja pri obeh razlistanjih (113,2 in 112,7 g/l za glukozo ter 108,4 in 106,0 g/l za fruktozo). Pri ukrepu DMR pa se vsebnost ni bistveno spremenila v primerjavi s kontrolo in je znašala za glukozo 103,7 g/l ter za fruktozo 97,9 g/l. Poni in sod. (2009) navajajo, da zgodnja odstranitev listov, kasneje ob zorenju povzroči hitrejše premeščanje sladkorjev v grozde, saj so slednji eni od glavnih porabnikov ogljikovih hidratov. Zgodnja odstranitev listov v predelu grozdja ter odstranitev listov nad drugim parom žic med zorenjem sta vplivali na značilno večjo vsebnost jabolčne kisline v prvem letu poskusa, medtem ko je obravnavanje DMR v drugem letu vplivalo na 0,8 g/l več vinske kisline ter 0,9 g/l več jabolčne kisline v grozdnem soku v primerjavi s kontrolo. Prav tako je pri DMR bilo več citronske kisline v drugem letu kot pri ostalih obravnavanjih. V naši raziskavi je bila povprečna temperatura zraka v času zorenja grozdja pod dolgoletnim povprečjem, kar je glede na trditve Bubola in sod. (2011) lahko razlog za manjše povečanje vsebnosti vinske kisline in nekoliko večjo vsebnost jabolčne kisline. Velja poudariti, da na primer Corso in sod. (2013) v raziskavi z obravnavanjem DMR niso ugotovili povečanja vsebnosti jabolčne in vinske kisline v grozdnem soku.

Sestava grozdja neposredno vpliva na sestavo in organoleptične lastnosti vina (Bindon in sod., 2013). Vsebnost sladkorja v grozdju neposredno vpliva na vsebnost alkohola v vinu (Liu in sod., 2006). Vina iz sorte 'Istrska malvazija' imajo običajno večjo vsebnost alkohola, le-ta pa pri potrošnikih ni vedno zaželena (Bindon in sod., 2014). Ne glede na obravnavanje je bila vsebnost alkohola v vinih iz prvega leta poskusa na splošno večja, med 13,6 in 14,0 % vol., v primerjavi z drugim letom. To bi lahko pripisali pretežno sušnemu obdobju v času zorenja in posledično večji sintezi sladkorjev v grozdju. Zgodnje razlistanje je značilno zmanjšalo vsebnosti skupnega ekstrakta ter sladkorja prostega ekstrakta v vinu. Predvsem pa je v drugem letu vplivalo na zmanjšanje skupnih kislin v vinu, manjša je bila tudi vsebnost jabolčne kisline in značilno večja vsebnost vinske kisline v vinu. Podobne ugotovitve so podali tudi Kozina in sod. (2008) v raziskavi z razlistanjem v predelu grozdja v času zorenja na sortah 'Sauvignon' ter 'Riesling'. Isti avtorji manjšo vsebnost jabolčne kisline v moštu in vinu iz sorte 'Sauvignon' pripisujejo razgradnji jabolčne kisline v grozdnih jagodah, kar navajajo kot posledico nenadne izpostavljenosti jagod soncu in višjim temperaturam. Največ jabolčne kisline v grozdju namreč nastaja pri temperaturah med 20 in 25 °C (Conde in sod., 2007).

Na drugi strani pa se je vsebnost jabolčne kisline v vinu povečala pri razlistanju nad drugim parom žic ter DMR. Sklepamo, da se pri razlistanju mlajših listov zmanjša sinteza sladkorjev v času zorenja, posledično je vsebnost kislin v grozdnih jagodah in vinu večja. Na drugi strani pri DMR pa Cargnello in sod. (2005) in Carbonneau in Murisier (2009) ne poročajo o povečanju ali zmanjšanju vsebnosti kislin kljub večji koncentraciji sladkorja. V vinu iz zgodnjega razlistanja v predelu grozdja je bila vsebnost reducirajočih sladkorjev značilno manjša, v primerjavi z ostalimi obravnavanji. Čeprav se vsebnost reducirajočih sladkorjev ni značilno razlikovala od kontrole pri drugem razlistanju in DMR. Kozina in sod. (2008) navajajo, da odstranitev listov v predelu grozdja lahko vpliva na večjo vsebnost reducirajočih sladkorjev v vinu, vendar v raziskavi niso zabeležili značilnih razlik v vinih, pridelanih iz grozdja z odstranitvijo listov in kontrole.

Na senzorične lastnosti vina pomembno vplivajo fenolne spojine. V jagodni kožici sorte 'Istrska malvazija' smo identificirali in kvantificirali 19 različnih fenolnih spojin iz skupin hidroksicimetnih kislin, flavanolov in flavonolov. Preračunali smo tudi delež posamezne spojine glede na vsoto vseh znotraj posamezne fenolne skupine. Znano je že, da ampelotehnični ukrepi vplivajo na sestavo in vsebnost fenolov v grozdju in vinu (Kozina in sod., 2008; Sternad Lemut in sod., 2013). V naši raziskavi so posamezna obravnavanja različno vplivala, ne samo na vsebnost posameznih fenolov, pač pa tudi na njihov delež. Izmed hidroksicimetnih kislin je bil največji vpliv obravnavanj viden pri heksozidu *p*-kumarne kisline, vsebnost omenjene spojine se je v drugem letu povečala pri vseh treh obravnavanjih v primerjavi s kontrolo. Zgodnje razlistanje je povečalo vsebnost in delež skupnih hidroksicimetnih kislin za približno 50 % v obeh letih. Bubola in sod. (2011) so analizirali vsebnost hidroksicimetnih kislin v grozdnem soku sorte 'Istrska malvazija' pri različnih terminih odstranitve listov. Največje povečanje vsebnosti hidroksicimetnih kislin so zabeležili pri odstranitvi treh do štirih listov v fazi nastanka grozdov (fenološka faza BBCH 71), medtem ko razlistanje pred cvetenjem in ob začetku zorenja ni imelo bistvenega vpliva na omenjene fenolne spojine. Povečano sintezo hidroksicimetnih kislin, Bubola in sod. (2011) povezujejo z izpostavljenostjo grozdja višjim temperaturam in svetlobi. Na drugi strani pa Sternad Lemut in sod. (2013) poročajo, da je izpostavljenost

grozdov sorte 'Modri Pinot' višjim temperaturam povzročila manjšo vsebnost hidroksicimetnih kislin, obenem pa sklepajo, da je daljša izpostavljenost grozdov svetlobi, dosežena z zgodnjim razlistanjem v fenofazi nastavka plodov (BBCH 71) pripomogla k povečanju vsebnosti omenjenih fenolnih kislin, kar bi lahko pojasnilo tudi naše ugotovitve. Izmed izvedenih ampelotehničnih ukrepov smo najmanjši vpliv na flavanole opazili pri odstranitvi mlajših listov v času zorenja grozdja. Ta pojav gre najbrž pripisati temu, da so grozdi pri tem obravnavanju ostali zasenčeni in močnejša svetloba ni vplivala na sintezo flavanolov, kot navajajo Feng in sod. (2015). Samo DMR je v obeh letih povečal vsebnost procianidin trimera 2. V prvem letu poskusa je največji delež k skupnim flavanolom prispeval procianidin dimer, v drugem letu pa je največji delež predstavljal procianidin trimer (med 26,5 in 31,4 %). Na splošno smo pri obravnavanju DMR ugotovili nekoliko večji vpliv na posamezne fenole, prav tako so bile vsebnosti fenolov v grozdju večje v prvem letu v primerjavi z drugim letom, kar je posledica predvsem različnih vremenskih razmer med letoma.

Castillo-Muñoz in sod. (2010) navajajo, da je sinteza flavonolov v grozdnih jagodah odvisna od svetlobe in večja v grozdih, ki so bolj osvetljeni. Flavonoli absorbirajo UV-A (325-400 nm) in UV-B (280-325 nm) sevanje in s tem ščitijo rastlino pred škodljivimi vplivi sevanja (Castillo-Muñoz in sod., 2007). Sinteza flavonolov se močno poveča ob nenadni spremembi v osvetlitvi grozdov, ki jo povzroči na primer odstranitev listov v coni grozdja (Sternad Lemut in sod., 2013). Opazili smo povečanje vsebnosti nekaterih glikozidov kamferola, kvercetin-3-glukuronida in heksozid apigenina pri zgodnjem razlistanju v coni grozdja. Sicer je bilo povečanje značilno samo v prvem letu poskusa, kar gre najverjetneje pripisati podpovprečnemu številu sončnih dni v drugem letu pred in med zorenjem grozdja. Podoben odziv smo zabeležili tudi pri razlistanju zgornjih, mlajših listov in pri obravnavanju DMR. Prav slednje je povečalo vsebnost in delež nekaterih glikozidov izoramnetina in kamferola v obeh letih, kar je najverjetneje povezano z venenjem in sušenjem listov po rezi DMR in posledično večjo ter nenadno osvetlitvijo grozdov. Na drugi strani pa Poni in sod. (2013) poročajo, da odstranitev listov nad predelom grozdja med zorenjem grozdja ni značilno vplivala na fenolno zrelost in fenolne spojine v grozdnih jagodah sorte 'Sangiovese', kar so povezali s tem, da ima razlistanje nad predelom grozdja majhen vpliv na mikroklimo grozdov. Podoben vpliv odstranitve fotosintezno aktivnejših listov na mladikah na vsebnost fenolov smo ugotovili tudi v naši raziskavi.

Celokupna vsebnost flavonolov je predstavljala največji delež glede na vse analizirane fenole in je ne glede na leto in obravnavanje znašal med 78,1 in 89,9 %. Glavni delež v skupini flavonolov v grozdju (kožici) sorte 'Istrska malvazija' je dajal kvercetin-3-glukozid (med 32,4 in 56,2 % vseh flavonolov), medtem ko je kvercetin-3-glukuronid predstavljal precej manjši delež (med 6,7 in 17,5 % vseh flavonolov). Sicer pa se deleži kvercetin-3-glukozida ter kvercetin-3-glukuronida iz naše raziskave (Reščič in sod., 2016) ujemajo z deleži iz raziskave Montealegre in sod. (2006), vendar so bile pri nas ugotovljene tudi do desetkrat večje vsebnosti, kar je zagotovo vpliv analitskih metod ter razmer v laboratoriju (Gómez-Alonso in sod., 2007).

V pridelanih vinih iz poskusa na sorti 'Istrska malvazija' smo s HPLC in HPLC-MS metodo identificirali in kvantificirali 15 različnih fenolnih spojin. V primerjavi s fenolnim profilom kože smo v vinu poleg hidroksicimetnih kislin, flavanolov ter flavonolov

identificirali še protokatehujsko kislino iz skupine hidroksibenzojskih kislin in resveratrol-3-glukozid iz skupine stilbenov. Kot razlog za različnost fenolnih spojin prisotnih v kožici in vinu, Conde in sod. (2007) navajajo sintezo in transformiranje določenih fenolnih snovi med procesom vinifikacije. Vsebnosti posameznih in skupnih hidroksicimetnih kislin v vinu so bile sicer manjše, kot so jih ugotovili Bubola in Peršurić (2012) v raziskavi z različnimi termini razlistanja na sorti 'Istrska malvazija'. Vpliv izvedenih ampelotehničnih ukrepov v naši raziskavi je pokazal, da sta zgodnje razlistanje in DMR v večji meri povečala vsebnosti nekaterih fenolov v vinu, v primerjavi z razlistanjem mlajših listov in kontrolo. Najbolj zastopana fenolna skupina v vinu so bile hidroksicimetne kisline, med katerimi smo identificirali in kvantificirali osem različnih derivatov.

Vpliv ampelotehničnih ukrepov na senzorične lastnosti vina smo v raziskavi dodatno preverili s senzorično analizo oziroma oceno, katero je izvedlo šest uradnih in usposobljenih pokaševalcev. Senzorično ocenjevanje je bilo izvedeno po 20-točkovni Buxbaumovi metodi, kjer sta bili možni maksimalno dve točki za bistrost, dve točki za barvo, štiri točke za vonj (aromo), šest točk za okus ter maksimalno šest točk za harmonijo (celotni vtis). Predvsem nas je zanimal vpliv posameznega ampelotehničnega ukrepa na okus ter harmonijo v poskusu pridelanih vin. Pokaševalci so ocenili vina, pridelana iz grozdja pod vplivom razlistanja zgornjih, mlajših listov in DMR, kot manj bogata na okusu ter manj harmonična, doseženo število točk je bilo pri obeh 15 od skupno 20. Na drugi strani pa je bilo vino, pridelano iz grozdja pod vplivom zgodnjega razlistanja, ocenjeno kot vino z več okusa, bolj harmonično in z več arome (16,5 točk od skupno 20), iz česar bi se lahko pridelalo lažje ter bolj všečno vino.

Vsaka sorta se drugače odziva na določen ampelotehnični ukrep, kar so potrdili tudi Cargnello (2000), Bavaresco in sod. (2008), Bubola in Peršurić (2012), Sternad Lemut in sod. (2013), Intrigliolo in sod. (2014) ter številni drugi. Predvsem pa na kakovost grozdja in vina poleg sorte (Prajitna in sod., 2007; Tardaguila in sod., 2010) in dejavnikov okolja (Jackson in Lombard, 1993) vplivajo tudi ampelotehnični ukrepi (Gil in sod., 2013; Šuklje in sod., 2013). Pri sortah, ki so bujne in dajejo velike pridelke sta običajno sestava in kakovost grozdja in vina slabši in nezadostni. Zato nas je v tretji raziskavi zanimal vpliv DMR grozdja na kemijsko sestavo grozdja in vina sort 'Rebula' in 'Vitovska grganja' (*Vitis vinifera* L.). Vplive smo vrednotili z analizo osnovnih parametrov zorenja v grozdnem soku, analizo parametrov vina, primarnih metabolitov (sladkorjev in organskih kislin) v grozdnem soku ter analizirali smo fenolni profil jagodne kožice in vina.

Bločni poskus je bil zasnovan z dvema obravnavanjema, in sicer kontrolo in dvojnimi namenskimi zorenjem grozdja (DMR). Rez šparona (enoletne rozge) za ukrep DMR smo izvedli, ko je grozdje doseglo želeno vsebnost topne suhe snovi, in sicer 18 °Brix za sorto 'Rebula' ter 17,5 °Brix za sorto 'Vitovska grganja'. Tri do štiri mladike na glavi debla smo pustili za nadaljnjo rast posamezne trte in zato niso bile odrezane. Po rezi je grozdje ostalo na trti vse do trgatve, ki pa je bila, ko je grozdje pri obravnavanju DMR doseglo približno 22 °Brix.

Izhlapljanje vode je posledično prispevalo h koncentriranju in povečanju topne suhe snovi v grozdnem soku, kar smo potrdili ne glede na sorto in leto. V povprečju je bilo povečanje vsebnosti topne suhe snovi v primerjavi s kontrolo večje pri sorti 'Vitovska grganja' (2,65

°Brix), v primerjavi s sorto 'Rebula' (1,45 °Brix), kar bi lahko povezali z dejstvom, da ima grozdje sorte 'Rebula' debelo kožico (Hrček in Koruza, 1996), ki, kot poročajo Rolle in sod. (2011), pripomore k manjšemu izhlapevanju vode iz jagod. DMR je pričakovano značilno povečal vsebnost glukoze in fruktoze, kar so za vsebnost skupnih sladkorjev ugotovili že Corso in sod. (2013). Corso in sod. (2013) poročajo tudi, da je ukrep DMR značilno zmanjšal vsebnost titracijskih kislin, ter za od 40 do 45 % zmanjšal vsebnost jabolčne kisline v moštu rdeče sorte 'Raboso Piave'. Kot razlog za takšno zmanjšanje vsebnosti jabolčne kisline navajajo povečanje oksidacije malata in pretvorbo jabolčne kisline v glukozo in fruktozo. V naši raziskavi smo opazili ravno obraten odziv. Pri obravnavanju DMR smo opazili značilno povečanje titracijskih kislin pri obeh sortah. Povečala se je tudi vsebnost jabolčne kisline pri obravnavanju DMR v primerjavi s kontrolnim obravnavanjem pri obeh sortah, razen pri sorti 'Rebula', kjer smo zabeležili rahlo zmanjšanje pri DMR v primerjavi s kontrolo v drugem letu poskusa. Prav tako smo ugotovili povečanje vsebnosti vinske kisline v moštu sort 'Rebula' in 'Vitovska grganja'. Dejstvo, da je prišlo pri obravnavanju DMR do povečanja vsebnosti obeh pomembnejših kislin najverjetneje nakazuje na delovanje encimov, ki sprožijo sintezo omenjenih kislin, čeprav je presnovna pot vinske in jabolčne kisline različna (Conde in sod., 2007). Izmed organskih kislin v moštu smo v raziskavi določili tudi citronsko kislino, katere vsebnost se je rahlo, a značilno povečala pri DMR obravnavanju sorte 'Rebula'. Ob povečanju vsebnosti skupnih titracijskih in posameznih organskih kislin v moštu se je pH le rahlo, v prvem letu tudi značilno, znižal.

Po analizi osnovnih parametrov v vinu smo ugotovili, da je DMR grozdja značilno povečalo vsebnost alkohola v pridelanih vinih iz sort 'Rebula' in 'Vitovska grganja' v letih 2012 in 2013. Ugotovitev je bila pričakovana glede na večjo vsebnost sladkorjev v grozdju, saj sladkor predstavlja substrat, ki ga kvasovke med alkoholno fermentacijo pretvorijo v alkohol (Conde in sod., 2007). Končna vsebnost alkohola je v pridelanih vinih, ne glede na sorto in leto znašala med 12,3 in 13,1 % vol. Garrido in Borges (2013) navajata, da alkohol vpliva na telo vina in da so vina z večjo vsebnostjo alkohola bolj kompleksna. Poleg alkohola, na kompleksnost vin vpliva tudi vsebnost skupnega ekstrakta (Panceri in sod., 2013), ki pa je bila v naši raziskavi značilno večja v vinih pridelanih iz grozdja DMR.

Prav tako je DMR značilno povečal vsebnost skupnih kislin v vinu sorte 'Rebula' v prvem letu (2012) za 1 g/l, medtem ko v drugem letu ter pri sorti 'Vitovska grganja' med obravnavanji nismo zabeležili značilnih razlik, kar sovпада z navedbami Carbonneau in Murisier (2009). Značilno pa se je povečala vsebnost jabolčne kisline v vinih iz DMR ne glede na sorto in leto, v vinu 'Rebula' v povprečju za 81,5 %, v vinu 'Vitovska grganja' pa za 47,1 % glede na vino iz kontrole, kar pa se ne ujema z ugotovitvami predhodnih raziskav Reščič in sod. (2016). Razlog za takšno povečanje jabolčne kisline je kot navajajo Mato in sod. (2005) v vsebnosti jabolčne kisline v grozdju, saj vinska, jabolčna in citronska kislina preidejo v mošt in vino iz grozdja v fazi predelave. Ugotovili smo tudi, da se je vsebnost mlečne kisline značilno zmanjšala pri obravnavanju DMR. Vsebnost jabolčne in mlečne kisline v vinih iz DMR v naši raziskavi nakazuje na dejstvo, da spontani biološki razkis, kjer se ob delovanju jabolčno-mlečno kislinskih bakterij jabolčna kislina pretvarja v mlečno (Bauer in Dicks, 2004; Mato in sod., 2005), ni potekel. V povezavi s tem, Bauer in Dicks (2004) navajajo, da je večja vsebnost alkohola (med 12 in

14 % vol.) omejujoč dejavnik biološkega razkisa, ki zavre delovanje mlečnokislinskih bakterij.

Z ukrepom DMR se spodbudi zorenje grozdja (Cargnello in sod., 2005; Corso in sod., 2013). Ob sočasni dehidraciji vode iz jagod, se povečuje razmerje med površino kože in volumnom jagode, kar v osnovi predstavlja pomemben vzvod za sintezo oziroma kopičenje fenolnih snovi v grozdju (Conde in sod., 2007). V splošnem smo izmerili večjo vsebnost posameznih in skupnih hidroksicimetnih kislin pri obravnavanju DMR, v primerjavi s kontrolo. Po poročanjih nekaterih avtorjev (Bonghi in sod., 2012; Corso in sod., 2013) je povečanje vsebnosti hidroksicimetnih spojin lahko posledica stresa po rezi šparona ter sočasne dehidracije, ki vpliva na spremembe vodnega potenciala, kar pa se lahko odraža v drugačnem delovanju encimov in prekursorjev fenilpropanoidne poti. Ob stresnih razmerah rastlina sproži določene obrambne mehanizme, na primer pred izgubo vode. V povezavi s tem Conde in sod. (2007) navajajo, da ima *p*-kumarna kislina vlogo pri lignifikaciji celične stene jagod, ki predstavlja pomembno obrambo pred izhlapevanjem vode. Izmed hidroksicimetnih kislin smo identificirali in kvantificirali heksozid *p*-kumarno kislino in kutarno kislino. Mozetič in sod. (2006) sicer ne poročajo o vsebnosti *p*-kumarne kisline v grozdnih jagodah sorte 'Rebula', kar pa se lahko pripiše tudi drugačnim analitskim metodam (Gómez-Alonso in sod., 2007).

Med flavanoli sta največjo vsebnost v jagodni kožici imela procianidin dimer 1 ('Rebula') in procianidin trimer 4 ('Vitovska grganja'). O procianidinih kot glavnih flavanolih poročajo tudi Montealegre in sod. (2006), katerih vsebnosti se ujemajo s podatki iz naše raziskave. DMR je vplivalo na značilno povečanje vsebnosti večine posameznih flavanolov v obeh letih, saj se je pri sorti 'Rebula' povečala vsebnost pri sedmih od devetih spojin in pri sorti 'Vitovska grganja' pri osmih od devetih spojin. Značilno povečanje posameznih flavanolov gre po navedbah Panceri in sod. (2013) pripisati izhlapevanju vode iz jagod ter zato koncentriranju vsebine jagod. Rolle in sod. (2009) in Bonghi in sod. (2012) navajajo, da izguba vode povzroči oksidacijo ter pospešeno aktivnost encima polifenoloksidaze, kar vodi v zmanjšanje vsebnosti flavanolov in ostalih fenolov v grozdju, kar pa z našimi rezultati ne moremo potrditi.

Večjo vsebnost flavonolov, podobno kot flavanolov, smo izmerili pri DMR obravnavanju, a vendar je bil vpliv DMR na vsebnosti in deleže flavonolov nekoliko večji pri sorti 'Rebula' v primerjavi s sorto 'Vitovska grganja'. Med flavonoli je imel kvercetin-3-glukozid (pri sorti 'Rebula' z med 52,1 in 62,4 % in pri sorti 'Vitovska grganja' z med 51,7 in 62,5 % deležem glede na celokupno vsebnost flavonolov), največjo vsebnost. Vsebnosti flavonolov iz naše raziskave se ujemajo z vsebnostmi, ki jih poročajo Deluc in sod. (2009) in Bonghi in sod. (2012), ki zatrjujejo, da je večja akumulacija flavonolov nekakšen odgovor na izzvane stresne razmere, ki sprožijo aktivacijo ligninske poti ter povečane ekspresije genov. Kot poročajo Castillo-Muñoz in sod. (2007), bi lahko povečanje vsebnosti flavonolov pripisali aktivaciji le-teh ob večji izpostavljenosti grozdja svetlobi, kar pa se doseže pri DMR, saj listi hitro ovenijo in zaradi tega se grozdi izpostavijo svetlobi.

V vinu pa smo poleg hidroksicimetnih kislin, flavanolov in flavonolov identificirali in kvantificirali še hidroksibenzojske kisline in stilbene. Kot poročajo Ružić in sod. (2011) so

v belih vinih najbolj zastopane hidroksicimetne kisline, kar smo potrdili tudi v naši raziskavi. Poleg tega, Ružič in sod. (2011) še poročajo, da je bila v belih maceriranih vinih najbolj zastopana kaftarna kislina, s povprečno vsebnostjo 53 mg/l. Pri nas sta bila izmed hidroksicimetnih kislin najbolj zastopana derivat 1 ('Rebula') in derivat 2 *p*-kumarne kisline ('Vitovska grganja'), ki sta ne glede na obravnavanje in leto predstavljala delež med 13,2 in 36,9 % vseh hidroksicimetnih kislin. Poleg tega smo v vinu iz grozdja sorte 'Rebula' določili šest različnih procianidinov, katehin, epikatehin in naringenin, medtem ko v vinu iz sorte 'Vitovska grganja' pa smo poleg katehina in epikatehina identificirali še tri različne procianidine. Največji delež sta predstavljala procianidin trimer 1 ('Rebula') in procianidin dimer 3 ('Vitovska grganja'). Značilen vpliv DMR smo zabeležili pri vsebnosti hidroksibenzojskih kislin in stilbenov. Izmed hidroksibenzojskih kislin smo v vinu iz sorte 'Rebula' identificirali galno in protokatehujsko kislino ter etil galat siringinske kisline, medtem ko v vinu iz sorte 'Vitovska grganja' pa samo protokatehujsko kislino, kar se lahko pripiše sortni lastnosti (Conde in sod., 2007). Poleg sorte pa so za različno fenolono sestavo vin iz določene sorte po navedbah Monagas in sod. (2005) in Conde in sod. (2007), razlog lahko tudi razmere med zorenjem grozdja.

DMR je vplivalo tudi na vsebnost nekaterih flavonolov v pridelanem vinu. Kljub temu, da Monagas in sod. (2005) navajajo, da flavonoli predstavljajo manjšo skupino fenolov v belih vinih, smo kvantificirali nekatere glikozide kamferola in kvercetina. Poleg tega smo v vinu iz sorte 'Rebula' identificirali dva glikozida laricitrina, v vinu iz sorte 'Vitovska grganja' pa glikozid siringetina. Sicer Garrido in Borges (2013) navajajo, da laricitrin in siringetin glikozida običajno nista prisotna v belih vinih, ker se encim 3',5'-hidroksilaza ne izraža v grozdju belih sort. Rezultati naše raziskave pa se ujemajo z rezultati raziskave, ki so jo opravili Jeffery in sod. (2008).

Stilbeni so bioaktivne spojine, ki imajo pozitiven vpliv na človekovo zdravje, saj zavirajo nastanek srčno-žilnih bolezni (Romero-Pérez in sod., 1999; Romero-Pérez in sod., 2001). Najbolj pogosto zastopana stilbena v vinu sta *cis*- in *trans*-resveratrol (Garrido in Borges, 2013). Omenjeni spojini smo identificirali in kvantificirali tudi v pridelanih vinih, njuna koncentracija je bila večja v vinih pridelanih iz grozdja DMR, kot v tistih iz kontrole, kar je bilo še izrazitejše pri sorti 'Vitovska grganja'. Garrido in Borges (2013) trdita, da je resveratrol prisoten tudi v jagodnih kožicah, vendar je ta vsebnost tako majhna, da ga običajno sistem HPLC ne zazna. Garrido in Borges (2013) povezujejo povečanje vsebnosti resveratrola v jagodah z okužbo z glivo *Botrytis cinerea* (Pers.), vendar pri delnem sušenju listi v okolici grozdja ovenijo in se delno posušijo, poveča se zračnost grozdov in je tako manjša možnost okužbe z omenjeno glivo. Da je za povečanje vsebnosti resveratrola v jagodah potreben stres razlagajo tudi Romero-Pérez in sod. (2001), ki kot stresne razmere poleg okužbe z glivama, ki povzročata sivo grozdno plesen in oidij vinske trte, navajajo še izpostavljenost grozdov UV svetlobi.

Vsebnost fenolov v vinu je odvisna od vsebnosti fenolov v jagodni kožici. Conde in sod. (2007) razlagajo, da DMR povzroči hitrejšo fenolno zrelost, kar vodi v večjo vsebnost fenolnih snovi v jagodah. Podobno hipotezo so potrdili tudi Río Segade in sod. (2008) in Hernández-Hierro in sod. (2014), ki poročajo, da že zelo kratka dehidracija jagod vpliva na strukturne, mehanske in kemijske lastnosti jagod. Posledično to lahko vodi v večjo ekstrakcijo fenolnih spojin iz grozdja v mošt. Conde in sod. (2007) navajajo tudi, da se

fenolne spojine modificirajo in sintetizirajo med ekstrakcijo in alkoholno fermentacijo, kar je morebiten razlog za pojav določenih fenolnih spojin samo v vinu.

Delna ali pa večja izguba vode povzroči številne spremembe v kemijski sestavi grozdne jagode, predvsem v sestavi in vsebnosti sekundarnih metabolitov. Zato smo v letu 2013 zastavili poskus z dvema različnima tehnikama delnega sušenja grozdja na sorti 'Refošk'; (1) dvojnimi namenskimi zorenjem grozdja (DMR) in (2) z delnim sušenjem (dehidracijo) grozdja v zaprti komori. Proučevali smo odziv sorte na obe obravnavanji v okviru sestave in vsebnosti osnovnih parametrov dozorevanja in primarnih metabolitov ter dinamiko sinteze sekundarnih metabolitov v grozdju in vinu. Izguba vode oziroma izhlapevanje je glavni promotor sprememb v kemijski sestavi, pomembno vlogo imajo tudi dejavniki okolja (Franco in sod., 2004).

V primerjavi z DMR, smo pri delnem sušenju v komori zabeležili večji delež izgube mase, kar je najverjetneje posledica dehidracije jagod pod kontroliranimi razmerami, kjer je izhlapevanje vode bolj konstantno in neodvisno od okoljskih, vremenskih razmer, ki dehidracijo jagod morebiti tudi upočasnijo. Obenem pa smo pri obravnavanju DMR zabeležili nekoliko večjo vsebnost topne suhe snovi v primerjavi z delnim sušenjem in kontrolo, čeprav razlike niso bile značilne. To bi lahko delno pripisali nadaljnji fotosintezni aktivnosti, ki so jo listi ohranili vsaj nekaj dni po rezi šparona. Corso in sod. (2013) navajajo, da pride pri DMR do povratnega toka ksilema iz jagod ter sočasno do slabšega delovanja floema, posledica pa je izguba vode in koncentriranje sladkorjev v jagodi. V nasprotju z pravkar omenjenim pa v naši raziskavi nismo zabeležili značilnih razlik v vsebnosti glukoze in fruktoze v grozdju iz DMR v primerjavi s kontrolo, vendar pa je bila vsebnost saharoze v delno sušenem grozdju iz obeh obravnavanj skoraj dvakrat večja kot pri kontroli. Obraten odziv kot pri sladkorjih smo ugotovili pri vsebnosti organskih kislin, saj je delno sušenje vplivalo na značilno večje vsebnosti vinske, jabolčne ter skupnih kislin v primerjavi s kontrolo. Glede na dejstvo, da se vsebnost sladkorjev z delnim sušenjem ni bistveno spremenila, bi pojav najbrž lahko pojasnili s koncentriranjem kislin v jagodi. Bellincontro in sod. (2016) poročajo, da se vsebnost kislin v začetni fazi sušenja najprej zmanjša, kasneje pa se z večjo izgubo vode zopet poveča. Zanimivo, kljub ne največji vsebnosti kislin, je bil pri DMR pH grozdnega soka najnižji (2,54), najvišjega pa smo zabeležili pri kontroli (2,96).

V pridelanih vinih nismo beležili značilnih razlik v pH, prav tako ne v vsebnosti alkohola. Slednja se je v vinih iz vseh treh obravnavanj gibala okrog 10 % vol., kar je tudi v skladu s Pravilnikom o pridelavi vina teran PTP (2000), za katerega se uporablja izključno sorto 'Refošk'. V vinih pridelanih iz delnega sušenja, glede na kontrolo, smo zabeležili značilno večjo vsebnost organskih kislin in skupnega ekstrakta. Iz rezultatov bi lahko sklepali, da bi imela vina pridelana s tehniko delnega sušenja več svežine, obenem pa bi bila bolj prijetna in ekstraktna.

Bolj izrazit, a hkrati različen vpliv tehnik delnega sušenja (dehidracije), smo opazili v fenolnem profilu jagodne kože in vina. Značilen vpliv DMR na fenolne spojine se je odrazil v kožici, medtem ko je delno sušenje v komori značilno vplivalo na fenolni profil vina. Ne glede na obravnavanje so antocijani predstavljali največji delež med analiziranimi fenolnimi spojinami v jagodni kožici sorte 'Refošk', sledili so jim flavonoli. Poleg

omenjenih skupin fenolov smo v kožici identificirali in kvantificirali še dimer procianidina ter derivat *p*-kumarne kisline ter dva predstavnika stilbenov. V vinu pa so poleg antocianov večji delež predstavljali flavanoli, hidroksicimetne kisline, flavonoli ter stilbeni.

Rezultati raziskave so pokazali, da je DMR grozdja v primerjavi s kontrolo značilno povečalo vsebnosti antocianov, flavonolov in stilbenov v kožici in vinu. Večja vsebnost antocianov in drugih fenolov v kožici, v primerjavi s kontrolo in delnim sušenjem v komori, je najverjetneje posledica izgube vode oziroma dehidracije. Ob tem se poveča razmerje med kožico in volumnom jagode, kar vpliva na vsebnost fenolov v kožici.

Corso in sod. (2013) poročajo, da je obravnavanje DMR značilno povečalo vsebnost fenolnih spojin v grozdju sorte 'Raboso Piave' in kot razlog navajajo, da je grozdje iz DMR med zorenjem doseglo bolj uravnotežen fenolni profil ter boljšo fenolno zrelost. Podobno kot pri sušenju, ob zrelosti grozdja se vsebnost fenolov začne zmanjševati (Frangipane in sod., 2012). Ta pojav bi lahko pojasnil manjšo vsebnost nekaterih posameznih in skupnih fenolov v kožici grozdja pod vplivom delnega sušenja v komori. Prej omenjenega pojava pa nismo opazili v kožici pri DMR, čeprav le-ta ukrep vpliva na hitrejšo zorenje. Možno je, da je bila fenolna zrelost ob tehnološki zrelosti grozdja na vrhuncu, kar bi lahko pojasnilo zmanjšanje vsebnosti nekaterih posameznih fenolov v vinu iz grozdja DMR, v primerjavi z vinom iz kontrole. Zmanjšanje vsebnosti fenolov v vinu pa bi lahko še pojasnili s teksturo kožice sorte 'Refošk', saj je ekstrakcija antocianov iz kožice v mošt odvisna tudi od sestave celične stene kožice (Hernández-Hierro in sod., 2014). Celična stena kožice se ob dolgotrajnejšem in intenzivnejšem izhlapevanju vode zmehča, kar kot navajajo Zsófi in sod. (2014) vodi v lažjo ekstrakcijo antocianov, vendar je pojav precej odvisen od stopnje in načina sušenja jagod. Obenem pa so isti avtorji izpostavili da rahel do zmeren sušni stres sproži različne regulacijske mehanizme sekundarnega metabolizma, kar se odraža v pospešeni sintezi antocianov. Stres bi lahko bil po navedbah Romero-Pérez in sod. (2001) razlog tudi za povečano sintezo stilbenov.

Prav redčenje grozdja in razlitanje vinske trte sta pogosta ampelotehnična ukrepa v vinogradih, ki neposredno vplivata na sestavo in kakovost grozdja in omogočata pridelavo različnih stilov vina. Poleg tega, se vsaka sorta na določen ukrep različno odziva v smislu kakovosti. Zato je potrebno praktične ukrepe prilagajati prav odzivom sort, česar pa je bilo do sedaj premalo. Vse večje povpraševanje po različnih stilih vina in pomanjkanje raziskav na temo sortne ampelotehnike - kakšne ukrepe in v kolikšni meri izvajati na določeni sorti vinske trte, sta bila osnovno vodilo za študijo. Z rezultati poskusov smo pokazali, da sta intenziteta redčenja ter čas in način izvedbe razlitanja in ukrepa DMR, ključna dejavnika pri regulaciji vsebnosti določenih metabolitov v grozdju in vinu. Predvsem pa disertacija predstavlja temelj raziskav na področju sortne ampelotehnike pri nas, saj je bilo v poskusih prvič izvedeno zgodnje razlitanje ter razlitanje mlajših listov in ukrep DMR na lokalnih, udomačenih sortah žlahtne vinske trte.

3.2 SKLEPI

Glede na poskuse, ki so bili opravljeni v okviru doktorske disertacije, smo odgovorili na vse zastavljene hipoteze:

- Potrdili smo hipotezo, da se pri večji intenziteti redčenja grozdja poveča vsebnost fenolov. Intenzivno redčenje je, v primerjavi z manj intenzivnim redčenjem in kontrolo, značilno povečalo vsebnost posameznih in skupnih antocianov, flavonolov in hidroksicimetnih kislin v grozdju sorte 'Portugalka', ter vsebnost in delež večine acetiliranih in kumaroiliranih glikozidov malvidina v vinu.
- Zgodnje razlitanje je značilno povečalo vsebnost topne suhe snovi ter posameznih in skupnih fenolnih spojin v grozdju in vinu sorte 'Istrska malvazija', kar v večini potrjuje zastavljeno hipotezo. A vendar nismo potrdili vsakoletnega, permanentnega vpliva na fenole. Prav tako nismo potrdili značilnega vpliva zgodnjega razlitanja na zmanjšanje vsebnosti kislin v grozdju in vinu. V skladu z zastavljeno hipotezo, pa je bilo vino iz grozdja z zgodnjim razlitanjem senzorično ocenjeno kot vino z več okusa in harmonije.
- V drugem poskusu smo na zastavljeno hipotezo v večini odgovorili negativno, razlitanje zgornjih, mlajših listov na mladikah je namreč vplivalo na značilno večjo vsebnost jabolčne kisline v grozdju sorte 'Istrska malvazija', medtem ko nismo ugotovili značilnega vpliva na ostale skupne in posamezne sladkorje ter organske kisline. Poleg tega je razlitanje mlajših listov, v nasprotju z zastavljeno hipotezo, značilno vplivalo na večjo vsebnost posameznih flavonolov v jagodni kožici, predvsem kvercetin-3-glukuronida in kvercetin-3-rutinozida, značilnega vpliva na vsebnost in delež posameznih fenolnih spojin v vinu pa nismo potrdili. Pozitiven odgovor pa je podala senzorična ocena vina, saj je bilo vino iz grozdja z razlitanjem mlajših, fotosintezno aktivnejših listov, ocenjeno kot vino z manj harmonije in svežine ter manj uravnoteženo.
- Z drugim in tretjim poskusom smo potrdili hipotezo, da dvojno namensko zorenje poveča vsebnost sladkorjev, organskih kislin ter fenolov v grozdju. DMR je namreč značilno povečal vsebnost posameznih sladkorjev in organskih kislin ter vsebnost posameznih fenolnih spojin v grozdju sort 'Istrska malvazija', 'Rebula' in 'Vitovska grganja'.
- Prav tako smo pozitivno odgovorili na hipotezo, da DMR vpliva na glavne kakovostne parametre vina. DMR je v vinu značilno povečal vsebnost alkohola, skupnega ekstrakta ter ekstrakta brez sladkorja, vino iz grozdja DMR sorte 'Istrska malvazija' pa je bilo senzorično ocenjeno kot vino z manj harmonije in manj svežine.
- V četrtem poskusu smo potrdili hipotezo, da bo grozdje in vino iz DMR trt imelo večjo vsebnost fenolov, predvsem antocianov. DMR je značilno vplival na večjo vsebnost antocianov, flavonolov in stilbenov v grozdju in vinu sorte 'Refošk', v primerjavi s kontrolo in sušenjem v komori, vendar je slednje značilno povečalo vsebnost hidroksicimetnih kislin, flavanolov ter skupnih fenolov v grozdju in vinu.
- Glede na ugotovitve, predvsem v fenolnem profilu grozdja in vina, bi se ukrep DMR zagotovo lahko priporočal pri sortah 'Rebula' in 'Vitovska grganja' za

pridelavo bolj polnega vina z večjo vsebnostjo alkohola, medtem ko pri sorti 'Istrska malvazija' zaradi sušnega vremena v prvem letu in obilnih padavin v drugem letu poskusa, vpliva DMR ne moremo z gotovostjo potrditi.

- Ukrep DMR je cenejši in enostavnejši v primerjavi s sušenjem grozdja v komori, zato bi se lahko priporočal pri pridelavi grozdja in vina iz sorte 'Refošk'.

S poskusi, vključenimi v doktorsko disertacijo, smo pridobili vpogled v odziv primarnega in sekundarnega metabolizma v grozdnih jagodah izbranih sort žlahtne vinske trte na nekatere ampelotehnične ukrepe, med katerimi bi izpostavili DMR. Prvič smo pridobili celoten fenolni profil grozdja in vina sort 'Portugalka', 'Refošk' in 'Vitovska grganja'. Pridobljeno znanje je v pomoč vinogradnikom in vinarjem, z namenom da se zagotovi boljšo kakovost grozdja in vina, da se pridelava določen stil vina, ki ga potrošnik želi ter da se zmanjšajo stroški pridelave, poveča pa se cena in prepoznavnost določenih sort in vin.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V okviru doktorske disertacije smo opravili štiri ločene poskuse, s katerimi smo želeli raziskati vpliv določenih ampelotehničnih ukrepov na kakovost grozdja in vina pri izbranih sortah vinske trte (*Vitis vinifera* L.). Poskusili smo preučiti značilne odzive trte oziroma vplive posameznega ampelotehničnega ukrepa in s tem potencialno zagotoviti pridelovalcem grozdja in vina možnost za izboljšanje kakovosti in pridelavo drugačnega stila vina.

Izvedli smo poskus z redčenjem grozdja na rdeči sorti 'Portugalka', v katerem smo uporabili dve intenzivnosti redčenja grozdja, prvo z 20-30 % odstranitvijo grozdov in drugo z 40-50 % odstranitvijo grozdov. Redčenje smo opravili v fenološki fazi, ko so bile jagode velikosti zrn graha (BBCH 75). Vplive redčenja na kakovost smo ugotavljali z merjenjem barve jagod in vina in v zadnjem letu poskusa tudi z določanjem sekundarnih metabolitov v grozdju in vinu. Največji vpliv smo opazili pri najbolj intenzivnem redčenju, saj se je samo pri tem zmanjšal pridelek na trto in povečala se je vsebnost topne suhe snovi ter zmanjšala vsebnost titracijskih kislin. Pri obeh redčenjih je bil CIRG indeks obarvanosti jagod večji kot pri kontroli, prav tako so imela vina pridelana iz redčenega grozdja značilno večjo intenziteto barve. V poskusu z redčenjem grozdja smo prvič prikazali celoten fenolni profil, tako grozdja kot vina sorte 'Portugalka', kar je še dodaten doprinos k znanosti. Intenzivno redčenje (40-50 %) je značilno povečalo posamezne in skupne fenole v jagodni kožici. Poleg skupnih hidroksicimetnih kislin, je redčenje vplivalo na večjo vsebnost kaftarne kisline in pentoze *p*-kumarne kisline v kožici in vinu. Povečala se je tudi vsebnost nekaterih flavanolov. Redčenje je vplivalo na značilno večjo vsebnost skupnih in nekaterih posameznih flavanolov, predvsem kvercetin-3-glukuronida in laricitrin-3-glukuronida v kožici. Redčenje je tudi vplivalo na fenolno sestavo v vinu, saj so bili določeni nekateri fenoli, ki jih v grozdju nismo našli. Prav tako je bilo opaziti značilno večjo vsebnost posameznih hidroksicimetnih kislin, flavanolov in nekaterih flavanolov.

V naslednjem poskusu nas je zanimal vpliv treh različnih ampelotehničnih ukrepov na osnovne parametre, primarne ter sekundarne metabolite grozdja in vina sorte 'Istrska malvazija'. Pričakovano se je pridelek značilno zmanjšal pri dvojnem namenskem zorenju (DMR) grozdja, medtem ko je bil največji pridelek v primerjavi s kontrolo, pri zgodnjem razlistanju. Prav tako sta zgodnje razlistanje in razlistanje zgornjih, mlajših listov vplivala na povečanje vsebnosti topne suhe snovi in titracijskih kislin. Vplive obeh razlistanj na posamezne sladkorje in kisline nismo mogli v celoti potrditi, saj je bilo povečanje ali zmanjšanje značilno samo v enem letu. Potrdili smo lahko le povečanje vsebnosti jabolčne kisline v grozdju pri odstranitvi mlajših listov v času zorenja. Kljub večji vsebnosti topne suhe snovi pri zgodnjem razlistanju smo določili manjšo vsebnost alkohola v vinu, v primerjavi s kontrolo. Poleg tega se je zmanjšal tudi pH, kar zavira razvoj neželenih in kvarljivih bakterij v vinu. V dvoletnem poskusu se ni pokazal značilen vpliv zgodnjega razlistanja na vsebnost fenolov v grozdju in vinu, kar bi lahko pojasnili s tem, da

razlistanje v predelu grozdja kmalu po cvetenju ne vpliva na spremembe in kasnejšo akumulacijo fenolnih snovi v jagodi.

Fenolne snovi se v grozdju tvorijo v času zorenja kot odgovor na stres in dejavnost okolja. Stresni situaciji s povečanimi vplivi okolja (večja osvetlitev) sta v našem poskusu predstavljala razlistanje zgornjih, mlajših listov ter DMR, kjer smo opazili tudi značilno povečanje vsebnosti posameznih fenolnih snovi v grozdju in vinu. Večjo vsebnost fenolov pri DMR lahko pripišemo tudi hitrejšemu zorenju. Vina, pridelana iz grozdja z razlistanjem mlajših listov ter DMR so bila v primerjavi z vini iz zgodnjega razlistanja in kontrole senzorično ocenjena z manj okusa in harmonije. Na drugi strani pa je bilo vino iz zgodnjega razlistanja v primerjavi z vini iz drugih obravnavanj ocenjeno in opisano kot lažje vino, z boljšim okusom in harmonijo. Glede na rezultate, bi sicer pričakovali, da bi bilo vino pridelano iz DMR bolj polno in kompleksno. Rezultati so uporabni za vinogradnike in vinarje, ki iščejo tehnološke smernice, kako pridelati različen stil vina, ki bi zadovoljil želje potrošnikov.

Tudi v tretjem poskusu smo se osredotočili na vplive dvojnega namenskega zorenja (DMR) na kemijsko sestavo grozdja in vina pri belih sortah. V dvoletni poskus sta bili vključeni dve lokalni in bujni sorti, 'Rebula' in 'Vitovska grganja' (*Vitis vinifera* L.). V primerjavi s kontrolnim obravnavanjem smo v grozdju obeh proučevanih sort določili manjšo maso 100 jagod pri obravnavanju DMR, večjo vsebnost topne suhe snovi ter tudi večjo vsebnost titracijskih kislin. Ugotovili smo, da je DMR grozdja vplivalo na primarne metabolite, saj smo opazili značilno večjo vsebnost glukoze in fruktoze v jagodah obeh sort, vinske kisline pri sorti 'Rebula' ter jabolčne kisline pri sorti 'Vitovska grganja'. Največjo vsebnost alkohola sta imeli vini iz grozdja DMR pri obeh sortah. V vinu, pridelanem iz grozdja DMR smo v primerjavi s kontrolo zabeležili tudi večje vsebnosti skupnega ekstrakta.

V raziskavi smo ugotovili, da so se vsebnosti posameznih fenolnih spojin značilno povečale v jagodni kožici iz grozdja DMR, kar smo opazili predvsem pri hidroksicimetnih kislinah in flavanolih, nekoliko manj pri flavonolih obeh sort. DMR je imel tudi manjši vpliv na skupne fenole pri sorti 'Rebula'. Prav tako se deleži posameznih fenolov v jagodni kožici glede na skupno vsebnost določene skupine pri obeh sortah niso bistveno spremenili. Med flavanoli je imelo obravnavanje DMR največji vpliv na procianidine ter na katehin, a manjši na epikatehin pri obeh sortah. Vsebnosti posameznih flavonolov v vinu pa so bile majhne.

V poskusu z obravnavanjem DMR in delnim sušenjem grozdja v komori smo ugotovili različen vpliv obeh obravnavanj predvsem na fenolni profil grozdja in vina rdeče sorte 'Refošk'. Vsebnosti sladkorjev se pri obravnavanjih v primerjavi s kontrolo niso bistveno spremenile. Ukrep DMR je značilno vplival na večjo vsebnost antocianov, flavanolov in stilbenov v kožici, v primerjavi s kontrolo. Na drugi strani pa smo opazili značilen vpliv delnega sušenja na fenole v vinu, v primerjavi s kontrolo in obravnavanjem DMR. Rezultati so pokazali, da ukrep DMR omogoča bolj uravnoteženo kakovost grozdja, z večjo vsebnostjo antocianov in drugih fenolnih spojin, kar zagotovo pripomore k intenzivnosti barve vina in okusu.

4.2 SUMMARY

Within the present framework, four separated experiments were carried out in order to investigate the influence of different canopy measures on grape and wine quality at different (*Vitis vinifera* L.) grapevine varieties. We tried to determine the most significant influence of each canopy measure and to provide to winegrowers and winemakers a potential to improve the quality and to produce a different wine style.

An experiment based on cluster thinning was conducted on a red 'Blauer Portugieser' grapevine variety with two different thinning intensities; first with 20-30 % of clusters removed and second with 40-50 % of removed clusters. The cluster thinning was always performed at the pea-size berry phenological stage (BBCH stage 75). The influences on the quality were assessed by measuring the colour parameters of berries and wine and in the last year of the experiment secondary metabolites were also determined in grape and wine.

The major impact was observed only at the severe (40-50 %) cluster thinning, as only this intensity reduced the yield on vine, augmented the soluble solids content and reduced the titratable acidity. Furthermore, we have observed the highest CIRG index of berries at both cluster thinning intensities in comparison with the control, as well the wines from thinned grape obtained significantly higher colour intensity, compared with control wines. In the present experimental study, for the first time the complete grape and wine phenolic profile was presented. The severe cluster thinning (40-50 % of removed clusters) significantly increased the contents of individual and total phenolic substances in the berry skin. Beside hydroxycinnamic acids, the thinning influenced also the content of caftaric acid and *p*-coumaric acid pentoside. Moreover, also the contents of epicatechin, procyanidin dimer and trimer were increased. Nevertheless, the content of the most represented flavonols, quercetin-3-glucuronide and laricitrin-3-glucuronide was increased by the severe cluster thinning. The thinning must have influenced also the composition of the phenolics in the wine, taking into account that certain phenolic compounds were only detected in the wine, in comparison to the berry skin. In addition, an increased content of individual hydroxycinnamic acids, flavanols and certain flavonols in the wine from thinned grape was observed.

In the following experiment, we focused on the impact of three different canopy measures on common characteristics and primary and secondary metabolites of 'Istrian Malvasia' grape and wine. Expectedly, the DMR significantly reduced the yield, while the highest yield, compared with the control, was obtained at early leaf removal. Also, both leaf removals impacted the augmentation of soluble solids and titratable acidity. In addition, the influences of both leaf removals on individual sugars and acids were not completely confirmed, since the augmentation or reduction of mentioned compounds was observed only in one year. All we could confirmed was only the increase of malic acid content at leaf removal above the second paired wire. Despite higher contents of soluble solids in grape berries at the early leaf removal and compared to the control, a lower content of alcohol was obtained in the wine. Furthermore, early leaf removal decreased the pH value, which is welcome to inhibit growth of adverse and spoilage bacteria in wine.

Phenolic compounds in grape berries are usually synthesized in the time of ripening as a response to environmental and stress conditions. In our experiment, the leaf removal above second paired wire represented stress conditions, where in general an increase in contents of phenolic compounds was observed. The increased contents of phenolics at DMR could be ascribed to faster ripening. The contents of individual phenolic compounds in the wine were increased at mentioned treatments, mostly due to higher phenolic content of the berries. Sensory evaluation resulted that the wines from leaf removal above second paired wire and DMR were less tasty and harmonic, compared with the wines from control and early leaf removal, where the latter was evaluated as a lighter wine with more taste and harmony. Regarding the results it would be expected, that the wine from DMR grape will be more complex and full bodied. The results are definitely useful for winegrowers and winemakers, who seek technological guidelines, how to produce a different wine style, that would satisfy consumer preferences.

In the third experiment we focused on the influences of Double Maturation Raisonnee (DMR) on chemical composition of grape and wine of white grapevine varieties. In a two-year experiment two local and vigorous varieties, 'Rebula' and 'Vitovska grganja' (*Vitis vinifera* L.) were included. In accordance to the control, DMR resulted in a lower 100-berries weight, higher soluble solids and titratable acidity at both varieties. We have found that DMR impacted primary metabolites, since significantly higher contents of glucose and fructose were determined in the must of both varieties, whereas higher content of tartaric acid at 'Rebula' and malic acid at 'Vitovska grganja'. The highest content of alcohol as well as of total extract were obtained in the wine from DMR grape, compared with the control wine. No differences in the contents of residual sugar were obtained in 'Rebula' wine, on the other hand, higher contents of residual sugar, compared to the control, were obtained in 'Vitovska grganja' DMR wine.

In the present experiment we have found that the contents of individual phenolic acids were significantly increased in the berry skins of DMR grape, which was especially observed at hydroxycinnamic acids and flavanols, but less at flavonols. DMR also had a lesser effect on total phenolic content of 'Rebula' grape. Also the percentages of individual phenolic compounds regarding the corresponding phenolic group did not significantly change. Among the flavanols DMR had the highest impact on procyanidins and catechin, but lesser on epicatechin. The contents of flavonols were minor and were not significantly impacted by DMR.

In the experiment with DMR and partial dehydration of grape in chamber we determined a different impact of both treatments, especially on grape and wine phenolic profile of a red variety 'Refošk'. The DMR significantly influenced and increased the content of anthocyanins, flavanols and stilbenoids in the skin, in comparison to the control. On the other hand, we have noticed significant impact of partial dehydration in chamber on the phenolic compounds in the wine, compared to the control and DMR. The results showed that DMR provides balanced quality of grape, with higher content of anthocyanins and other phenolic compounds, which lead to an improved colour intensity and taste.

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PRILOGA A

Dovoljenje za uporabo članka Reščič in sod. (2015) reviji Journal International des Sciences de la Vigne et du Vin

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Subject: Paper published in the JISVV

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The impact of cluster thinning on fertility and berry and wine composition of 'Blauer Portugieser' (*Vitis vinifera* L.) grapevine variety.

by:

Reščič J., Mikulič-Petkovšek M., Štampar F., Zupan A., Rusjan D.

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Full names of all authors (in order to appear on manuscript): Maja Mikulič-Petkovšek, Tjaša Jug, Jan Reščič, Denis Rusjan
Name, address etc. of corresponding author: Dr. Maja Mikulič-Petkovšek, Department of Agronomy, Biotechnical faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
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