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**Karotenoidy a fenolické látky v pšenících s barevným zrnem**  
doktorská disertační práce

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### **Prohlášení**

Prohlašuji, že jsem disertační práci na téma: „Karotenoidy a fenolické látky v pšenících s barevným zrnem“ vypracoval samostatně a použil jen pramenů, které cituji a uvádím v příloženém seznamu literatury.

V Praze dne .....

Podpis

## Poděkování

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## 1. Úvod

Pšenice (*Triticum spp.*) je jednou z nejvýznamnějších zemědělských plodin na světě, která podstatným dílem přispívá k saturaci nutričních potřeb světové populace. V rámci Evropského kontinentu je nejvíce využívanou obilovinou pro výrobu mouky a následně celé řady potravin, jako je chléb, pečivo nebo těstoviny. Botanicky spadá pšenice do třídy jednoděložných rostlin (*Liliopsida*), řádu lipnicotvarých (*Poales*) a čeledi lipnicovitých (*Poaceae*). Plodem pšenice je obilka s výraznou podélnou rýhou. Základními morfologickými částmi obilky jsou obalové vrstvy s vysokým podílem vlákniny (celulóza, hemicelulózy), škrobnatý endosperm představující největší podíl zrna a klíček, který se vyznačuje vysokým obsahem lipidů.

Jedním ze současných trendů ve šlechtění pšenice je mj. snaha o zvýšení obsahu nutričně významných biologicky aktivních látek, tzv. fytochemikálií. Jedná se zejména o obnovení schopnosti intenzivní syntézy karotenoidů (lipofilních rostlinných barviv), jež je typická pro staré, neprošlechtěné druhy pšenice (diploidní jednozrnky či tetraploidní dvouzrnky). Dále pak o introdukci zcela nových genů podmiňujících produkci a ukládání antokyanů v obilkách do genomu pšenice. Výsledkem takového šlechtitelského úsilí jsou tzv. pšenice s barevným zrnem. Dle typu a místa uložení pigmentů jsou to především pšenice se žlutým endospermem, modrým aleuronem či purpurovým perikarpem. Karotenoidy, antokyany, ale i další fenolické látky vykazují značnou antioxidační aktivitu a pro člověka, jakožto konzumenta, tak přinášejí mnohé zdravotní benefity. Antioxidanty v organismu účinně napomáhají předcházet rozvoji oxidačního stresu a následnému poškození mnohých biomolekul (nukleových kyselin, proteinů nebo lipidů buněčných membrán), které je považováno za hlavní příčinu rozvoje tzv. civilizačních onemocnění - nádorových, kardiovaskulárních či jiných chorob - spojených se stárnutím organismu. I proto v současné době roste zájem odborné i laické veřejnosti o nutričně hodnotné potraviny. Do popředí zájmu se tak dostávají starší (původní) druhy pšenice, jako je špalda, pšenice tvrdá, jednozrnka, dvouzrnka nebo také nově vyšlechtěné pšenice s barevným zrnem charakteristické zvýšeným obsahem karotenoidů či antokyanů.

## 2. Literární rešerše

### 2.1 Obiloviny ve výživě člověka

Obiloviny jsou stěžejní součástí lidské stravy, jelikož představují bohatý zdroj sacharidů, proteinů, vlákniny a dalších nutričně hodnotných látek, z nichž mnohé se vyznačují vysokou antioxidační aktivitou (Liu 2007). Tyto látky jsou uloženy především v obalových vrstvách zrna (otrubách) či klíčku, a proto je člověk přijímá zvláště konzumací celozrnných cereálních výrobků. Adekvátní příjem antioxidantů představuje preventivní opatření proti rozvoji mnoha chronických civilizačních chorob jako kardiovaskulární onemocnění, diabetes 2. typu, některé typy rakoviny (tlustého střeva). Mezi tyto antioxidanty patří široké spektrum molekul z tříd karotenoidů, fenolických látek (fenolických kyselin, antokyanů, proantokyanidinů, lignanů, flavonoidů), vitaminů E, fytátů, sterolů, betaglukanů a dalších (Ma et al. 2014; Laddomada et al. 2015; Liu 2007). Většina z nich patří mezi tzv. rostlinné sekundární metabolity, tedy sloučeniny, které nejsou přímo nezbytně nutné pro průběh základního metabolismu, růst a vývoj, ale pomáhají rostlinám přežít v přírodě, jelikož jsou významným prostředkem interakce s okolním prostředím (Cheynier et al. 2013).

V rámci evropského kontinentu je nejvíce využívanou obilovinou pšenice, jež slouží k výrobě mouky a následně celé řady potravin, jako je chléb, pečivo nebo těstoviny (Laknerová et al. 2014; Ficco et al. 2014). Celosvětově pak z hlediska roční produkce (za rok 2018 činila 758 mil. tun) zaujímá druhé místo hned za kukuřicí s celkovou průměrnou spotřebou 66,9 kg/os./rok (Statista 2019). V České republice v roce 2016 dosáhla spotřeba pšenice podle ČSÚ 122,7 kg/os./rok v hodnotě zrna (tj. 95,7 kg/os./rok v hodnotě mouky) (ČSÚ 2018). V posledních letech je diskutován obsah látek s antioxidačními vlastnostmi zvláště v odrůdách pšenic (ale i jiných obilovin jako například ječmene, triticales či tritordea, tedy kříženců pšenice s žitem a pšenice s ječmenem) s netradičně zbarveným zrnem. Tyto jsou oproti tradičním odrůdám navíc zdrojem karotenoidů a antokyanů, dieteticky cenných přírodních pigmentů (Li et al. 2007; Hosseinian et al. 2008; Lachman et al. 2017).

Většina v Evropě běžně pěstovaných odrůd hexaploidní pšenice seté (*Triticum aestivum* L.) má obilky tzv. červené barvy, která je způsobena přítomností různých derivátů katechinů a taninů odvozených od biosyntézy flavonoidů (Martinek et al. 2014). Mezi netradiční barevné pšenice patří pšenice se žlutým, purpurovým, modrým nebo černým zbarvením zrna (viz obrázek č. 1). Žluté



standardní barva



žlutý endosperm



purpurový perikarp



modrý aleuron



modrý aleuron +  
purpurový perikarp

Obrázek č. 1 Pšenice s různým zbarvením zrna

zbarvení endospermu způsobené přítomností karotenoidů (především luteinu a zeaxantinu) je typické pro diploidní pšenice jednozrnky (*Triticum monococcum* L.), tetraploidní dvouzrnky (*T. dicoccum* Schöbl.) a pšenice tvrdé (*T. durum* Desf.) (Martinek & Vyhnánek 2014). Purpurová barva zrna je dána přítomností antokyanů (např. kyanidin-3-glukosidu či kyanidin-3-rutinosidu) v perikarpu. Schopnost syntetizovat tato barviva byla do pšenice seté přenesena křížením s tetraploidní pšenicí *T. turgidum* L. subsp. *abbyssinicum* Vavilov), která pochází z Abyssinské oblasti z Etiopie (Martinek et al. 2014; Martinek & Vyhnánek 2014). Modré zbarvení je způsobeno antokyany (především delfinidin-3-glukosidem a delfinidin-3-rutinosidem) uloženými v aleuronové vrstvě. Geny pro tuto schopnost byly do pšenice přeneseny z různých divoce rostoucích rostlin: pýru pontického (*Thynopyrum ponticum* Podp.), *T. monococcum* ssp. *aegilopoides* (syn. *T. boeoticum*) a *Thynopyrum bessarabicum* (Martinek & Vyhnánek 2014; Burešová et al. 2015). Černé (někdy též označované jako tmavě purpurové) zbarvení zrna je způsobeno rovněž přítomností antokyanů a je dáno kombinací genů pro modrý aleuron a purpurový perikarp (Böhmendorfer et al. 2018). Různé zbarvení zrna je známo také u dalších obilovin – ječmene, rýže, kukuřice, ovsa či tritikale (Martinek et al. 2016).

První komerčně využitelné odrůdy pšenice s barevným zrnem byly vyšlechtěny na Novém Zélandu a jednalo se o jarní odrůdy s purpurovým perikarpem (Pp, z angl. Purple pericarp) Charcoal (registrována r. 1977), Konini (r. 1981) a Amethyst (r. 1988). Z barevných pšenic jsou v České republice od roku 2011 registrovány dvě pšenice se žlutým endospermem (Ye, z angl. Yellow endosperm), odrůdy Citrus a Luteus (Martinek & Vyhnánek 2014), od roku 2018 také pšenice AF Jumiko s Pp (ÚKZÚZ 2018). Nejnovější v ČR registrovanou odrůdou s barevným zrnem je AF Oxana (r. 2019) s modrou aleuronovou vrstvou (Ba, z angl. Blue aleurone) (ÚKZÚZ 2019). Na Slovensku jsou registrovány ozimé pšenice Bona Dea (r. 2006) a Bona Vita (r. 2011) obě s Ye, dále pak od roku 2014 také pšenice PS Karkulka s Pp. V Rakousku je registrována ozimá pšenice Yello s Ye (r. 2008), hned několik ozimých pšenic s Pp: Indigo (r. 2006), Rosso (r. 2011), Ceraso (r. 2014) a Merlot (r. 2015) a rovněž ozimá pšenice s Ba Skorpion (r. 2011). Tyto odrůdy sice nedosahují výnosů srovnatelných s běžně pěstovanými odrůdami tradiční barvy obilí (Martinek et al. 2016), ale jejich zrno představuje zajímavou surovinu potenciálně využitelnou pro výrobu tzv. funkčních potravin, tedy potravin s pozitivním přínosem pro zdraví konzumentů (Martinek et al. 2014).

## 2.2 Karotenoidy

### **2.2.1 Obecná charakteristika karotenoidů**

Karotenoidy jsou lipofilní organické látky ze skupiny tetraterpenoidů složené z osmi izoprenových (2-methyl-buta-1,3-dienových) jednotek. Všechny karotenoidy mohou být považovány za deriváty lykopenu, od něhož jsou odvozovány hydrogenací, dehydrogenací, cyklizací, začleněním atomu kyslíku do molekuly, posunem dvojné vazby či methylového zbytku a prodloužením nebo zkrácením řetězce (Delgado-Vargas et al. 2000). Dodnes bylo popsáno více než 750 karotenoidů, které lze systematicky rozdělit do dvou základních skupin na karoteny (např.  $\alpha$ -karoten,  $\beta$ -karoten, lykopen), červeně a oranžově zbarvené uhlovodíky a xantofyly (např. lutein, zeaxantin), jež jsou žlutě zbarvenými oxidačními deriváty karotenů a v jejich molekule se nachází kyslík ve formě hydroxy-, keto- nebo epoxy-skupiny (Nisar et al. 2015; Lachman et al. 2017). Karotenoidy tvoří rozmanitou skupinu pigmentů hojně



rozšířených v přírodě, přičemž barva těchto sloučenin je dána přítomností systému konjugovaných dvojných vazeb v uhlíkatém řetězci (Lu & Li 2008). Primárně se jedná o akcesorní pigmenty ve světelné fázi fotosyntézy a dále pak tyto látky chrání rostlinné buňky před poškozením v důsledku oxidačního stresu. Vytvářením barvy květů, plodů a semen lákají opylovače i další živočichy, kteří šířením pylu či semen napomáhají rostlinám v reprodukci (Delgado-Vargas et al. 2000; Hidalgo et al. 2006). Naprostá většina karotenoidů se v přírodě nachází primárně v *all-trans* konfiguraci, která je oproti *cis* konfiguraci více stabilní. *Cis* izomery mají odlišné biologické funkce (např. nižší aktivitu provitaminu A) (Nisar et al. 2015). Naprosto klíčovou roli pak hrají karotenoidy (zvláště 9-*cis*-violaxantin nebo 9-*cis*-neoxantin) jako prekurzory v syntéze abscisové kyseliny (ABA), rostlinného růstového hormonu (Auldrige et al. 2006; Nisar et al. 2015). Pro člověka je také velmi významná biologická aktivita, resp. silný antioxidační účinek a role tzv. provitaminů A (Hidalgo et al. 2006).

### 2.2.2 Biosyntéza karotenoidů

Karotenoidy jsou v rostlinách syntetizovány *de novo* téměř ve všech typech plastidů (především na membránách chloroplastů a thylakoidů) a k jejich akumulaci dochází hlavně v chloroplastech a chromoplastech (Howitt & Pogson 2006; Nisar et al. 2015). Biosyntéza karotenoidů je regulována v průběhu celého životního cyklu rostlin v závislosti na momentálních environmentálních podmínkách a požadavcích rostliny např. během klíčení, při fotosyntéze, kvetení, tvorbě a dozrávání plodů a semen nebo v reakci na různé vnější podněty, resp. biotické a abiotické stresové faktory. Vlivem působení uvedených faktorů dochází k dynamickým změnám kvalitativního spektra i kvantitativního zastoupení jednotlivých karotenoidů (Howitt & Pogson 2006).

Samotné biosyntéze karotenoidů předchází syntéza prekurzorů, dvou izomerů odvozených od izoprenu, a sice izopentenylidifosfátu (IPP) a dimethylallyldifosfátu (DMAPP) (Cuttriss & Pogson 2004; Cazzonelli 2011). Ke vzniku IPP a DMAPP mohou vést dvě nezávislé biosyntetické dráhy, přičemž první, známá jako MEP dráha vychází z 2-C-methyl-D-erythritol-4-fosfátu (MEP), druhá, tzv. MVA dráha se odvíjí od mevalonové kyseliny (MVA) (Cazzonelli 2011). Schéma MEP i MVA biosyntetické dráhy je znázorněno na obrázku č. 2. Ačkoliv většina organismů schopná syntézy karotenoidů využívá buď plastidickou MEP, nebo cytozolickou MVA biochemickou dráhu, existuje i několik druhů bakterií, které jsou schopné syntetizovat karotenoidy prostřednictvím obou možných biosyntetických drah. Rostlinné karotenoidy jsou tvořeny ze svých izoprenoidních prekurzorů převážně cestou MEP. Původ této biosyntetické dráhy u rostlin, tedy eukaryotních organismů, je přičítán dávné endosymbióze kyanobakterie, z níž se později vyvinul buněčný plastid (Moise et al. 2014).

### 2.2.3 Biosyntéza prekurzorů: MEP dráha

MEP dráha začíná reakcí glyceraldehyd-3-fosfátu a pyruvátu, tedy látek pocházejících z Calvinova cyklu, popřípadě z glykolýzy, za vzniku 1-deoxy-D-xylulózy-5-fosfátu (DXP). Syntéza DXP je katalyzována enzymem 1-deoxy-D-xylulózo-5-fosfát syntázou (DXS) (Cazzonelli 2011). Dále 1-deoxy-D-xylulózo-5-fosfát reduktoizomeráza (DXR) katalyzuje

redukci DXP na MEP za přítomnosti NADPH. DXP může být také přeměněn v další esenciální metabolity, pyridoxalfosfát nebo thiamin trifosfát. Tento krok je proto uzlem v syntéze vitamínu B<sub>1</sub>, B<sub>6</sub> a izoprenoidů (Moise et al. 2014).

Dalším enzymem MEP dráhy je 2-C-methyl-D-erythritol-4-fosfát cytidyltransferáza (MCT), která katalyzuje přeměnu MEP na 4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol (CDP-ME). CDP-ME je fosforylován enzymem CDP-ME kinázou (CMK) na 4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol-2-fosfát (CDP-ME-2P), který je dále intramolekulární transfosforylací, činností enzymu 2-C-methyl-D-erythritol-2,4-cyklodifosfát (MECDP) syntázy (MDS), převeden na MECDP (Moise et al. 2014).

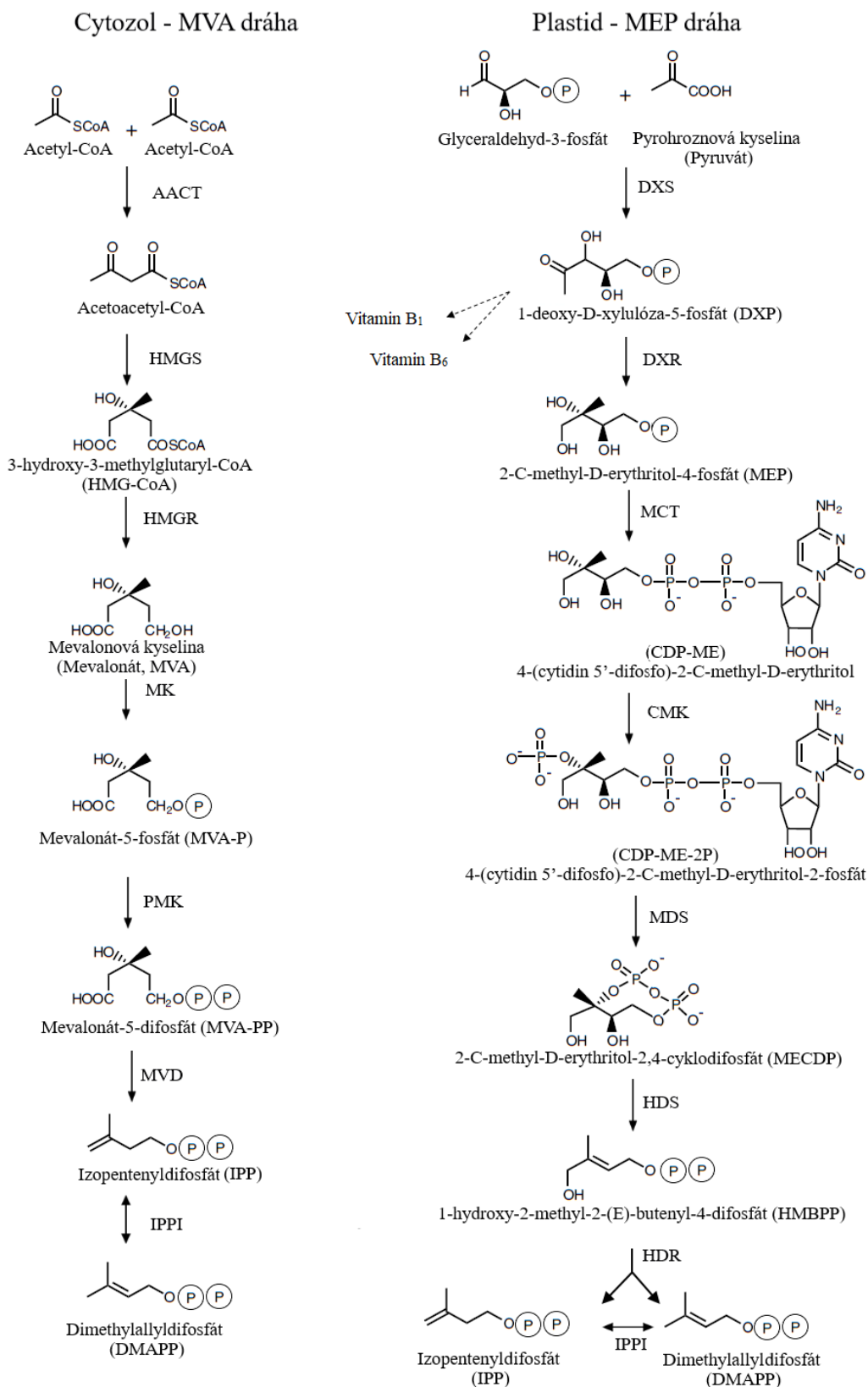
Posledními enzymy MEP syntetické dráhy jsou 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-difosfát (HMBPP) syntáza (HDS) a HMBPP reduktáza (HDR). HDS řídí přeměnu MECDP na HMBPP, který je následně redukován enzymem HDR. Mechanismus redukce HMBPP spočívá v postupném přenosu dvou elektronů z enzymu na substrát. Přenosem prvního elektronu dojde k vytvoření allyl radikálu, přenos druhého pak umožňuje vytvoření přechodného allyl karbanionu. Následnou protonací C1, nebo C3 uhlíku vzniká DMAPP, nebo IPP (Bouvier et al. 2005; Moise et al. 2014). Mezi IPP a DMAPP je možná vzájemná reverzibilní enzymatická přeměna katalyzovaná izopentenyl-difosfát izomerázou (IPPI) (Moise et al. 2014).

#### **2.2.4 Biosyntéza prekurzorů: MVA dráha**

MVA dráha přispívá k produkci karotenoidů především u organismů z říše *Archaeobacteria*, Houby a méně často pak *Eubacteria*. Prvním krokem je kondenzace dvou molekul acetyl-CoA za vzniku acetoacetyl-CoA, tato reakce je katalyzována enzymem acetoacetyl-CoA thiolázou (AACT). Navázání třetí molekuly acetyl-CoA je katalyzováno 3-hydroxyl-3-methylglutaryl-CoA syntázou (HMGS), vzniká tak 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA je dále redukován HMG-CoA reduktázou (HMGR) na mevalonovou kyselinu (MVA). MVA je následně postupně fosforylována, nejprve mevalonát kinázou (MK) za vzniku mevalonát-5-fosfátu (MVA-P) a fosfomevalonát kinázou (PMK) na mevalonát-5-difosfát (MVA-PP). Dekarboxylací řízenou difosfomevalonát dekarboxylázou (MVD) pak vzniká IPP (Moise et al. 2014).

#### **2.2.5 Vlastní biosyntéza karotenoidů**

Biosyntézu karotenoidů lze rozdělit do několika základních fází. Prvně je to vznik geranylgeranyldifosfátu (GGPP), dále syntéza a následná cyklizace lykopenu a postupná oxygenace karotenů za vzniku xantofylů (Smolikova & Medvedev 2015).



Obrázek č. 2 Schéma biosyntézy IPP a DMAPP – MEP a MVA dráha (Bouvier et al. 2005; upraveno)

Legenda k obrázku č. 2

AACT (acetoacetyl-CoA thioláza; EC 2.3.1.9), HMGS (3-hydroxyl-3-methylglutaryl-CoA syntáza; EC 2.3.3.10), HMGR (3-hydroxy-3-methylglutaryl-CoA reduktáza; EC 1.1.1.34), MK (mevalonát kináza; EC 2.7.1.36), PMK (fosfomevalonát kináza; EC 2.7.4.2), MVD (difosfomevalonát dekarboxyláza; EC 4.1.1.33), IPPI (izopentenyl-difosfát izomeráza; EC 5.3.3.2), DXS (1-deoxyxylulózo-5-fosfát syntáza; EC 2.2.1.7), DXR (1-deoxy-D-xylulózo-5-fosfát reduktoizomeráza; EC 1.1.1.267), MCT (2-C-methyl-D-erythritol-4-fosfát cytidyltransferáza; EC 2.7.7.60), CMK (4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol kináza; EC 2.7.1.148), MDS (2-C-methyl-D-erythritol-2,4-cyklo-difosfát syntáza; EC 4.6.1.12), HDS (1-hydroxy-2-methyl-2-(*E*)-butenyl-4-difosfát syntáza; EC 1.17.7.3), HDR (1-hydroxy-2-methyl-2-(*E*)-butenyl-4-difosfát reduktáza; EC 1.17.7.4)

#### 2.2.5.1 Syntéza GGPP

Karotenoidy jsou syntetizovány postupnými kondenzačními reakcemi aktivovaných izoprenových jednotek IPP a DMAPP. Jelikož je HDR schopna syntetizovat IPP i DMAPP, není vzájemná přeměna těchto dvou izoprenoidních prekurzorů pro organismy využívající MEP dráhu nijak esenciální. Avšak pro organismy závislé na MVA dráze, je tato schopnost nepostradatelná. Uvedená reakce je katalyzována enzymem IPPI (Berthelot et al. 2012).

Kondenzací tří molekul IPP a jedné molekuly DMAPP, jež je řízena enzymem geranylgeranyldifosfát syntázou (GGPPS) vzniká geranylgeranyldifosfát (GGPP) (Cazzonelli 2011; Smolikova & Medvedev 2015), klíčový prekurzor pro biosyntézu karotenoidů, tokoferolů, plastochinonů, postranních řetězců chlorofylů nebo giberelinů (Bouvier et al. 2005; Lu & Li 2008).

#### 2.2.5.2 Syntéza lykopenu

Následuje kondenzace dvou molekul GGPP katalyzovaná enzymem fytoen syntázou (PSY), při níž dochází ke vzniku 15-*cis*-fytoenu, prvního karotenoidu, který je však bezbarvý (Cazzonelli 2011; Smolikova & Medvedev 2015) a je výchozí látkou pro syntézu všech ostatních karotenoidů (Moise et al. 2014).

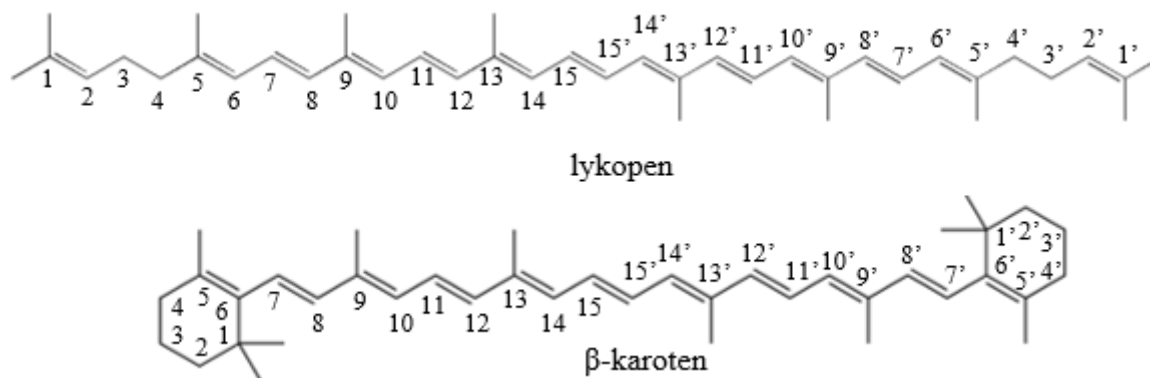
Od 15-*cis*-fytoenu k *all-trans*-lykopenu vedou dvě různé biochemické dráhy. První, využívaná všemi karotenoidy syntetizujícími bateriemi, s výjimkou kyanobakterií, je založena na jediném enzymu, fytoen desaturáze bakteriálního typu (CRTI). CRTI katalyzuje desaturaci fytoenu v pozicích 7, 11, 11' a 7' a izomeraci na dvojně vazbě mezi C15 a C15'. Druhá, tzv. poly-*CIS*-dráha, využívaná vyššími rostlinami, zelenými řasami a kyanobakteriemi, zahrnuje čtyři po sobě jdoucí enzymatické reakce (Yu et al. 2011; Moise et al. 2014) katalyzované fytoen desaturázou (PDS), ζ-karoten izomerázou (ZISO), ζ-karoten desaturázou (ZDS) a karotenoid izomerázou (CRTISO), důležitá je též světlem zprostředkovaná fotoizomerace (Breitenbach & Sandmann 2005). Každá z uvedených desaturáz (PDS a ZDS) řídí vytvoření dvou dvojných vazeb (Yu et al. 2011).

Poly-*CIS*-dráha začíná postupnou desaturací (resp. dehydrogenací) 11 a 11' vazeb fytoenu prostřednictvím enzymu PDS, čímž je umožněna volná rotace. Postupná izomerace na přilehlých dvojných vazbách 9 a 9' z *trans* na *cis* dává vzniknout nejprve 9,15-*di-cis*-fytofluenu

a dále, již barevnému, 9,15,9'-*tri-cis*- $\zeta$ -karotenu. Následnou izomerací centrální dvojně vazby, jež je řízena enzymem ZISO, vzniká 9,9'-*di-cis*- $\zeta$ -karoten. Dále ZDS řídí postupnou stereospecifickou dehydrogenaci na C7 a C7'. Tímto způsobem dochází ke vzniku dalších dvou dvojných *cis* vazeb, nejprve 7,9,9'-*tri-cis*-neurosporen (proneurosporen) a poté 7,9,9',7'-*tetra-cis*-lykopen, tzv. prolykopen (Yu et al. 2011; Smolikova & Medvedev 2015). Vytvořený 7,9,9',7'-*tetra-cis*-lykopen však není substrátem zpracovatelným  $\beta$ - nebo  $\epsilon$ -cyklázami. Pokračování biosyntézy proto vyžaduje izomeraci přítomného poly-*cis* meziprojektu na *all-trans*-lykopen, tedy sloučeninu vhodnou pro následnou cyklizaci (Breitenbach & Sandmann 2005). Reakce přeměny všech *cis*-dvojných vazeb 7,9,9',7'-*tetra-cis*-lykopenu vytvořených enzymy PDS a ZDS na *trans*-vazby je řízena enzymem CRTISO (Yu et al. 2011; Moise et al. 2014). PDS, ZDS a CRTISO rozeznávají pouze polovinu molekuly karotenoidů, a proto postupná desaturace a izomerace může probíhat nezávisle na každé polovině molekuly zvlášť (Breitenbach & Sandmann 2005).

### 2.2.5.3 Cyklizace lykopenu

Cyklizace lykopenu je předpokladem tvorby cyklických karotenoidů. Tato fáze je místem větvení metabolické dráhy biosyntézy karotenoidů na  $\alpha$ - a  $\beta$ -karoten a jejich deriváty (Cuttriss & Pogson 2004; Moise et al. 2014; Smolikova & Medvedev 2015). Struktura lykopenu a  $\beta$ -karotenu je znázorněna na obrázku č. 3., schéma biosyntézy karotenoidů zachycuje obrázek č. 4.



Obrázek č. 3 Struktura lykopenu a  $\beta$ -karotenu

(Fraser & Bramley 2004; upraveno)

Oba  $\psi$ -acyklické konce lykopenu mohou podléhat cyklizačním reakcím za vzniku  $\beta$ -,  $\epsilon$ - nebo  $\gamma$ -jononových kruhů. Typ a počet substituentů na jononových kruzích je nejdůležitějším kritériem, které určuje funkci daného karotenoidu. Druh a počet modifikací polyenového řetězce je naproti tomu podstatně více omezený (Moise et al. 2014). Karotenoidy obsahující alespoň jeden nesubstituovaný  $\beta$ -jononový cyklus mohou být potenciálně substrátem pro tvorbu retinolu, tedy vitamínu A, který může být dále využit k syntéze signálních molekul, jako je retinová kyselina či 11-*cis*-retinal, zrakový pigment obratlovců (Cunningham & Gantt 2001).

Cyklizaci lykopenu u rostlin a kyanobakterií zajišťují enzymy ze skupiny lykopen cykláz (LCY), jež lze rozdělit dle typu vytvářeného cyklu na tři skupiny. Lykopen cyklázy, které tvoří  $\beta$ -jononové cykly (LCY B),  $\epsilon$ -jononové cykly (LCY E) (Cuttriss & Pogson 2004)

a třetí typ tzv. bifunkčních lykopen cykláz, tedy enzymů, které tvoří  $\beta$ - nebo  $\varepsilon$ -jononové cykly (LCY B/E) (Moise et al. 2014).

Syntézu  $\beta$ -karotenu zajišťuje enzym LCY B, který katalyzuje vznik cyklických karotenoidů s jononovým cyklem typu  $\beta$  (dvojná vazba je lokalizována mezi pátým a šestým uhlíkem). Vytvořením jednoho takového cyklu vzniká  $\gamma$ -karoten, následně syntézou druhého, na opačném konci řetězce, vzniká  $\beta$ -karoten (Cunningham & Gantt 2001; Smolikova & Medvedev 2015).

Syntéza  $\alpha$ -karotenu probíhá taktéž ve dvou krocích. V první řadě dochází činností LCY E, jež řídí vytvoření  $\varepsilon$ -jononového cyklu (dvojná vazba se nachází mezi čtvrtým a pátým uhlíkem), ke vzniku  $\delta$ -karotenu. Následně LCY B vytvoří  $\beta$ -jononový cyklus na opačném konci řetězce za vzniku  $\alpha$ -karotenu (Cunningham & Gantt 2001; Smolikova & Medvedev 2015).

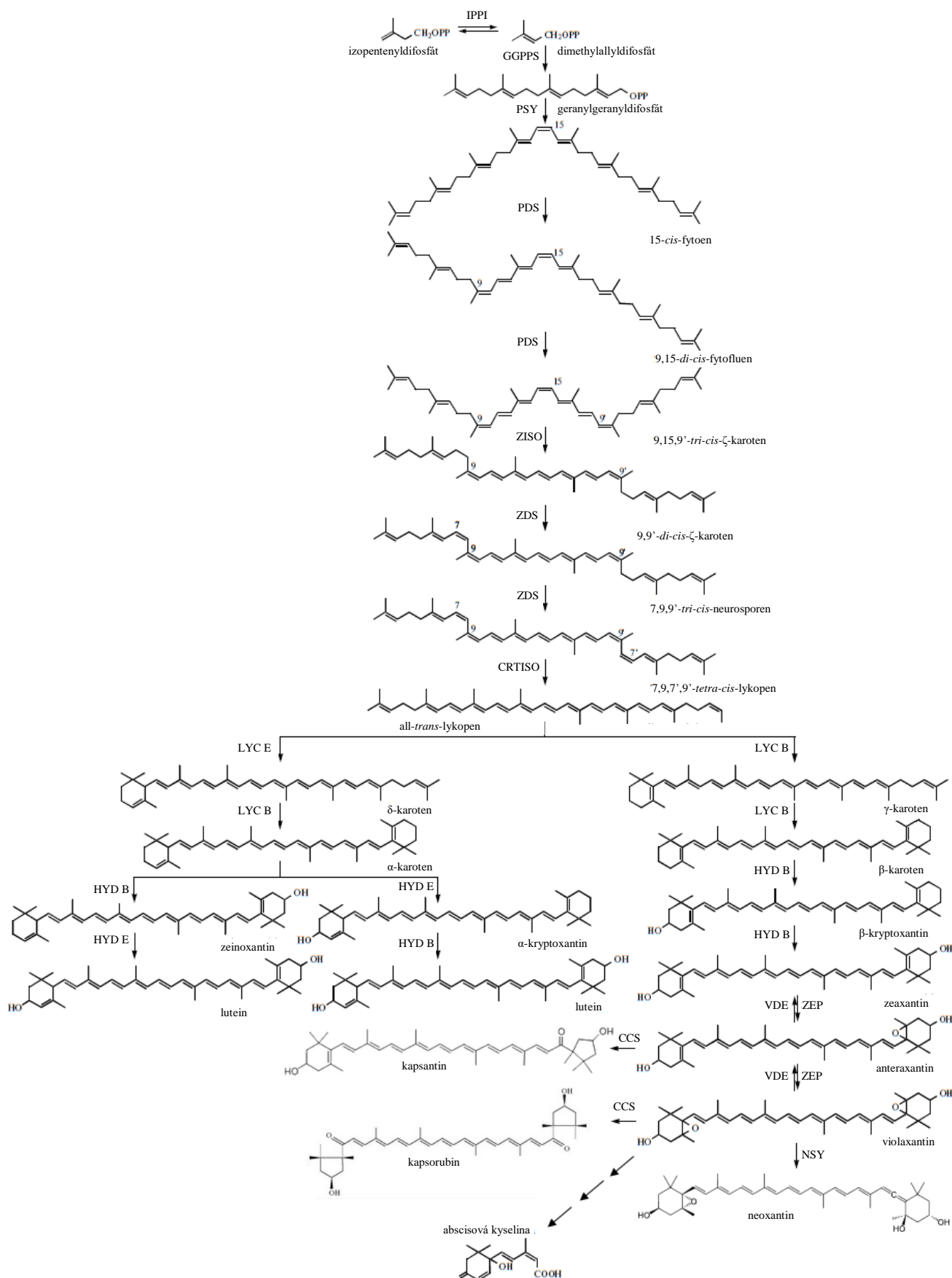
#### 2.2.5.4 Postupná oxygenace karotenů za vzniku xantofylů

Diverzita skupiny xantofylů je dána vytvářením různých funkčních skupin obsahujících kyslík (aldehydická, ketonická, hydroxy-, methoxy-, epoxy- nebo karboxy-skupina) (Cuttriss & Pogson 2004). Nejčastějšími substitucemi na cyklech je vytváření hydroxy-skupin (Davison et al. 2002). Různými biochemickými drahami tak mohou vznikat stovky různých karotenoidů lišících se počtem dvojných vazeb, obsahem kyslíku či prostorovou konfigurací (Cuttriss & Pogson 2004).

Hydroxylace  $\beta$ - a  $\varepsilon$ -cyklu  $\alpha$ - a  $\beta$ -karotenu je katalyzována  $\beta$ -karoten hydroxylázami (HYD B) a  $\varepsilon$ -karoten hydroxylázami (HYD E) (Tanaka et al. 2008). Z  $\beta$ -karotenu tak vzniká nejprve  $\beta$ -kryptoxantin a dále zeaxantin. Analogicky potom z  $\alpha$ -karotenu vzniká zeinoxantin a následně lutein (Fraser & Bramley 2004; Lu & Li 2008). Epoxidací 3-hydroxy- $\beta$ -cyklu zeaxantinu v pozici C5,6 činností zeaxantin epoxidázy (ZEP) vzniká anteraxantin, jehož další epoxidací v pozici C5',6' druhého  $\beta$ -cyklu je tvořen violaxantin (Tanaka et al. 2008). Violaxantin je dále neoxantin syntázou (NSY) přeměňován na neoxantin (Cuttriss & Pogson 2004).

Karotenoidy obsahující ketoskupinu na  $\beta$ -jononovém cyklu, často syntetizované kyanobakteriemi vznikají činností karotenoidních ketoláz (CrtO/CrtW). Vytvořením 4-ketoskupiny na  $\beta$ -jononovém cyklu  $\beta$ -karotenu je produkován echinenon, následně pak začleněním další ketoskupiny do  $\beta$ -jononového cyklu na opačném konci molekuly vzniká kantaxantin (Moise et al. 2014).

Kapsantin a kapsorubin, hlavní karotenoidy červené papriky, obsahují ketoskupinu v řetězci a cyklopentanový cyklus ( $\kappa$ -cyklus), k jehož vytvoření dochází z původního 3-hydroxy-5,6-epoxy  $\beta$ -cyklu anteraxantinu nebo violaxantinu činností enzymů kapsantinsyntázy (CCS) resp. kapsorubin syntázy (Fraser & Bramley 2004).



Obrázek č. 4 Biosyntéza karotenoidů

(Moise et al. 2014; Smlikova & Medvedev 2015; upraveno)

Legenda k obrázku č. 4

IPPI (izopentenylidifosfát izomeráza; EC 5.3.3.2), GGPPS (geranylgeranylidifosfát syntáza; EC 2.5.1.29), PSY (fytoen syntáza; EC 2.5.1.32), PDS (fytoen desaturáza; EC 1.3.5.5), ZISO ( $\zeta$ -karoten izomeráza; EC 5.2.1.12), ZDS ( $\zeta$ -karoten desaturáza; EC 1.3.5.6), CRTISO (karotenoid izomeráza; EC 5.2.1.13), LCY B (lykopen  $\beta$ -cykláza; EC 5.5.1.19), LCY E (lykopen  $\epsilon$ -cykláza; EC 5.5.1.18), HYD B ( $\beta$ -karoten hydroxyláza; EC 1.14.15.24), HYD E ( $\epsilon$ -karoten hydroxyláza; EC 1.14.14.158), VDE (violaxantin deepoxidáza; EC 1.23.5.1), ZEP (zeaxantin epoxidáza; EC 1.14.15.21), CCS (kapsantin/kapsorubin syntáza; EC 5.3.99.8), NSY (neoxantin syntáza; EC 5.3.99.9)

### 2.2.6 Akumulace karotenoidů

Ke kumulaci karotenoidů dochází v různých typech plastidů (Howitt & Pogson 2006; Nisar et al. 2015), chloroplastech, chromoplastech (barevných plastidech), amyloplastech (plastidech určených pro skladování škrobu), elaioplastech (plastidech skladujících lipidy), leukoplastech (bezbarvých plastidech) a etioplastech (za tmy tvořených prekurzorech chloroplastů). Obecně nejdůležitějšími místy skladování karotenoidů jsou chloroplasty a chromoplasty, v případě obilovin, tedy semen s vysokým obsahem škrobu, pak amyloplasty (Howitt & Pogson 2006). Thylakoidní membrány chloroplastů poskytují vysokou kapacitu pro izolaci a skladování syntetizovaných karotenoidů v chloroplastech. Karotenoidy jsou zde vázány v podobě pigment-proteinových komplexů ve fotosynteticky aktivních membránách (Zhai et al. 2016), což je příčinou relativně vysokého obsahu karotenoidů v zelených částech rostlin. V chromoplastech naproti tomu vytvářejí interakci s proteiny a polárními lipidy karotenoid-lipoproteinové struktury (globuly, krystaly, membrány, fibrily či tubuly), jež jsou efektivním způsobem ukládání velkého množství karotenoidů (Li & Yuan 2013; Nisar et al. 2015; Zhai et al. 2016). Tento systém zároveň zabraňuje hromadění reakčních produktů (karotenoidů) na membránách plastidů, tedy v místě vlastní biosyntézy. Jde tak o preventivní opatření proti vzniku negativní zpětné vazby, resp. inhibice biochemické reakce jejím produktem (Lu & Li 2008; Mellado-Ortega et al. 2015).

### 2.2.7 Degradace karotenoidů

Karotenoidy přirozeně podléhají katabolickým přeměnám, které jsou zapříčiněny buď nespecifickými mechanismy jako například (foto)chemickou oxidací nebo mohou být řízeny nespecifickými enzymy lipoxygenázami či peroxidázami (Walter & Strack 2011; Nisar et al. 2015). Enzymy, jež štěpí specifické dvojně vazby karotenoidů, jsou souhrnně označovány jako karotenoidy štěpící oxygenázy (CCOs; Carotenoid Cleavage Oxygenases) (Walter & Strack 2011). Produkty zmíněných štěpných reakcí karotenoidů jsou nazývány apokarotenoidy (Lu & Li 2008), někdy též degradované karotenoidy. Tyto sloučeniny proto obsahují méně než čtyřicet atomů uhlíku a v rostlinách zastávají různé biologické funkce (Šivel et al. 2013). Je-li totiž v molekule apokarotenoidu zachován dostatečný počet konjugovaných dvojných vazeb, pak má daná sloučenina rovněž charakter barviva a může například sloužit jako rostlinný pigment. Přítomnost terminální karboxylové skupiny navíc zvyšuje intenzitu barvy a stabilitu chromoforu. Mezi významná rostlinná barviva skupiny apokarotenoidů patří bixin ( $C_{24}$ ) odvozený od lykopenu rozštěpením vazeb mezi uhlíky 5-6 a 5'-6' nebo krocin ( $C_{20}$ ) odvozený



od zeaxantinu rozštěpením vazeb 7-8/7'-8' (Walter & Strack 2011). Pro rostliny je též velice významná abscisová kyselina (ABA; C<sub>15</sub>), která je důležitým fytohormonem, jenž řídí například dozrávání či tvorbu semen a je zároveň součástí rostlinných stresových reakcí. ABA je produktem štěpení 9-*cis*-violaxantinu a nebo 9-*cis*-neoxantinu enzymem 9-*cis*-epoxykarotenoid dioxygenázou (NCED) za vzniku patnáctiuhlíkatého xantoxinu. Následně dalšími přeměnami přes aldehyd abscisové kyseliny vzniká ABA (Auldridge et al. 2006; Tanaka et al. 2008; Ohmiya 2009).

Z hlediska lidské výživy je nejvýznamnějším apokarotenoidem vitamin A<sub>1</sub> (*all-trans*-retinol; C<sub>20</sub>), jak je podrobněji uvedeno v kapitole 2.2.8 (Fraser & Bramley 2004).

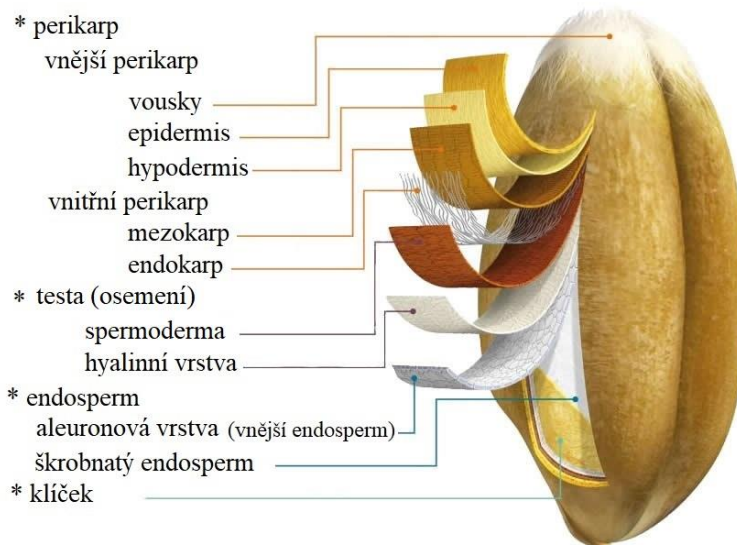
### 2.2.8 Nutriční význam karotenoidů

Karotenoidy jsou díky své polyenové struktuře efektivními lapači volných radikálů (Berman et al. 2015). Vykazují tedy vysokou antioxidační aktivitu, která se odvíjí od chemické struktury, přičemž roste s počtem dvojných vazeb, keto-skupin a cyklopentanových kruhů v molekule (Delgado-Vargas et al. 2000). Karotenoidy jsou proto často spojovány s prevencí mnohých nádorových onemocnění a kardiovaskulárních onemocnění. Některé z těchto sloučenin rovněž vykazují provitaminovou aktivitu (jsou označovány jako tzv. provitaminy A), jež mohou být metabolicky přeměněny až na retinol (vitamin A). Tato funkce je omezena pouze na cca 50 karotenoidů, které se vyznačují přítomností alespoň jednoho nesubstituovaného β-iononového kruhu, jako jsou například β-karoten nebo β-kryptoxantin (Fraser & Bramley 2004). Enzym β-karoten 15,15'-dioxygenáza způsobuje symetrické štěpení molekuly β-karotenu na dvě molekuly *all-trans*-retinalu, který je vlivem retinol dehydrogenázy reverzibilně redukován na *all-trans*-retinol. Následnou izomerací vzniká 11-*cis*-retinol a jeho enzymatickou oxidací dále 11-*cis*-retinal, který vazbou s opsinem dává vzniknout fotoreceptoru rodopsinu (Šivel et al. 2013). Například jedna molekula β-karotenu tak může poskytnout dvě molekuly vitamínu A, α-karoten pouze jednu, naproti tomu lutein a zeaxantin kvůli hydroxylaci obou iononových kruhů aktivitu provitaminu A nemají. Vitamin A je nezbytný pro správnou funkci imunitního systému a světločivných buněk oční sítnice (Berman et al. 2015). Nutriční deficit vitamínu A se projevuje mnohými závažnými zdravotními poruchami od retardace růstu, přes sníženou imunitní odpověď a vyšší náchylnost k infekčním chorobám až po šeroslepost či úplnou slepotu (Cuttriss & Pogson 2004). Význam luteinu a zeaxantinu tkví rovněž v ochraně žluté skvrny oční sítnice (makuly), kde jsou hojně koncentrovány. Uvedené karotenoidy zde působí fotoprotektivně a antioxidačně proti volným radikálům (Fraser & Bramley 2004).

### 2.2.9 Karotenoidy v pšeničném zrně

Různé druhy pšenice se vzájemně výrazně liší celkovým obsahem karotenoidů v zrně. Konvenční pšenice obsahují od 1,36 μg/g (Lachman et al. 2013) do 2,12 μg/g (Hidalgo et al. 2006). Podstatně vyšší množství karotenoidů pak uvádí studie provedená Abdel-Aalem et al. (2007) v pšenici špaldě (4,01 μg/g), dvouzrnce (5,76 μg/g) nebo pšenici tvrdé (6,27 μg/g) a nejvyšší množství byla naměřena v pšenici jednozrnce (9,62 μg/g). Ndolo & Beta (2013) dále uvádějí celkový obsah karotenoidů v zrně purpurové pšenice 2,62 μg/g (vyjádřeno jako ekvivalent luteinu). V rámci zrna (struktura pšeničného zrna znázorněna na obrázku č. 5) se největší koncentrací karotenoidů vyznačuje klíček, kde Ndolo & Beta (2013) naměřili

průměrně 2,16 µg/g luteinu (tedy čtyř- a pětinasobek jeho obsahu v endospermu a aleuronové vrstvě) a 3,09 µg/g zeaxantinu (čtyřikrát vyšší koncentrace v porovnání s aleuronem). V endospermu nebyl uvedenými autory zeaxantin detekován. Klíček však představuje pouze 3-5 % hmotnosti (Mellado-Ortega & Hornero-Méndez 2015), a tak je v této části uloženo přibližně jen 5-10 % z celkového obsahu karotenoidů v obilce. Zatímco karotenoidy obsažené v otrubách tvoří cca 20-40 % a největší podíl s cca 60-70 % nese endosperm (Ndolo & Beta 2013).

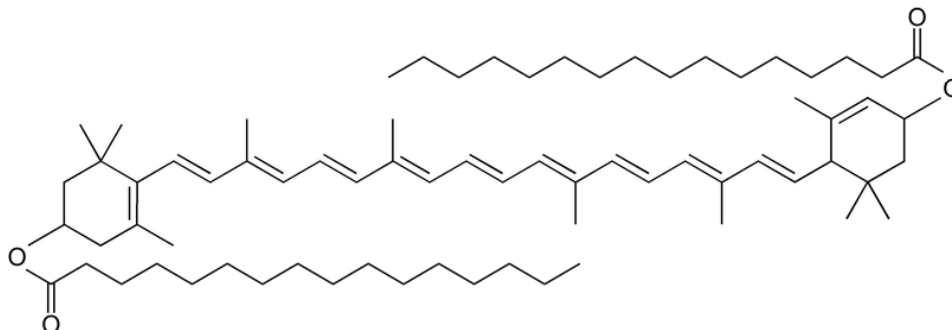


Obrázek č. 5 Řez pšeničnou obilkou  
(Michaelhannan 2018; upraveno)

Nejhojněji se vyskytujícím karotenoidem v pšeničném zru je lutein (Ndolo & Beta 2013; Luthria et al. 2015; Lachman et al. 2017) nejčastěji ve formě *all-trans* izomeru, avšak Mellado-Ortega et al. (2015) v pšenici tvrdé zaznamenali i některé *cis*-izomery (9-*cis* a 13-*cis* lutein). Dále bývá zastoupen také zeaxantin, v menším množství potom β-kryptoxantin, β-karoten či anteraxantin (Luthria et al. 2015; Lachman et al. 2017). Lutein představuje dle výsledků Mellado-Ortegy et al. (2015) více než 85 % celkového obsahu karotenoidů v zru pšenice tvrdé, podstatně menší podíl zaujímá zeaxantin (10,7 %) a β- a α-karoten (1,8 a 1,4 %). Karotenoidy (resp. xantofyly) se v pšeničném zru vyskytují jednak jako volné sloučeniny anebo v podobě esterů s různými mastnými kyselinami, jejichž vznik je umožněn přítomností jedné nebo více hydroxylových skupin na jononových kruzích (Mellado-Ortega et al. 2015; Ziegler et al. 2015).

Esterované xantofyly vznikající acylací prostřednictvím acyl-CoA se vyznačují vyšší lipofilitou oproti svým neesterovaným analogům, což umožňuje lepší integraci uvnitř buněčných membrán a plastoglobulů (Howitt & Pogson 2006). Esterifikace xantofylů je efektivním způsobem ochrany před degradací vlivem oxidace a UV záření, umožňuje dlouhodobé skladování karotenoidů uvnitř rostlinných buněk (Atienza et al. 2007; Ahmad et al. 2013; Ahmad et al. 2015) a zvyšuje jejich stabilitu v procesu výroby potravin (Fu et al. 2010; Mattera et al. 2017). Výzkumem esterovaných xantofylů v obilovinách se zabývala celá řada prací (Mellado-Ortega & Hornero-Méndez 2012; Mellado-Ortega et al. 2015; Zeigler et al. 2015; Mattera et al. 2017). V pšenici tvrdé a tritordeu tak byly nalezeny mono- i di-estery luteinu. Z monoesterů byly identifikovány především lutein-3'-O-palmitát, lutein-3-O-palmitát, lutein-3'-O-linoleát a lutein-3-O-linoleát, z diesterů pak lutein dipalmitát (viz obrázek č. 6), lutein linolylpalmitát a lutein dilinoleát (Mellado-Ortega et al. 2015; Mattera et al. 2017). Ziegler et al. (2015) uvádí navíc kyseliny olejovou a linolenovou jakožto další vyšší mastné

kyseliny schopné tvorby esterů s luteinem. Tito autoři v pšenici jednozrnce identifikovali mj. lutein-3-O-oleát, lutein-3'-O-oleát, lutein-linolyllinolenát. Přítomnost esterů luteinu a stupeň esterifikace jsou geneticky řízeny (Ahmad et al. 2015; Ziegler et al. 2015) a vykazují značnou mezidruhovou ale i vnitrodruhovou variabilitu (Atienza et al. 2007; Ziegler et al. 2015).



Obrázek č. 6 Struktura lutein dipalmitátu

Schopnost určitých genotypů tvořit a ukládat velký podíl karotenoidů ve formě esterů by tak mohla být významným faktorem pro výběr genetického materiálu v rámci mnohých šlechtitelských programů.

## 2.3 Fenolické látky

### **2.3.1 Obecná charakteristika fenolických látek**

Pojmem rostlinné fenolické látky rozumíme přírodní sekundární metabolity biogeneticky odvozené jednak od šikimát-fenylpropanoidové metabolické dráhy (vznik fenylpropanoidů) nebo „polyketidové“ acetát-malonátové dráhy (vznik jednoduchých fenolů). Uvedené metabolické dráhy produkují obrovské množství monomerních a polymerních struktur, jež v rostlinném organismu plní množství fyziologických funkcí v rámci reprodukce a růstu (Cheynier et al. 2013). Fenolické látky jsou sloučeniny obsahující jedno nebo více aromatických jader s jednou nebo více hydroxylovými skupinami (Liu 2007; Goleniowski et al. 2013) a je možné je rozdělit podle chemické struktury na: C<sub>6</sub> (jednoduché fenoly, benzochinony), C<sub>6</sub>-C<sub>1</sub> (fenolické kyseliny a aldehydy), C<sub>6</sub>-C<sub>2</sub> (acetofenony, fenylactové kyseliny), C<sub>6</sub>-C<sub>3</sub> (hydroxyskořicové kyseliny, kumariny, fenylpropany, chromony), C<sub>6</sub>-C<sub>4</sub> (naftochinony), C<sub>6</sub>-C<sub>1</sub>-C<sub>6</sub> (xantony), C<sub>6</sub>-C<sub>2</sub>-C<sub>6</sub> (stilbeny, antrachinony), C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> (flavonoidy, které dále zahrnují flavonoly, flavony, isoflavony, flavanoly, flavanony a antokyany), (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>)<sub>2,3</sub> (bi-, tri-flavonoidy, dimery a trimery proantokyanidinů), (C<sub>6</sub>-C<sub>3</sub>)<sub>2</sub> (lignany, neolignany), (C<sub>6</sub>-C<sub>3</sub>)<sub>n</sub> (ligniny), (C<sub>6</sub>)<sub>n</sub> (katechol melaniny, florotaniny), (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>)<sub>n</sub> (kondenzované taniny). Nízkomolekulární fenolické látky se vyskytují ve všech vyšších rostlinách, jiné jsou typické pro určité skupiny a některé jsou velmi druhově specifické (Crozier et al. 2009; Dinelli et al. 2009; Cheynier et al. 2013). Podílejí se na zbarvení květů a plodů rostlin (Liu 2007). V potravinách jsou fenoly zodpovědné za chuť, vůni, texturu či oxidační stabilitu (Kaur et al. 2014).

Mezi nejvýznamnější fenoly vyskytující se v cereáliích patří fenolické kyseliny (Liu 2007; Boz 2015). V obilovinách jsou z větší části přítomny ve své vázané formě, tedy estericky poutané na složky buněčných stěn jako jsou polysacharidy a proteiny (Shao et al. 2014;

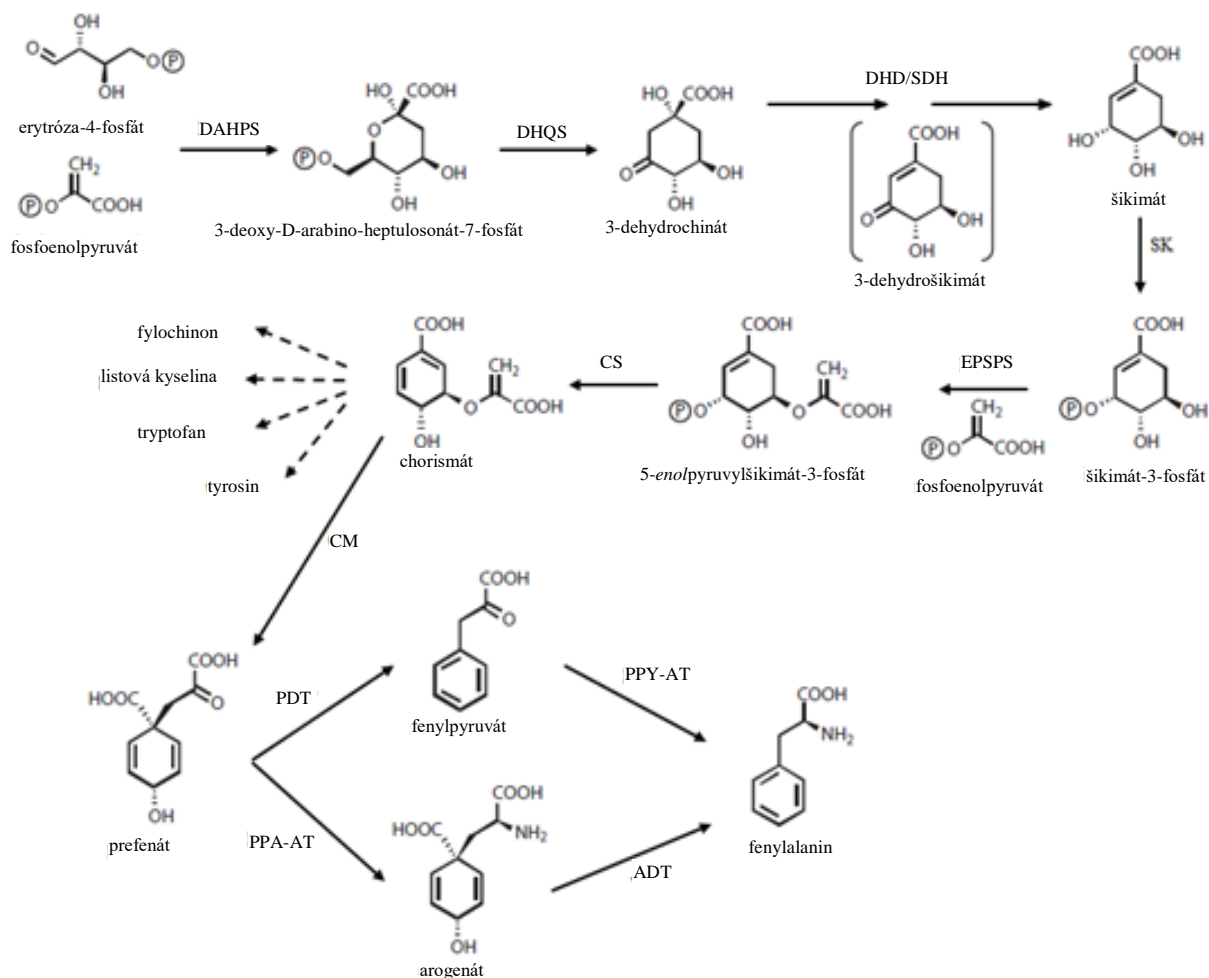
Boz 2015), vykazují vysokou antioxidační aktivitu a mají mnoho terapeutických účinků v prevenci chronických nemocí jako je diabetes, neurodegenerativní choroby anebo různé druhy nádorových onemocnění (Shao et al. 2014).

### 2.3.2 Fenolické kyseliny

Fenolické kyseliny (PAs; Phenolic Acids) lze rozdělit do dvou základních skupin, na deriváty kyselin hydroxybenzoové (tj. gallová, protokatechová, gentisová, *p*-hydroxybenzoová, vanilová a syringová) a hydroxyskořicové (*p*-kumarová, chlorogenová, kávová, sinapová a ferulová) (Robbins 2003; Dykes & Rooney 2007; Kaur et al. 2014; Luthria et al. 2015; Heleno et al. 2015). PAs odvozené od hydroxyskořicové kyseliny jsou v potravinách obecně běžnější, přičemž největší koncentrace se vyskytují ve vnějších vrstvách zralého ovoce, v zelenině a obilovinách (Robbins 2003). Mnoho vědeckých prací pojednávajících o fenolických látkách se zaměřuje především na ty obsažené v ovoci, zelenině anebo čaji, přičemž obiloviny, jakožto další významný zdroj těchto látek, zůstávají často opomenuty (Dykes & Rooney 2007).

#### 2.3.2.1 Biosyntéza fenolických kyselin

Na začátku biosyntézy PAs stojí tzv. šikimátová dráha (viz obrázek č. 7), jejíž první reakcí je aldolová kondenzace erytrózy-4-fosfátu (E4P) a fosfoenolpyruvátu (PEP) katalyzovaná enzymem 3-deoxy-D-arabino-heptulosonát-7-fosfát syntázou (DAHPS), kdy vzniká 3-deoxy-D-arabino-heptulosonát-7-fosfát (DAHP) (Tohge et al. 2013). Druhá reakce je řízena enzymem 3-dehydrochinát syntázou (DHQS) a dochází při ní k intramolekulární výměně atomu kyslíku (heteroatomu pyranózového cyklu) se sedmým uhlíkem DAHP za vzniku 3-dehydrochinátu. Třetí a čtvrtá reakce je katalyzována bifunkčním enzymem 3-dehydrochinát dehydratázou/šikimát 5-dehydrogenázou (DHD/SDH) a jedná se o dehydrataci 3-dehydrochinátu na 3-dehydrošikimát (vytvoření první dvojné vazby v cyklu) a následnou reverzibilní redukci 3-dehydrošikimátu na šikimát za účasti NADPH jakožto kofaktoru (Maeda & Dudareva 2012; Tohge et al. 2013). V pořadí pátou reakci představuje fosforylace C3 hydroxylové skupiny šikimátu na šikimát-3-fosfát (S3P) vyžadující přítomnost ATP, jež je katalyzována šikimát kinázou (SK). V dalším kroku je na hydroxylovou skupinu na pátém uhlíku S3P navázán enolpyruvát, k čemuž je zapotřebí již druhé molekuly PEP a enzymu 5-enolpyruvylšikimát-3-fosfát syntázy (EPSPS) a vzniká tak 5-enolpyruvylšikimát-3-fosfát (EPSP). Posledním krokem šikimátové dráhy je vytvoření chorismátu eliminací fosfátu a odtržením vodíku (vytvoření druhé dvojné vazby), jež řídí enzym chorismát syntáza (CS) (Maeda & Dudareva 2012; Tohge et al. 2013). V tomto místě dochází k větvení metabolické dráhy, jelikož z chorismátu může být vytvořena řada významných látek jako např. fylochinon (vitamin K<sub>1</sub>), kyselina listová (vitamin B<sub>9</sub>) nebo aromatické aminokyseliny tryptofan (následně serotonin, indolové alkaloidy či auxiny), fenylalanin (následně pak flavonoidy, antokyany, suberin, lignin, lignany a fenolické kyseliny) a tyrosin (dále betalainy, tokoferoly, aj.). Syntéza PAs dále pokračuje izomerací chorismátu působením enzymu chorismát mutázy (CM) na prefenát. Pro syntézu PAs je důležitý fenylalanin, jehož je možné z prefenátu získat dvěma různými cestami, které se však od sebe liší pouze pořadím probíhajících přeměn. U rostlin převažující tzv. arogenátová cesta začíná transaminací prefenátu činností prefenát aminotransferázy (PPA-AT) na L-arogenát, jež je následně dehydratován a dekarboxylován na fenylalanin působením enzymu arogenát dehydratázy (ADT). Méně obvyklá tzv.



Obrázek č. 7 Šikimátová biosyntetická dráha

(Maeda & Dudareva 2012; upraveno)

DAHPS (3-deoxy-D-arabino-heptulosonát-7-fosfát syntáza; EC 2.5.1.54), DHQS (3-dehydrochinát syntáza; EC 4.2.3.4), DHD/SDH (3-dehydrochinát dehydratáza; EC 4.2.1.10/šikimát 5-dehydrogenáza; EC 1.1.1.25), SK (šikimát kináza; EC 2.7.1.71), EPSPS (5-enolpyruvylšikimát-3-fosfát syntáza; EC 2.5.1.19), CS (chorismát syntáza; EC 4.2.3.5), CM (chorismát mutáza; EC 5.4.99.5), PDT (prefenát dehydratáza; EC 4.2.1.51), PPY-AT (fenylpyruvát aminotransferáza; EC 2.6.1.5), PPA-AT (prefenát aminotransferáza; EC 2.6.1.78), ADT (arogenát dehydratáza; EC 4.2.1.91)

fenylpyruvátová cesta začíná dehydratací a dekarboxylací prephenátu činností prephenát dehydratázy (PDT) za vzniku fenylpyruvátu, z něhož transaminací řízenou fenylpyruvát aminotransferázou (PPY-AT) vzniká fenylalanin (Maeda & Dudareva 2012).

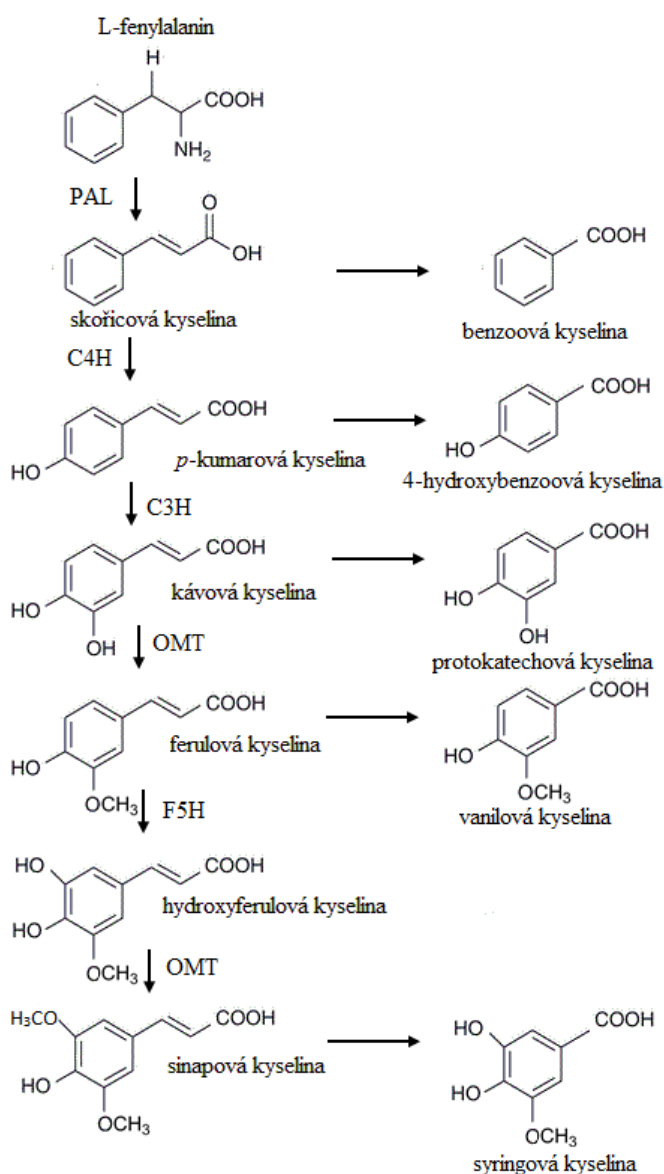
Schéma biosyntézy fenolických kyselin z aminokyseliny fenylalaninu je uvedeno na obrázku č. 8. Z molekuly fenylalaninu je enzymem fenylalanin amoniaklyázou (PAL) eliminována aminoskupina za vzniku *trans*-skořicové kyseliny. Ta je dále *trans*-cinamát monooxygenázou (C4H) hydroxylována v poloze 4 za vzniku *p*-kumarové kyseliny. Enzym *p*-kumarát 3-hydroxyláza (C3H) poté hydroxylací v poloze 3 vytváří kávovou kyselinu. Ferulová kyselina vzniká z kávové kyseliny methylací hydroxylové skupiny v poloze 3, jež je řízena *o*-methyl transferázou (OMT). Ferulová kyselina je dále hydroxylována v poloze 5

enzymem ferulát 5-hydroxylázou (F5H) na hydroxyferulovou kyselinu, následnou methylací nově vzniklé -OH skupiny vzniká sinapová kyselina (Goleniowski et al. 2013; Shahidi & Yeo 2016).

Biosyntéza derivátů hydroxybenzoové kyseliny může vést mnoha různými cestami. Gallová či 4-hydroxybenzoová kyselina mohou být například tvořeny z dehydrošikimové kyseliny – meziprojektu šikimátové dráhy (Goleniowski et al. 2013; Widhalm & Dudareva 2015). Hydroxybenzoové kyseliny mohou též vznikat jako degradační produkty hydroxyskořicových kyselin (Goleniowski et al. 2013; Widhalm & Dudareva 2015; Shahidi & Yeo 2016), dále je možná hydroxylace a methylace hydroxybenzoových kyselin podobným způsobem, jako je tomu při syntéze hydroxyskořicových kyselin z fenylalaninu. Hydroxybenzoáty mohou vznikat rovněž jako produkty degradace flavonoidů (Goleniowski et al. 2013). Hlavní cestou biosyntézy derivátů benzoové kyseliny je degradace postranního řetězce (ztráta acetátu) odpovídajících derivátů hydroxyskořicové kyseliny (Robbins 2003; Heleno et al. 2015). Alternativní cestu pak představuje série enzymatických přeměn vycházející z 3-dehydrošikimátu, tedy meziprojektu šikimátové dráhy (Robbins 2003).

### 2.3.2.2 Nutriční význam fenolických kyselin

Pro člověka jsou PAs významnými antioxidanty, jejichž konzumace je spojována s prevencí mnoha chronických chorob, jako jsou diabetes, neurodegenerativní, kardiovaskulární a nádorová onemocnění (Verma et al. 2008; Goleniowski et al. 2013; Shao et al. 2014). Boz (2015) a Zuchowski et al. (2011) uvádějí, že fenolické látky, zvláště pak PAs, jsou hlavními



Obrázek č. 8 Biosyntéza fenolických kyselin

(Goleniowski et al. 2013; Widhalm & Dudareva 2015; upraveno)

PAL (fenylalanin amoniaklyáza; EC 4.3.1.5),  
 C4H (*trans*-cinamát monooxygenáza; EC 1.14.13.11),  
 C3H (*p*-kumarát 3-hydroxyláza; EC 1.14.18.1),  
 OMT (*o*-methyl transferáza; EC 2.1.1.68),  
 F5H (ferulát 5-hydroxyláza; EC 1.14.13.B32)

látkami zodpovědnými za antioxidační aktivitu celozrnných cereálních výrobků. V pšenici je nejvýznamnější fenolickou kyselinou ferulová kyselina, která je velmi efektivním lapačem volných radikálů (peroxidových, superoxidových i hydroxylových) a vyznačuje se i antimikrobiálními účinky proti některým gram-pozitivním i gram-negativním bakteriím, čímž ovlivňuje spektrum lidské střevní mikrobioty (Boz 2015).

Převážná část PAs je esterově vázána k polymerům buněčných stěn rostlin (Verma et al. 2008). Lidský trávicí trakt však není dostatečně enzymaticky vybaven ke štěpení těchto esterových vazeb, a tedy uvolnění poutaných fenolických kyselin (Heleno et al. 2015). Acosta-Estrada et al. (2014) uvádějí, že nerozpustné vázané fytochemikálie odolávají trávicím procesům v žaludku a tenkém střevě, a proto je pouze malé množství ferulové kyseliny uvolněno již v této části trávicího traktu (2,6 %). Potravou přijaté rozpustné volné a rozpustné konjugované fenolické kyseliny jsou v porovnání s vázanými vstřebávány rychleji (již v žaludku a tenkém střevě) (Acosta-Estrada et al. 2014; Heleno et al. 2015) a následně podléhají řadě enzymaticky řízených reakcí jako například metylace, sulfatace či glukuronidace (Heleno et al. 2015). Naprostá většina však prochází až do tlustého střeva, kde je činností bakteriálních enzymů střevní mikrobioty (esteráz a xylanáz) uvolněna z vazeb na složky obilné vlákniny (Liu 2007; Acosta-Estrada et al. 2014; Heleno et al. 2015). Následně se tak projevuje jednak jejich lokální pozitivní přínos a někteří autoři píší i o vstřebávání do oběhového systému (Liu 2007; Zhang et al. 2012). Antioxidační aktivita vázaných (a posléze uvolněných) fenolických kyselin se tak projevuje především v koncové části trávicího systému. Právě vázané fenolické kyseliny obsažené v celozrnných cereálních výrobcích jsou často označovány za účinný nástroj prevence rozvoje rakoviny tlustého střeva (Liu 2007; Acosta-Estrada et al. 2014; Shao & Bao 2015).

Bylo však také prokázáno, že v důsledku některých způsobů technologického zpracování (fermentace, nakličování, sladování, extruze či vaření) dochází k uvolnění vázaných fenolických látek včetně fenolických kyselin (Boz 2015; Acosta-Estrada et al. 2014).

### 2.3.2.3 Fenolické kyseliny v pšeničném zrně

Fenolické kyseliny jsou nejrozšířenějšími fenolickými látkami v obilovinách (Boz 2015). Různé obiloviny se vzájemně významně liší obsahem PAs. Největší obsah PAs byl nalezen v kukuřici > ječmeni > pšenici > ovsu (Ndolo & Beta 2014). PAs se v pšeničném zrně nacházejí především ve vnějších obalových vrstvách zrna (Ndolo & Beta 2014; Mazzoncini et al. 2015), například v aleuronové vrstvě, kde je jejich obsah mnohonásobně (30-40×) vyšší oproti endospermu (průměrně 4309 µg/g vs. 122 µg/g) (Ndolo & Beta 2014). Během mlýnského zpracování většinou dochází k odstraňování těchto obalů, které přecházejí do otrub, tedy odpadního produktu výroby bílé mouky. Se stupněm vymletí tak v mouce klesá obsah PAs (Mazzoncini et al. 2015). Využitím otrub při výrobě potravin je tak možné zvýšit příjem vlákniny, antioxidantů z řad fenolických sloučenin a dalších nutričně významných látek v populaci (Li et al. 2008; Dykes & Rooney, 2007).

Průměrný obsah PAs v zrně pšenice (standardní červené barvy) se běžně pohybuje od 547 µg/g (Zuchowski et al. 2011) do 676 µg/g (Li et al. 2008). Výrazně vyšší hodnoty (až o 50 %) pak dosáhly červené pšenice analyzované autory Ma et al. (2016) (842 µg/g) nebo Fernandez-Orozco et al. (2010) (728-900 µg/g). Li et al. (2008) zjistili, že celkový obsah

fenolických kyselin v různých genotypech pšenice je značně variabilní (326-1171 µg/g). Tito autoři v rámci Evropského projektu Healthgrain analyzovali celkem 175 genotypů pšenice, z toho 130 ozimých a 20 jarních pšenice (*Triticum aestivum* var. *aestivum*; 664 a 612 µg/g), 10 tvrdých pšenice (*T. turgidum* var. *durum*; 699 µg/g), 5 genotypů pšenice špaldy (*T. aestivum* var. *spelta*; 579 µg/g), 5 jednozrnků (*T. monococcum*; 615 µg/g) a 5 dvouzrnků (*T. turgidum* var. *dicoccum*; 779 µg/g).

Barevné genotypy pšenice obsahují vyšší množství fenolických kyselin oproti tradičně pěstovaným (Li et al. 2005; Ndolo et al. 2013; Ndolo & Beta 2014; Zhang et al. 2018). Ndolo et al. (2013) našli v perikarpu pšenice s purpurovým zrnem o pětinu vyšší obsah PAs oproti nepigmentované pšenici (3815 vs. 3194 µg/g). Dále pak lze dle výsledků autorů Li et al. (2005) seřadit skupiny barevných pšenice sestupně dle PAs v otrubách následovně: purpurové, modré, černé a bílé pšenice (3084 > 2842 > 2824 > 1984 µg/g).

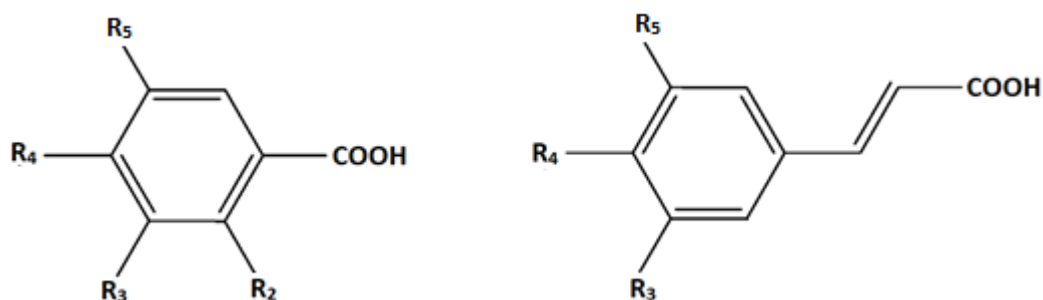
V obilovinách se PAs vyskytují ve třech různých frakcích: rozpustné volné (FPAs; Free Phenolic Acids), rozpustné konjugované (CPAs; Conjugated Phenolic Acids) a nerozpustné vázané (BPAs; Bound Phenolic Acids) přičemž pro jednotlivá stanovení je třeba použít odlišný způsob extrakce. FPAs a CPAs je možné extrahovat polárními rozpouštědly (obvykle jsou využívány 80% směsi metanolu nebo etanolu s vodou), přičemž konjugáty lze působením hydroxidů hydrolyzovat a uvolnit tak danou fenolickou kyselinu. Pro uvolnění nerozpustných vázaných fenolických kyselin je hydrolyzační činidlo (kyselina nebo hydroxid) aplikováno přímo na obilnou matrici (Li et al. 2008; Okarter et al. 2010; Fernandez-Orozco et al. 2010; Boz 2015; Martini et al. 2015; Shao & Bao 2015). FPAs obvykle představují pouze velmi malou část (0,1-1,0 %) z celkových fenolických kyselin (TPAs; Total Phenolic Acids) v zrna (Li et al. 2008; Martini et al. 2015). CPAs jsou sdruženy s menšími organickými molekulami jako např. s cukry (glukózou), organickými kyselinami (maleinovou, chinovou nebo vinnou kyselinou) a dalšími přírodními látkami (např. terpeny, steroly) (Robbins 2003; Goleniowski et al. 2013; Brandolini et al. 2013). Tato frakce v pšeničném zrna obvykle představuje cca 13-22 % z TPAs (Fernandez-Orozco et al. 2010). PAs mohou být vázány i na antokyanová barviva uložená v obalových vrstvách zrn barevných (Pp a Ba) pšenice (Lachman et al. 2017). Samotný chromofor (antokyanidin) je často stabilizován prostřednictvím glykosylace různými sacharidy (glukózou, galaktózou, arabinózou, aj.) a případně další acylace organickými kyselinami (octovou, malonovou, *p*-kumarovou, kávovou, ferulovou). Tím je umožněno propojení jednotlivých částí molekuly antokyanu a tedy vytvoření tzv. intramolekulární kopigmentace, která zvyšuje stabilitu barviva vůči změnám pH nebo vysokým teplotám (Delgado-Vargas et al. 2000; Escribano-Bailón et al. 2004). BPAs tvoří jednoznačně největší podíl z TPA – obvykle více než 70 % (Fernandez-Orozco et al. 2010). Tyto PAs jsou kovalentně etherovými můstky (díky přítomnosti hydroxylových funkčních skupin na aromatických jádrech) nebo esterovými vazbami (jejich vytvoření umožňuje karboxylová funkční skupina) vázány na strukturální složky rostlinných buněčných stěn, jako je celulóza, hemicelulózy (např. arabinoxylany, arabinogalaktany), lignin či pektin (Brandolini et al. 2013; Acosta-Estrada et al. 2014). PAs vázané v buněčných stěnách tvoří fyzickou i chemickou bariéru chránící před invazí patogenů či napadáním hmyzem a jinými býložravými živočichy (Liu 2007).

V pšeničném zrna byla různými autory identifikována celá řada PAs (ferulová, kávová, syringová, *p*-kumarová, vanilová, sinapová, 4-hydroxybenzoová, gallová, chlorogenová,



salicylová; strukturu jednotlivých PAs znázorňuje obrázek č. 9), avšak naprosto dominantní je ferulová kyselina (Dykes & Rooney 2007; Li et al. 2008; Dinelli et al. 2009; Fernandez-Orozco et al. 2010; Hernández et al. 2011; Zhang et al. 2012; Brandolini et al. 2013; Ndolo & Beta 2014; Boz 2015; Ma et al. 2016; Zhang et al. 2018), jež tvoří asi 79 % (Okarter et al. 2010) až 89 % z TPAs (Zuchowski et al. 2011).

Dle výsledků, které publikoval Zuchowski et al. (2011) byla ferulová kyselina (87,1 %) doprovázena sinapovou, *p*-kumarovou, vanilovou a 4-hydroxybenzoovou kyselinou (5,7 %; 2,9 %; 2,4 % a 1,9 %). V zrna se vyskytuje ve třech různých frakcích, jako volná rozpustná, konjugovaná a vázaná (Li et al. 2008; Martini et al. 2015), přičemž Ma et al. (2016) uvádějí obvyklý poměr těchto frakcí 0,1:1:100.



deriváty benzoové kyseliny

$R_2 = \text{OH}$ , salicylová k.

$R_3 = R_4 = R_5 = \text{OH}$ , gallová k.

$R_4 = \text{OH}$ , 4-hydroxybenzoová k.

$R_3 = \text{OCH}_3$ ,  $R_4 = \text{OH}$ , vanilová k.

$R_3 = R_5 = \text{OCH}_3$ ,  $R_4 = \text{OH}$ , syringová k.

deriváty skořicové kyseliny

$R_4 = \text{OH}$ , *p*-kumarová k.

$R_3 = R_4 = \text{OH}$ , kávová k.

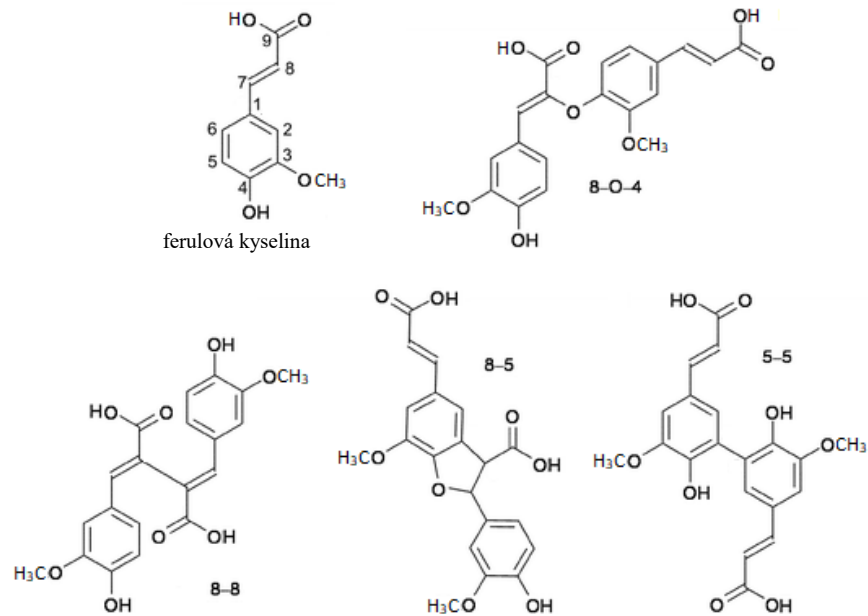
$R_3 = \text{OCH}_3$ ,  $R_4 = \text{OH}$ , ferulová k.

$R_3 = R_5 = \text{OCH}_3$ ,  $R_4 = \text{OH}$ , sinapová k.

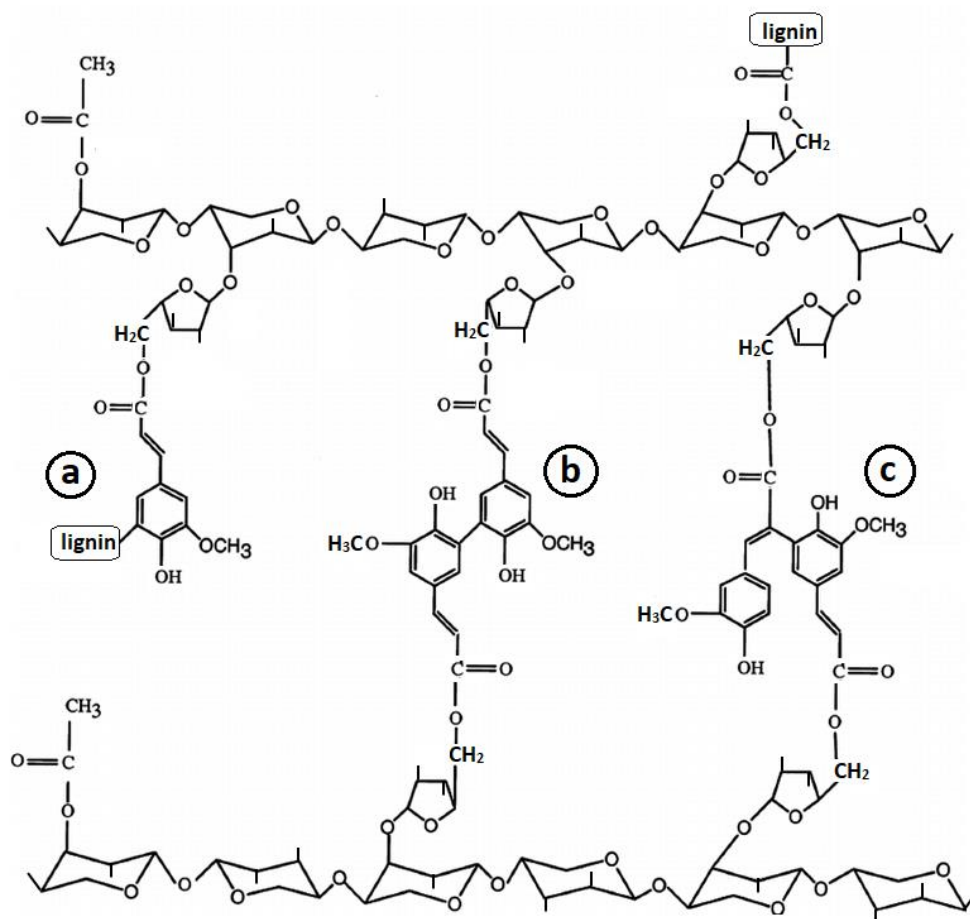
Obrázek č. 9 Fenolické kyseliny identifikované v pšeničném zrna

(Li et al. 2008; upraveno)

V obilovinách se ferulová kyselina vyskytuje i ve formě různých dimerů (5-5-; 8-*O*-4-; 8-5- a 8-8-; viz obrázek č. 10) (Li et al. 2008; Boz 2015; Shao & Bao 2015), trimerů nebo vyšších oligomerů (Pedersen et al. 2015). V obalových vrstvách zrna zprostředkovává intra- nebo intermolekulární propojení polysacharidových řetězců či polysacharidů s ligninem (Renger & Steinhart 2000), čímž dochází k posílení buněčných stěn a zvýšení odolnosti buněk vůči patogenům (Goleniowski et al. 2013; Pedersen et al. 2015). Vzájemné propojení řetězců je znázorněno na obrázku č. 11. Li et al. (2008) uvádí podíl diferulátů na celkovém obsahu ferulové kyseliny v rozmezí 3-9 %.



Obrázek č. 10 Ferulová kyselina a její dimery identifikované v pšeničném zru (Mathew & Abraham 2004; upraveno)



a) 5-O-ferulloyl lignin, b) spojení přes 5-5 dimer, c) spojení přes 8-5 dimer

Obrázek č. 11 Propojení arabinoxylanových řetězců dimery ferulové kyseliny (Mathew & Abraham 2004; upraveno)

## 2.4 Vliv podmínek prostředí a systému pěstování na obsah sekundárních metabolitů

Karotenoidy a fenolické kyseliny patří do skupiny tzv. sekundárních metabolitů, tedy látek, které jsou prostředky rostlinné interakce s okolním prostředím (Hidalgo et al. 2006; Goleniowski et al. 2013). Karotenoidy fungují jako atraktanty, lákají opylovače nebo další živočichy, kteří například napomáhají šíření semen (Hidalgo et al. 2006), dále slouží rostlině jako antioxidanty či prekurzory pro syntézu abscisové kyseliny (Ohmiya 2009; viz kap. 2.2.7). Fenolické látky (fenolické kyseliny) jakožto stavební složky buněčných stěn tvoří fyzickou i chemickou bariéru chránící rostlinné buňky (Dixon & Paiva 1995; Acosta-Estrada et al. 2014), dále se rovněž vyznačují antibakteriálními a antioxidačními vlastnostmi (Acosta-Estrada et al. 2014).

Produkce karotenoidů i fenolických látek je ovlivňována jednak vnitřními faktory (geneticky) a dále rovněž vnějšími vlivy, resp. přírodními podmínkami (Abdel-Aal et al. 2007; Van Hung & Hatcher 2011; Fratianni et al. 2013; de Leonardis et al. 2015; Martini et al. 2015) i systémem pěstování (Zuchowski et al. 2011). Podmínky prostředí pak představují různou úroveň výskytu stresových faktorů, ať už biotických či abiotických, jimž jsou rostliny vystaveny, a na které musí reagovat (Zuchowski et al. 2011; Ma et al. 2014; Mazzoncini et al. 2015). Obecně tedy stresové faktory indukují syntézu sloučenin s antioxidačním potenciálem (de Leonardis et al. 2015).

Syntéza a kumulace fenolických látek je například charakteristickou reakcí na vysokou intenzitu UV záření nebo napadání patogeny, parazity či býložravci (Dixon & Paiva 1995; Robbins 2003; Liu 2007; Acosta-Estrada et al. 2014; Martini et al. 2015; Dwivedi et al. 2016). Tyto sloučeniny mohou na napadající organismus působit buď přímo toxicky, nebo například vyvolávat svíravou chuť v ústní dutině (Shahidi & Yeo 2016). Některé druhy rostlin dokonce využívají fenolických látek jako prostředků v konkurenčním boji s ostatními rostlinami, tzv. alelopatie (Goleniowski et al. 2013; Heleno et al. 2015). Příkladem takových látek mohou být kyseliny kávová či ferulová (Heleno et al. 2015). Syntéza PAs je důsledkem zvýšené aktivity některých enzymů, jako například fenylalanin amoniaklyázy, chalkon syntázy a fosfoenolpyruvát karboxylázy, které jsou katalyzátory biosyntetické dráhy PAs (jak je podrobně uvedeno v kapitole 2.3.2.1). V konečném důsledku je tak snižována produkce tzv. primárních metabolitů (např. sacharidů) ve prospěch tvorby obranných sloučenin a látek podporujících hojení rostlinných pletiv (Cheynier et al. 2013).

Zemědělské plodiny pěstované v tzv. ekologickém režimu, který nepoužívá průmyslová hnojiva ani pesticidy, jsou obecně vzato oproti rostlinám konvenční produkce vystaveny vyšší úrovni působení rozličných stresových faktorů. Vlivu systému pěstování (ekologický vs. konvenční) na obsah fenolických kyselin, karotenoidů i dalších fytochemikálií v různých produktech (zelenina, ovoce, brambory) tak byla věnována značná pozornost (Rembiałkowska 2007; Mitchell et al. 2007; Stracke et al. 2009b; Lairon 2010; Hamouz et al. 2013; Hallmann et al. 2019).

Barański et al. (2014) ve svém review uvádí, že produkty ekologického zemědělství se vyznačují výrazně vyššími obsahy nejrůznějších fenolických látek (celkových flavonoidů, fenolických kyselin, flavonů, flavonolů, flavanonů, antokyanů). Vyšší obsahy fenolických sloučenin v produktech ekologického zemědělství jsou přičítány změnám rostlinného

metabolismu v důsledku nedostatečného zásobení dusíkem (zákaz aplikace průmyslových hnojiv). Dusíkový deficit vede k aktivaci fenyylpropanoidové biosyntetické dráhy, a tedy intenzivnější produkci bezdusíkatých sekundárních metabolitů jako například flavonoidů či fenolických kyselin (Dixon & Paiva 1995; Zuchowski et al. 2011). Podobně pak mohou rostliny reagovat na nedostatek fosforu či železa (Dixon & Paiva 1995).

### 3. Cíle práce a hypotézy

- Cíl 1. Stanovit obsah celkových polyfenolů v obilkách vybraných odrůd barevných pšeníc spektrofotometricky.
- Cíl 2. Identifikovat chromatograficky jednotlivé fenolické kyseliny charakteristické pro barevné pšenice a jejich zastoupení v jednotlivých odrůdách.
- Cíl 3. Charakterizovat chromatograficky jednotlivé karotenoidy a stanovit jejich obsah v barevných odrůdách pšeníc.
- Cíl 4. Stanovit antioxidační aktivitu v nových odrůdách pšenice a vyhodnotit vztah mezi antioxidační aktivitou a zastoupením jednotlivých antioxidantů v barevných pšenicích.
- Cíl 5. Vytipovat vhodné kultivary barevných pšeníc s vysokým obsahem antioxidantů a antioxidační aktivitou pro další šlechtění a vhodnosti pro potravinářské účely
- Hypotéza 1. Vyvinuté HPLC-DAD metody budou vhodné pro analýzu karotenoidů a fenolických sloučenin v obilkách barevných odrůd pšenice.
- Hypotéza 2. Obilky barevných odrůd pšenice obsahují karotenoidy (uložené především v endospermu) a fenolické sloučeniny, které se nacházejí hlavně v otrubách a svrchních částech zrna.

## 4. Publikované práce

### 4.1 Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains

Paznocht L, Kotíková Z, Šulc M, Lachman J, Orsák M, Eliášová M, Martinek P. 2018. Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains. Food Chemistry **240**: 670-678.

### 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

Paznocht L, Kotíková Z, Orsák M, Lachman J, Martinek P. 2019. Carotenoid changes of colored-grain wheat flours during bun-making. Food Chemistry **277**: 725-734.

### 4.3 Total phenolic content and antioxidant activity of tritordeum wheat and barley

Eliášová M, Paznocht L. 2017. Total phenolic content and antioxidant activity of tritordeum wheat and barley. Agronomy Research **15(S2)**: 1287-1294.

### 4.4 The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain

Zrcková M, Capouchová I, Eliášová M, Paznocht L, Pazderů K, Dvořák P, Konvalina P, Orsák M, Štěrbá Z. 2018. The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain Plant, Soil and Environment **64**: 530-538.

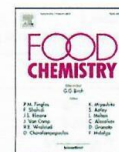
### 4.5 Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year

Zrcková M, Capouchová I, Paznocht L, Eliášová M, Dvořák P, Konvalina P, Janovská D, Orsák M, Bečková L. 2019. Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year. Plant, Soil and Environment **65**: 260-266.



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## Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains



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β-Carotene (PubChem CID: 5280489)  
Antheraxanthin (PubChem CID: 5281223)  
Lutein (PubChem CID: 5281243)  
Zeaxanthin (PubChem CID: 5280899)

### ABSTRACT

Carotenoids are important phytonutrients responsible for the yellow endosperm color in cereal grains. Free carotenoids, namely lutein, zeaxanthin, antheraxanthin, α- and β-carotene, were quantified by HPLC-DAD-MS in fourteen genotypes of wheat, barley and tritordeum harvested in Czechia in 2014 and 2015. The highest carotenoid contents were found in yellow-grained tritordeum HT 439 (12.16 μg/g DW), followed by blue-grained wheat V1-131-15 (7.46 μg/g DW), and yellow-grained wheat TA 4024 (7.04 μg/g DW). Comparing carotenoid contents, blue varieties had lower whereas purple ones had the same or higher levels than conventional bread wheat. Lutein was the main carotenoid found in wheat and tritordeum while zeaxanthin dominated in barley. The majority of cereals contained considerable levels of esterified forms (up to 61%) of which lutein esters prevailed. It was assessed that cereal genotype determines the proportion of free and esterified forms. High temperatures and drought during the growing season promoted carotenoid biosynthesis.

### 1. Introduction

Cereals are grasses cultivated for their edible grains, which play an important role in the human diet, supplying almost 70–80% of energy requirements. World production of wheat has been steadily increasing, making it the second most-produced cereal after corn (735.6 million tons in 2016) with its global consumption of 67 kg/capita/year (FAO, 2017; Statista, 2017). It was not until the last decade that researchers began to recognize wheat and other cereals as a good source of phytonutrients; among them, phenolic and terpenoid compounds being the most representative (Borrelli & Trono, 2016). Carotenoids are yellow, orange and red pigments responsible for the color of most fruits and vegetables. They are C<sub>40</sub> isoprenoids with a long conjugated polyene chain that is responsible for their color and biological activities. Carotenoids play an important role in plants in both photosynthetic and non-photosynthetic tissues, where they assist in light harvesting, photoprotection, or act as signaling molecules, precursors of volatile compounds, colorants and pollinator attractants (Nisar, Li, Lu,

Khin, & Pogson, 2015). Carotenoids are divided into two classes: carotenes (which are hydrocarbons) and xanthophylls (oxygen derivatives of carotenes). Carotenes and xanthophylls are both located in various types of plastids. They accumulate in high levels in chloroplasts (photosynthetic tissue) and chromoplasts found in mature fruit, vegetable and flower tissues. In chloroplasts, carotenoids are associated with proteins in pigment-protein complexes located in thylakoid membranes. In chromoplasts, carotenoids are deposited in multiple lipoprotein structures, which differ among plant species and tissues. Fibrillar and tubular lipoprotein structures contain xanthophylls in the form of fatty acid esters located on the α- or β-ionone ring (Howitt & Pogson, 2006). The esterification (i.e. sequestration of carotenoids) does not affect the chromophore properties, protects carotenoids from degradation and is a common and effective mechanism to increase their accumulation in plant tissues (Atienza, Ballesteros, Martín, & Hornero-Méndez, 2007; Saini, Nile, & Park, 2015).

In cereals, carotenoids occur naturally either in free or esterified forms (mostly with palmitic and linoleic acid) depending on the cereal

**Abbreviations:** Ba, blue aleurone; BHT, butylated hydroxytoluene; DW, dry weight; FW, fresh weight; Pp, purple pericarp; TAC, total antheraxanthin content; TALC, total α-carotene content; TBC, total β-carotene content; TCC, total carotenoid content; TLC, total lutein content; TZC, total zeaxanthin content; Ye, yellow endosperm

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**Table 1**  
Description of cereal genotypes.

Variety	Species	Growth type	Country of origin <sup>d</sup>	Variety status	Grain color
Bohemia	<i>Triticum aestivum</i> L.	winter	CZE	released variety	conventional red grain (control)
TA 4024	<i>Triticum aestivum</i> L.	spring	ISR	genetic resource	yellow endosperm
Purple Feed	<i>Triticum aestivum</i> L.	spring	AUS	research germplasm	purple pericarp
RU 687-12	<i>Triticum aestivum</i> L.	spring	CZE	breeding line	purple pericarp
Konini	<i>Triticum aestivum</i> L.	spring	NZL	research germplasm	purple pericarp
Purple	<i>Triticum aestivum</i> L.	spring	CND	research germplasm	purple pericarp
PS Karkulka	<i>Triticum aestivum</i> L.	winter	SVK	released variety	purple pericarp
UC 66049	<i>Triticum aestivum</i> L.	spring	USA	genetic resource	blue aleurone
Tschermak's B.S <sup>a</sup>	<i>Triticum aestivum</i> L.	spring	AUT	research germplasm	blue aleurone
Xiao Yian	<i>Triticum aestivum</i> L.	spring	CHN	genetic resource	blue aleurone
V1-131-15 <sup>b</sup>	<i>Triticum aestivum</i> L.	winter	CZE	breeding line	blue aleurone
V1-133-15 <sup>c</sup>	<i>Triticum aestivum</i> L.	winter	CZE	breeding line	blue aleurone
AF Cesar	<i>Hordeum vulgare</i> L.	spring	CZE	released variety	standard
HT 439	× <i>Tritordeum martinii</i> A. Pujadas nothosp. nov.	spring	ESP	breeding line	yellow endosperm

<sup>a</sup> Tschermak's blaukörniger Sommerweizen.

<sup>b</sup> V1-131-15: (RU-440 × V1-702) × (Citrus × Bona Dea).

<sup>c</sup> V1-133-15: RU-440 × UC 66049.

<sup>d</sup> AUS Australia, AUT Austria, CHN China, CND Canada, CZE Czech Republic, ESP Spain, ISR Israel, NZL New Zealand, SVK Slovakia, USA United States of America.

genotype (Lachman, Martinek, Kotíková, Orsák, & Šulc, 2017; Mellado-Ortega & Hornero-Méndez, 2012; Ziegler et al., 2015). Abdel-Aal, Young, Rabalski, Hucl, and Fregeau-Reid (2007) reported low levels of total carotenoids in conventional bread wheat flour (1.94 µg/g), compared to higher values found in spelt (4.01 µg/g), emmer (5.76 µg/g), durum (6.27 µg/g) and einkorn wheat (9.62 µg/g). Spring and winter wheat contains predominantly lutein (2.17 µg/g DW) and zeaxanthin (0.50 µg/g DW), followed by (9Z)-lutein (0.13 µg/g DW), α- and β-carotene (0.04 and 0.07 µg/g DW) (Konopka, Czaplíckí, & Rotkiewicz, 2006). In addition, some authors like Adom, Sorrells, and Liu (2003) mention β-cryptoxanthin (0.01–0.13 µg/g of grain) as being contained in wheat.

The new cereal species tritordeum (× *Tritordeum martinii* A. Pujadas nothosp. nov.), which is rich in total carotenoids, is a hexaploid hybrid derived from a cross between wild barley (*Hordeum chilense* Roem. & Schult.) and durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.). It is the *Hordeum* parent that causes the high total carotenoid content. Lutein predominates among xanthophylls found in this species with levels being 5–8 fold higher than those found in durum wheat (Mellado-Ortega & Hornero-Méndez, 2015). A high proportion of lutein is bound in mono- and diesters, which cause improved stability compared to the free forms. The increased stability of esterified forms has been shown during post-harvest storage of grain (Mattera, Hornero-Méndez, & Atienza, 2017; Mellado-Ortega, Atienza, & Hornero-Méndez, 2015), and heat processing of foods, such as baking of bread (Abdel-Aal, Young, Akhtar, & Rabalski, 2010).

Barley (*Hordeum vulgare* L.) is an ancient grain traditionally grown in temperate climates and used mostly malted to brew beer. Among cereals, barley is rather low in total carotenoids with its levels comparable to conventional bread wheat. Yellow barley grain (the most typical form) contains 2.25 µg/g of total carotenoids whereas its purple form contains twice this content (4.54 µg/g by Ndolo & Beta, 2013). These authors also reported zeaxanthin as being the major carotenoid in barley.

In terms of human health, there is evidence that carotenoids are antioxidants that protect cells against reactive oxygen species and free radicals, hence possessing powerful health benefits. Carotenoids may prevent retinal degradation, sunburn (Stahl & Sies, 2005), liver cancer (Nishino, Murakoshi, Tokuda, & Satomi, 2009), and enhance the immune system. Further, they have shown some antiapoptotic and anti-inflammatory properties and take part in intracellular signaling cascades by influencing transcription factors (Kaulmann & Bohn, 2014). Carotenoids containing unsubstituted β-ionone ring possess vitamin A activity (Fernández-García et al., 2012). However, neither humans nor animals are able to synthesize carotenoid compounds; therefore they

need to include them in the diet to be able to gain any health benefits (Fernandez-Orozco, Gallardo-Guerrero, & Hornero-Méndez, 2013).

In recent years, many studies have dealt with carotenoids in various cereals such as diploid wheat *Triticum monococcum* L., tetraploid wheat *T. dicoccum* S. and hexaploid wheat *T. aestivum* L. (Digesù, Platani, Cattivelli, Mangini, & Blanco, 2009; Lachman, Hejtmánková, & Kotíková, 2013), tritordeum (Atienza et al., 2007; Mellado-Ortega & Hornero-Méndez, 2012), barley (Masís et al., 2015; Siebenhandl et al., 2007), and corn (Žilić, Serpen, Akilioğlu, Gökmen, & Vančetović, 2012). Only a few papers (De Leonardis et al., 2015; Ficco et al., 2016; Ndolo & Beta, 2013; Siebenhandl et al., 2007) have specifically dealt with carotenoid profiles in purple/blue wheat grains. In addition to this fact, the majority of published studies take into account only the free forms of carotenoids (Abdel-Aal et al., 2007; Ndolo & Beta, 2013; Siebenhandl et al., 2007).

Keeping in mind the important health benefits of carotenoids and high cereal consumption per capita, the objective of this study was (1) to quantify free carotenoids and the carotenoid moieties found in esters in pigmented cereal grains; (2) to compare the less traditional, pigmented purple- and blue-wheat varieties that are rich in anthocyanins with traditional red winter wheat and, to a lesser extent, yellow endosperm wheat; and last (3) to assess the impact of environment on carotenoid levels.

## 2. Material and methods

### 2.1. Plant material

Wheat, barley and tritordeum varieties and breeding lines (Table 1) were harvested in 2014 and 2015 at the Agricultural Research Institute (Agrotest Fyto, Ltd.) in Kroměříž, Czech Republic. Experimental field parameters: GPS location 49.2851172 N, 17.3646269E, 235 m above sea level, luvis chernozem/loamic soils, long-term annual average temperature 9.2 °C and precipitations averaging 576 mm. Table 2 shows the exact weather conditions during the two growing seasons. Plants were grown on small experimental plots (10 m<sup>2</sup>) using conventional growing technology. After harvesting, samples were stored in paper bags in a box in the dark at room temperature (25 °C) for 2 months before being analyzed.

### 2.2. Chemicals

Lutein and zeaxanthin standards (UV, ≥95%, ≥98%) were obtained from Extrasynthese, Genay, France. β-Carotene standard (HPLC, ≥95%), ethanol absolute (puriss., ≥99.8%), butylated hydroxytoluene (BHT, ≥99% FG), tert-butyl methyl ether (HPLC grade), diethyl ether



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**Table 2**  
Weather conditions in Kroměříž, Czech Republic.

Season	Parameter	IX.	X.	XI.	XII.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
2013/2014	Average temperature [°C]	13.4	10.8	5.7	2.3	1.3	3.8	8.1	11.3	14.0	17.8	21.0	18.0
	Deviation from long-term standard [°C]	-0.9	+1.5	+1.7	+2.2	+2.6	+3.4	+3.8	+1.9	-0.5	+0.5	+1.8	-0.8
	Monthly precipitation [mm]	94.1	36.8	26.1	9.4	23.8	19.3	5.2	15.5	74.9	63.9	78.1	69.4
	Percentage of long-term standard	174	96	65	28	96	73	16	38	113	79	106	106
2014/2015	Average temperature [°C]	15.7	10.9	7.5	2.2	1.7	1.3	9.7	9.7	13.9	18.1	22.1	23.1
	Deviation from long-term standard [°C]	+1.4	+1.6	+3.5	+2.1	+3.0	+0.9	+1.2	+0.3	-0.6	+0.8	+2.9	+4.3
	Monthly precipitation [mm]	119.9	42.0	27.8	35.7	41.7	18.9	39.6	13.6	41.9	47.0	42.0	65.1
	Percentage of long-term standard	221	109	70	107	167	71	121	33	63	58	57	99

(cont. BHT as inhibitor, puriss.) were purchased from Sigma-Aldrich, St. Louis, USA. Antheraxanthin and  $\alpha$ -carotene standards (HPLC, 95%, 97%) were purchased from CarotenNature, GmbH, Münsingen, Switzerland. Methanol (HPLC grade), acetone (GR grade), ethanol (GR grade), hexane (GR grade) and potassium hydroxide (GR grade) were purchased from Lachner s.r.o., Neratovice, Czech Republic. HPLC grade water was prepared using Simplicity UV (Merck Millipore, KGaA, Darmstadt, Germany).

### 2.3. Analysis of free carotenoids

Carotenoids were extracted according to the method by Kotřková, Šulc, Lachman, Pivec, Orsák, and Hamouz (2016) modified for the cereal grain matrix. 2 g of finely ground sample were extracted with 12 mL ethanol/acetone/hexane mixture (1:1:2, v/v/v), vortexed and left to stand for 24 h in a refrigerator (4 °C). Then, the sample was vortexed for 1 min (Basic 3, IKA, KG, Staufen, Germany), sonicated for 10 min in an ultrasonic bath (PS 04, Powersonic-Notus, Ltd., Vrábce, Slovakia) and finally centrifuged at 8228 rcf for 10 min (5810 R, Eppendorf, Hamburg, Germany). 9 mL of the supernatant were transferred into a 50-mL glass evaporation flask and the sediment re-extracted with 12 mL of the extraction mixture. Both supernatants were combined and evaporated under vacuum at 40 °C (Rotavapor R-200, Büchi Labortechnik, AG, Flawil, Switzerland). The dry residue was reconstituted with 2 mL ethanol/acetone (3:2, v/v) solution containing 0.2% BHT and filtered through a syringe filter (PVDF, 0.45  $\mu$ m) into an amber HPLC vial.

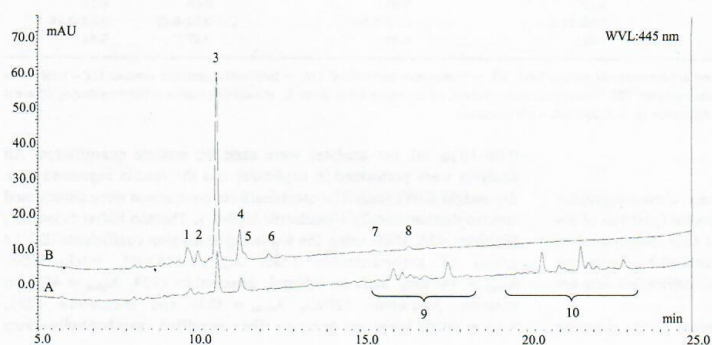
### 2.4. Hydrolysis of xanthophyll esters

The xanthophyll esters were obtained by the same extraction process as described above and subjected to a slightly modified hydrolysis procedure by Schiedt and Liaen-Jensen (1995). The dry residue (paragraph 2.3.) was reconstituted in 6 mL diethyl ether and 6 mL of the hydrolysis medium (50% aqueous KOH and ethanol 1:9, v/v), eventually resulting in 2.5% ethanolic KOH and shaken (GFL 3006,

Burgwedel, Germany) for 2 h at room temperature in the dark. 6 mL of the hydrolysate were then transferred into another falcon tube and 6 mL diethyl ether/hexane (1:1, v/v) and 6 mL water were successively added. The mixture was shaken for 10 min followed by centrifugation at 4 °C and 2057 rcf for 10 min. Then, the lower (aqueous) phase containing KOH was removed by a syringe with a needle. To wash out the alkali completely, 6 mL of water were added to the remaining organic phase, shaken for 10 min and centrifuged. The organic layer was quantitatively transferred into a 25 mL evaporation flask and evaporated under vacuum at 30 °C. The dry residue was reconstituted with 1 mL ethanol/acetone (3:2, v/v) containing 0.2% BHT and filtered through a syringe filter (PVDF, 0.45  $\mu$ m) into an amber HPLC vial.

### 2.5. HPLC-DAD-APCI<sup>+</sup>-MS analysis

The analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a quaternary pump, autosampler, column heater and diode array detector. The analytes were separated by gradient elution on an YMC Carotenoid C30 analytical column (150 mm  $\times$  3.0 mm, particle size = 3  $\mu$ m, YMC Co., Kyoto, Japan). The operating conditions were as follows: flow rate 0.6 mL/min; column temperature 25 °C; autosampler temperature 10 °C; injection volume 10  $\mu$ L; detection at  $\lambda$  = 445 nm (spectral acquisition 300–700 nm). The tertiary mobile phase consisted of methanol (A), water (B) and tert-butyl methyl ether (C). Gradient: initial conditions of 90% A, 10% B and 0% C were kept for 1 min, then increased to 90% A, 0% B and 10% C at 6 minutes, reaching 40% A, 0% B and 60% C at 22 min following column flush and re-equilibration for 11 min. An example chromatogram is shown in Fig. 1. The mass analysis was performed on a triple quadrupole mass spectrometer (3200 QTRAP, AB Sciex, Framingham, MA, USA) with atmospheric pressure chemical ionization (APCI) in positive mode (curtain gas 30 psi, nebulizer gas 45 psi, turbo gas 50 psi, nebulizer current 3  $\mu$ A, source temperature 300 °C, declustering potential 36 V, entrance potential 7 V).



**Fig. 1.** Chromatogram of the TA 4024 (Ye) variety depicting differences between unhydrolyzed (A) and hydrolyzed (B) grain sample. 1 – antheraxanthin, 2 – (13'Z)-lutein, 3 – lutein, 4 – zeaxanthin, 5 – (9'Z)-lutein, 6 – (9'Z)-lutein, 7 –  $\alpha$ -carotene, 8 –  $\beta$ -carotene, 9 – xanthophyll monoesters, 10 – xanthophyll diesters.

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**Table 3**  
Identification parameters of carotenoids detected in cereal grains.

Peak <sup>a</sup>	Compound	Rt [min] <sup>b</sup>	$\lambda_{\max}$ [nm]	%A <sub>III</sub> /A <sub>II</sub> <sup>c</sup>	%A <sub>B</sub> /A <sub>II</sub> <sup>d</sup>	[M + H] <sup>+5</sup>	Fragments <sup>e</sup>
1	Antheraxanthin	09.96	421, 444, 472	60	0	585.4 (100%)	567.5 [M + H-18] <sup>+</sup> (11%)
2	(13Z)-Lutein	10.14	330, 416, 439, 466	40	28	569.5 (8%)	551.5 [M + H-18] <sup>+</sup> (100%)
3	Lutein	10.69	420, 444, 471	60	0	569.5 (10%)	551.5 [M + H-18] <sup>+</sup> (100%), 533.4 [M + H-18-18] <sup>+</sup> (4%)
4	Zeaxanthin	11.40	424, 450, 476	28	0	569.5 (100%)	551.5 [M + H-18] <sup>+</sup> (14%)
5	(9Z)-Lutein	11.55	332, 416, 438, 466	72	5	569.5 (11%)	551.5 [M + H-18] <sup>+</sup> (100%)
6	(9Z)-Lutein	12.27	332, 416, 440, 467	64	4	569.5 (25%)	551.5 [M + H-18] <sup>+</sup> (100%)
7	$\alpha$ -Carotene	15.47	420, 445, 472	61	0	537.6	
8	$\beta$ -Carotene	16.54	425, 450, 477	26	0	537.6	

<sup>a</sup> Peak number as shown in Fig. 1.

<sup>b</sup> Retention time.

<sup>c</sup> The height ratio between the highest absorption wavelength (A<sub>III</sub> peak) and the middle absorption wavelength (A<sub>II</sub> peak) from the peak valley between A<sub>III</sub>/A<sub>II</sub>.

<sup>d</sup> The height ratio of the Z-peak to the middle absorption peak (the so-called Q-ratio).

<sup>e</sup> in APCI<sup>+</sup> ionization.

**Table 4**  
Carotenoid profiles in cereal grains.

Season	Variety	Grain color	TAC [ $\mu$ g/g DW]	Free form [% of total]	TLC [ $\mu$ g/g DW]	Free form [% of total]	TZC [ $\mu$ g/g DW]	Free form [% of total]	TALC [ $\mu$ g/g DW]	TBC [ $\mu$ g/g DW]	
2014	Bohemia	Red	0.06 $\pm$ 0.01 <sup>ef</sup>	100	2.23 $\pm$ 0.04 <sup>f</sup>	100	1.19 $\pm$ 0.02 <sup>b</sup>	100	n.d.	n.d.	
	TA 4024	Ye	0.28 $\pm$ 0.02 <sup>b</sup>	11	5.03 $\pm$ 0.19 <sup>b</sup>	49	0.74 $\pm$ 0.05 <sup>d</sup>	100	0.06 $\pm$ 0.01 <sup>a</sup>	0.22 $\pm$ 0.00 <sup>a</sup>	
	Purple Feed	Pp	0.07 $\pm$ 0.01 <sup>def</sup>	0	1.96 $\pm$ 0.05 <sup>f</sup>	34	0.26 $\pm$ 0.02 <sup>f</sup>	100	n.d.	n.d.	
	RU 687-12	Pp	0.12 $\pm$ 0.01 <sup>d</sup>	16	2.75 $\pm$ 0.08 <sup>e</sup>	69	0.32 $\pm$ 0.03 <sup>f</sup>	100	n.d.	0.05 $\pm$ 0.01 <sup>c</sup>	
	Konini	Pp	0.22 $\pm$ 0.00 <sup>c</sup>	12	4.01 $\pm$ 0.05 <sup>c</sup>	58	0.31 $\pm$ 0.02 <sup>f</sup>	100	0.04 $\pm$ 0.01 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>b</sup>	
	Purple	Pp	0.12 $\pm$ 0.00 <sup>d</sup>	16	3.23 $\pm$ 0.06 <sup>d</sup>	76	0.29 $\pm$ 0.05 <sup>d</sup>	100	n.d.	0.03 $\pm$ 0.00 <sup>d</sup>	
	PS Karkulka	Pp	0.09 $\pm$ 0.01 <sup>de</sup>	36	1.85 $\pm$ 0.06 <sup>gh</sup>	55	0.71 $\pm$ 0.08 <sup>d</sup>	100	n.d.	0.06 $\pm$ 0.00 <sup>c</sup>	
	UC 66049	Ba	0.05 $\pm$ 0.00 <sup>ef</sup>	0	1.07 $\pm$ 0.01 <sup>f</sup>	36	0.28 $\pm$ 0.00 <sup>f</sup>	100	n.d.	0.05 $\pm$ 0.02 <sup>c</sup>	
	Tscheramak's B.S.	Ba	0.12 $\pm$ 0.01 <sup>d</sup>	0	2.23 $\pm$ 0.10 <sup>f</sup>	43	0.29 $\pm$ 0.02 <sup>f</sup>	100	n.d.	0.07 $\pm$ 0.00 <sup>c</sup>	
	Xiao Yian	Ba	0.03 $\pm$ 0.00 <sup>f</sup>	0	0.76 $\pm$ 0.06 <sup>ij</sup>	58	0.15 $\pm$ 0.02 <sup>f</sup>	100	n.d.	n.d.	
	V1-131-15	Ba	0.22 $\pm$ 0.01 <sup>c</sup>	100	5.23 $\pm$ 0.06 <sup>b</sup>	100	1.96 $\pm$ 0.06 <sup>a</sup>	100	n.d.	n.d.	
	V1-133-15	Ba	n.d.		1.63 $\pm$ 0.02 <sup>h</sup>	100	0.48 $\pm$ 0.06 <sup>e</sup>	100	n.d.	n.d.	
	AF Cesar	Std	0.08 $\pm$ 0.01 <sup>def</sup>	62	0.56 $\pm$ 0.01 <sup>j</sup>	66	0.86 $\pm$ 0.04 <sup>c</sup>	100	n.d.	0.03 $\pm$ 0.00 <sup>d</sup>	
	HT 439	Ye	0.56 $\pm$ 0.00 <sup>a</sup>	15	9.30 $\pm$ 0.25 <sup>a</sup>	75	0.94 $\pm$ 0.07 <sup>c</sup>	100	0.07 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>c</sup>	
	Mean			0.14		2.99		0.67		0.06	0.07
	Range			0.03–0.56		0.56–9.30		0.15–1.96		0.04–0.07	0.03–0.22
	2015	Bohemia	Red	0.21 $\pm$ 0.01 <sup>de</sup>	100	2.62 $\pm$ 0.14 <sup>c</sup>	100	0.81 $\pm$ 0.06 <sup>def</sup>	100	n.d.	n.d.
TA 4024		Ye	0.59 $\pm$ 0.00 <sup>b</sup>	34	5.29 $\pm$ 0.03 <sup>b</sup>	31	1.60 $\pm$ 0.01 <sup>ab</sup>	55	0.05 $\pm$ 0.01 <sup>ab</sup>	0.22 $\pm$ 0.03 <sup>ab</sup>	
Purple Feed		Pp	0.14 $\pm$ 0.03 <sup>c</sup>	70	3.19 $\pm$ 0.02 <sup>c</sup>	50	0.82 $\pm$ 0.02 <sup>defg</sup>	100	n.d.	n.d.	
RU 687-12		Pp	0.18 $\pm$ 0.00 <sup>de</sup>	100	1.95 $\pm$ 0.02 <sup>efg</sup>	85	0.69 $\pm$ 0.04 <sup>efg</sup>	100	n.d.	0.03 $\pm$ 0.00 <sup>d</sup>	
Konini		Pp	0.52 $\pm$ 0.01 <sup>bc</sup>	35	5.29 $\pm$ 0.22 <sup>b</sup>	45	1.43 $\pm$ 0.09 <sup>bc</sup>	85	0.05 $\pm$ 0.00 <sup>ab</sup>	0.24 $\pm$ 0.05 <sup>a</sup>	
Purple		Pp	0.18 $\pm$ 0.00 <sup>de</sup>	82	2.19 $\pm$ 0.02 <sup>def</sup>	84	0.41 $\pm$ 0.03 <sup>h</sup>	100	n.d.	n.d.	
PS Karkulka		Pp	0.27 $\pm$ 0.00 <sup>de</sup>	57	2.60 $\pm$ 0.22 <sup>cde</sup>	51	0.64 $\pm$ 0.11 <sup>fg</sup>	92	n.d.	0.11 $\pm$ 0.01 <sup>c</sup>	
UC 66049		Ba	0.27 $\pm$ 0.01 <sup>de</sup>	52	1.70 $\pm$ 0.08 <sup>fg</sup>	39	0.79 $\pm$ 0.02 <sup>defg</sup>	69	n.d.	0.08 $\pm$ 0.01 <sup>c</sup>	
Tscheramak's B.S.		Ba	0.31 $\pm$ 0.04 <sup>d</sup>	71	2.32 $\pm$ 0.52 <sup>def</sup>	67	0.89 $\pm$ 0.03 <sup>de</sup>	100	0.02 $\pm$ 0.01 <sup>b</sup>	0.15 $\pm$ 0.01 <sup>bc</sup>	
Xiao Yian		Ba	0.19 $\pm$ 0.01 <sup>de</sup>	59	1.92 $\pm$ 0.02 <sup>efg</sup>	28	0.63 $\pm$ 0.00 <sup>g</sup>	82	n.d.	n.d.	
V1-131-15		Ba	0.45 $\pm$ 0.01 <sup>c</sup>	100	5.80 $\pm$ 0.16 <sup>b</sup>	100	1.26 $\pm$ 0.04 <sup>c</sup>	100	n.d.	n.d.	
V1-133-15		Ba	0.16 $\pm$ 0.01 <sup>e</sup>	100	2.42 $\pm$ 0.16 <sup>de</sup>	100	0.72 $\pm$ 0.04 <sup>efg</sup>	100	n.d.	n.d.	
AF Cesar		Std	0.27 $\pm$ 0.03 <sup>de</sup>	100	1.32 $\pm$ 0.17 <sup>g</sup>	80	1.65 $\pm$ 0.02 <sup>a</sup>	100	n.d.	0.03 $\pm$ 0.00 <sup>d</sup>	
HT 439		Ye	0.77 $\pm$ 0.14 <sup>a</sup>	75	11.6 $\pm$ 0.10 <sup>a</sup>	67	0.94 $\pm$ 0.05 <sup>d</sup>	100	0.06 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>d</sup>	
Mean				0.32		3.59		0.92		0.05	0.11
Range				0.14–0.77		1.32–11.6		0.41–1.65		0.02–0.06	0.03–0.24
Mean				0.23		3.29		0.80		0.05	0.10
Range			0.03–0.77		0.56–11.6		0.15–1.96		0.02–0.07	0.03–0.24	
h <sup>2</sup> b			0.19		0.83		0.34		0.97	0.82	

The data were expressed as total content of each component and as percentage of the free form; h<sup>2</sup>b – broad-sense heritability; TAC – total antheraxanthin content; TLC – total lutein content; TZC – total zeaxanthin content; TALC – total  $\alpha$ -carotene content; TBC – total  $\beta$ -carotene content; values expressed as mean  $\pm$  standard deviation of three replicates; different superscript letters within the same column express statistical difference ( $p < 0.05$ ); n.d. – not detected).

### 2.6. Identification and quantification

The analytes were identified based on their chromatographic properties (retention time), UV–Vis absorption spectra (position of absorption maxima, %A<sub>III</sub>/A<sub>II</sub> and %A<sub>B</sub>/A<sub>II</sub>) and mass data (fragmentation patterns, ion ratio). Z-isomers of lutein were identified by comparing their spectral properties with literature data. All identification data are summarized in Table 3.

Peak area and external calibration (ten different levels spanning

0.05–10  $\mu$ g/mL per analyte) were used for analyte quantitation. All analyses were performed in triplicates and the results expressed on a dry weight (DW) basis. The carotenoid concentrations were determined spectrophotometrically (Spectronic Helios  $\gamma$ , Thermo Fisher Scientific, Waltham, MA, USA) using the following extinction coefficients (EC, L/g/cm) of antheraxanthin (235,  $\lambda_{\max}$  = 446 nm), lutein (255,  $\lambda_{\max}$  = 445 nm) both in ethanol; zeaxanthin (234,  $\lambda_{\max}$  = 452) in acetone;  $\beta$ -carotene (259.2,  $\lambda_{\max}$  = 453) and  $\alpha$ -carotene (271,  $\lambda_{\max}$  = 445) both in hexane. The esterified xanthophylls were

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quantified as the difference between the intrinsic free xanthophylls and the xanthophylls released after alkaline hydrolysis. The sum of all carotenoid components is further referred to as total carotenoid content (TCC).

### 2.7. Statistical analysis

The data were processed by Chromeleon (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Excel (Microsoft, Redmond, WA, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, OK, USA). Genetic effects on carotenoid content were evaluated by one-way ANOVA ( $p < 0.05$ ; separately for each year). Genetic effects, environmental effects and their interactions were evaluated by the factorial ANOVA ( $p < 0.05$ ). Tukey's Post Hoc HSD test was used for detailed evaluation. Broad-sense heritability ( $h^2b$ ) was calculated using the formula  $h^2b = \sigma^2g / (\sigma^2g + \sigma^2e)$  where  $\sigma^2g$  is the genetic variance and  $\sigma^2e$  is the environmental variance. Pearson correlation coefficients were calculated between total carotenoid content and individual carotenoids ( $p < 0.05$ ).

### 3. Results and discussion

In total, fourteen genotypes comprising wheat, barley and tritordeum were harvested in 2014 and 2015 (Table 1). The wheat genotypes included five lines with purple pericarp (Pp), five with blue aleurone layer (Ba), one with yellow endosperm (Ye) and one conventional red wheat (bread type) used as a control. Those cereal grains were analyzed for their carotenoid profile – total antheraxanthin content (TAC), total lutein content (TLC), total zeaxanthin content (TZC), total  $\alpha$ -carotene content (TALC), total  $\beta$ -carotene content (TBC), and total carotenoid content (TCC). Individual results can be taken from Table 4 and the TCC in Fig. 2.

#### 3.1. Total carotenoid content

The average TCC in Pp wheat was 3.60  $\mu\text{g/g}$  DW (3.13  $\mu\text{g/g}$  DW in 2014 and 4.07  $\mu\text{g/g}$  DW in 2015). The 2014 harvest has shown a lower average TCC ranging from 2.29  $\mu\text{g/g}$  DW (Purple Feed) up to 4.70  $\mu\text{g/g}$  DW (Konini) compared with the following season when it ranged from 2.78  $\mu\text{g/g}$  DW (Purple) up to 7.53  $\mu\text{g/g}$  DW (Konini).

Ba wheat contained on average 2.41  $\mu\text{g/g}$  DW of total carotenoids.

Like in Pp wheat, the 2014 harvest has shown lower contents of TCC averaging 1.74  $\mu\text{g/g}$  DW (ranging from 0.94  $\mu\text{g/g}$  DW in Xiao Yan to 2.71  $\mu\text{g/g}$  DW found in Tschermak's B.S.). In the following year's harvest, the average TCC was 3.08  $\mu\text{g/g}$  DW, which ranged from 2.74  $\mu\text{g/g}$  DW (Xiao Yan) to 3.69  $\mu\text{g/g}$  DW (Tschermak's B.S.). These TCC values for Ba wheat do not include the unusual line V1-131-15, which is specific in that it exhibits a very deep aleurone color (caused by anthocyanin pigments) and contains high amounts of free carotenoids. Thus, it seems that the elevated content of anthocyanins and carotenoids would predispose this line to be an excellent source of them. The 2–4 fold higher total carotenoid content (7.41  $\mu\text{g/g}$  DW in 2014 and 7.51  $\mu\text{g/g}$  DW in 2015) is attributed to its genealogy (P1: RU-440  $\times$  V1-702 and P2: Citrus  $\times$  Bona Dea), which is rather untypical in Ba wheat.

High TCC (7.04  $\mu\text{g/g}$  DW; average for both seasons) was detected in the Ye variety TA 4024 as well. This variety of Israeli origin contained a significant portion of carotenoids bound in esters.

The conventional red winter wheat Bohemia (control), typically used by the industry to make bread, had the average TCC of 3.56  $\mu\text{g/g}$  DW (3.48  $\mu\text{g/g}$  DW in 2014 and 3.64  $\mu\text{g/g}$  DW in 2015). Our values are higher compared to those reported by other authors like Hidalgo, Brandolini, Pompei, and Piscozzi (2006), who found 2.12  $\mu\text{g/g}$  DW of total carotenoids in wheat, 1.94  $\mu\text{g/g}$  DW by Abdel-Aal et al. (2007), 1.36  $\mu\text{g/g}$  DW by Lachman et al. (2013) and 0.63  $\mu\text{g/g}$  DW, which was so far the lowest content reported by Mattered et al. (2017). In addition, these authors (except for Hidalgo et al., 2006) report levels that represent free forms of carotenoids, which might eventually underestimate the TCC if the varieties contain esterified forms. Comparing Bohemia (control) with the other pigmented wheat varieties, Pp lines showed comparable or slightly higher TCC levels (3.60  $\mu\text{g/g}$  DW), thus allowing the conclusion that Pp varieties are equal in TCC to traditional wheat varieties. This finding became especially pronounced when comparing Konini (Pp), which achieved comparable TCC values in 2015, with the high-carotenoid variety TA 4024 (Ye) (Fig. 2). On the other hand, Ba varieties reached lower TCC (2.41  $\mu\text{g/g}$  DW; average for both seasons) and thus are inferior to the commonly grown red winter wheat. Pp lines in our experiment showed 1.7–5.7 times higher values than the authors above have found in conventional wheat. Surprisingly, the TCC in Pp lines compares even to that found in *Triticum durum*, which is known for high TCC (3.02  $\mu\text{g/g}$  DW by Hidalgo et al., 2006), leading to the ultimate conclusion that Pp lines would be valuable

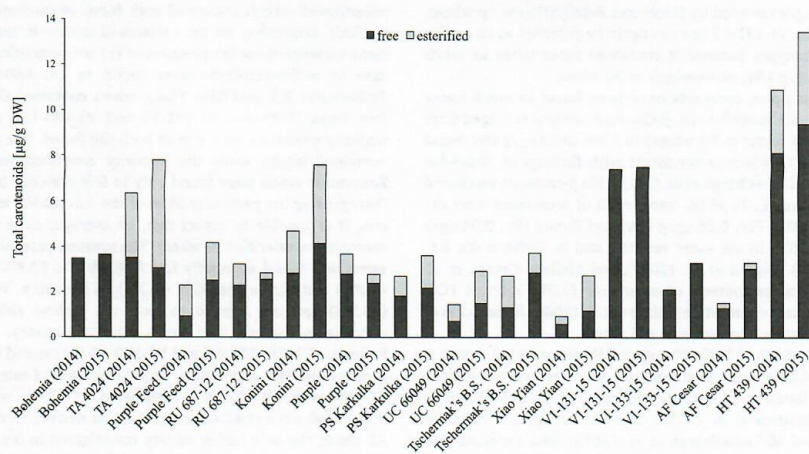


Fig. 2. Total carotenoid content (TCC) in cereal grains showing the proportion of free and esterified forms.

sources of carotenoids and, as Lachman et al. (2017) mentioned, also the anthocyanin pigments.

The barley variety AF Cesar contained 2.40 µg/g DW of total carotenoids (average for both seasons), which made it comparable to Ba wheat (2.41 µg/g DW). Our results correspond well with those reported for barley (2.25 µg/g DW in Ndolo & Beta, 2013) even though being the half of what has been reported by the same authors for purple barley (4.54 µg/g DW).

The highest TCC (12.16 µg/g DW; average for both seasons) of our results was observed in tritordeum HT 439 (similar values reported by Mellado-Ortega & Hornero-Méndez, 2012). In general, tritordeum is known to contain very high levels of carotenoids. This property had been introduced into it from *Hordeum chilense*, which has on average 5.2 times more carotenoids than durum wheat, thus assuming the role of a functional food (Atienza et al., 2007). However, the result we report is nearly twice as high as that of Mellado-Ortega et al. (2015) who found only 6.50 µg/g FW.

### 3.2. Carotenoid profiles

The HPLC-DAD-APCI<sup>+</sup>-MS analysis confirmed the presence of eight carotenoids (Table 3). Antheraxanthin, lutein, zeaxanthin, α-carotene and β-carotene (Table 4) were quantified in cereals in our study. The xanthophylls have been analyzed both in free and esterified forms. Carotenoid profiles of the investigated grains were similar in most species, except for barley. Carotenoid profiles revealed lutein to be the predominant carotenoid in wheat and tritordeum. Lutein comprised 68.1% (of TCC) in red wheat used as the control, 71.5% in Ba, 75.5% in Ye and 79.5% in Pp wheat. Similar proportions were found in durum wheat by Abdel-Aal et al. (2007), Mellado-Ortega et al. (2015) and Ziegler et al. (2015). Our TLC results (comprising both free and esterified forms) averaging 2.91 and 1.76 µg/g DW in Pp and Ba wheat varieties, were much higher when compared to 0.60 µg/g DW found in Pp wheat by Ndolo and Beta (2013). Very high lutein content (5.51 µg/g DW, on average) was found in the already mentioned unusual Ba line V1-131-15 (Table 4).

Zeaxanthin was the second most abundant xanthophyll identified in wheat. Its share in TCC varied from 11.8% to 19.8% in Pp and Ba wheat, which is actually comparable to that found in durum wheat by Mellado-Ortega et al. (2015). Zeaxanthin's share of 28.1% of TCC found in the control was higher than in the other pigmented wheat varieties investigated in our experiment. The zeaxanthin concentration in Pp and Ba wheat averaged 0.59 and 0.53 µg/g DW, respectively, which is similar to the 0.54 µg/g reported by Ndolo and Beta (2013) in Pp wheat. On the contrary, the V1-131-15 line can again be regarded as an outlier within the Ba genotypes because it contained three times as much zeaxanthin (1.61 µg/g DW, on average) as Ba wheat.

In our wheat samples, carotenes have been found in much lower levels compared to the xanthophylls. β-Carotene content averaged from 2.2% (0.09 µg/g DW found in Pp wheat) to 3.3% (0.22 µg/g DW found in Ye wheat) of TCC, which is consistent with findings of Abdel-Aal et al. (2007) and Mellado-Ortega et al. (2015). No β-carotene was found in the control (Bohemia). In 2014, trace levels of α-carotene were detected only in TA 4024 (Ye; 0.06 µg/g DW) and Konini (Pp; 0.04 µg/g DW), whereas in 2015 in the same varieties and in Tschermak's B.S. (Ba; 0.02 µg/g DW). Digesù et al. (2009) and Mellado-Ortega et al. (2015) also found minor contents of α-carotene (0.5%, 1.4% of TCC, respectively) in durum wheat while Hidalgo et al. (2006) found 5.2% of each of α- and β-carotene in conventional wheat.

By comprising 86.0% of TCC (10.4 µg/g DW, on average), lutein is the most dominant carotenoid in tritordeum and expresses the highest lutein proportion found in all investigated varieties. This finding is in accordance with Mattera et al. (2017), Mellado-Ortega and Hornero-Méndez (2012) and Mellado-Ortega et al. (2015) who reported that lutein represents 84%–99% of TCC in tritordeum. Other carotenoids that were detected in tritordeum are zeaxanthin (7.7%; 0.94 µg/g DW)

and antheraxanthin (5.5%; 0.67 µg/g DW), followed by the minor ones – α- and β-carotene (0.5% and 0.3%; 0.07 and 0.04 µg/g DW, respectively).

Interestingly, when Mellado-Ortega and Hornero-Méndez (2012) and Mellado-Ortega et al. (2015) studied tritordeum, they found neither zeaxanthin nor α-carotene. They hypothesized that over-activation of the β, ε-branch of the carotenoid biosynthetic pathway leads to the formation of lutein at the expense of the β, β-branch, which leads to zeaxanthin. In addition, the absence of α-carotene may be explained by its rapid hydroxylation to form lutein and the subsequent esterification. Our results for zeaxanthin and α-carotene, however, do not seem to support this hypothesis.

As mentioned above, barley's carotenoid profile was found to be different from that of wheat and tritordeum. Zeaxanthin was the predominant carotenoid (49.7% of TCC; 1.26 µg/g DW) in barley followed by lutein (41.5%; 0.94 µg/g DW) and antheraxanthin (7.6%; 0.18 µg/g DW). We agree with Ndolo and Beta (2013) that zeaxanthin is the major carotenoid, although their zeaxanthin content in barley was twice as high (0.65 µg/g) as that of lutein (0.30 µg/g).

### 3.3. Carotenoid esters

Xanthophyll esterification is known to prevent carotenoid degradation and enables their long-term storage in plant cells (Atienza et al., 2007). Xanthophylls modified by a relatively easy esterification process via acyl-CoA are more lipophilic, which facilitates their integration into plant membranes and lipid-rich plastoglobules (Howitt & Pogson, 2006). The xanthophyll ester composition in cereals has been investigated by authors such as Mattera et al. (2017), Mellado-Ortega and Hornero-Méndez (2012) and Mellado-Ortega et al. (2015). In our study, we focused on finding out what percentage of the particular xanthophyll moiety constitutes the esters (disregarding the fatty acid part).

Our study proved the presence of lutein, zeaxanthin and antheraxanthin esters based on sample hydrolysis by KOH after which the peaks of esterified xanthophylls disappeared (Fig. 1) and free forms increased in quantity. Even though the free forms of carotenoids might be susceptible to degradation in an alkaline environment we have evidence that the alkaline hydrolysis does not affect them much. For instance, in Bohemia (which contains only free forms), lutein content has changed after 2-h exposure to the alkaline medium by +1.94%, zeaxanthin by -5.06% and antheraxanthin by -5.59%.

From the results given in Fig. 2, it is evident that the majority of investigated cereals contained both forms of carotenoids (free and esterified). Depending on the carotenoid moiety in the ester (Table 4), some varieties show the presence of (a) only esterified forms (as in the case of antheraxanthin esters found in UC 66049, Purple Feed, Tschermak's B.S. and Xiao Yian); others contained (b) exclusively the free forms (Bohemia, V1-131-15 and V1-133-15), and the absolute majority contained (c) a mix of both the forms. The majority of esters contained lutein, while the minority contained zeaxanthin moiety. Zeaxanthin esters were found only in five varieties in 2015 (Table 4). Disregarding the particular form of the carotenoid and the cereal species, it is possible to expect that, on average, close to a third of carotenoids are esterified in wheat. The greatest percentage of carotenoid esters was found especially in TA 4024 (Ye; 52.8%), UC 66049 (Ba; 49.8%) and Purple Feed (Pp; 49.7%). In summary, Ye (52.8%) and Pp (33.5%) varieties seemed to have the highest ability to store carotenoids in the esterified forms. On the contrary, wheat genotypes Bohemia, V1-131-15 (Ba) and V1-133-15 (Ba) stored only free forms of carotenoids. Still, a relatively high proportion of esterified carotenoids (27.9%) were also found in tritordeum HT 439 in which lutein esters dominated, yet a small ester fraction was derived from antheraxanthin. AF Cesar, the only barley variety investigated in our study, contained only 11.4% of esters mostly derived from lutein which was similar to the other studied cereals.

The lack of zeaxanthin esters in most cereals could be explained by its different distribution within the grain kernel. According to Acquistucci, Melini, Carbonaro, and Finotti (2013) lutein is mostly stored in the inner part of the kernel, whereas zeaxanthin is exclusively found in the outer part. Ndolo and Beta (2013) and Masisi et al. (2015) did not find any zeaxanthin in the endosperm but in the aleurone layer, where zeaxanthin constituted the major form. This finding suggests that zeaxanthin (present in the aleurone layer in free form) may play a different role in plant physiology than lutein, which is present in endosperm.

By looking closer at Fig. 1, several new peaks (designated as 2, 5, 6) appeared nearby lutein after the alkaline hydrolysis. Those peaks were first thought to be various Z-isomers of lutein, which was later confirmed by performing lutein isomerization by iodine as described by Kotiková et al. (2016) and by the mass spectra matching that of lutein. Z-isomers were identified according to the following criteria: (a) the presence of the typical Z-peak in the absorption spectrum ( $A_B$ ), the maximum of which was 330–332 nm (Gentili & Caretti, 2011; Zhong et al., 2016); (b) the hypsochromic shift of absorption maxima by 2–6 nm in mono-Z, 10 nm in di-Z and 50 nm in poly-Z isomers (Gentili & Caretti, 2011); (c) by the  $A_B/A_{II}$  ratio, which identified the position of the Z-double bond in the carotenoid molecule, and finally (d) by the  $A_{II}/A_{III}$  ratio (Aman et al., 2005). Based on those criteria the three newly formed peaks were identified as (13'Z)-, (9Z)- and (9'Z)-lutein. Due to the lack of available analytical standards, it was not possible to quantify the Z-isomers. Due to the appearance of the peaks 2, 5 and 6 in the hydrolyzed sample, we might conclude that a certain part of esters was made up of lutein Z-isomers.

Similarly to our results confirming lutein as the major xanthophyll ester, other authors (Ziegler et al., 2015) have shown that lutein mono- and diesters (with linoleic, palmitic, oleic and linolenic acid) are dominant in cereals as for instance lutein dilinoleate, lutein linoleate-palmitate and lutein dipalmitate (Mellado-Ortega & Hornero-Méndez, 2012). The presence of lutein esters and the degree of lutein esterification varies strongly among and within cereal species (Atienza et al., 2007; Ziegler et al., 2015) and is genetically controlled (Ahmad et al., 2015; Ziegler et al., 2015). This statement is supported by our results for Bohemia, V1-131-15 and V1-133-15, in which only free forms of carotenoids were found in both years. These varieties seem to have no genetic predispositions to synthesize xanthophyll esters, although this hypothesis still needs to be confirmed on a genetic basis. Similarly, Ziegler et al. (2015) reported that all of his investigated emmer, durum and only seven of the bread wheat genotypes contained zero lutein esters, whereas eight of his bread wheat genotypes contained from 22.7 to 38.3% of lutein esters. Our results indicate that the degree of xanthophyll esterification is not dependent on the TCC in cereals ( $r = -0.186$  in 2014 and  $r = 0.162$  in 2015). This is vastly different from potato tubers where a positive correlation between TCC and xanthophyll esters was reported (Fernandez-Orozco et al., 2013; Kotiková et al., 2016).

Because esterification protects xanthophylls against oxidation, high temperatures, UV light, degradation during storage and processing (Ahmad et al., 2015; Fu et al., 2010; Subagio, Wakaki, & Morita, 1999), a high portion of xanthophyll esters in cereals makes them more stable during long-term storage, and culinary or industrial processing. A decrease in the lutein monoesters fraction during storage was observed in the tritordeum and whole-grain durum flours, coinciding with an increase in the diesterified forms (Mellado-Ortega & Hornero-Méndez, 2016). The formation of lutein dilinoleate, lutein dipalmitate and lutein palmitate-linoleate explains the slower carotenoid degradation in tritordeum whole-grain flour. Thus, the ability of cereals to form esters and their quantity may play an important role in breeding programs.

### 3.4. Environmental effects

Carotenoid profiles gathered over the course of two years were

researched to find out the impact of the year (environmental factors) and the interaction of year  $\times$  genotype. The 2015 season was marked by extraordinarily low precipitations and higher average temperatures compared to 2014 (Table 2). In 2015, the temperature increased by 2.9 °C in July and by 4.3 °C in August in comparison to the long-term average, whereas precipitation was down by 37–67% between April and July of 2015. As all experimental conditions remained equal in both years, we might conclude that the changes in TCC were likely caused by environmental factors. In our study, ten out of fourteen tested varieties have shown increased TCC in response to higher temperatures (as well as probably higher solar radiation) and lower precipitation (Fig. 2). A major increase in TCC was recorded in Xiao Yan (+194.2%), AF Cesar (+117.3%), UC 66049 (+102.3%), which are all known to be typically low in carotenoids, followed by Purple Feed (+81.9%), Konini (+61.6%), V1-133-15 (+56.1%), and others. Two varieties (Bohemia and V1-131-15) achieved similar levels (+4.8% and +1.2%, resp.) in both seasons and only Purple and RU 687-12 have both recorded a decrease (–24.3% and –12.0%, resp.) in TCC in 2015. Individual carotenoids have shown a similar trend. Except for  $\alpha$ -carotene, in which the changes were not significant, the other analyte levels were higher in 2015. In addition, in 2015 higher levels of esterified xanthophylls were detected and maintained a similar proportion to TCC as in 2014 (27.0% vs. 29.2%).

Our results above are supported by Fratianni et al. (2013), who observed an increased carotenoid synthesis in durum wheat (var. Simeto) grown under water deficit by 20% in whole meal as well as by 15% in semolina flour. Considering single carotenoids, lutein and zeaxanthin increased by about 20%, while the  $\beta$ -carotene level has not been affected.

In contrast to our results, Abdel-Aal et al. (2007), who investigated einkorn wheat in a 6-year field experiment, found that a cooler and wetter climate increased the lutein content. Mattera et al. (2017) observed that high temperature during grain filling causes lower total carotenoid and free lutein content. Regarding the esters, those authors could not unambiguously confirm their decrease but found out that increased temperature enhanced the accumulation of lutein esters with linoleic acid in tritordeum. Considering the works of the authors above and our own experiences we might conclude that total carotenoid content in cereal grains is influenced by a multitude of factors among which the most important ones are actually expressed in the interactions between many intrinsic and extrinsic factors such as genotype/variety, environment, weather, biotic stress or the variety's origin.

Except for  $\alpha$ -carotene, significant interactions between year  $\times$  genotype were found for all individual carotenoids (TAC, TLC, TZC, TBC) as well as TCC (Supplementary Material S1 A–F). This interaction was most pronounced in the case of zeaxanthin (S1 D). Varieties which were the most influenced by the environmental factors were Konini, AF Cesar, Purple Feed and Xiao Yan.

We suppose that varieties originating in temperate climates, which can be found in the Czech Republic (RU 687-12, V1-131-15 and Bohemia) or Canada (Purple), might react differently to increased temperature and decreased precipitation (thus being stressed in harsher weather conditions). All varieties mentioned above did show similar or lower levels of TCC whereas varieties that were bred in subtropical climates (tritordeum HT 439, wheat varieties TA 4024 and Purple Feed) have shown increased TCC. This phenomenon may indicate that the subtropical cereal varieties might be better adapted to warmer and drier climates. We suggest that farmers and breeders bear in mind the origin of cereals and choose those that are best adapted to the local weather conditions to harvest phytonutrient-rich cereals.

### 3.5. Heritability and regression analysis

Broad-sense heritability ( $h^2_b$ ) of all analytes was calculated using ANOVA mean square values. The highest heritability was found in the  $\beta$ , e-branch (0.97 for  $\alpha$ -carotene and 0.83 for lutein). On the other hand,

the lowest heritability was found for  $\beta$ ,  $\beta$ -branch carotenoids like zeaxanthin (0.34) and antheraxanthin (0.19). These results suggest that both branches have different roles in the cereal grain. The carotenoids synthesized by the  $\beta$ ,  $\epsilon$ -branch are stored in the endosperm and their content is influenced mainly by the genotype. The latter branch is active mostly in the outer layer of the caryopsis where it produces carotenoids which are remnants of the grain's photosynthetic activity. Both zeaxanthin and antheraxanthin are pigments of the xanthophyll cycle, which protects the photosynthetic tissue from excessive radiation (Zhai, Xia, & He, 2016). Thus, their content will be rather influenced by the environmental factors.  $\beta$ -Carotene being the precursor of zeaxanthin and antheraxanthin revealed high heritability (0.82), which is a puzzling result. Relatively high heritability, which has likely been caused by the lutein dominance was also found for TCC (0.66) and for xanthophyll esters (0.71). In accordance with our results, Digesù et al. (2009) found high heritability for  $\beta$ ,  $\epsilon$ -branch, i.e. for  $\alpha$ -carotene (0.79) and lutein (0.93) and low one for  $\beta$ ,  $\beta$ -branch represented by zeaxanthin (0.48). With the respect to other analytes, they found a higher value for TCC (0.94) and a lower one for  $\beta$ -carotene (0.57).

The regression analysis ( $p < 0.05$ ) revealed strong correlation between TCC/lutein ( $r = 0.98$ ), TCC/antheraxanthin ( $r = 0.79$ ) and TCC/ $\alpha$ -carotene ( $r = 0.76$ ), medium correlation for TCC/zeaxanthin ( $r = 0.54$ ) and low correlation for TCC/ $\beta$ -carotene ( $r = 0.27$ ).

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#### Conflict of interest

All authors declare that they have no conflict of interest.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.07.151>.

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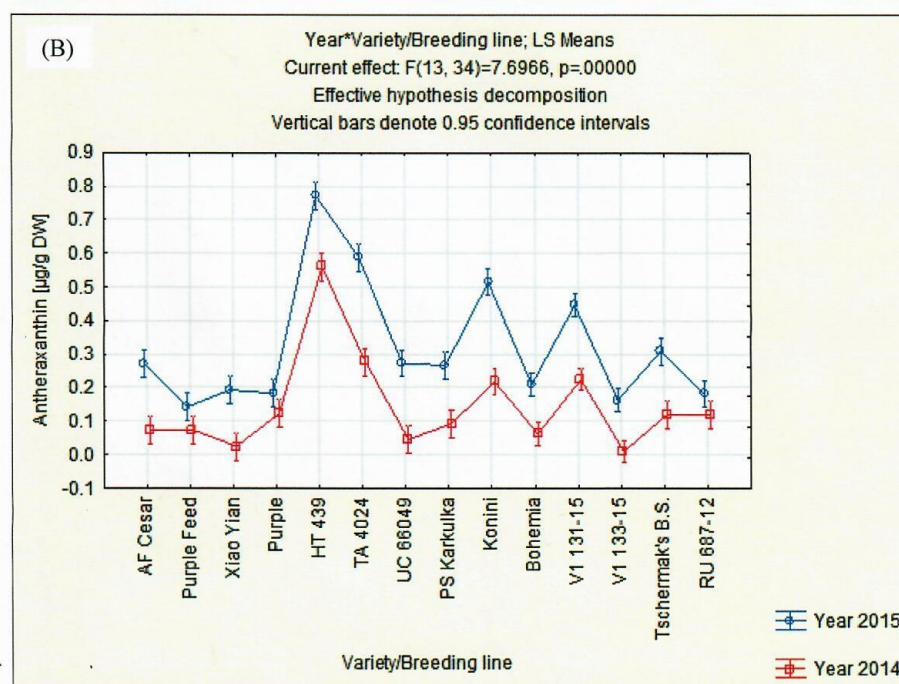
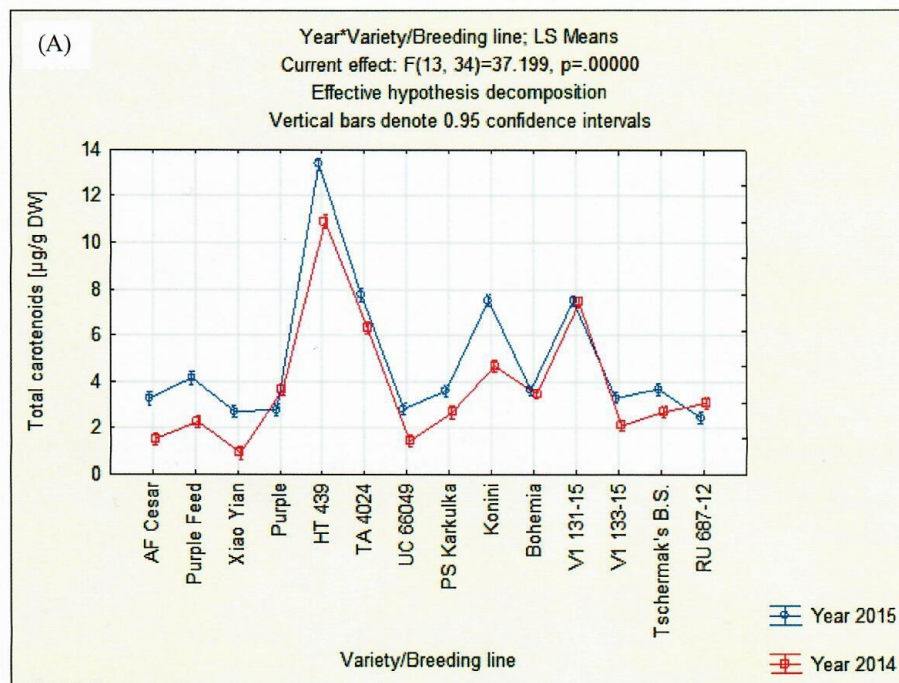
## 4.1 Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains

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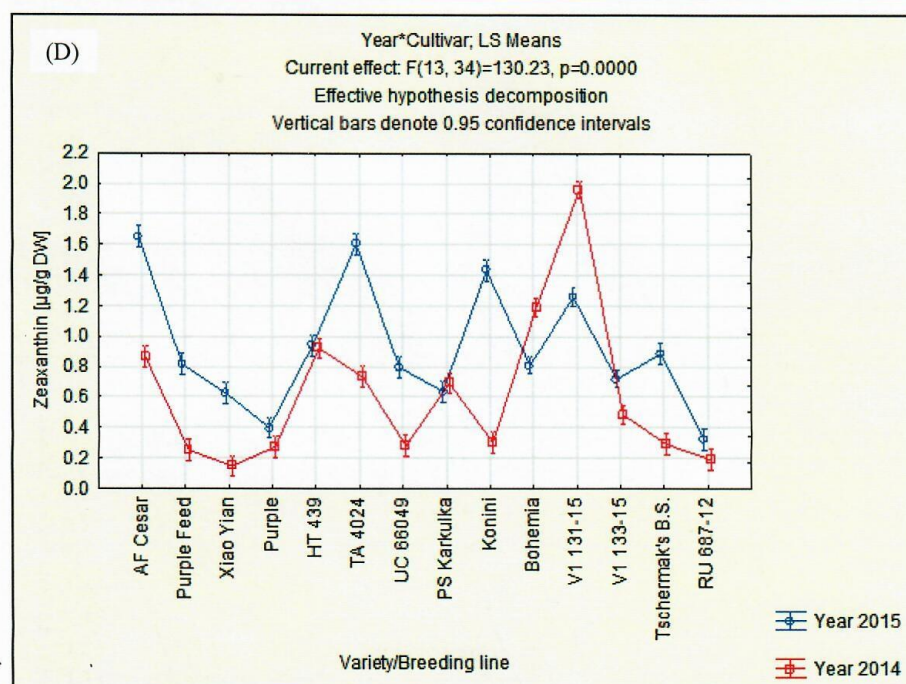
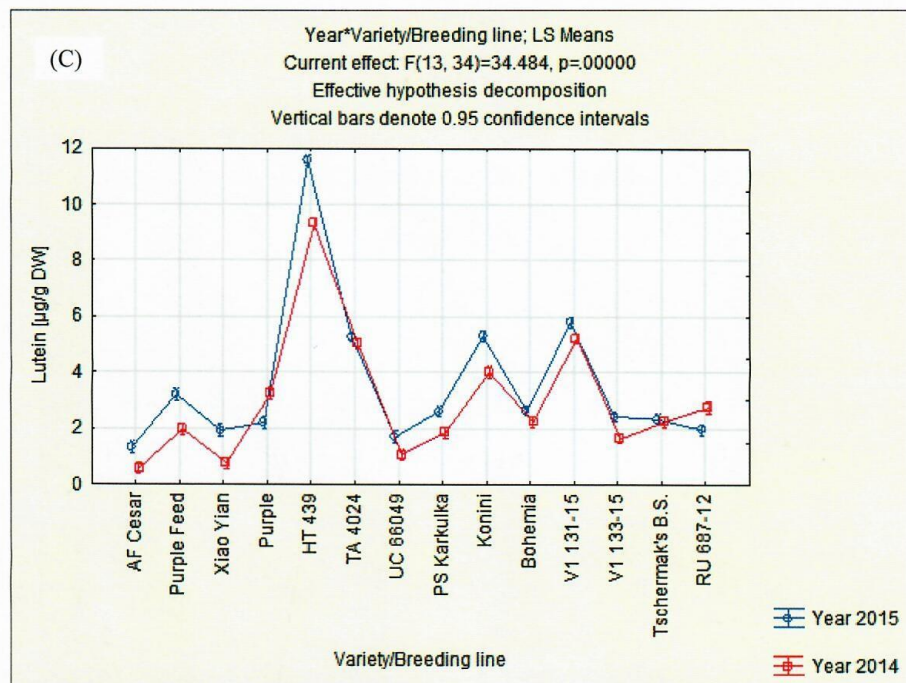
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#### 4.1 Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains

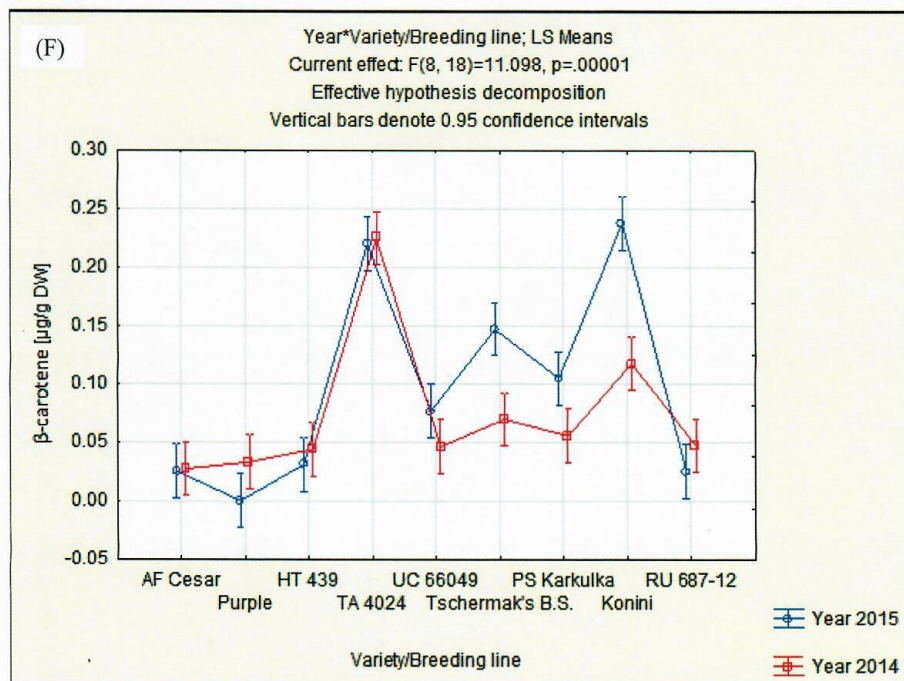
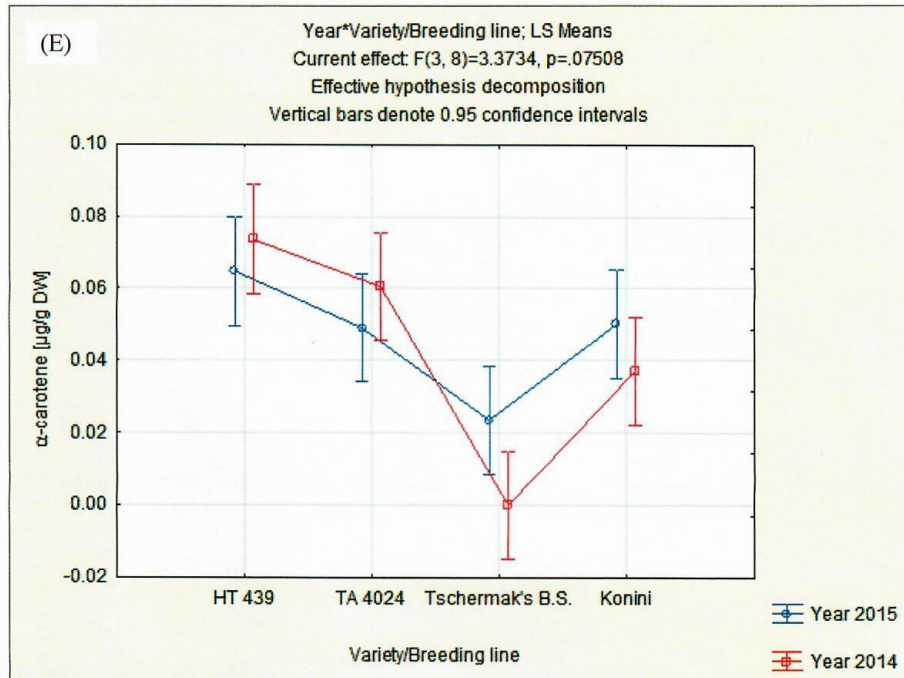




#### 4.1 Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains

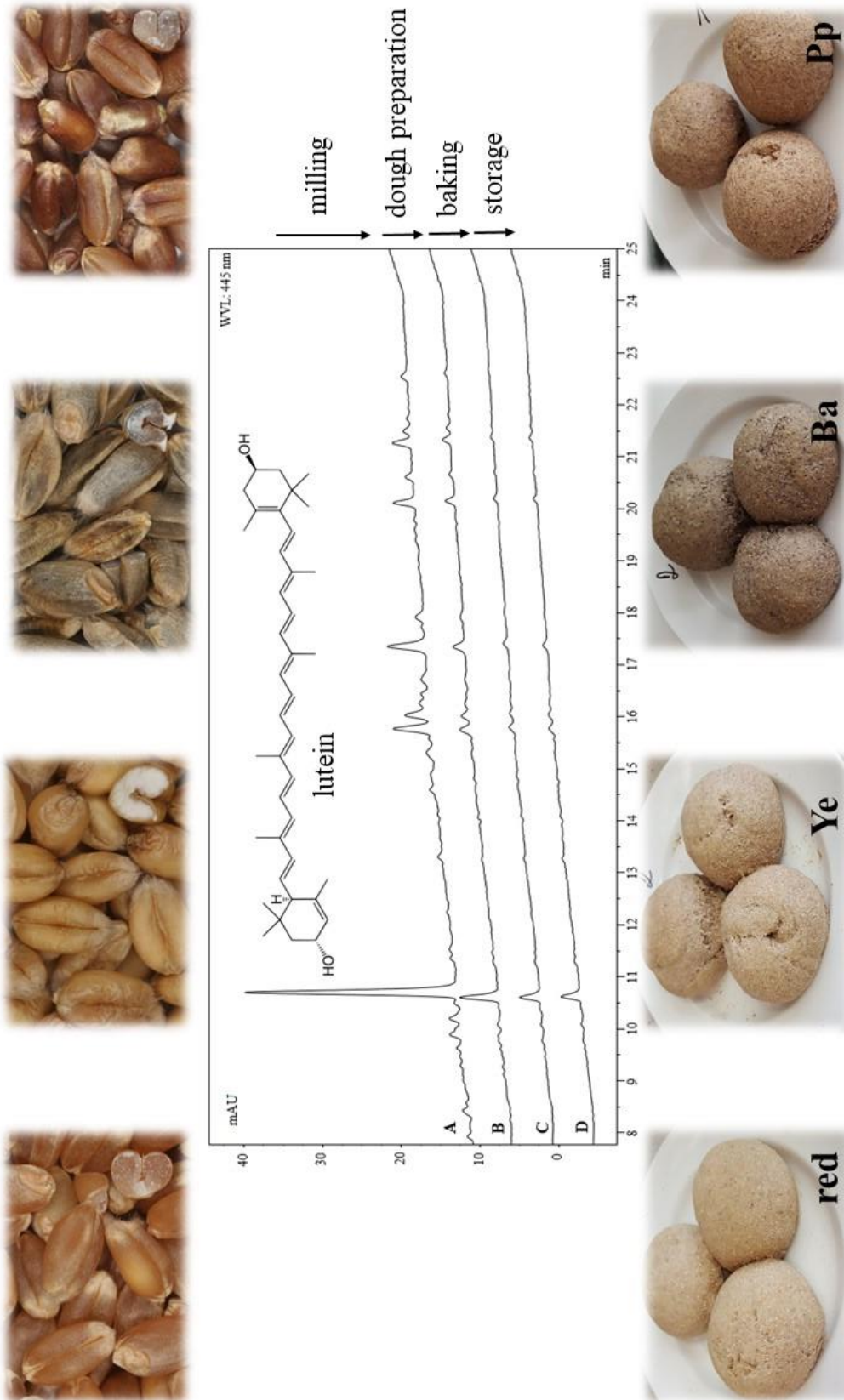


#### 4.1 Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains



## 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

### Grafický abstrakt



## 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

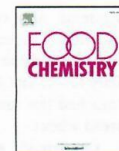
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### Carotenoid changes of colored-grain wheat flours during bun-making

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#### ARTICLE INFO

##### Chemical compounds:

$\alpha$ -Carotene (PubChem CID: 4369188)  
Antheraxanthin (PubChem CID: 5281223)  
 $\beta$ -Carotene (PubChem CID: 5280489)  
Lutein (PubChem CID: 5281243)  
Zeaxanthin (PubChem CID: 5280899)

##### Keywords:

Esterified carotenoids  
Non-esterified carotenoids  
Colored-grain wheat genotypes  
Flour  
Dough  
Baked buns  
Short-term stored buns

#### ABSTRACT

Colored-grain wheat genotypes were used in the preparation of flour, dough, buns, and buns stored for a short period of time. The main carotenoid in all genotypes was lutein, followed by its esters, zeaxanthin, and  $\beta$ -carotene, while antheraxanthin and  $\alpha$ -carotene occurred only at negligible levels. The highest carotenoid contents were observed in yellow- and purple-grained genotypes. After the preparation of dough, total carotenoid content (TCC) decreased significantly by an average of 61.5%. Zeaxanthin was shown to be stable, whereas  $\alpha$ -carotene was destroyed. In baked buns, the average decrease of TCC and all-*E*-lutein was lower than in unbaked dough. Greater decreases were recorded for esters, antheraxanthin, and  $\beta$ -carotene. After storing buns for 24 h at room temperature, approximately one-quarter of TCC observed in the original flour was preserved. *Z*-Isomers of lutein occurred in minor concentrations, but the degradation of this component, and that of zeaxanthin, was low, suggesting *E*- to *Z*-isomerization.

#### 1. Introduction

Cereals play an essential role in the human diet, especially wheat, which is the second most produced cereal on earth. Around 750 million metric tons are produced annually, corresponding to a yearly consumption of 67 kg per capita (FAOSTAT, 2018). In Europe, wheat is the most commonly used cereal for the production of flour and its resulting food products – breads, pastries, and pastas. Due to the increasing worldwide prevalence of lifestyle diseases, the overall demand for nutritionally valuable foodstuffs is increasing. In addition to traditional cereal crops, there is also an opportunity for the cultivation and processing of non-traditional modern and ancient cereals such as einkorn, emmer, spelt, and colored-grain wheat. Such crops are valuable not only as a source of essential nutrients, but also as a source of a wide range of biologically active compounds, including anthocyanins, carotenoids, and phenolic acids. These compounds are notable for their biological functions, particularly their high antioxidant activity, thanks to which they can help prevent oxidative stress and lifestyle diseases (Ficco et al., 2014; Giordano et al., 2017; Luthria, Lu, & John, 2015).

A wide range of carotenoids may be present in wheat grain

including lutein,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin, antheraxanthin, taraxanthin (lutein 5,6-epoxide), triticoxanthin, and flavoxanthin (Barnes, 2012). In general, lutein is the most abundant carotenoid, followed by zeaxanthin, antheraxanthin,  $\alpha$ -carotene, and  $\beta$ -carotene, while  $\beta$ -cryptoxanthin is a minor component or it occurs at non-detectable levels (Adom, Sorrells, & Liu, 2003; Paznocht et al., 2018). Zeaxanthin in wheat is interconverted to the mono-epoxidated xanthophyll antheraxanthin, but the further product of the xanthophyll cycle, violaxanthin, has not been detected in wheat grain (Paznocht et al., 2018). Total carotenoid content (TCC) in wheat falls in the range of 3.0–10.2  $\mu\text{g/g}$  DW depending on wheat species (Hidalgo, Fongaro, & Brandolini, 2017). These authors reported the highest carotenoid content in einkorn wheat (*Triticum monococcum* ssp. *monococcum*), followed in descending order by Polish wheat (*Triticum turgidum* ssp. *polonicum*), Khorasan wheat (*Triticum turgidum* ssp. *turanicum*), durum wheat (*Triticum turgidum* ssp. *durum*), spelt (*Triticum aestivum* ssp. *spelta*), bread wheat (*Triticum aestivum* ssp. *aestivum*), and emmer wheat (*Triticum turgidum* ssp. *dicoccum*).

Colored-grain wheat genotypes, which are currently an object of interest to breeders and consumers, can in addition to anthocyanin

**Abbreviations:** Ba, blue aleurone; BHT, butyl hydroxytoluene; DW, dry weight; Pp, purple pericarp; Red, red grain; TCC, total carotenoid content; TLC, total lutein content; Ye, yellow endosperm

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pigments, also contain meaningful (not negligible) amounts of carotenoids. A recent study (Paznocht et al., 2018) deals with the content and composition of carotenoids in unusual colored wheat varieties. They found that blue wheat genotypes had lower TCC, whereas purple ones had the same or higher levels of carotenoids than conventional bread wheat.

Carotenoids and anthocyanins are natural pigments that degrade due to both external factors (e.g., high temperature, extreme pH values, oxygen presence, UV light) and internal factors (e.g., enzymatic activity) (Leenhardt et al., 2006). The characteristic highly unsaturated structure of carotenoids is the source of their beneficial effects but also of their particular instability, especially toward light and oxygen. Carotenoid degradation is a complex process involving many factors linked to the food matrix and the type of carotenoid (Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010). Carotenoids occur in foods predominantly as *E*-isomers, though *Z*-isomers are formed during thermal processing and show different biological properties, bioavailability, and antioxidant activity. In cereals, carotenoids are present either in free or esterified forms (mostly with palmitic and linoleic acid) depending on the cereal genotype (Ziegler et al., 2015). It has been shown that esterified forms of xanthophylls are more stable in the presence of heat than their free forms (Subagio, Wakaki, & Morita, 1999). The potential health benefits of these susceptible substances is therefore influenced by grain processing (from milling to final technological processing), which can significantly reduce the amount of the nutritionally valuable compounds in foodstuffs (Hidalgo, Brandolini, & Pompei, 2010).

The effect of processing (boiling, baking, etc.) on the content of carotenoids and anthocyanins has been discussed in many studies. Some of these describe the degradation of carotenoids or anthocyanins in cereal grains and consequent cereal, baked, or puffed products (Abdel-Aal, & Hucl, 2014; Hidalgo et al., 2010; Hidalgo, Scuppa, & Brandolini, 2016).

Currently, newly bred genotypes of colored-grain cereals seem to be potentially valuable sources of these phytochemicals in the human diet (Paznocht et al., 2018).

Therefore, the aim of this study was to compare carotenoid content and profile in differently colored wheats and their products. We focused on colored wheat because it is a novel and apparently healthier alternative to conventional wheat. Keeping in mind the important health benefits of carotenoids and high per capita wheat consumption, the objectives of this study were: i) to establish the carotenoid profile and content in flour from each genotype; ii) to investigate the changes in carotenoids in dough prepared from colored-grain wheat flours; iii) to establish the changes of carotenoid content in buns after baking and after a short 24-h storage.

### 2. Material and methods

#### 2.1. Plant material

A total of nine colored-grain wheat varieties and breeding materials were grown in 2016/2017 at the Agricultural Research Institute in Kroměříž, Czech Republic (49.2851172N, 17.3646269E). The experimental field is located 235 m above sea level, has Luvic Chernozem (Loamic) soil, an average annual temperature 9.2 °C, mild winters, and annual precipitation averaging 576 mm. The plants were grown on small experimental plots (10 m<sup>2</sup>) using conventional growing technology. Samples were stored after the harvest in paper bags in a box in the dark at room temperature of 21 °C for 2 months before being analyzed. The main characteristics of the selected wheat genotypes are listed in Table 1.

#### 2.2. Preparation of wheat flour, dough, baking of buns and their storage after baking

##### 2.2.1. Preparation of wholemeal wheat flour

The grains were cleaned on a Labofix (C.W. Brabender® Instruments, Inc.; South Hackensack, NJ) over a 2-mm sieve, and impurities were removed manually. The grain was milled on a YM1 Wet Wheat Grinding Machine Y-10 (Yucebas Machine Analytical Equipment Industry, Izmir, Turkey), and three fractions were obtained in total: coarse bran (> 35 mesh), fine bran (50–35 mesh), and flour (< 50 mesh). All the fractions were mixed together resulting in total recovery of the grain in so-called wholemeal flour.

##### 2.2.2. Preparation of dough, baking, and short-term storage of baked buns for 24 h

For the preparation of the doughs, 300 g wholemeal flour, 4.8 g table salt, 6 g compressed commercial baker's yeast, and 180 mL warm (30 °C) water were used. The dough-making process was as follows: 30 min kneading, 1 h rising, and short kneading (2 min) in the middle of the rising period (Alaska BM 2000; SIG GmbH, Düsseldorf, Germany). After rising, the dough was divided into 3 buns. Two of them were baked at 240 °C for 14 min in a forced air oven Venticell 111 (BMT Medical Technology, Ltd., Brno, Czech Republic); the oven also contained a water bath containing 70 mL of distilled water. After baking, one bun was frozen immediately after cooling and the other after 24-h storage at room temperature (21 °C) under a cloth. The unbaked third bun was frozen immediately after separation from the other parts of dough. All samples were then freeze-dried (Lyovac GT2; Steris, Hürth, Germany) in the dark for 120 h and then subjected to further analyses. The bun experiments were performed in three replicates per genotype.

#### 2.3. Chromatographic analysis of carotenoids

##### 2.3.1. Chemicals

Lutein and zeaxanthin standards (UV, ≥95%, ≥98%) were obtained from Extrasynthèse, Genay, France. β-Carotene standard (HPLC, ≥95%), ethanol absolute (puriss., ≥99.8%), butylated hydroxytoluene (BHT, ≥99% FG), and *tert*-butyl methyl ether (HPLC grade) were purchased from Sigma-Aldrich, St. Louis, MO. Antheraxanthin and α-carotene standards (HPLC, 95%, 97%) were purchased from CaroteNature, GmbH, Lupsingen, Switzerland. Methanol (HPLC grade), acetone (GR grade), ethanol (GR grade), and hexane (GR grade) were purchased from Lachner Ltd., Neratovice, Czech Republic. Ultra-pure HPLC water was prepared using Simplicity UV (Merck Millipore, KGaA, Darmstadt, Germany).

##### 2.3.2. Sample extraction

Carotenoids were extracted according to the method modified for the grain matrix (Kotlková et al., 2016). Finely ground sample (2 g) was placed into a 50-mL plastic Falcon tube with 12 mL ethanol/acetone/hexane mixture (1:1:2, v/v/v), vortexed, and left to stand for 24 h in a refrigerator (4 °C). The sample was then vortexed for 1 min (Basic 3; IKA Werke GmbH & Co. KG, Staufen, Germany), sonicated for 10 min in an ultrasonic bath (PS 04; Powersonic-Notus, Ltd., Vráble, Slovakia), and centrifuged at 8228 rcf for 10 min (5810R; Eppendorf, Hamburg, Germany). Then 9 mL of the supernatant were transferred into a 50-mL glass evaporation flask and the sediment re-extracted with 12 mL of the extraction mixture. Both supernatants were combined and evaporated under vacuum at 40 °C (Rotavapor R-200; Büchi Labortechnik, AG, Flawil, Switzerland). The dry residue was reconstituted with 2 mL ethanol/acetone (3:2, v/v) containing 0.2% BHT and filtered through a syringe filter (PVDF, 0.45 μm) into an amber HPLC vial.

##### 2.3.3. Chromatographic separation by HPLC-DAD

Analyses were carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA) with a quaternary pump,

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**Table 1**  
Description of bread wheat (*Triticum aestivum* L.) varieties and genotypes.

Variety/genotype	Growth type	Country of origin (1)	Variety status	Grain color
Bohemia	Winter	CZE	Released variety	Red (standard)
Annie	Winter	CZE	Released variety	Red (standard)
Citrus	Winter	DEU	Released variety	Yellow endosperm
Bona Vita	Winter	SVK	Released variety	Yellow endosperm
AF Jumiko	Winter	CZE	Released variety	Purple pericarp
V1 131-15 (2)	Winter	CZE	Breeding line	Blue aleurone
UC 66049	Spring	USA	Genetic resource	Blue aleurone
Konini	Spring	NZL	Research germplasm	Purple pericarp
ANK-28A	Spring	RUS	Research germplasm	Purple pericarp

(1) CZE – Czech Republic, DEU – Germany, SVK – Slovak Republic, NZL – New Zealand, RUS – Russian Federation, USA – United States of America.

(2) V1 131-15: (Skorpion × PS Karkulka) × (Citrus × Bona Dea).

autosampler, column heater, and diode array detector. The analytes were separated by gradient elution on an YMC C30 Carotenoid Column (150 mm × 3.0 mm, S-3 µm; YMC Co., Kyoto, Japan). The operating conditions were as follows: flow rate 0.6 mL/min; column temperature 25 °C; autosampler temperature 10 °C; injection volume 10 µL; detection at  $\lambda = 445$  nm (spectral acquisition 300–700 nm). The tertiary mobile phase consisted of methanol (A), water (B), and *tert*-butyl methyl ether (C). Gradient: initial conditions of 90% A, 10% B, and 0% C were sustained for 1 min, then increased to 90% A, 0% B, and 10% C at 6 min, reaching 40% A, 0% B, and 60% C at 22 min following column flush and re-equilibration for 11 min.

### 2.3.4. Identification and quantification

The carotenoids were identified by comparing retention times and absorption spectra with those of analytical standards. The quantification of free forms was based on peak area and external calibration (concentration range 0.01–10 µg/mL per analyte). Analyses were performed in triplicate. The exact concentration of carotenoid stock solutions was determined spectrophotometrically (Spectronic Helios  $\gamma$ ; Thermo Fisher Scientific, Waltham, MA) using the following extinction coefficients (EC, L/g/cm): antheraxanthin (235,  $\lambda_{\text{max}} = 446$  nm), lutein (255,  $\lambda_{\text{max}} = 445$  nm), both in ethanol; zeaxanthin (234,  $\lambda_{\text{max}} = 452$  nm) in acetone;  $\beta$ -carotene (259.2,  $\lambda_{\text{max}} = 453$  nm) and  $\alpha$ -carotene (271,  $\lambda_{\text{max}} = 445$  nm), both in hexane. The presence of lutein esters was confirmed by alkaline hydrolysis of the sample extract (Paznocht et al., 2018). Since esterification with fatty acids does not affect chromophore properties (Mellado-Ortega & Hornero-Méndez, 2017), lutein esters were quantified using the all-*E*-lutein calibration curve. Since most of the esters in wheat are lutein esters with only a minor proportion of antheraxanthin and zeaxanthin esters (Paznocht et al., 2018), we have expressed them in this article summarily as lutein esters. The *Z*-isomers of lutein were confirmed by photoisomerization of lutein by iodine. Particular *Z*-forms were identified by exploring their absorption spectra (Paznocht et al., 2018). Their quantification was based on the calibration curve of all-*E*-lutein. Limits of detection (LOD) for antheraxanthin, lutein, zeaxanthin,  $\alpha$ -carotene, and  $\beta$ -carotene (0.004, 0.006, 0.012, 0.010, and 0.015 µg/g DW, respectively) were calculated using the formula  $3.3 \times (\sigma/S)$  (Q2B CH, 1996). An illustrative chromatogram of the Bona Vita variety (yellow endosperm) showing differences between flour, dough, bun and bun stored for 24 h is shown in Fig. 1.

### 2.3.5. Dry weight (DW)

Dry weight (calculated as loss of water) was determined by drying 10 g of wholemeal flour at 105 °C for 24 h in the forced-air oven Venticell 111.

### 2.4. Statistical analysis

The data were processed by Chromeleon (Thermo Fisher Scientific,

Inc., Waltham, MA) and Excel (Microsoft, Redmond, WA). Statistical evaluation was performed using STATISTICA software (ver. 12; StatSoft, Inc., Tulsa, OK). The effects of variety and technological processes on the carotenoid content were evaluated by one-way and two-way ANOVA ( $p \leq 0.05$ ). Tukey's Post Hoc HSD test was used for detailed statistical evaluation.

## 3. Results and discussion

The profiles of individual carotenoids in flour, dough, baked buns, and buns stored for 24 h after baking are reported in Table 2.

### 3.1. Carotenoid content and profile in wheat flour

The average total carotenoid content (TCC) in wholemeal flour in all analyzed genotypes was 1.05 µg/g DW and ranged from  $0.378 \pm 0.004$  µg/g DW (var. Bohemia) to  $2.24 \pm 0.044$  µg/g DW (var. Citrus). The highest TCC was observed in winter yellow-grained Citrus and Bona Vita flours. Elevated contents were also found in flour from purple spring wheat Konini and purple winter wheat AF Jumiko. TCC in flour from varieties and breeding materials with blue aleurone (V1 131-15 and UC 66049) and red-grained control varieties (Annie and Bohemia) was relatively low.

Wholemeal flour is composed of 10–14% bran, 2.5–3.0% germ and 80–85% endosperm. The bioactive compounds are distributed within these parts and are concentrated in the outer layer of the grain. Thus, wholemeal flour contains substantially more phytonutrients, including carotenoids, than refined wheat flour (Mellado-Ortega & Hornero-Méndez, 2016). Our TCC values for wholemeal flour originating from conventional winter Annie and Bohemia varieties were on average 23% higher than those measured by Lv et al. (2012) in soft red winter wheat flours and almost twice as high as the values for refined flours reported by Konopka, Czaplinski, and Rotkiewicz (2006). Similarly, Ficco et al. (2016), who used different types of conventional and purple durum wheat flours to produce fresh pasta, found the highest TCC in wholemeal flour (7.38 µg/g DW), followed by semi-wholemeal (6.30 µg/g DW), and the lowest in semolina (5.25 µg/g DW). They reported that TCC in purple durum wheat (7.38 µg/g DW) was slightly lower in comparison with yellow durum wheat wholemeal flour (7.68 µg/g DW). However, some of our pigmented varieties, especially purple ones, reached comparable or even higher values of TCC than conventional red varieties. Ndolo and Beta (2013) also found in their study higher TCC in wholegrain fraction of purple wheat (2.62 µg/g) than the average of three non-colored wheat varieties (2.56 µg/g). The authors found a more obvious difference between purple and non-pigmented barleys: TCC of purple barley (4.54 µg/g) was twice as high as its non-pigmented form (2.25 µg/g). These results, as well as the results of our previous study (Paznocht et al., 2018), revealed that some purple pericarp (Pp) genotypes would be valuable sources of carotenoids as well as anthocyanins, which are also important bioactive compounds in

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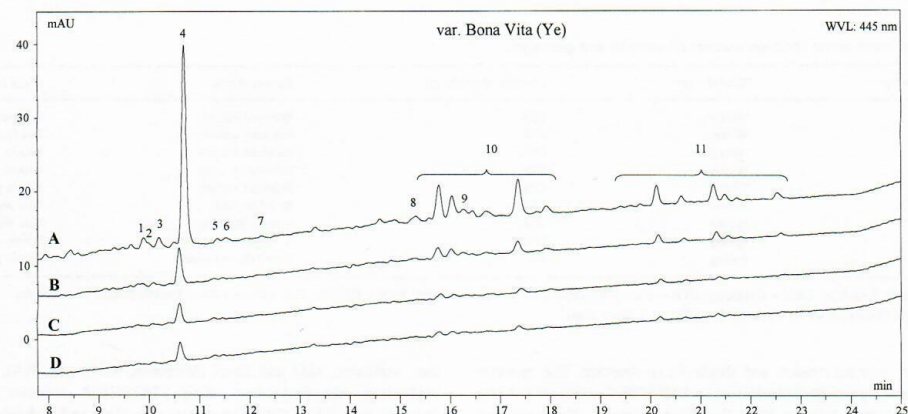


Fig. 1. Chromatogram of Bona Vita wheat variety (Ye) showing differences between flour (A), dough (B), bun (C), and bun after 24 h of storage (D). 1 – (13-Z)-Lutein (9.89 min), 2 – Antheraxanthin (10.00 min), 3 – (13-Z)-Lutein (10.20 min), 4 – All-E-Lutein (10.70 min), 5 – Zeaxanthin (11.35 min), 6 – (9-Z)-Lutein (11.53 min), 7 – (9'-Z)-Lutein (12.24 min), 8 –  $\alpha$ -Carotene (15.30 min), 9 –  $\beta$ -Carotene (16.25 min), 10 – Lutein monoesters, 11 – Lutein diesters.

purple and blue bread wheat varieties (Ficco et al., 2014). The increased content of carotenoids in purple wheat grain could be explained by the genetic origin of the plants. Purple grain color is conferred by the Pp genes, which were transferred to common wheat from tetraploid wheat (*Triticum turgidum* L. subsp. abyssinicum Vavilov, genome AABB) originating from the East Africa region (Lachman, Martinek, Kotíková, Orsák, & Šulc, 2017). According to older data, the purple pericarp genes are located on chromosomes 7B (*Pp1*), 6A (*Pp2*), and 2A (*Pp3a* and *Pp3b*) (Dobrovolskaya, Arbuzova, Lohwasser, Röder, & Börner, 2006). More recent studies indicate that the purple pericarp is determined by complementary genes *Pp3* (on 2A in bread and durum wheat), *Pp-B1* (on 7B in bread and durum wheat), or *Pp-D* (on chromosome 7D in bread wheat) (Tereshchenko, Gordeeva, Arbuzova, Börner, & Khlestkina, 2012; Tereshchenko, Pshenichnikova, Salina, & Khlestkina, 2012). Phytoene synthase enzymes are responsible for the synthesis of yellow compounds, which are conditioned by genes located on the long arms of the homoeologous group 7 chromosomes (7AL, 7BL, and 7DL) (Komugi - Wheat Genetic Resources Database, 2017). In the AF Jumiko variety, the donor of purple pericarp was ANK-28A, in which the anthocyanins were conditioned by the genes *Pp1* (7B) and *Pp2* (6A) (Watanabe, Koval, & Koval, 2003). It is possible to theorize that there could be a genetic link between the *Pp1* gene and some of the genes of the *Psy1-B1* locus (at 7BL) for phytoene synthase enzymes. Because most of the studied donors of wheat with purple pericarp carry the *Pp1* gene and differ from each other in other Pp genes, it can be assumed a relation between increased anthocyanins and an increased content of yellow carotenoids. This hypothesis needs to be confirmed by other experiments.

The composition of individual carotenoids in wholemeal flours of the analyzed genotypes was very similar. The highest content was typical for total lutein (the sum of all-E-lutein and other detected Z-isomers) (on average 0.695  $\mu\text{g/g}$  DW), followed by its esters, zeaxanthin,  $\beta$ -carotene,  $\alpha$ -carotene, and antheraxanthin (0.244, 0.061, 0.033, 0.010, and 0.010  $\mu\text{g/g}$  DW, respectively). The lutein fraction has been separated into all-E-lutein (0.615  $\mu\text{g/g}$  DW), followed by 13-Z-lutein, 13-Z-lutein, 9-Z-lutein, and 9'-Z-lutein (0.031, 0.030, 0.014, and 0.005  $\mu\text{g/g}$  DW, respectively) in wholemeal flour on average in all analyzed genotypes. While some carotenoids, such as all-E-lutein, its Z-isomers, and zeaxanthin, were detected in all samples, others were detected only in some specific accessions, as in the case of antheraxanthin,  $\beta$ -carotene, and  $\alpha$ -carotene (Table 2). Total lutein content (TLC) correlated positively with TCC. The highest TLC was found in yellow-

grained Citrus and Bona Vita varieties (1.85 and 1.03  $\mu\text{g/g}$  DW, respectively), followed by purple-grained varieties. Higher TLC was also determined in the V1 131-15 genotype (Ba) where the yellow ancestors Citrus and Bona Dea can contribute to higher TLC content (Skorpion  $\times$  PS Karkulka)  $\times$  (Citrus  $\times$  Bona Dea). Lower TLC was observed in flours from blue-grained UC 66049 and standard red-grained varieties.

The second most represented zeaxanthin ranged from 0.034 to 0.110  $\mu\text{g/g}$  DW with the highest content in Konini (Pp) and V1 131-15 (Ba) flours and the lowest in Bona Vita (Ye) flour. Antheraxanthin,  $\alpha$ -carotene and  $\beta$ -carotene represented only minor components of the carotenoid profile not exceeding 1.9% (var. Bohemia), 2.1% (var. Bona Vita) and 6.6% (var. Bona Vita) of the TCC.

Giordano et al. (2017) compared the main carotenoids – lutein and zeaxanthin – contained in wholemeal flour of red-, white-, yellow-, purple-, and blue-grained wheat varieties. Also in their study, lutein was the major carotenoid followed by zeaxanthin. Similar carotenoid profiles in wheat species have been identified by a number of other authors (Abdel-Aal, Young, Rabalski, Hucl, & Fregeau-Reid, 2007; Adom et al., 2003; Digesú, Platani, Cattivelli, Mangini, & Blanco, 2009; Hidalgo et al., 2010; Hidalgo & Brandolini, 2014; Konopka et al., 2006). Most of these authors also identified minor amounts of  $\beta$ -cryptoxanthin, which was not identified in our samples. On the other hand, none of the cited authors found antheraxanthin across different wheat species. The absolute lutein predominance in wheat grains observed in our samples, as well as in samples of all the above-mentioned authors, is probably due to the increased activity of enzymes involved in the  $\beta$ , $\epsilon$ -branch carotenoid biosynthesis. The carotenoid biosynthesis pathway is split from all-*trans*-lycopene into two competing tracks. One track ( $\beta$ , $\epsilon$ -branch) leads to  $\alpha$ -carotene and finally to lutein, while the other ( $\beta$ , $\beta$ -branch) leads to  $\beta$ -carotene and then to the oxygenated products  $\beta$ -cryptoxanthin, zeaxanthin, violaxanthin, and neoxanthin (Sun et al., 2018). The higher activity of two specific  $\beta$ , $\epsilon$ -branch enzymes, lycopene *e*-cyclase (LCY-*e*) and heme-containing cytochrome P450 hydroxylase (CYP97C), catalyzing the cyclization of all-*trans*-lycopene and the final hydroxylation of zeinoxanthin to lutein, can be responsible for higher lutein content in wheat grains.

It follows from the results of our study that most of the investigated wheat genotypes (with the exception of Bohemia, which contained only free xanthophylls) produced esterified forms of xanthophylls, which in some cases represent a significant portion of total carotenoids stored in wheat flour (Table 2). A high portion of esterified xanthophylls were

## 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

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**Table 2**  
Content of carotenoids  $\pm$  STD ( $\mu\text{g/g DW}$ ) in wheat flour, dough, bun after baking, and bun after 24 h of storage and their decrease (%) as compared to the content of wholemeal flour.

Variety/genotype	Technological step	Antheraxanthin	all-E-lutein	13-Z-lutein	13-Z-lutein	9-Z-lutein
Bohemia (Red)	Flour	0.007 $\pm$ 0.002a	0.260 $\pm$ 0.002a	0.014 $\pm$ 0.000a	0.013 $\pm$ 0.000a	0.013 $\pm$ 0.001a
	Dough	n.d.	0.081 $\pm$ 0.005b	n.d.	0.008 $\pm$ 0.001b	0.007 $\pm$ 0.003b
	Bun after 24 h	n.d.	0.060 $\pm$ 0.003c	n.d.	0.006 $\pm$ 0.001b	0.006 $\pm$ 0.002b
Annis (Red)	Flour	0.005 $\pm$ 0.001a	0.320 $\pm$ 0.010a	0.015 $\pm$ 0.002a	0.016 $\pm$ 0.000a	0.010 $\pm$ 0.001a
	Dough	0.004 $\pm$ 0.001a	0.232 $\pm$ 0.009b	0.014 $\pm$ 0.003a	0.018 $\pm$ 0.004a	0.009 $\pm$ 0.002a
	Bun after 24 h	n.d.	0.133 $\pm$ 0.006c	0.011 $\pm$ 0.003a	0.010 $\pm$ 0.001b	0.007 $\pm$ 0.001a
Citrus (Ye)	Flour	0.038 $\pm$ 0.001a	1.637 $\pm$ 0.032a	0.094 $\pm$ 0.005a	0.074 $\pm$ 0.002a	0.037 $\pm$ 0.005a
	Dough	0.015 $\pm$ 0.001b	0.343 $\pm$ 0.013b	0.022 $\pm$ 0.001b	0.020 $\pm$ 0.004b	0.017 $\pm$ 0.002b
	Bun after 24 h	0.009 $\pm$ 0.000c	0.255 $\pm$ 0.008c	0.023 $\pm$ 0.001b	0.019 $\pm$ 0.003b	0.018 $\pm$ 0.003b
Bona Vita (Ye)	Flour	0.008 $\pm$ 0.001c	0.238 $\pm$ 0.009c	0.019 $\pm$ 0.002b	0.016 $\pm$ 0.001b	0.014 $\pm$ 0.000b
	Dough	0.018 $\pm$ 0.003a	0.915 $\pm$ 0.016a	0.046 $\pm$ 0.002a	0.044 $\pm$ 0.003a	0.019 $\pm$ 0.001a
	Bun after 24 h	0.007 $\pm$ 0.001b	0.157 $\pm$ 0.010b	0.032 $\pm$ 0.000b	0.016 $\pm$ 0.004b	0.007 $\pm$ 0.000b
V1 131-15 (Ba)	Flour	n.d.	0.093 $\pm$ 0.001c	0.011 $\pm$ 0.002b	0.007 $\pm$ 0.001c	0.007 $\pm$ 0.001b
	Dough	n.d.	0.084 $\pm$ 0.003c	0.009 $\pm$ 0.003b	0.008 $\pm$ 0.001c	0.007 $\pm$ 0.002b
	Bun after 24 h	n.d.	0.491 $\pm$ 0.021a	0.015 $\pm$ 0.001a	0.034 $\pm$ 0.001b	0.014 $\pm$ 0.002b
UC 66049 (Ba)	Flour	0.006 $\pm$ 0.000a	0.361 $\pm$ 0.009b	0.012 $\pm$ 0.002ab	0.044 $\pm$ 0.003a	0.028 $\pm$ 0.006a
	Dough	n.d.	0.239 $\pm$ 0.007c	0.011 $\pm$ 0.001bc	0.022 $\pm$ 0.001c	0.015 $\pm$ 0.004b
	Bun after 24 h	n.d.	0.203 $\pm$ 0.002d	0.008 $\pm$ 0.001c	0.022 $\pm$ 0.002c	0.012 $\pm$ 0.004b
AF Jumiko (Pp)	Flour	0.005 $\pm$ 0.000a	0.196 $\pm$ 0.006a	0.007 $\pm$ 0.000a	0.014 $\pm$ 0.001a	n.d.
	Dough	n.d.	0.056 $\pm$ 0.002b	n.d.	0.010 $\pm$ 0.002b	0.006 $\pm$ 0.003a
	Bun after 24 h	n.d.	0.047 $\pm$ 0.003bc	n.d.	n.d.	n.d.
Konini (Pp)	Flour	0.007 $\pm$ 0.001a	0.606 $\pm$ 0.030a	0.030 $\pm$ 0.002a	0.030 $\pm$ 0.001a	0.012 $\pm$ 0.000a
	Dough	0.006 $\pm$ 0.001a	0.315 $\pm$ 0.021b	0.013 $\pm$ 0.002b	0.026 $\pm$ 0.001b	0.013 $\pm$ 0.002a
	Bun after 24 h	0.004 $\pm$ 0.000b	0.216 $\pm$ 0.007c	0.016 $\pm$ 0.001b	0.012 $\pm$ 0.002c	0.011 $\pm$ 0.002a
ANK28A (Pp)	Flour	0.010 $\pm$ 0.002a	0.216 $\pm$ 0.006c	0.013 $\pm$ 0.000b	0.014 $\pm$ 0.002c	0.013 $\pm$ 0.001a
	Dough	0.004 $\pm$ 0.000b	0.781 $\pm$ 0.020a	0.035 $\pm$ 0.001a	0.033 $\pm$ 0.001a	0.015 $\pm$ 0.002a
	Bun after 24 h	n.d.	0.144 $\pm$ 0.001b	0.007 $\pm$ 0.002b	0.011 $\pm$ 0.002b	0.007 $\pm$ 0.001b
Average of all analyzed varieties	Flour	0.005 $\pm$ 0.001a	0.332 $\pm$ 0.006a	0.017 $\pm$ 0.001a	0.020 $\pm$ 0.001a	0.008 $\pm$ 0.002a
	Dough	0.004 $\pm$ 0.000a	0.178 $\pm$ 0.010b	n.d.	0.023 $\pm$ 0.002a	0.010 $\pm$ 0.002a
	Bun after 24 h	n.d.	0.119 $\pm$ 0.002c	n.d.	0.009 $\pm$ 0.002b	0.007 $\pm$ 0.001a
Average of all analyzed varieties	Flour	0.010 $\pm$ 0.012a	0.615 $\pm$ 0.045a	0.030 $\pm$ 0.027a	0.031 $\pm$ 0.019a	0.014 $\pm$ 0.010a
	Dough	0.005 $\pm$ 0.004b	0.205 $\pm$ 0.114b	0.008 $\pm$ 0.008b	0.020 $\pm$ 0.011b	0.011 $\pm$ 0.007b
	Bun after 24 h	0.002 $\pm$ 0.004bc	0.145 $\pm$ 0.076b	0.009 $\pm$ 0.008b	0.011 $\pm$ 0.007c	0.009 $\pm$ 0.005bc
		0.001 $\pm$ 0.003c	0.132 $\pm$ 0.071b	0.007 $\pm$ 0.006b	0.010 $\pm$ 0.006c	0.007 $\pm$ 0.005c

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## 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

Table 2 (continued)

Variety/genotype	Technological step	Antheraxanthin	all-E-lutein	13-Z-lutein	13-Z-lutein	13-Z-lutein	9-Z-lutein	Sum of carotenoids
*Decrease to [%] (related to 100% in flour)	Dough	53.6	33.3	26.4	63.5	34.4	80.5	0.378 ± 0.004a
	Bun	22.8	23.6	28.4	34.4	34.4	62.4	0.120 ± 0.004b
	Bun after 24 h	13.9	21.5	23.1	34.0	34.0	51.7	0.112 ± 0.004bc
*Decrease by [%] (related to 100% in flour)	Dough	46.4	66.7	73.6	36.5	36.5	19.5	0.683 ± 0.002a
	Bun	77.2	76.4	71.6	65.6	65.6	37.6	0.508 ± 0.018b
	Bun after 24 h	86.1	78.5	76.9	66.0	66.0	48.3	0.301 ± 0.012c
Bohemian (Red)	0.011 ± 0.001a	0.060 ± 0.004a	n.d.	n.d.	n.d.	n.d.	0.312 ± 0.002a	0.378 ± 0.004a
	n.d.	0.024 ± 0.002c	n.d.	n.d.	n.d.	n.d.	0.096 ± 0.006b	0.120 ± 0.004b
Annie (Red)	n.d.	0.040 ± 0.003b	n.d.	n.d.	n.d.	n.d.	0.072 ± 0.002c	0.112 ± 0.004bc
	n.d.	0.035 ± 0.003b	n.d.	n.d.	n.d.	n.d.	0.061 ± 0.003c	0.096 ± 0.005c
Citrus (Ye)	0.008 ± 0.000a	0.054 ± 0.003ab	n.d.	n.d.	n.d.	n.d.	0.361 ± 0.011a	0.683 ± 0.002a
	n.d.	0.050 ± 0.003bc	n.d.	n.d.	n.d.	n.d.	0.281 ± 0.010b	0.508 ± 0.018b
V1 131-15 (Ba)	n.d.	0.059 ± 0.002a	n.d.	n.d.	n.d.	n.d.	0.161 ± 0.006c	0.301 ± 0.012c
	n.d.	0.042 ± 0.004c	n.d.	n.d.	n.d.	n.d.	0.129 ± 0.004d	0.237 ± 0.005d
Bona Vita (Ye)	0.012 ± 0.002a	0.074 ± 0.011a	0.025 ± 0.002a	0.053 ± 0.011a	0.194 ± 0.012a	1.854 ± 0.035a	2.237 ± 0.044a	2.237 ± 0.044a
	0.008 ± 0.002a	0.038 ± 0.008b	n.d.	0.025 ± 0.002b	0.168 ± 0.004b	0.411 ± 0.016b	0.557 ± 0.030b	0.557 ± 0.030b
UC 66049 (Ba)	0.011 ± 0.003a	0.065 ± 0.011a	n.d.	n.d.	n.d.	n.d.	0.446 ± 0.026c	0.446 ± 0.026c
	0.010 ± 0.000a	0.063 ± 0.004a	n.d.	0.017 ± 0.003b	0.047 ± 0.002c	0.325 ± 0.014c	0.431 ± 0.020c	0.431 ± 0.020c
AF Jumiko (Pp)	0.007 ± 0.001a	0.034 ± 0.003a	0.044 ± 0.010a	0.140 ± 0.003a	0.843 ± 0.045a	1.030 ± 0.017a	2.109 ± 0.041a	2.109 ± 0.041a
	0.008 ± 0.001a	0.024 ± 0.002b	n.d.	0.047 ± 0.011b	0.264 ± 0.007b	0.200 ± 0.010b	0.541 ± 0.021b	0.541 ± 0.021b
ANK28A (Pp)	n.d.	0.027 ± 0.001ab	n.d.	0.016 ± 0.002c	0.109 ± 0.001c	0.119 ± 0.001c	0.272 ± 0.003c	0.272 ± 0.003c
	n.d.	0.019 ± 0.005b	n.d.	0.017 ± 0.000c	0.089 ± 0.005c	0.109 ± 0.002c	0.234 ± 0.010c	0.234 ± 0.010c
Average of all analyzed varieties	0.014 ± 0.003a	0.078 ± 0.009b	n.d.	0.020 ± 0.003a	0.081 ± 0.005a	0.554 ± 0.020a	0.734 ± 0.020a	0.734 ± 0.020a
	0.009 ± 0.001b	0.054 ± 0.003c	n.d.	0.015 ± 0.002a	0.090 ± 0.003a	0.459 ± 0.010b	0.624 ± 0.015b	0.624 ± 0.015b
Sum of carotenoids	0.010 ± 0.002ab	0.111 ± 0.011a	n.d.	n.d.	0.063 ± 0.002b	0.295 ± 0.011c	0.469 ± 0.022c	0.469 ± 0.022c
	n.d.	0.087 ± 0.001b	n.d.	n.d.	0.048 ± 0.004c	0.255 ± 0.008d	0.390 ± 0.008d	0.390 ± 0.008d
Sum of carotenoids	n.d.	0.035 ± 0.007a	n.d.	n.d.	0.144 ± 0.006a	0.217 ± 0.006a	0.400 ± 0.015a	0.400 ± 0.015a
	n.d.	0.018 ± 0.002b	n.d.	n.d.	0.056 ± 0.001b	0.071 ± 0.004b	0.145 ± 0.004b	0.145 ± 0.004b
Sum of carotenoids	n.d.	0.026 ± 0.001ab	n.d.	n.d.	0.021 ± 0.002c	0.047 ± 0.003c	0.094 ± 0.003c	0.094 ± 0.003c
	n.d.	0.022 ± 0.005b	n.d.	n.d.	0.021 ± 0.003c	0.044 ± 0.002c	0.088 ± 0.009c	0.088 ± 0.009c
Sum of carotenoids	0.006 ± 0.000a	0.039 ± 0.004a	n.d.	n.d.	0.067 ± 0.002a	0.685 ± 0.029a	0.798 ± 0.023a	0.798 ± 0.023a
	0.006 ± 0.003a	0.020 ± 0.001b	n.d.	n.d.	0.055 ± 0.004b	0.372 ± 0.026b	0.453 ± 0.027b	0.453 ± 0.027b
Sum of carotenoids	n.d.	0.028 ± 0.006b	n.d.	n.d.	0.021 ± 0.001d	0.256 ± 0.009c	0.309 ± 0.013c	0.309 ± 0.013c
	0.006 ± 0.001a	0.025 ± 0.002b	n.d.	n.d.	0.039 ± 0.001c	0.262 ± 0.004c	0.330 ± 0.004c	0.330 ± 0.004c
Sum of carotenoids	0.006 ± 0.001a	0.110 ± 0.006a	0.019 ± 0.001a	0.084 ± 0.006a	0.438 ± 0.004a	0.870 ± 0.018a	1.531 ± 0.026a	1.531 ± 0.026a
	n.d.	0.036 ± 0.004c	n.d.	0.015 ± 0.001b	0.115 ± 0.011b	0.141 ± 0.009b	0.311 ± 0.011b	0.311 ± 0.011b
Sum of carotenoids	n.d.	0.087 ± 0.002b	n.d.	0.015 ± 0.002b	0.071 ± 0.003c	0.169 ± 0.006b	0.345 ± 0.007b	0.345 ± 0.007b
	n.d.	0.086 ± 0.006b	n.d.	n.d.	0.074 ± 0.013c	0.147 ± 0.011b	0.308 ± 0.029b	0.308 ± 0.029b
Sum of carotenoids	n.d.	0.063 ± 0.001a	n.d.	n.d.	0.170 ± 0.008a	0.377 ± 0.007a	0.610 ± 0.013a	0.610 ± 0.013a
	n.d.	0.033 ± 0.001b	n.d.	n.d.	0.146 ± 0.013b	0.211 ± 0.011b	0.394 ± 0.023b	0.394 ± 0.023b
Sum of carotenoids	n.d.	0.043 ± 0.006b	n.d.	n.d.	0.066 ± 0.002c	0.135 ± 0.006c	0.247 ± 0.004c	0.247 ± 0.004c
	n.d.	0.044 ± 0.012b	n.d.	n.d.	0.071 ± 0.002c	0.135 ± 0.006c	0.250 ± 0.011c	0.250 ± 0.011c
Sum of carotenoids	0.005 ± 0.005a	0.061 ± 0.0025a	0.010 ± 0.016a	0.033 ± 0.0050a	0.244 ± 0.0258a	0.695 ± 0.512a	1.053 ± 0.718a	1.053 ± 0.718a
	0.005 ± 0.005a	0.033 ± 0.013c	n.d.	0.011 ± 0.016b	0.107 ± 0.078b	0.249 ± 0.140b	0.406 ± 0.180b	0.406 ± 0.180b
Sum of carotenoids	0.002 ± 0.004b	0.054 ± 0.029ab	n.d.	0.003 ± 0.007b	0.053 ± 0.034b	0.175 ± 0.097b	0.288 ± 0.129b	0.288 ± 0.129b
	0.003 ± 0.004b	0.047 ± 0.026b	n.d.	0.004 ± 0.007b	0.050 ± 0.028b	0.160 ± 0.091b	0.266 ± 0.119b	0.266 ± 0.119b

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## 4.2 Carotenoid changes of colored-grain wheat flours during bun-making

Table 2 (continued)

Variety/genotype	9-Z-Lutein	Zeaxanthin	α-Carotene	β-Carotene	Esters	Total lutein	Sum of carotenoids
*Decrease to [%] (related to 100% in flour)	98.3 47.7 66.3	54.0 89.0 77.6	n.d. n.d. n.d.	34.2 10.4 11.2	43.9 21.7 20.6	35.8 25.2 23.0	38.5 27.4 24.9
*Decrease by [%] (related to 100% in flour)	1.7 52.3 33.7	46.0 11.0 22.4	n.d. n.d. n.d.	65.8 89.6 88.8	56.1 78.3 79.4	64.2 74.8 77.0	61.5 72.6 75.1

n.d. – not detectable; STD – standard deviations; values in columns marked with different letters are statistically different at  $p \leq 0.05$ ; \* average of all analyzed varieties and genotypes.

contained in Bona Vita (0.843 µg/g DW, 43.8%), Konini (0.438 µg/g DW, 30.7%), and Annie (0.263 µg/g DW, 38.6%) varieties. In our previous study (Paznocht et al., 2018), we found that esterified xanthophylls are represented mainly by lutein esters, accompanied by minor amounts of antheraxanthin and zeaxanthin esters. Based on this finding, we attributed xanthophyll esters to lutein and expressed them simply as the sum of lutein esters.

Xanthophyll esterification is a common method of sequestering carotenoids in plants and it is presupposed to be an effective mechanism in increasing their accumulation (Atienza, Ballesteros, Martín, & Hornero-Méndez, 2007). Some authors have observed that xanthophyll esterification varies widely among and within wheat species and is subject to genetic control (Ahmad et al., 2015; Ziegler et al., 2015). Therefore, the Bohemia variety (which contained only free carotenoids) seems to have no genetic predisposition to synthesize xanthophyll esters. Similarly, Ziegler et al. (2015) reported an absence of lutein esters in seven out of fifteen investigated bread wheat *T. aestivum* genotypes, whereas in the remaining genotypes lutein esters accounted for 22.7–38.3% of TLC. Einkorn genotypes contained lutein esters from 4.4% to 58.3% of TLC. In contrast, no durum wheat varieties contained lutein esters. Particularly interesting is the fact that higher TCC in cereals seems to have no direct influence on the final portion of xanthophyll esters (Paznocht et al., 2018; Ziegler et al., 2015).

### 3.2. Degradation of carotenoids during dough preparation

Results of the degradation of TCC, TLC, and other carotenoids during dough preparation are given in Table 2. Percentage losses of TCC and TLC in individual varieties and breeding materials during baking production are shown in Fig. 2. In the doughs, TCC in all analyzed wheat genotypes decreased to 38.5% on average compared to its original content in wholemeal flour. The preparation of dough was the most devastating step regarding the degradation of carotenoids. The largest decreases in TCC during dough preparation were recorded in the yellow wheat varieties Citrus and Bona Vita (to 24.9% and 25.7%, respectively). In Pp genotypes, various losses were observed among the individual accessions. The most considerable of these decreases occurred in the Konini variety (to 20.3%), which was characterized by the highest beginning TCC among Pp genotypes. In other Pp genotypes, moderate losses in TCC were recorded: in AF Jumiko, TCC decreased to 56.9% and in ANK-28A to 64.7% of the original content in wholemeal flour. A similar situation was observed in Ba genotypes. A significant decrease in TCC was recorded in the UC 66049 genotype (to 36.3%), while in the V1 131-15 genotype, carotenoid losses were significantly smaller (to 85.0%). Conventional wheat varieties were also characterized by different carotenoid losses during dough preparation; in variety Bohemia TCC decreased to 31.7%, and in variety Annie to 74.5% (Fig. 2).

Among individual carotenoids, the highest losses were recorded for α-carotene and β-carotene. Originally contained in the flours only in trace amounts, α-carotene totally decomposed and was not detected in doughs (Table 2). However, not a total loss, β-carotene content dropped to 34.2% of its initial value in flour. A significant decrease also occurred in TLC (to 35.8%). Lutein esters appeared to be more stable compared to free lutein, as evidenced by their smaller decrease (to 43.9%). Zeaxanthin and antheraxanthin were evaluated as the carotenoids most resistant to the dough preparation process, with the lowest reduction in their content (to 54.0% and 53.6%, respectively). Focusing on lutein forms, the major all-*E*-lutein decreased to a considerable extent (to 33.3%) of the original content. In particular, the behavior of *Z*-isomers was different during the preparation of dough. The content of 13-*Z*-lutein dropped even more than its all-*E* counterpart (to 26.4%), whereas losses in other *Z*-isomers were significantly lower. 13-*Z*-Lutein and 9-*Z*-lutein decreased moderately (to 63.5% and 80.5%, respectively), and the decrease of the least-contained 9-*Z*-lutein was only negligible (to 98.3%). These results suggest an enhanced stability of certain *Z*-isomers

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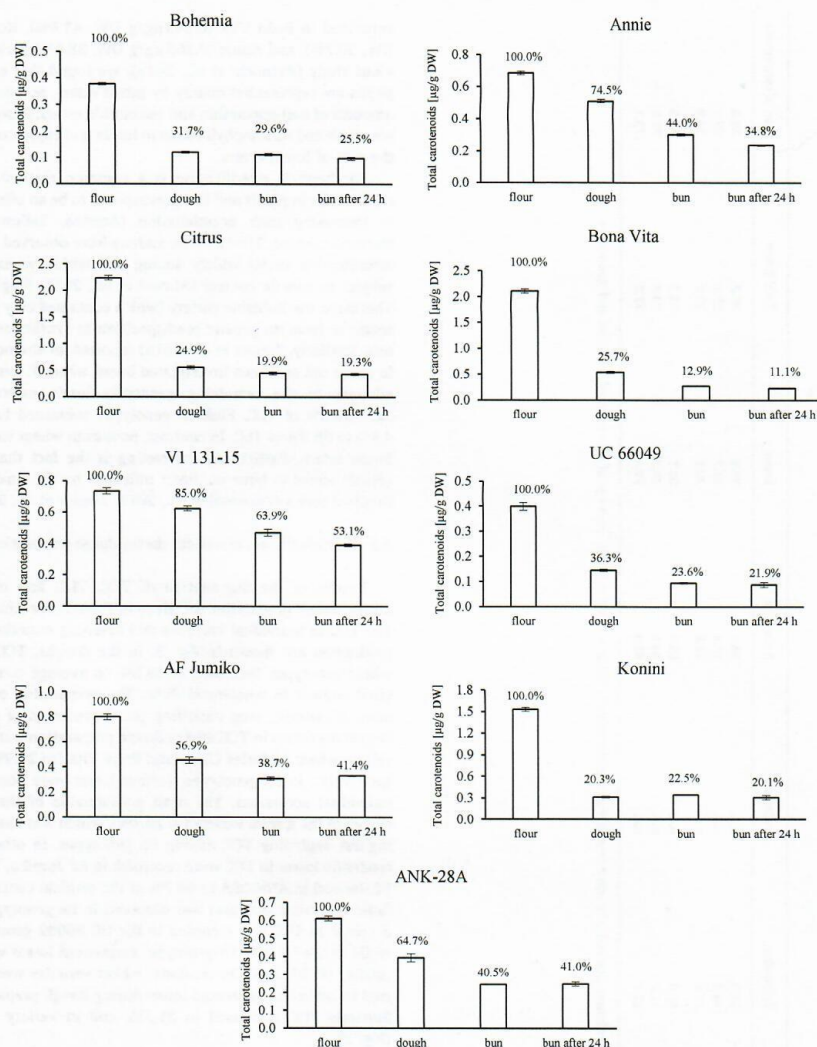


Fig. 2. Percentage loss of total carotenoid content (TCC) during baking production for each variety and genotype (%).

of lutein and/or ongoing isomerization of all-*E*-lutein to its particular *Z*-counterparts during the dough preparation process.

Carotenoids are susceptible to a number of exogenous factors, such as light, oxygen, heat, and extreme pH values, as well as to endogenous enzymatic activity. Their losses during dough preparation take place due to the presence of oxidative enzymes, such as lipoxygenase, peroxidase, and polyphenol oxidase, in the flour, which become active when water is added and oxygen is incorporated during the process (Ficco et al., 2016; Leenhardt et al., 2006; Luthria et al., 2015). In our study, TCC decreased by 61.5% during dough preparation, which is comparable to the significant decrease in TCC (by 66.0%) of bread wheat wholemeal flour during kneading previously reported by Leenhardt et al. (2006). Fratianni, Di Criscio, Mignogna, and Panfil (2012) also reported significant reduction in TCC (by 48.9%) in dough

during egg pasta-making process. The above cited authors found high correlation between carotenoid losses and the lipoxygenase (LOX) activity, while peroxidase was far less active and had little impact on carotenoid degradation and related dough bleaching (Leenhardt et al., 2006). LOX catalyzes the oxidation of polyunsaturated fatty acids, which leads to the subsequent oxidation of carotenoid pigments. LOX activity is highly dependent on wheat species (Hidalgo et al., 2010; Hidalgo & Brandolini, 2012; Leenhardt et al., 2006). Hidalgo and Brandolini (2012) reported the highest lipoxygenase activity in flour of *T. aestivum* (8.02  $\mu\text{mol}/\text{min}/\text{g DW}$ ), followed by *T. turgidum* (3.48  $\mu\text{mol}/\text{min}/\text{g DW}$ ) and *T. monococcum* (0.45  $\mu\text{mol}/\text{min}/\text{g DW}$ ). Similarly, Hidalgo et al. (2010) observed different damage levels between wheat species during kneading associated with their different LOX activity (in descending order, bread wheat, durum wheat, and einkorn). These

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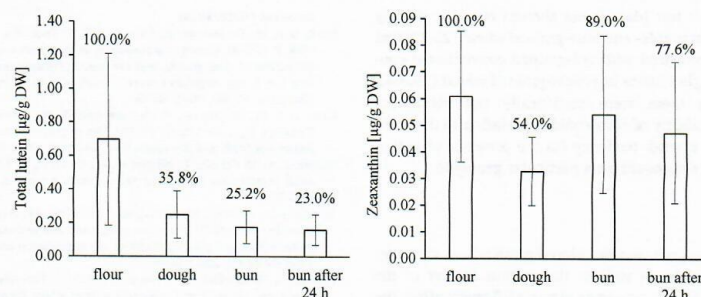


Fig. 3. Evolution of total lutein and zeaxanthin content on average of all analyzed varieties and genotypes during baking production (%).

results indicate a high lipoxygenase activity in bread wheat and lower activity in high-carotenoid varieties. In our high-carotenoid varieties Citrus, Bona Vita, and Konini, there were significant decreases in TCC, probably because they are *T. aestivum* varieties. Other important factors influencing LOX activity are the time, intensity, and temperature of dough kneading (Hidalgo et al., 2010), the pH value of the dough – enzymatic activity of LOX is highest over the pH range 5–6 (Hidalgo, & Brandolini, 2012) – and the percentage and composition of lipids in the grain. Higher proportions of unsaturated fatty acids promote the production of peroxides and other free radicals that can react with the carotenoid compounds (Cueto, Farroni, Schoenlechner, Schleining, & Buera, 2017). Therefore, to improve carotenoid stability during the dough preparation process, it is crucial to choose appropriate wheat species and varieties with low LOX activity and appropriate lipid composition and to minimize the time and intensity of kneading.

### 3.3. Degradation of carotenoids during heat treatment (baking) and short-term bun storage

Heat treatment during bun baking caused further carotenoid degradation (by 11.1%), decreasing TCC to 27.4% of the original content in flour. This means that baking led to a substantially smaller reduction of carotenoids as compared to dough preparation (Table 2; Fig. 2). The most significant relative decreases of TCC were recorded in the red wheat Annie (by nearly one-third, to 44.0%) and the Pp wheat ANK-28A (by nearly one-fourth, to 40.5%). However, the least significant TCC decreases were observed in the red wheat Bohemia and yellow-grained wheat Citrus (by 2.1% and 5.0%, respectively). In buns formed with the Pp wheat Konini, a slight increase (of 2.2%, to 22.5%) was recorded. Significantly higher TCC reduction (by 29%) occurred in the bread crust during baking, but carotenoids present in the bread crumb were substantially more stable (experiencing a decrease of only 3%) as reported by Hidalgo et al. (2010). According to these authors, heat treatment had a major effect on overall TCC.

Total lutein decreased by 10.6% (Table 2, Fig. 3). All-*E*-lutein remained the major carotenoid despite its amount falling by 9.7%. Considerable decrease of approximately one-third was found for 13'-*Z*-lutein (by 29.1%). In the case of two lutein *Z*-isomers, 9-*Z*- and 9'-*Z*-lutein, their amounts were reduced by around one-quarter and one-half, respectively, though despite these substantial losses, 62.4% and 47.7% of the original values remained. Hence, we assume a higher stability of 9-*Z*- and 9'-*Z*-lutein in comparison to other lutein isomers and/or other reactions resulting in isomerization from *E*- to certain *Z*-isomers. Baking caused significant decreases of lutein esters (by 22.2%),  $\beta$ -carotene and antheraxanthin (by 23.8% and 30.8%, respectively). On the other hand, an increase was recorded in individual carotenoids such as zeaxanthin, which increased to 89% (an increase of 35%) of its original content in flour and 13-*Z*-lutein, which increased to 28.4% (an increase of 2%). It is understood that thermal processing induces carotenoid isomerization

of *E*-isomers to particular *Z*-forms (Cueto et al., 2017; Updike & Schwartz, 2003). Hidalgo et al. (2010) further hypothesize the hydroxylation of carotenes took place caused by high temperatures, due to the significant increase of  $\beta$ -cryptoxanthin content in bread crust observed in their experiment.

After the short-term storage of buns for 24 h, only a small additional decrease in TCC was observed (reaching 24.9% of wholemeal flour, a decrease of 2.5%). The largest storage-related losses were found in wheats V1 131-15 and Annie (by 10.8% and 9.2%, respectively). Regardless, in buns made of wheat V1 131-15 (blue, crosses with Citrus ancestor), the highest TCC remained at the end of the baking and storage process (53.1%). However, the V1 131-15 grain buns retained their relatively highest TCC after the production and storage processes. From the viewpoint of the final TCC measurement in our study, this genotype occupies second place behind the yellow-grained wheat Citrus (0.431  $\mu\text{g/g DW}$ ). The high TCC preserved in buns after storage was mainly due to the high carotenoid content in the original flour. The mildest fall of TCC was observed in wheat Citrus (a decrease of 0.6%). Two samples of Pp wheat (ANK-28A and AF Jumiko) showed no decrease or only a very slight increase (of 0.5% and 2.7%, respectively).

Similarly, concentrations of individual analytes did not change significantly during the 24 h of storage. The major carotenoid lutein dropped by only 2.2%. The largest decreases were observed in zeaxanthin (a drop of 11.4%) and 9-*Z*-lutein (a drop of 10.7%) levels. On the contrary, 9'-*Z*-lutein increased by 18.6%. Decline of TCC during short-term storage was probably caused by the presence of atmospheric oxygen (Hidalgo et al., 2010).

In total, during the full process from flour to final product, carotenoids degraded by 75.1% (i.e., 24.9% of TCC was preserved). Distinctly higher carotenoid losses (92%) in wheat (*Triticum aestivum*) bread have been reported by Leenhardt et al. (2006). Hidalgo et al. (2010) reported TCC decrease in bread made of Blasco wheat (*Triticum aestivum*) by 24% and 55% in bread crumb and crust, respectively. The differences between obtained results may be caused by using different bread-making protocols.

In our study, the highest degradation was characteristic of  $\alpha$ -carotene and the most resistant to decomposition was zeaxanthin (Fig. 3), likely due to possible isomerization of lutein to zeaxanthin or increased hydroxylation of carotenes at higher temperatures (Hidalgo et al., 2010). In the present study, bound esterified carotenoids degraded to a similar extent as free carotenoids, and there was not observed any significant protective effect of carotenoid esterification on stabilization and resistance to heat thermal treatment, as was reported previously in some studies (Ahmad et al., 2015; Subagio et al., 1999). Adding a layer of complexity, *Z*-isomers of lutein in our samples showed a lesser decrease in final buns compared with all-*E*-lutein. This can be explained by the isomerization of *E*-forms to *Z*-forms through thermal processing, as has also been observed in vegetables (Updike, & Schwartz, 2003).

Although the results of two-way statistical analysis of variance

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ANOVA using Tukey's HSD test (data is not shown) revealed slightly lower average TCC losses in purple- and blue-grained wheat (25.7% and 27.2%, respectively) as compared with red-grained conventional wheat (28.7%) and higher losses in yellow-grained wheat (39.3%), the differences between them were statistically not significant ( $p > 0.05$ ). That is, the stability of carotenoids in relation to the color of the grain showed only a weak tendency (in the presence of anthocyanins), but were highly dependent on a particular genotype.

### 4. Conclusion

In conclusion, the genotypes of new colored-grain wheat varieties, lines, and breeding materials, as well as the various factors of the preparation, storage, and baking processes may significantly affect the carotenoid levels and other health components in wheat flour and final baked products. These results may serve as a background for wheat breeding efforts to produce wheat flours and bakery products beneficial to human health. Therefore, it may be possible for wheat breeders to select genotypes that optimize the levels of selected health components. Future investigations should examine two paths: first, breeding approaches that can be used to select high-carotenoid and high-anthocyanin cultivars with low activity of oxidative enzymes and appropriate composition of grain lipids; and second, new technologically optimized approaches, like air-classification, vacuum dough making, and optimal durations of kneading, baking, heat treatment, extrusion, and puffing.

### Conflicts of interest

The authors have declared no conflicts of interest regarding this work.

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### Total phenolic content and antioxidant activity of tritordeum wheat and barley

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**Abstract.** Whole grains are a source of numerous antioxidant compounds such as phenolic compounds, anthocyanins, phenolic acids, proanthocyanidins, lignans and others which are able to scavenge free radicals. Thus cereals seem to be very useful in preventing chronic diseases like metabolic syndrome (obesity, high blood pressure, high blood triglyceride and glucose levels), diabetes, neurodegenerative diseases, cancer and chronic inflammatory diseases. Recently, there has been an increased consumer demand for cereal based foods, especially whole cereals. Such demand provides scope for innovations of which an important one is introduction of a completely new cereal cross called tritordeum. This alternative cereal, which is presented as a good source of health beneficial compounds, was assessed in this study and compared with wheat and barley. The total phenolic content (TPC) and related total antioxidant activity (TAA) were investigated via two spectrophotometric methods using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and Folin-Ciocalteu reagent respectively. Both TPC and related TAA values of tritordeum and wheat were similar but were significantly lower compared to barley. Results have also revealed a close relationship between TAA and TPC ( $R^2 = 0.93$ ,  $p < 0.05$ ), which might suggest that increased antioxidant activity in those grains is caused by phenolic compounds contained in them. Tritordeum seems to represent a new cereal with good prospects; nevertheless more detailed analysis of its health related compounds is required.

**Key words:** cereals, DPPH, spectrophotometry, total antioxidant activity, total phenolic content, tritordeum

#### INTRODUCTION

Cereals represent a staple food group in the human diet. They are a major source of energy, essential and health promoting components (Tayyem et al., 2016). Especially whole grain products are a potential source of numerous antioxidant compounds, which can be found in bran or germ of kernels (Abozed et al., 2014; Laddomada et al., 2015). Yet they are very heterogenic groups of compounds belonging to several classes such as phenolic compounds, anthocyanins, phenolic acids, proanthocyanidins, lignans and others (Okarter et al., 2010). Thanks to their antioxidant properties they can significantly contribute to the total antioxidant activity of cereal based foods (Liu, 2007; Shao et al., 2014). Nowadays, such foods are gaining importance while they seem to be very useful for prevention of human chronic diseases. Due to the increasing prevalence of metabolic

syndrome (obesity, high blood pressure, high blood triglyceride and glucose levels), diabetes, neurodegenerative diseases, cancer, chronic inflammatory diseases, insufficient fibre intake and only minimal physical activity, the overall demand for nutritionally beneficial products is growing. Consumers are interested in buying new sources (or in re-discovering the old ones) of such foods, which can help them to maintain good health. Since whole cereal products represent such commodities, grain breeders all over the world are seeking and breeding various cereal varieties/lineages with increased health benefits (Espín et al., 2007; Dykes and Rooney, 2007; Syed Jaafar et al., 2013).

Genetic breeding led to creation of, besides many others, tritordeum, a cross between durum wheat (*Triticum durum* L.) and wild barley (*Hordeum chilense* L.). While wheat and barley have been common crops worldwide for millennia, tritordeum is a relatively new crop presented as an alternative cereal with unique properties and major health benefits (www.tritordeum.com). These are associated with content of nutritionally significant constituents, such as carotenoids and phenolic acids. Above all, tritordeum shows high content of carotenoids, especially lutein, which is estimated to be 5-6 times higher than in common wheat (Mellado-Ortega & Hornero-Méndez, 2015). Furthermore, another recent study identified the composition of phenolic acids in tritordeum, suggesting their contribution to health benefits of tritordeum (Navas-Lopez et al., 2014). Nonetheless, little is known about other nutritionally important constituents in tritordeum or its health associated characteristics.

A possible way of assessing cereals' health benefits is analysis of compounds contained in grains or potential health effects evaluation. Although these effects are difficult to determine they can be reflected in some detail by certain methods. For example measuring of total phenolic content (TPC) or total antioxidant activity (TAA) represents a simple way to evaluate potential nutritional significance. Total phenolic content quantifies phenolic compounds using Folin-Ciocalteu reagent assay, which is based on electron transfer and measures the ability of an extract to reduce the reagent (Margraf et al., 2015). Total antioxidant activity can be measured by various methods, for example common antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). It is based on the significant ability of phenolic compounds to scavenge the DPPH free radical (Antolovich et al., 2002).

So far, no study quantifying phenolic compounds (TPC) in tritordeum and their antioxidant activity (TAA) has been conducted. Therefore the first aim of this study was to assess the overall amount of phenolic compounds in tritordeum by determination of TPC and to obtain the TAA value of phenolic compounds. The subsequent aim of this study was to verify the correlation between TPC and TAA as presented in some studies (Ivanišová et al., 2014; Zhao et al., 2006) and to compare these values to those measured in wheat and barley.

### MATERIALS AND METHODS

#### Grain samples

This study evaluated several various cultivars and genotypes of three types of cereal grains – wheat, barley and tritordeum (Table 1). The cereals included red wheat, blue wheat, purple wheat, light-coloured barley, dark-coloured barley (*Hordeum vulgare* var. *nudimelanocrithon* L.) and tritordeum. The description of all cereal genotypes used in

#### 4.3 Total phenolic content and antioxidant activity of tritordeum wheat and barley

this study is shown in Table 1. The cereal varieties were grown in the Agricultural Research Institute Kromeriz (Czech Republic) over the same period and were harvested in 2014. The analyses were performed using ground samples prepared according to Hosseinian et al. (2008). Briefly, the grain samples were ground in an IKA analytical mill (Janke & Kunkel Co., Stanfen, Germany) to pass through 0.5 mm screen (35 mesh) and homogenised well.

**Table 1.** Description of investigated cereal genotypes

Cultivar	Species	Growth type	Country of origin*	Variety status	Grain colour
JB 1	× <i>Tritordeum</i> Ascherson et Graebner	spring	ESP	released variety	yellow endosperm
JB 3	× <i>Tritordeum</i> Ascherson et Graebner	spring	ESP	released variety	yellow endosperm
HT 439	× <i>Tritordeum</i> Ascherson et Graebner	spring	ESP	breeding line	yellow endosperm
UC 66049	<i>Triticum aestivum</i> L.	spring	USA	genetic resource	blue aleurone
Tschermaks B.S.	<i>Triticum aestivum</i> L.	spring	AUT	research germplasm	blue aleurone
KM 131-15**	<i>Triticum aestivum</i> L.	winter	CZE	breeding line	blue aleurone
Skorpion	<i>Triticum aestivum</i> L.	winter	CZE	released variety	blue aleurone
RU 687-12	<i>Triticum aestivum</i> L.	spring	CZE	breeding line	purple pericarp
Konini	<i>Triticum aestivum</i> L.	spring	NZL	research germplasm	purple pericarp
Vanessa	<i>Triticum aestivum</i> L.	winter	CZE	released variety	red (standard)
PS Karkulka	<i>Triticum aestivum</i> L.	winter	SVK	released variety	purple pericarp
Bohemia	<i>Triticum aestivum</i> L.	winter	CZE	released variety	red (standard)
Hordeum nudimelanocrithon	<i>Hordeum vulgare</i> L.	spring	ETH	genetic resource	black grain
AF Cesar***	<i>Hordeum vulgare</i> L.	spring	CZE	released variety	standard
AF Lucius***	<i>Hordeum vulgare</i> L.	spring	CZE	released variety	standard

\* CZE Czech Republic, ESP Spain, AUS Austria, NZL New Zealand, SVK Slovakia, USA United States, AUT Austria, ETH Ethiopia

\*\* origin of KM 131-15 is: (Skorpion × PS Karkulka) × (Citrus × Bona Dea)

\*\*\* barley varieties have naked (hulless) caryopsis

##### Total phenolic content

The TPC was evaluated according to Lachman et al. (2011) with minor modifications. Briefly, 2.5 g of ground grain were extracted with 25 ml of 0.1% HCl in methanol overnight at -20 °C. Each extract was centrifuged at 8,000 rpm for 10 min and 2 ml volume was reacted with 2.5 ml of Folin-Ciocalteu reagent with addition of 7.5 ml



#### 4.3 Total phenolic content and antioxidant activity of tritordeum wheat and barley

of 20% sodium carbonate and was filled up with pure water to 50 ml. After 2 hours the solution was centrifuged at 8000 rpm for 2 min and absorbance at 765 nm was measured. The results were expressed as mg of gallic acid per 100 g of dry matter (mg GA 100 g<sup>-1</sup> DM).

##### **Total antioxidant activity using DPPH**

The DPPH radical cation scavenging activity of methanolic extracts was evaluated according to Lachman et al. (2012) with minor modifications. Briefly, 2.5 g of ground grain were extracted with methanol and shaken for 2 hours at room temperature. The extracts were filled up with methanol to 25 ml and stored in darkness at room temperature for one week. The extracts were then centrifuged at 8000 rpm for 2 min. The solution of DPPH was prepared in methanol in a concentration responding to absorbance of 0.600 ± 0.01 AU at 515 nm. 100 µl of extract was mixed with 1 ml of DPPH solution, incubated for 20 min and measured at 515 nm. The results were expressed as mg of Trolox equivalent antioxidant activity per 100 g of dry matter (mg TEAC 100 g<sup>-1</sup> DM).

##### **Statistical analysis**

All experimental data were analysed using Statistica software, version 12 (Dell Software). Analysis of variance (ANOVA) was performed with 5 replicates for each sample. Statistical significance was declared at  $p < 0.05$ .

#### **RESULTS AND DISCUSSION**

Total phenolic content (TPC) and total antioxidant activity (TAA) were evaluated in varieties of tritordeum as well as wheat and barley. The measured values of TPC and TAA proved distinctions among cereal types (Fig. 1).

Tritordeum, the new cereal with possible major health benefits, showed the lowest values of both TPC and TAA. Their average values were 65.86 ± 2.68 mg GA 100 g<sup>-1</sup> DM and 16.30 ± 0.21 mg TEAC 100 g<sup>-1</sup> DM. Contrary to the JB 1 and JB 3 released varieties, the HT 439 breeding line reached higher values of TPC and TAA. Its higher content of health beneficial compounds therefore offers potential for subsequent breeding. Overall, evaluation of tritordeum showed just a little difference between tritordeum and wheat varieties, which gave slightly higher results of 73.68 ± 3.87 and 21.98 ± 1.23 for TPC and TAA respectively. These results indicate that the amount of phenolic compounds possessed by tritordeum is similar to that in wheat. Such finding therefore supplements the study of Navas-Lopez et al. (2014) who reported that the composition of phenolic compounds in tritordeum and wheat is alike. Nevertheless, as mentioned before, Mellado-Ortega & Hornero-Méndez (2015) stated that tritordeum has above all a significant content of carotenoids, particularly lutein, the amount of which can reach 5-6 times that of the amount in wheat. Since the extraction method used in this study reflects only the antioxidant activity of phenolic compounds, we can still assume that carotenoid content in tritordeum can contribute to overall antioxidant activity more than phenolic compounds.

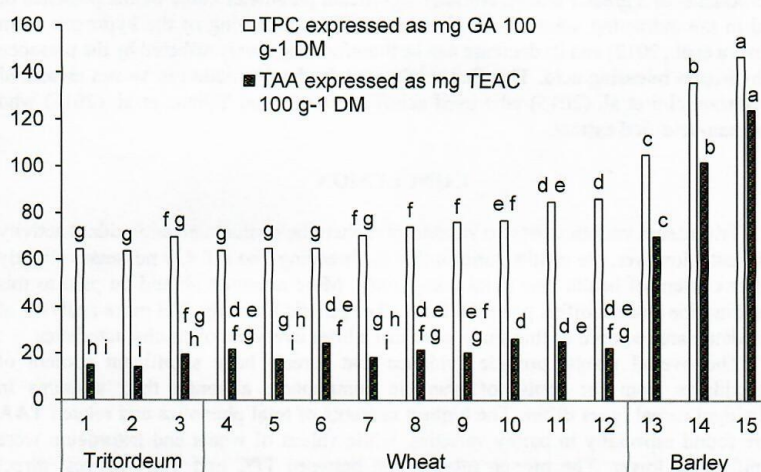
Compared to these results, barley reached substantially higher values of both TPC ( $p = 0.039$ ,  $\alpha = 0.05$ ) and TAA ( $p = 0.028$ ,  $\alpha = 0.05$ ) than tritordeum and wheat. The average TPC of barley was 129.49 ± 5.81 mg GA 100 g<sup>-1</sup> DM and 98.86 ± 2.82 mg

#### 4.3 Total phenolic content and antioxidant activity of tritordeum wheat and barley

TEAC 100 g<sup>-1</sup> DM. The TPC and TAA values were significantly different not only between barley on one side and wheat and tritordeum on the other side, but also among single barley varieties.

Moreover, the results showed a noticeable trend among TPC and TAA values, which were assessed by Fisher's linear correlation analysis to evaluate their relationship. The measurement proved a very close correlation between TPC and TAA as the correlation analysis gave  $R^2 = 0.93$  ( $p < 0.05$ ).

These results suggest that phenolic compounds in cereals directly affect antioxidant capacity. This fact supports the previous findings of Ivanišová et al. (2014) who reported significant correlation between TCP and AOA ( $R^2 = 0.87$ ,  $p < 0.05$ ) as well as those of Zhao et al. (2006) who determined an even closer relationship ( $R^2 = 0.94$ ,  $p < 0.01$ ) very similar to the one published in this study.



**Figure 1.** TPC and TAA values of specific cereals and their varieties. Values with the same letters are significantly different ( $P < 0.05$ ). Different small letters indicate significant difference among samples. Tritordeum: 1 – JB 1, 2 – JB 3, 3 – HT 439. Wheat: 4 – UC 66049, 5 – Tschermak B.S., 6 – KM 131-15, 7 – Skorpion, 8 – RU 687-12, 9 – Konini, 10 – Vanessa, 11 – PS Karkulka, 12 – Bohemia. Barley: 13 – *Hordeum nudimelanocrithon*, 14 – AF Cesar, 15 – AF Lucius.

TPC values measured in this study are similar to results published by other researchers. Wheat varieties showed value of  $73.68 \pm 3.87$  mg GA 100 g<sup>-1</sup> DM, which correspond well with Moore et al. (2005) and Lachman et al. (2011) who reported mean value of TPC in wheat 60.0 and 55.2 respectively, but are slightly lower than the values of Adom et al. (2002) and Abozed et al. (2014) who measured 136.0 and 112.0 respectively. This distinction is not substantial and might be caused by the differences between analysed varieties and the extraction methods used in these studies. The

distinction of TPC between barley and wheat, which was demonstrated in this study, was also published by Fogarasi et al. (2015).

The literature reports significant discrepancies in cereals' TAA measured using DPPH. Some researchers such as Li et al. (2007), Fogarasi et al. (2015) and Mazzoncini et al. (2015) obtained TAA mean values of wheat (as mg TEAC 100 g<sup>-1</sup> DM) to be 147.0, 250.0, and 2753.2 respectively. On the contrary, other authors such as Brandolini et al., Lachman et al. (2012) Yilmaz et al. (2015) reported TAA mean values of wheat as 13.8, 16.4 and 30.0 respectively. The last stated results correspond with those measured in this study. Such distinctions could be caused by diverse sample preparation or by a different method procedure. Particularly the time of incubation of the extract with DPPH can significantly affect the results – for example Fogarasi et al. (2015) let DPPH react for 60 min while Brandolini et al. (2013) for only 30 min. The shorter time of incubation quantifies rather fast reacting antioxidants while a longer time reflects the slow reacting antioxidants to a greater extent. Another significant parameter could be the presence of acid in the extracting solution. DPPH is reduced by accepting of the hydrogen atom (Mishra et al., 2012) and its decrease can be therefore negatively affected by the presence of hydrogen releasing acid. This fact might explain the distinction in values measured by Mazzoncini et al. (2015) who used acidified extract and Yilmaz et al. (2015) who used non-acidified extract.

#### CONCLUSION

Tritordeum varieties showed similar total phenolic content and antioxidant activity to wheat. However, the results indicate that the breeding line HT 439 possesses slightly higher content of health beneficial compounds. More attention should be paid to this breeding line since it offers potential for further genetic breeding. Yet more analyses of tritordeum are required as this study gave just a brief overview of its characteristics.

The overall results provide evidence that cereals have significant content of antioxidants from the group of phenolic compounds, although their amounts in individual cereal types differ. The highest contents of total phenolics and related TAA were found especially in barley varieties, while values of wheat and tritordeum were significantly lower. The proven relationship between TPC and TAA suggest direct responsibility of phenolic compounds for antioxidant activity. It can be assumed that cereals are an important source of phenolics and other antioxidant compounds and that their consumption can considerably contribute to consumers' health through increase of antioxidant intake.

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## 4.4 The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain

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### The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain

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#### ABSTRACT

Zrcková M., Capouchová I., Eliášová M., Paznocht L., Pazderů K., Dvořák P., Konvalina P., Orsák M., Štěrba Z. (2018): The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain. *Plant Soil Environ.*, 64.

The aim of the study was to evaluate total antioxidant activity (TAA) and total content of carotenoids (TCC), anthocyanins (TAC), phenolics (TPC) and phenolic acids (PAs) in grain of selected pigmented wheat genotypes and traditional control cultivar cultivated under organic and conventional cropping systems in two-year trials. All of the evaluated parameters were significantly affected both by genotype and evaluated environmental factors. While in TPC, PAs and TCC the effect of years prevailed, TAC was affected mainly by genotype. The effect of genotype and year in TAA was comparable. TPC ranged from 581.71 mg/kg (control cv. Annie) to 723.60 mg/kg (cultivar with purple pericarp PS Karkulka), total PAs content from 711.77 mg/kg (cv. PS Karkulka) to 849.47 mg/kg (cv. Skorpion with blue aleurone). TCC varied from 1.56 mg/kg (cv. PS Karkulka) to 5.32 mg/kg (cv. Citrus with yellow endosperm). The highest TAC (63.23 mg/kg) was found in cv. Skorpion, the lowest (12.70 mg/kg) in cv. AF Jumiko with purple pericarp. Anthocyanins were not detected in cvs. Annie and Citrus. TAA varied from 162.68 mg/kg in cv. Annie to 226.71 mg/kg in breeding line KM 53-14 with blue aleurone. Higher TAA and antioxidants contents and lower grain yields were observed in organic cropping system and in drier year 2016.

**Keywords:** *Triticum aestivum* L.; cereals; phytochemical; abiotic stress; water deficit

Wheat is the most widely grown cereal crop in the world and bread wheat represents a staple food for human nutrition. The consumption of grain and especially whole grain products is associated with a number of health benefits which may be

related in part to the contents of different phytochemicals (Shewry and Hey 2015). They can act as antioxidants and help in prevention of cardiovascular diseases, diabetes, inflammation, cancer, obesity and aging (Garg et al. 2016). Some of these

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phytochemicals significantly influence the grain colour. The purple colour is due to anthocyanins in the pericarp layer, while blue colour is due to anthocyanins in the aleurone layer (Lachman et al. 2017). Carotenoids are responsible for the yellow endosperm colour in cereal grains (Paznocht et al. 2018). Phenolic acids (PAs) represent the most common phenolic compounds in cereal grains (Martini et al. 2015). PAs are secondary metabolites which, as many other antioxidants, are synthesized as a part of multifunctional defence system against biotic and abiotic stresses in plants (Lattanzio et al. 2006). They are, similarly as other phenolic compounds, mainly present in the outer layers of grains (Žilić et al. 2011) and apparently act as a defence against herbivores, microbes, viruses or competing plants as well as they protect the plant from oxidation (Lattanzio et al. 2006).

In recent years, many studies have dealt with antioxidant compounds in various cereal species such as common wheat (Stracke et al. 2009a, Žilić et al. 2011, Zuchowski et al. 2011), durum wheat (Žilić et al. 2011, Ficco et al. 2014, Martini et al. 2015), einkorn wheat and emmer wheat (Lachman et al. 2013). The most of them have also shown a significant impact of genotype on the occurrence of antioxidants and, therefore, on the antioxidant activity of cereals. However, only a few papers have specifically dealt with these phytochemicals in wheat with coloured grain (Abdel-Aal et al. 2016, Garg et al. 2016, Lachman et al. 2017, Paznocht et al. 2018).

Despite relatively high genetic weighing, some environmental factors influence the antioxidant compounds development, too (Ficco et al. 2014). Many of the antioxidants are known to be produced by plants in response to abiotic (e.g. wounding and heat, water and nutrient) and biotic (pest attacks and disease) stress (Barański et al. 2014). Regarding to weather conditions, some authors observed an increased antioxidants synthesis in different cereals grown under water deficit and higher average temperatures during the grain filling (Fратиanni et al. 2013, Paznocht et al. 2018), others registered a negative correlation between high temperatures and antioxidants contents (Mattera et al. 2017). Paznocht et al. (2018) suppose that cultivars originating in climate that can be found in the Czech Republic might react differently to increased temperature and decreased precipitation compared to cultivars of different origin. These findings might

be useful for selecting wheat genotypes naturally rich in antioxidant compounds, also in relation to the choice of the more suitable growing areas (Martini et al. 2015).

Cultivation system may also affect antioxidants contents in crops. There is evidence that differences in fertilization regimes between organic and conventional production systems (and, in particular, the non-use of high mineral N fertilizer inputs) are significant drivers for higher antioxidant concentrations in organic crops. Many studies dealt with the effect of organic and conventional cropping systems on the contents of antioxidants in different crops, including cereals (Zuchowski et al. 2011, Almuayrifi 2013, Barański et al. 2014). However, data related to the effect of organic and conventional cropping systems on concentrations of different antioxidant compounds in grain of less traditional, pigmented wheat are still scarce.

Wheat breeders are currently attempting to develop new types of colour-grained wheat cultivars with improved properties including quality and yield (Martinek et al. 2013). Nevertheless, more data are needed regarding the antioxidant compounds in this wheat, as this could lead to new opportunities for breeding and commercial production of value-added colour-grained cultivars rich in health-beneficial components. Therefore, the objective of this study was to compare the less traditional, pigmented wheat genotypes with traditional common wheat and to assess the impact of weather conditions and cropping system on evaluated antioxidant compounds contents.

#### MATERIAL AND METHODS

**Plant material.** The exact field plot trials with collection of 6 winter wheat genotypes (Table 1) were carried out during the 2015/2016 and 2016/2017 growing seasons at the experimental station of the Czech University of Life Sciences in Prague-Uhřetěves (central part of Bohemia, 295 m a.s.l., average annual temperature 8.4°C, average sum of precipitation 575 mm). The field trials were established using the random blocks, in 3 replicates, with experimental plot average area of 10 m<sup>2</sup>. The trials were carried out under organic and conventional cropping systems. Red clover was used as a preceding crop of wheat in both cropping systems. Treatment of the wheat

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Table 1. Basic characteristics of evaluated wheat genotypes

Genotype	Country of origin	Genotype character	Grain colour
Annie	CZE	cultivar	standard commercial cultivar (check cultivar)
KM 53-14	CZE	breeding line	blue aleurone
Skorpion	AUT	cultivar	blue aleurone
AF Jumiko	CZE	cultivar	purple pericarp
PS Karkulka	SVK	cultivar	purple pericarp
Citrus	DEU	cultivar	yellow endosperm

stands by weeding harrows was used during the vegetation; no fertilizers and pesticides were applied to wheat cultivated under organic cropping system. Nitrogen fertilization in the total dose of 120 kg N/ha and treatment by herbicide (Agritox 50SL; 1.0 L/ha), fungicide (Amistar XTRA; 2.5 L/ha) and insecticide (Nurelle D; 0.6 L/ha) were applied to wheat cultivated conventionally.

As for the weather conditions (Table 2), the period of grain formation and maturing both in 2016 and 2017 was similar in the average temperatures. With regard to precipitation, year 2016 was drier and reached only 60% of precipitation in the evaluated period compared to 2017.

**Grain samples.** Grain samples obtained after the field plot trials harvest were ground using the IKA analytical mill (Janke & Kunkel Co., Stanfen, Germany) to pass through 0.5 mm screen (35 mesh) and were homogenised well. Dry matter (DM) was determined by drying of meal at 105°C for 24 h. Three replicates were made in all of the following analyses.

**Total phenolics content (TPC).** The TPC was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of meal was extracted with

25 mL of 0.1% HCl in methanol. 2 mL of extract were reacted with 2.5 mL of the Folin-Ciocalteu reagent with addition of 7.5 mL of 20% sodium carbonate and filled up with pure water to 50 mL. After 2 h the solution was measured spectrophotometrically at 765 nm. The results were quantified using external calibration and expressed as mg of gallic acid per kg of DM.

**Total antioxidant activity using DPPH (TAA).** The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical cation scavenging activity of methanolic extracts was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of meal was extracted with methanol. The extracts were filled up with methanol to 25 mL and stored in darkness at the room temperature for one week. 100 µL of extract was mixed with 1 mL of DPPH methanolic solution, incubated for 20 min and measured at wavelength 515 nm. The results were quantified using external calibration and expressed as mg of Trolox equivalent antioxidant activity (TEAC) per kg of DM.

**Total phenolic acids content (PAs).** For extraction and chromatographic separation, a method published by Martini et al. (2015) with some modi-

Table 2. Average temperature and sum of precipitation from anthesis to grain maturity

Decade	Month	Average temperature (°C)		Σ of precipitation (mm)	
		2016	2017	2016	2017
1 <sup>st</sup>	June	19.97	18.00	17.00	23.60
2 <sup>nd</sup>		19.95	18.95	10.40	25.20
3 <sup>rd</sup>		20.52	20.91	18.00	51.80
1 <sup>st</sup>	July	20.55	19.90	3.60	9.40
2 <sup>nd</sup>		19.14	19.40	19.00	15.40
3 <sup>rd</sup>		20.24	21.56	32.30	41.80
Average temperature		20.06	19.79		
Σ of precipitation				100.30	167.20
Average temperature (long-term standard)		17.25			
Σ of precipitation (long-term standard)				148.00	



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fications was used. Briefly, 0.25 g of meal was hydrolysed with 14 mL of 2 mol/L aqueous sodium hydroxide for 1 h at the room temperature. 7 mL of 4 mol/L HCl was added to adjust acidic pH (1–2). Two mL of hydrolysate were transferred into 8 mL glass vial and twice extracted with 2 mL of ethylacetate. Combined supernatants of upper organic phase were removed to another glass vial, evaporated to dryness under the nitrogen stream and reconstituted with 1 mL of 70% aqueous methanol, filtered through a syringe filter into an amber HPLC vial and analysed by HPLC-DAD. The analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with diode array detector. The analytes were separated by an Omnispher C18 HPLC column (250 × 4.6 mm; particle size 5 µm; Agilent, Inc., Santa Clara, USA) and detected at two different wavelengths, 280 nm and 325 nm. The results were expressed in mg per kg of DM.

**Total anthocyanin content (TAC).** The TAC was determined using a method described by Syed Jaafar et al. (2013) with minor modifications. Briefly, 2.5 mL of 1 mol/L HCl/MeOH (15:85, v/v) was added to 400 mg of meal and was shaken for 30 min. Then the sample was centrifuged and the supernatant was removed. The extraction was repeated three more times and all the four supernatants were collected. The extract volume was adjusted to 10 mL with extraction solvent. The TAC was determined spectrophotometrically at 529 nm. The total anthocyanin content was quantified using external calibration and expressed as mg of cyanidin-3-glucoside equivalent per kg of DM.

**Total carotenoids content (TCC).** The TCC was determined according to Paznocht et al. (2018).

Briefly, 2 g of meal were twice extracted with 12 mL of ethanol/acetone/hexane mixture (1:1:2, v/v/v), centrifuged and combined supernatants were evaporated to dryness. The dry residue was reconstituted with 2 mL ethanol/acetone (3:2, v/v) containing 0.2% BHT and filtered through a syringe filter into an amber HPLC vial. The HPLC-DAD analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with diode array detector. The analytes were separated on an YMC C30 Carotenoid Column (150 × 3.0 mm, S-3 µm, YMC Co., Kyoto, Japan) and detected at wavelength 445 nm. The TAC was expressed in mg per kg of DM.

**Statistical analysis.** The results were statistically analysed by the analysis of variance (ANOVA) method, with expression of the Fisher's *F*-value. The differences between mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS program (version 9.4, SAS Institute, Cary, USA) at the level of significance *P* = 0.05.

#### RESULTS AND DISCUSSION

**The effect of genotype on the content of evaluated antioxidant compounds.** The results of ANOVA related to six wheat genotypes, grown over two years in two different cropping systems (organic and conventional) are given in Table 3. The analysis shows that genotype (G) and its interactions with crop year (Y) and cropping system (S) significantly affect all the parameters under study. Nevertheless, only the TAC was mostly affected by G factor and impact of Y and S factors was lesser. In case of TAA, similar impacts of G

Table 3. The effect of genotype, cropping system and year on the content of evaluated antioxidants in the wheat grain and yield of grain (ANOVA, Fisher's *F* values)

	Total phenolics content	Phenolic acids	Total carotenoids content	Total anthocyanin content	Total antioxidant activity	Grain yield
Genotype (G)	34.19***	22.64***	7698.97***	1826.75***	38.82***	55.57***
Year (Y)	178.66***	30.74***	9540.05***	1379.07***	36.71***	207.43***
Cropping system (S)	23.39***	6.00**	27.55***	490.99***	18.91***	834.23***
G × Y	3.40*	11.74***	1040.32***	29.79***	5.07**	13.07***
G × S	5.47**	8.09***	8.28***	8.62**	0.96	3.59**
Y × S	30.97***	12.15**	3.52	8.57**	2.59	5.01**
G × Y × S	1.88	7.53**	6.64***	4.35*	1.38	1.14

*P* < 0.05\*; *P* < 0.01\*\*; *P* < 0.001\*\*\*

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and Y were observed. The impact of S was lesser but still statistically significant. The TPC, PAs and TCC appear mostly influenced by the Y factor and less by G, S and their interactions.

According to Martini et al. (2015), yellow-coloured pigments and TAA in durum wheat were mainly affected by genotype, differently from the content of PAs and TPC, which appears to be mostly affected by environmental factors. Our results are in accordance with these findings, with exception of TCC, where the prevailing effect of year was observed. Lachman et al. (2017) reported that anthocyanin levels are highly significantly affected by genotype, but the effects of environment are significant, too. Our results confirm the high heritability for anthocyanins as well as significant effects of year and cropping system.

The results of Tukey's test describing significance between the mean values of genotypes, cropping systems and years are shown in Table 4, a more detailed view on individual genotypes is given in Table 5.

Control cv. Annie reached the lowest TPC of all the evaluated genotypes. However, the difference in TPC between cv. Annie and cv. Skorpion with blue aleurone was statistically insignificant. These results indicate that TPC in traditional wheat

cultivars may be on the same or similar level as in wheat with coloured grain. Our results of TPC in wheat correspond with the results of Eliášová and Paznocht (2017), but are significantly lower compared to the results presented by Abozed et al. (2014). The PAs content was higher in total compared to TPC. Cv. Annie, belonging to the genotypes with lower total PAs content, did not differ statistically from genotypes with purple pericarp. Cv. Annie also reached comparable values of TCC as genotypes with blue aleurone and purple pericarp, with the exception of cv. AF Jumiko with purple pericarp and especially, in accordance with Paznocht et al. (2018), cultivar with yellow endosperm where TCC was significantly higher. According to Garg et al. (2016) and Syed Jaafar et al. (2013), the highest contents of anthocyanin are present in black grained wheats, followed by blue-grained wheats and wheats with purple pericarp. However, some purple wheat may contain higher anthocyanin levels than blue wheats (Abdel-Aal et al. 2016). Our results show that the TAC was substantially higher in genotypes with blue aleurone compared to the purple pericarp ones. In cvs. Annie and Citrus, anthocyanins were not detected. The highest TAA was determined in genotypes with purple pericarp and blue aleurone,

Table 4. The content of evaluated antioxidant compounds, antioxidant activity and yield of grain in the wheat genotypes, years and cropping systems (Tukey's HSD (honestly significant difference) test)

		TPC	PAs	TCC	TAC	TAA	Yield (t/ha)
		(mg/kg dry matter)					
Genotype	Annie/control	581.71 <sup>d</sup>	714.61 <sup>d</sup>	1.61 <sup>cd</sup>	nd	162.68 <sup>d</sup>	8.20 <sup>a</sup>
	Citrus	659.81 <sup>c</sup>	778.85 <sup>bc</sup>	5.32 <sup>a</sup>	nd	195.12 <sup>c</sup>	7.78 <sup>b</sup>
	AF Jumiko	695.50 <sup>b</sup>	751.72 <sup>cd</sup>	2.18 <sup>b</sup>	12.70 <sup>d</sup>	212.70 <sup>b</sup>	8.21 <sup>a</sup>
	PS Karkulka	723.60 <sup>a</sup>	711.77 <sup>d</sup>	1.56 <sup>d</sup>	20.47 <sup>c</sup>	201.17 <sup>bc</sup>	7.40 <sup>c</sup>
	KM 53-14	645.09 <sup>c</sup>	818.49 <sup>ab</sup>	1.62 <sup>cd</sup>	57.25 <sup>b</sup>	226.71 <sup>a</sup>	7.75 <sup>b</sup>
	Skorpion	596.83 <sup>d</sup>	849.47 <sup>a</sup>	1.64 <sup>c</sup>	63.23 <sup>a</sup>	206.31 <sup>c</sup>	7.06 <sup>d</sup>
	HSD <sub>0.05</sub>	39.44	49.17	0.07	2.87	12.96	0.25
Year	2016	701.70 <sup>a</sup>	810.65 <sup>a</sup>	3.00 <sup>a</sup>	42.41 <sup>a</sup>	210.09 <sup>a</sup>	7.37 <sup>b</sup>
	2017	599.15 <sup>b</sup>	730.99 <sup>b</sup>	1.64 <sup>b</sup>	34.41 <sup>b</sup>	191.48 <sup>b</sup>	8.09 <sup>a</sup>
	HSD <sub>0.05</sub>	15.43	19.23	0.03	1.52	5.07	0.10
Cropping system	ECO	670.51 <sup>a</sup>	796.96 <sup>a</sup>	2.36 <sup>a</sup>	39.59 <sup>a</sup>	205.45 <sup>a</sup>	7.01 <sup>b</sup>
	CONV	630.34 <sup>b</sup>	744.67 <sup>b</sup>	2.28 <sup>b</sup>	37.22 <sup>b</sup>	196.12 <sup>b</sup>	8.45 <sup>a</sup>
	HSD <sub>0.05</sub>	15.43	19.23	0.03	1.52	5.07	0.10

nd – non detected; TPC – total content of phenolics; PAs – total content of phenolic acids; TCC – total content of carotenoids; TAC – total content of anthocyanins; TAA – total antioxidant activity; ECO – organic; CONV – conventional cropping system

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Table 5. The content of evaluated antioxidant compounds, antioxidant activity and yield of grain in individual wheat genotypes (Tukey's *HSD* (honestly significant difference) test)

		Annie	Citrus	AF Jumiko	PS Karkulka	KM 53-14	Skorpion
Total content of phenolics (mg/kg DM)	2016	642.40 <sup>a</sup>	699.67 <sup>a</sup>	776.85 <sup>a</sup>	757.75 <sup>a</sup>	694.45 <sup>a</sup>	639.08 <sup>a</sup>
	2017	521.02 <sup>b</sup>	619.95 <sup>b</sup>	614.15 <sup>b</sup>	689.45 <sup>b</sup>	595.73 <sup>b</sup>	554.58 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	16.39	49.53	26.72	52.22	23.05	23.28
	ECO	593.68 <sup>a</sup>	663.43 <sup>a</sup>	700.10 <sup>a</sup>	780.75 <sup>a</sup>	659.40 <sup>a</sup>	625.70 <sup>a</sup>
	CONV	569.73 <sup>b</sup>	656.18 <sup>a</sup>	690.90 <sup>a</sup>	666.45 <sup>b</sup>	630.78 <sup>b</sup>	567.97 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	16.39	49.53	26.72	52.22	23.05	23.28
Total content of phenolic acids (mg/kg DM)	2016	767.60 <sup>a</sup>	793.49 <sup>a</sup>	769.16 <sup>a</sup>	780.58 <sup>a</sup>	843.78 <sup>a</sup>	909.26 <sup>a</sup>
	2017	661.61 <sup>b</sup>	764.21 <sup>a</sup>	734.29 <sup>a</sup>	642.95 <sup>b</sup>	793.19 <sup>a</sup>	789.69 <sup>a</sup>
	<i>HSD</i> <sub>0.05</sub>	27.28	45.08	51.03	31.08	51.18	40.76
	ECO	736.39 <sup>a</sup>	795.07 <sup>a</sup>	751.86 <sup>a</sup>	775.32 <sup>a</sup>	839.99 <sup>a</sup>	883.15 <sup>a</sup>
	CONV	692.82 <sup>b</sup>	762.63 <sup>a</sup>	751.59 <sup>a</sup>	648.21 <sup>b</sup>	796.99 <sup>a</sup>	815.80 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	27.28	45.08	51.03	31.08	61.18	40.76
Total content of carotenoids (mg/kg DM)	2016	2.16 <sup>a</sup>	7.10 <sup>a</sup>	2.72 <sup>a</sup>	1.96 <sup>a</sup>	2.07 <sup>a</sup>	1.97 <sup>a</sup>
	2017	1.07 <sup>b</sup>	3.54 <sup>b</sup>	1.64 <sup>b</sup>	1.15 <sup>b</sup>	1.16 <sup>b</sup>	1.31 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	0.07	0.16	0.03	0.05	0.05	0.04
	ECO	1.62 <sup>a</sup>	5.29 <sup>a</sup>	2.29 <sup>a</sup>	1.63 <sup>a</sup>	1.64 <sup>a</sup>	1.66 <sup>a</sup>
	CONV	1.60 <sup>a</sup>	5.34 <sup>a</sup>	2.06 <sup>b</sup>	1.48 <sup>b</sup>	1.59 <sup>b</sup>	1.62 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	0.07	0.16	0.03	0.05	0.05	0.03
Total content of anthocyanins (mg/kg DM)	2016	nd	nd	14.02 <sup>a</sup>	22.60 <sup>a</sup>	63.21 <sup>a</sup>	69.81 <sup>a</sup>
	2017	nd	nd	11.38 <sup>b</sup>	18.34 <sup>b</sup>	51.29 <sup>b</sup>	56.65 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	nd	nd	1.79	2.16	3.49	3.87
	ECO	nd	nd	13.09 <sup>a</sup>	21.09 <sup>a</sup>	59.00 <sup>a</sup>	65.17 <sup>a</sup>
	CONV	nd	nd	12.31 <sup>a</sup>	19.84 <sup>a</sup>	55.48 <sup>b</sup>	61.27 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	nd	nd	1.79	2.16	3.49	3.87
Total antioxidant activity (mg/kg DM)	2016	169.40 <sup>a</sup>	202.92 <sup>a</sup>	217.72 <sup>a</sup>	211.42 <sup>a</sup>	236.48 <sup>a</sup>	222.57 <sup>a</sup>
	2017	155.97 <sup>b</sup>	187.32 <sup>b</sup>	207.68 <sup>b</sup>	190.92 <sup>b</sup>	216.93 <sup>b</sup>	190.05 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	5.79	9.62	8.38	5.69	6.60	20.74
	ECO	166.35 <sup>a</sup>	196.83 <sup>a</sup>	212.82 <sup>a</sup>	207.32 <sup>a</sup>	232.32 <sup>a</sup>	217.05 <sup>a</sup>
	CONV	159.02 <sup>b</sup>	193.40 <sup>a</sup>	212.58 <sup>a</sup>	195.02 <sup>b</sup>	221.10 <sup>b</sup>	195.57 <sup>b</sup>
	<i>HSD</i> <sub>0.05</sub>	5.79	9.62	8.38	5.69	6.60	20.74
Grain yield (t/ha)	2016	7.83 <sup>b</sup>	7.56 <sup>b</sup>	8.07 <sup>b</sup>	7.23 <sup>b</sup>	7.12 <sup>b</sup>	6.44 <sup>b</sup>
	2017	8.57 <sup>a</sup>	8.00 <sup>a</sup>	8.36 <sup>a</sup>	7.57 <sup>a</sup>	8.38 <sup>a</sup>	7.67 <sup>a</sup>
	<i>HSD</i> <sub>0.05</sub>	0.37	0.22	0.37	0.16	0.23	0.27
	ECO	7.53 <sup>b</sup>	7.06	7.57 <sup>b</sup>	6.82 <sup>b</sup>	6.86 <sup>b</sup>	6.24 <sup>b</sup>
	CONV	8.87 <sup>a</sup>	8.49	8.86 <sup>a</sup>	7.97 <sup>a</sup>	8.63 <sup>a</sup>	7.87 <sup>a</sup>
	<i>HSD</i> <sub>0.05</sub>	0.37	0.22	0.37	0.16	0.23	0.27

DM – dry matter; nd – non detected; ECO – organic; CONV – conventional cropping system

but differences between them and cultivar with yellow endosperm were insignificant in some of cases. The TAA in the control cv. Annie was the lowest and significantly different from the others.

**The effect of weather conditions.** Besides the genotype, a significant impact of weather conditions on all evaluated antioxidant compounds contents was determined and it prevailed in TPC,

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PAs and TCC. The 2016 season was marked by slightly higher average temperatures and substantially lower precipitation during the time from anthesis to wheat maturity compared to 2017. Therefore, it could be possible to suppose that in 2016, when the contents of all evaluated antioxidant compounds were higher, evaluated wheat genotypes were exposed to higher weather stress. The fact that many antioxidants are produced by plants in response to abiotic stress, like water stress and/or heat stress has been known (Barański et al. 2014). Our results are in agreement with findings of Paznocht et al. (2018) and Fratianni et al. (2013) who observed an increased carotenoid synthesis in wheat with coloured grain grown under water deficit or Alexieva et al. (2001) who registered an increase in soluble phenols and anthocyanins in wheat subjected to drought.

**The effect of the cropping system.** Our results (Table 3) show that cropping system affects significantly the content of all evaluated antioxidant compounds, although at lower level compared to year and genotype. It is evident from the results (Table 4) that there were statistically significant differences among the cropping systems in the content of all evaluated antioxidant compounds – higher contents of antioxidants in organic cropping system were determined. Organic and conventional wheat usually differed significantly in concentrations of the determined compounds even in individual genotypes, although not in all the cases (Table 5).

The results generally confirm the conclusions of Barański et al. (2014) that organic crops are usually richer in antioxidant compounds and are in accordance with the findings of some other authors, too. Zuchowski et al. (2011) evaluated the content of selected phenolic acids in wheat from organic and conventional cropping systems. Their study demonstrated statistically significant influence of cropping system on the level of total phenolic acid content in favour of organic cultivation, although no differences in some of phenolic acids were observed. Levels of phenolics in organic crops were reported to be significantly higher in some publications concerning fruits and vegetables (Mitchell et al. 2007, Stracke et al. 2009b). On the other hand, the study of Stracke et al. (2009a) was performed to evaluate the concentrations of carotenoids and phenolic acids in wheat cultivars grown under organic and conventional conditions.

The results indicate that climate factors have a greater impact on the phytochemical concentrations in the wheat grain than the production method (organic/conventional).

Elevated concentrations of antioxidant compounds in organic products can be explained by changes in plant metabolism caused by differences in the soil nitrogen availability in organic and conventional management methods (carbon/nutrient balance hypothesis) (Bloksma et al. 2007, Massad et al. 2012). In organic production, in which no synthetic fertilizers are allowed, nitrogen availability is usually expected to be lower. This leads to intensification of biosynthesis of carbon-containing compounds, including non-nitrogen secondary metabolites. When nitrogen is more readily available, plants will more intensively synthesise proteins and other nitrogen-containing compounds (Zuchowski et al. 2011). However, it should be also taken into account that the probable N deficiency of organically cultivated fields can lead to organic cereal grains with lower values of TKW. As smaller wheat kernels have a higher surface/volume ratio, they also have a higher percentage of pericarp and aleurone layer, parts containing the majority of antioxidant compounds (Zuchowski et al. 2011).

In our experiments, the same preceding crop (red clover) was used in both cropping systems. In the conventional system, the total dose of nitrogen of 120 kg N/ha applied in mineral fertilizer (nitrate form) in two partial doses of 60 kg N/ha was used. Despite the fact there are not sufficient data to discuss the potential effect of nitrogen availability on evaluated antioxidant compounds in wheat grain on the basis of soil parameters, it is possible to presume that the availability of nitrogen in the conventional cropping system was higher. This indicates the fact that the yields of grain in the conventional cropping system were by 17% in genotypes cvs. AF Jumiko and PS Karkulka, 18% in cv. Annie, 20% in cv. Citrus, 26% in cvs. Skorpion and KM 53-14 higher compared to the genotypes cultivated organically.

Moreover, it is well known that many of antioxidants found in higher concentrations in organic crops are produced by plants in response to biotic (pest attacks and disease) stress and form a part of plant's constitutive and inducible resistance mechanisms to pest and diseases (Nicholson and Hammerschmidt 1992). In our field trials in comparison with conventional cultivation, wheat genotypes grown under organic cropping system were

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much more damaged by the pests of *Oulema* spp. and fungal diseases caused by *Puccinia* spp. and *Phaeosphaeria nodorum*. Therefore, it is possible to assume they were exposed to higher biotic stress, compared to genotypes cultivated conventionally, with fungicide and insecticide protection. However, Almuayrifi (2013) demonstrated that non-use of synthetic pesticides and fungicides had no effect on phenolic acid and flavonoid concentrations. According to Barański et al. (2014) there are no sound published data for a causal link between higher pest/disease incidence and antioxidant concentrations in organic crops.

**Grain yield.** The successful introduction of cultivars with coloured grain into practice will depend on the level of yield and agronomic properties comparable to commercially used cultivars. At present, yields of coloured wheat genotypes are usually lower compared to traditional commercial cultivars. However, selected pigmented lines with commercial potential are able to give yield equivalent to the high yielding cultivars (Garg et al. 2016). In Austria, a commercial cv. Skorpion with blue grain was released with about 25% lower yield in comparison with the control cultivars (Martinek et al. 2014). Cv. Skorpion, included even to our wheat set, reached the yield only about 14% lower in comparison with the control cultivar. Moreover, cv. AF Jumiko with purple pericarp reached the same yield as the control cv. Annie.

In conclusion, variation of antioxidant compounds in the wheat grain depends on genotype, weather conditions and cultivation system. Organic cultivation may help increase the level of antioxidants in wheat grain. Coloured wheat cultivars are the important source of colour components and therefore they can be used for specific production of baking products, mainly made from the whole grain. Moreover, some of them are able to give the same or almost the same grain yields as traditional wheat cultivars.

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## 4.5 Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year

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### Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year

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**Abstract:** The study aimed at evaluating the total content of polyphenols (TPC) and phenolic acids (TPA) in grain of 4 spring einkorn, 4 emmer, 4 spelt and 4 common wheat genotypes cultivated under organic cropping system in two-year trials. The TPC and TPA were significantly affected both by genotype, wheat species and crop year (weather conditions). At the same time, TPC and TPA were mainly affected by the crop year while the effect of genetic factors was lesser. The TPC ranged from 618 mg/kg DM (dry matter) (common wheat cv. Annie) to 792.37 mg/kg DM (*Triticum monococcum* GEO) and TPA from 700.66 mg/kg DM (cv. Annie) to 874.74 mg/kg DM (Schwedishes einkorn) in an average of two-year results. Related to the wheat species, total content of polyphenols was in order einkorn > emmer > common wheat > spelt, total content of phenolic acids in order einkorn > spelt > emmer > common wheat. Higher TPC and TPA were observed in the very dry year 2018.

**Keywords:** hulled wheat; organic farming; antioxidant compounds; cereal; phytochemicals

Wheat is the most widely grown food crop. The consumption of wheat grain and especially whole grain products is associated with several health benefits which may be related in part to the contents of different phytochemicals. They can act as antioxidants and belong to chemically different groups of antioxidant compounds such as polyphenols, carotenoids, phytosterols (Lachman et al. 2012) or phenolic acids (Liu et al. 2008). Phenolic compounds are considered

as a major group of compounds that contribute to the antioxidant activity of cereals (Fogarasi et al. 2015). They are excellent oxygen radical scavengers, with an electron reduction potential lower than the oxygen radicals. Strong antioxidant activity of phenolic compounds leads to beneficial anti-inflammatory, anti-microbial, anti-thrombotic, anti-atherogenic, vasodilatory and cardio-protective effects on human health (Brandolini et al. 2008). In recent years,

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many studies have dealt with antioxidant compounds in common wheat or durum wheat (Stracke et al. 2009, Ficco et al. 2014, Martini et al. 2015).

Current trends towards organic and low-impact agriculture, as well as an increase in the utilization of organic food products, provide wider possibilities also for use of hulled wheat species (Brandolini et al. 2008). Hulled wheat species *Triticum dicoccum* (Schränk) Schuebl, *T. monococcum* L. and *T. spelta* L., also known as emmer, einkorn and spelt, respectively, were among the earliest *Triticeae* domesticated by a human (Chrprová et al. 2013). Despite their limited commercial availability, consumer's demand for einkorn, emmer and spelt is currently increasing because bakery and other cereal products derived thereof are widely perceived as 'healthy' alternatives to those made of bread wheat (Ziegler et al. 2016). Moreover, these wheat species could be an alternative to common wheat especially in organic farming with a wider diversity of crops. They are cultivated in organic farms in Europe not only because they are believed to have a higher nutritive value in comparison with common wheat, but also due to their higher resistance to unfavourable environmental conditions as well as lower fertilization and soil requirements (Konvalina et al. 2012). However, information regarding to antioxidant compounds in hulled wheat species cultivated under organic cropping system is still scarce, although this knowledge is important both for breeding and for organic farming.

Despite relatively high genetic weighing, some environmental factors such as the specific climate parameters (rainfall and temperature) influence the antioxidant compounds development, too (Ficco et al. 2014). Some authors have observed an increased antioxidants synthesis in different cereals grown under water deficit and higher average temperatures during the grain filling (Paznocht et al. 2018). The results of Lu et al. (2015) showed that environment factors (E), including precipitation and temperature stress, had a stronger influence on the selected health-beneficial components and antioxidant properties of soft winter wheat than genotype (G) or G × E interaction. Related to the phenolic compounds, they are secondary metabolites synthesized during plant development in response to stress conditions. Therefore, environmental stresses that induce oxidative damage often promote the synthesis of phenolic metabolites, which act as phytoalexins and safeguard cell wall integrity (Brandolini et al. 2013).

The objective of this study was to determine total contents of polyphenols and phenolic acids in the

collection of spring einkorn, emmer, spelt and, for comparison, common wheat genotypes grown under organic cropping system to identify the richest sources for potential improving the nutritional value of different wheat products. The understanding of the genotype, year (weather conditions) and G × Y effects on evaluated antioxidant compounds could also be used for improving the breeding efforts to produce hulled wheats grain rich in selected health components. Therefore, in addition to the effect of genotypes and wheat species on polyphenols and phenolic acids content, the effect of weather conditions (i.e. temperature and precipitation) was investigated, too.

### MATERIAL AND METHODS

**Plant material.** The collection of 12 spring hulled wheat genotypes was cultivated in the exact field plot trials, carried out during the 2017 and 2018 growing seasons at the experimental station of the Czech University of Life Sciences in Prague-Uhřetěves (central part of Bohemia, 295 m a.s.l., average annual temperature 8.4°C, average sum of precipitation 575 mm). The collection involved 4 einkorn wheat, 4 emmer wheat and 4 spelt wheat genotypes (both present cultivars, old landraces and accessions obtained from the Gene Bank of the Crop Research Institute Prague). The collection was supplemented by 4 common wheat genotypes (as a control).

The field trials with evaluated wheat genotypes were established using random blocks, in 3 replicates, with an experimental plot average area of 10 m<sup>2</sup>. The trials were carried out under an organic cropping system. Red clover was used as a preceding crop of wheat. Treatment of the wheat stands by weeding harrows was used during the vegetation; no fertilizers nor pesticides were applied. After the harvest, the yield of a grain of evaluated genotypes was observed, and hulled spikelets of einkorn, emmer and spelt were dehulled using a dehulling laboratory machine.

As for the weather conditions (Table 1), the period of wheat heading, flowering, grain filling and maturing both in 2017 and 2018 was generally similar in temperatures. As to precipitation, the year 2018 was very dry and reached only 33% of precipitation in the evaluated period compared to 2017.

**Grain samples.** Grain samples obtained after the field plot trials harvest were ground using IKA analytical mill (Janke & Kunkel Co., Staufen, Germany) to pass through 0.5 mm screen (35 mesh) and ho-



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Table 1. Weather conditions in decades from heading to grain maturity

Decade	Month	2017		2018	
		average temperature (°C)	Σ of precipitation (mm)	average temperature (°C)	Σ of precipitation (mm)
1 <sup>st</sup>	June	20.0	13.2	17.7	11.4
2 <sup>nd</sup>	June	19.8	62.8	18.6	6.6
3 <sup>rd</sup>	July	20.5	13.2	19.4	15.0
4 <sup>th</sup>	July	19.2	12.6	22.5	6.0
5 <sup>th</sup>	July	19.8	41.8	25.7	9.6
6 <sup>th</sup>	August	24.8	3.0	21.6	0.0
Average temperature and Σ of precipitation in the evaluated period		20.7	146.6	20.9	48.6
Long-term standard				17.3	74.0

mogenised well. Dry matter (DM) was determined by drying of a meal at 105°C for 24 h. Three replicates were made in all of the following analyses.

**Total polyphenols content (TPC).** The TPC was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of the meal was extracted with 25 mL of 0.1% HCl in methanol. 2 mL of extract was reacted with 2.5 mL of Folin-Ciocalteu reagent with the addition of 7.5 mL of 20% sodium carbonate and filled up with pure water to 50 mL. After 2 h the solution was measured spectrophotometrically at 765 nm. The results were quantified using external calibration and expressed as mg of gallic acid equivalent per kg of DM.

**Total phenolic acids content (TPA).** For extraction and chromatographic separation, a method published by Martini et al. (2015) with some modifications was used. Briefly, 0.25 g of the meal was hydrolysed with 14 mL of 2 mol/L aqueous sodium hydroxide for 1 h at the room temperature. 7 mL of 4 mol/L HCl was added to adjust acidic pH (1–2). Two mL of hydrolysate were transferred into 8 mL glass vial and twice extracted with 2 mL of ethylacetate. Combined supernatants of upper organic phase were removed to another glass vial, evaporated to dryness under the nitrogen stream and reconstituted with 1 mL of 70% aqueous methanol, filtered through a syringe filter into an amber glass vial and analysed by HPLC-DAD. The analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with a diode array detector. The analytes were separated by an Omnispher C18 HPLC column (250 × 4.6 mm; particle size 5 µm; Agilent, Inc., Santa Clara, USA) and detected at two different wavelengths 280 nm and 325 nm. The results were expressed in mg of the analyte per kg of DM.

**Statistical analysis.** The results were statistically analysed by the analysis of variance (ANOVA) method. The differences between mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS program (SAS Institute, Carry, USA), version 9.4 at the level of significance  $P = 0.05$ .

### RESULTS AND DISCUSSION

**Total polyphenols content.** The results of ANOVA related to the 16 wheat genotypes belonging to the four wheat species and grown over two trial years are given in Table 2. The analysis shows that the TPC was significantly affected both by genotype, wheat species (S), crop year (Y) and interaction ( $G \times Y$ ). However, differences in the impact of individual factors were observed. The TPC was mostly affected by Y (58.44%) and wheat species (20.92%), while the effects of genotype (12.97%) and interaction  $G \times Y$  (7.68%) were lesser.

Table 2. The effect of genotype, wheat species, crop year and interaction  $G \times Y$  (% of the total mean square) on the content of evaluated antioxidants in the wheat grain (ANOVA)

	TPC	TPA
	(%)	
Genotype (G)	12.97***	1.23***
Wheat species (S)	20.92***	28.57***
Year (Y)	58.44***	69.25***
$G \times Y$	7.68***	0.95***

\*\*\* $P < 0.001$ ; TPC – total content of polyphenols; TPA – total content of phenolic acids

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The results of the Tukey's *HSD* test describing significance between the mean values of genotypes, wheat species and years is shown in Table 3, a more detailed view on individual genotypes is given in Table 4. The TPC ranged from 556.57 mg/kg DM (common wheat cv. Annie, 2017) to 849.73 mg/kg DM (*T. monococcum* GEO, 2018) (Table 4).

The differences for the TPC of the wheat genotypes in previous studies might be due to differences of genotypes as well as differences in environmental conditions and environment × genotype interaction. Our results are similar to those of Lachman et al. (2012) who recorded the TPC from 502 to 748 mg/kg DM in the collection of einkorn, emmer and bread wheat cultivars and accessions. On the other hand,

total polyphenol contents in the collection of *T. monococcum*, *T. dicoccum*, *T. durum*, *T. spelta* and *T. aestivum* genotypes evaluated by Brandolini et al. (2013) were higher in comparison with our results and varied from 1075 to 1374 mg/kg DM. On the contrary, the TPC values in grain samples of different cereal species, including einkorn, were relatively low and ranged from 349 to 593 mg/kg DM (Fogarasi et al. 2015).

Our results confirmed the existence of differences among wheat species – einkorn reached the highest content of total polyphenols (744.97 mg/kg DM), followed by emmer (705.28 mg/kg DM) common wheat (702.15 mg/kg DM) and spelt (694.99 mg/kg DM). However, the only einkorn differed from other wheat species significantly (Table 3).

Table 3. The content of total polyphenols (TPC) and phenolic acids (TPA) in the wheat genotypes, species and years (means ± standard deviation and the Tukey's *HSD* test results)

	TPC	TPA
	(mg/kg DM)	
<i>Triticum monococcum</i> 44*	757.93 ± 56.92 <sup>ab</sup>	779.43 ± 109.64 <sup>efg</sup>
<i>T. monococcum</i> 38*	792.37 ± 59.07 <sup>a</sup>	817.54 ± 43.29 <sup>bcd</sup>
<i>T. monococcum</i> No. 8910*	721.07 ± 19.36 <sup>bcde</sup>	837.44 ± 53.00 <sup>b</sup>
Schwedisches einkorn*	708.50 ± 12.35 <sup>cde</sup>	874.74 ± 76.03 <sup>a</sup>
<i>T. dicoccum</i> Brno**	695.95 ± 35.02 <sup>cde</sup>	792.66 ± 35.87 <sup>cdef</sup>
<i>T. dicoccum</i> Dagestan**	692.62 ± 43.42 <sup>de</sup>	773.29 ± 49.06 <sup>fg</sup>
Weiser Sommer**	724.80 ± 24.25 <sup>bcde</sup>	816.29 ± 24.29 <sup>bcde</sup>
Rudico**	707.75 ± 17.29 <sup>cde</sup>	777.49 ± 37.46 <sup>fg</sup>
<i>T. spelta</i> No. 8930***	715.47 ± 25.88 <sup>bcde</sup>	823.26 ± 62.83 <sup>bc</sup>
Špaldá bílá jarní***	695.60 ± 26.64 <sup>cde</sup>	802.46 ± 57.17 <sup>bcdef</sup>
<i>T. spelta</i> Kew***	682.77 ± 16.03 <sup>e</sup>	783.89 ± 20.97 <sup>def</sup>
<i>T. spelta</i> VIR St. Petersburg***	686.13 ± 18.38 <sup>e</sup>	805.74 ± 40.08 <sup>bcdef</sup>
Izzy****	736.70 ± 45.45 <sup>bc</sup>	742.62 ± 52.44 <sup>gh</sup>
Jara****	718.17 ± 12.45 <sup>bcde</sup>	705.07 ± 44.87 <sup>i</sup>
Astrid****	734.90 ± 46.92 <sup>bcd</sup>	715.48 ± 62.72 <sup>hi</sup>
Annie****	618.83 ± 62.92 <sup>f</sup>	700.66 ± 62.57 <sup>i</sup>
<i>HSD</i> <sub>0.05</sub>	43.82	37.19
Einkorn	744.97 ± 53.79 <sup>a</sup>	827.29 ± 82.50 <sup>a</sup>
Emmer	705.28 ± 34.03 <sup>b</sup>	789.93 ± 39.95 <sup>c</sup>
Spelt	694.99 ± 25.60 <sup>b</sup>	803.84 ± 50.10 <sup>b</sup>
Common wheat	702.15 ± 66.83 <sup>b</sup>	715.96 ± 58.62 <sup>d</sup>
<i>HSD</i> <sub>0.05</sub>	16.21	13.76
2017	693.05 ± 24.85 <sup>b</sup>	734.60 ± 45.46 <sup>b</sup>
2018	730.65 ± 63.41 <sup>a</sup>	833.91 ± 60.36 <sup>a</sup>
<i>HSD</i> <sub>0.05</sub>	8.68	7.37

\*einkorn genotypes; \*\*emmer genotypes; \*\*\*spelt genotypes; \*\*\*\*common wheat genotypes; *HSD* – honestly significant difference; DM – dry matter

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Table 4. The effect of crop year on the content of total polyphenols (TPC) and phenolic acids (TPA) in a grain of individual wheat genotypes (means  $\pm$  standard deviation and Tukey's *HSD* test results)

Genotype	Origin	Year	TPC		TPA		<i>HSD</i> <sub>0.05</sub>	
			(mg/kg DM)		TPC	TPA	TPC	TPA
<i>Triticum monococcum</i> 44	ALB	2017	731.87 $\pm$ 12.70 <sup>b</sup>	670.29 $\pm$ 8.96 <sup>b</sup>	42.50	29.13		
		2018	784.00 $\pm$ 70.72 <sup>a</sup>	888.56 $\pm$ 11.82 <sup>a</sup>				
<i>T. monococcum</i> 38	GEO	2017	735.00 $\pm$ 19.71 <sup>b</sup>	777.05 $\pm$ 7.83 <sup>b</sup>	39.14	42.51		
		2018	849.73 $\pm$ 3.03 <sup>a</sup>	858.03 $\pm$ 20.19 <sup>a</sup>				
<i>T. monococcum</i> No. 8910	DNK	2017	707.00 $\pm$ 7.87 <sup>a</sup>	786.14 $\pm$ 15.97 <sup>b</sup>	36.92	37.01		
		2018	735.13 $\pm$ 17.08 <sup>a</sup>	888.74 $\pm$ 10.02 <sup>a</sup>				
Schwedisches einkorn	SWE	2017	711.10 $\pm$ 9.49 <sup>a</sup>	802.03 $\pm$ 23.82 <sup>b</sup>	33.53	48.65		
		2018	705.90 $\pm$ 14.20 <sup>a</sup>	947.46 $\pm$ 20.47 <sup>a</sup>				
<i>T. dicoccum</i> Brno	CSK	2017	662.37 $\pm$ 3.39 <sup>b</sup>	735.66 $\pm$ 2.19 <sup>b</sup>	27.50	21.66		
		2018	729.53 $\pm$ 13.62 <sup>a</sup>	849.67 $\pm$ 5.52 <sup>a</sup>				
<i>T. dicoccum</i> Dagestan	RUS	2017	649.73 $\pm$ 5.43 <sup>b</sup>	711.86 $\pm$ 3.98 <sup>b</sup>	18.98	26.65		
		2018	735.50 $\pm$ 7.94 <sup>a</sup>	834.73 $\pm$ 7.49 <sup>a</sup>				
Weiser Sommer	DEU	2017	703.53 $\pm$ 14.33 <sup>b</sup>	767.56 $\pm$ 8.51 <sup>b</sup>	32.37	26.63		
		2018	746.07 $\pm$ 8.15 <sup>a</sup>	865.02 $\pm$ 10.56 <sup>a</sup>				
Rudico	CZE	2017	692.90 $\pm$ 2.01 <sup>b</sup>	734.40 $\pm$ 3.08 <sup>b</sup>	24.57	24.08		
		2018	722.60 $\pm$ 12.36 <sup>a</sup>	820.57 $\pm$ 11.87 <sup>a</sup>				
<i>T. spelta</i> No. 8930	DNK	2017	693.40 $\pm$ 9.55 <sup>b</sup>	762.15 $\pm$ 18.59 <sup>b</sup>	37.56	40.56		
		2018	737.53 $\pm$ 16.58 <sup>a</sup>	884.36 $\pm$ 9.01 <sup>a</sup>				
Špalda bílá jarní	CSK	2017	677.57 $\pm$ 21.35 <sup>b</sup>	746.73 $\pm$ 4.58 <sup>b</sup>	31.15	35.42		
		2018	713.63 $\pm$ 17.70 <sup>a</sup>	858.19 $\pm$ 17.45 <sup>a</sup>				
<i>T. spelta</i> Kew	GBR	2017	675.03 $\pm$ 6.11 <sup>a</sup>	769.80 $\pm$ 10.22 <sup>b</sup>	38.98	31.12		
		2018	690.50 $\pm$ 18.89 <sup>a</sup>	797.97 $\pm$ 19.45 <sup>a</sup>				
<i>T. spelta</i> VIR St. Petersburg	CSK	2017	676.27 $\pm$ 8.20 <sup>a</sup>	769.18 $\pm$ 15.07 <sup>b</sup>	37.65	45.63		
		2018	696.00 $\pm$ 20.33 <sup>a</sup>	842.29 $\pm$ 17.69 <sup>a</sup>				
Izzy	CZE	2017	692.10 $\pm$ 4.81 <sup>b</sup>	694.12 $\pm$ 19.66 <sup>b</sup>	27.53	43.79		
		2018	781.30 $\pm$ 13.17 <sup>a</sup>	791.11 $\pm$ 19.08 <sup>a</sup>				
Jara	CSK	2017	710.60 $\pm$ 13.36 <sup>a</sup>	668.62 $\pm$ 15.51 <sup>b</sup>	27.44	34.81		
		2018	725.73 $\pm$ 4.08 <sup>a</sup>	741.51 $\pm$ 8.60 <sup>a</sup>				
Astrid	CZE	2017	689.17 $\pm$ 3.54 <sup>b</sup>	679.86 $\pm$ 18.33 <sup>b</sup>	29.12	42.59		
		2018	780.63 $\pm$ 14.40 <sup>a</sup>	751.10 $\pm$ 32.11 <sup>a</sup>				
Annie	CZE	2017	556.57 $\pm$ 6.16 <sup>b</sup>	620.66 $\pm$ 13.57 <sup>b</sup>	25.05	33.03		
		2018	681.10 $\pm$ 11.17 <sup>a</sup>	780.66 $\pm$ 9.94 <sup>a</sup>				

*HSD* – honestly significant difference; DM – dry matter

Our results are in agreement with those of Fogarasi et al. (2015) who observed higher TPC in einkorn compared to other wheat species. Also, the results of Şahin et al. (2017) showed that TPC of einkorn was significantly higher than TPC of bread and durum wheat. Lachman et al. (2012) recorded that the highest TPC was found for emmer genotypes, but also some einkorn and bread wheat genotypes were rich in the TPC. Similarly, Serpen et al. (2008) stated

that emmer had higher TPC than einkorn and the bread wheat controls. Abdel-Aal and Rabalski (2008) reported that einkorn, emmer and spelt had similar values, significantly higher than those of bread wheat.

Significantly, the prevailing impact of weather conditions on TPC values was determined. The 2018 season was marked by similar temperatures and substantially lower precipitation during the time from heading to the grain maturity compared to 2017

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(Table 1). Therefore, it could be possible to suppose that in 2018, when the TPC was significantly higher, evaluated wheat genotypes were exposed to higher weather stress. The fact that many antioxidants are produced by plants in response to abiotic stress, like water stress and heat stress, is known (Lu et al. 2015). Our results are in agreement with the findings of Lachman et al. (2012) who recorded significant changes between two cropping years in their study of emmer, einkorn and bread wheat genotypes and concluded that the superior total polyphenol contents were a consequence of lower rainfall and higher temperatures during the ripening stages of cereals. Similarly, Stracke et al. (2009) registered significant differences in TPC of the wheat grain in dependence on the year (weather conditions).

**Total phenolic acids content.** Similarly to total polyphenols content, total phenolic acids content was significantly affected by genotype, wheat species, crop year and interaction ( $G \times Y$ ) (Table 2). The results confirmed a strong effect of the year (69.25%) and wheat species (28.57%), while the effect of genotype (1.23%) was relatively low, probably due to lower genotypes variability within individual wheat species; the low effect was observed in interaction  $G \times Y$  (0.95%), too.

Phenolic acids represent the most common phenolic compounds in cereal grains (Martini et al. 2015). Our results showed that TPA content was in total higher compared to the total polyphenols content and ranged from 700.66 mg/kg DM (common wheat cv. Annie) to 874.74 mg/kg DM (Schwedishes einkorn) in an average of two-year results (Table 3). In total, the lowest TPA value (620.66 mg/kg DM) was observed in cv. Annie (2017), the highest (947.46 mg/kg DM) in Schwedishes einkorn (2018) (Table 4). Brandolini et al. (2013), on the basis of their investigation of different wheat species, reported that phenolic acids (conjugated + bound fractions) content varied from 477 mg/kg DM to 687 mg/kg DM. The total content of conjugated and bound phenolic acids evaluated by Hidalgo and Brandolini (2017) in three einkorn accessions during three years ranged from 524.00 to 672.20 mg/kg DM. Li et al. (2008) recorded the highest phenolic acids content in emmer ( $779 \pm 109$  mg/kg DM), durum ( $699 \pm 51$  mg/kg DM) and bread wheat ( $664 \pm 15$  mg/kg DM); nevertheless, these values did not differ significantly from those of spelt ( $579 \pm 57$  mg/kg DM) and einkorn ( $615 \pm 74$  mg/kg DM). The content of total phenolic acids (sum of free, conjugated and bound phenolic acids) in 10 durum wheat cultivars grown during 3 crop

years ranged from 856.6 to 1464.0 mg/kg DM (Martini et al. 2015). The method for TPA content evaluation described by Martini et al. (2015) was used in our study as well. Slightly lower TPA values of our wheat samples compared to those of Martini et al. (2015) could be probably connected with different environmental conditions as well as different wheat species and genotypes.

Our results confirmed the existence of differences among wheat species – einkorn reached the highest content of total phenolic acids (827.29 mg/kg DM), followed by spelt (803.84 mg/kg DM), emmer (789.93 mg/kg DM) and common wheat (715.96 mg/kg DM). There were significant differences among all of the wheat species (Table 3). Contradictory results related to the TPA content in different wheat species are described in different studies. Brandolini et al. (2013) reported that *T. durum* and *T. aestivum*, followed by *T. spelta*, *T. monococcum* and *T. dicoccum* showed the highest total phenolic acids content, while *T. turanicum* reached the lowest TPA concentration. Serpen et al. (2008) stated that emmer has more total phenolic acids than einkorn and two bread wheat controls.

Significantly prevailing impact of weather conditions on TPA was determined; higher TPA content in the very dry year 2018 was observed (Tables 3 and 4). The influence of the crop year on phenolic acids concentration has been scantily studied. A strong influence of the year on the content of phenolic acids was reported by Lachman et al. (2011) for *T. monococcum*, *T. dicoccum* and *T. aestivum*. Also, Stracke et al. (2009) studying the effects of two production methods (traditional and organic) for three years on the TPA content stated that the year effect was the most important.

In conclusion, the results indicate a high antioxidant potential of the hulled wheat species. Thus, they could be an opportunity for wheat breeders as well as commercial organic wheat growers. As to the stress factors, the usually decrease yield; however, on the other hand they provide opportunities for production of nutritionally profitable secondary metabolites. Therefore, further studies on these subjects need to be carried out.

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## 4.5 Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year

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## 5. Sumární diskuse

Publikace *Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains* (Paznocht et al. 2018) byla prvotním screeningem dostupných genotypů obilovin z hlediska obsažených karotenoidních barviv. Cílem bylo identifikovat a kvantifikovat jednotlivé karotenoidy v zrnech tzv. barevných genotypů pšenice a odhalit genotypy schopné syntézy a ukládání xantofylových esterů. Na základě získaných dat porovnat pigmentované pšenice s pšenicí běžně pěstovanou (tedy standardní barvou zrna označovanou jako *red*). Dále pak odhalit vliv prostředí na obsah karotenoidů srovnáním vzorků ze dvou sklizňových ročníků s velmi odlišným průběhem počasí.

V rámci studie bylo sledováno pět genotypů pšenice s Pp, pět s Ba, jedna s Ye a pro srovnání byly zařazeny vždy po jednom genotypu konvenčních odrůd pšenice (*red*), ječmene a tritordea. Kontrolní pšenice Bohemia se standardní barvou zrna celkově dosáhla průměrného obsahu karotenoidů (TCC; Total Carotenoid Content) 3,56 µg/g sušiny zrna, tedy hodnoty lehce převyšující tradiční pšenice seté 2,33 µg/g (Giordano et al. 2017) i pšenice tvrdé zkoumané jinými autory 3,02 µg/g (Hidalgo et al. 2006) nebo 3,10 µg/g (Digesù et al. 2009), která je i pro svůj obsah karotenoidů tradičně využívána pro výrobu těstovin (Hidalgo et al. 2010). Podobný obsah byl naměřen taktéž ve skupině Pp pšenic (3,60 µg/g). Nutno však zmínit značné rozdíly v TCC pozorované v rámci skupiny Pp i mezi oběma sklizňovými ročníky (sklizeň 2014: 2,29-4,70 µg/g; sklizeň 2015: 2,78-7,53 µg/g). Hodnoty TCC v Pp pšenicích, které uvádí jiné literární prameny, jsou rovněž poněkud rozdílné: 2,62 µg/g (Ndolo & Beta 2013) vs. 7,38 µg/g (Ficco et al. 2016), obě hodnoty jsou vyjádřeny jako ekvivalent luteinu, nejhojnějšího z karotenoidů v pšeničném zrně. Variabilita uváděných hodnot je pravděpodobně dána použitím odlišných genotypů, různými podmínkami prostředí jakož i analytickou metodou stanovení. Nižším průměrným TCC (2,41 µg/g) se oproti Pp vyznačovaly Ba pšenice (sklizeň 2014: 0,94-2,71 µg/g; sklizeň 2015: 2,74-3,69 µg/g). Průměrně nižší množství karotenoidů v Ba pšenicích (1,53 µg/g) v porovnání s Pp (2,01 µg/g) zjistili také Giordano et al. (2017). Zvláštním zástupcem skupiny pšenic s modrým aleuronem byla pšenice s pracovním označením V1 131-15 vytvořená v Zemědělském výzkumném ústavu Kroměříž, dosahující několikanásobně (2-4×) vyššího obsahu karotenoidů (průměrně 7,46 µg/g) oproti ostatním genotypům z téže barevné skupiny. Vysokým TCC se dle původních předpokladů vyznačovala rovněž pšenice TA 4024 (Ye; 7,04 µg/g), což je téměř dvojnásobně více oproti žluté pšenici (3,96 µg/g) již zmiňují Giordano et al. (2017). Tato hexaploidní pšenice setá se žlutým endospermem je tak srovnatelná například i s diploidními jednozrnkami známými vysokým obsahem karotenoidů, jenž se pohybuje okolo hodnot 5,79 µg/g (Lachman et al. 2013) až 8,41 µg/g (Hidalgo et al. 2006). Jednoznačně nejvyšší TCC byl naměřen v tritordeu HT 439 (12,16 µg/g), což představuje troj- až pětinašobek průměrného TCC v Pp a Ba pšenicích. Tritordeum vzniklo jakožto kříženec pšenice a ječmene čilského (Martín et al. 1999) mj. za účelem produkce nutričně hodnotné potravinářské suroviny (Mellado-Ortega & Hornero-Méndez 2015). TCC naměřené v zrně tritordeí uvedené v literatuře jsou značně různorodé. Například Mellado-Ortega & Hornero-Méndez (2016) zmiňují 10,8 µg/g. Výrazně nižší (ve srovnání s našimi výsledky cca poloviční) průměrný TCC v tritordeích (6,6 µg/g) pak naměřili Atienza et al. (2007).

V pšenicích sledovaných v našich experimentech byly identifikovány následující karotenoidy: anteraxantin, lutein, zeaxantin,  $\alpha$ - a  $\beta$ -karoten. V publikacích zabývajících se karotenoidy v pšeničném zrně bývá ještě zmiňován také  $\beta$ -kryptoxantin, který však představuje pouze minimální podíl z TCC (Abdel-Aal et al. 2007; Digesù et al. 2009; Hidalgo et al. 2010). Z hlediska procentuálního podílu jednotlivých karotenoidů na TCC nebyly mezi barevnými skupinami pšenice shledány významné rozdíly. Naprosto majoritním karotenoidem v obilovinách je lutein, na čemž se shoduje většina publikovaných prací (Abdel-Aal et al. 2007; Mellado-Ortega et al. 2015; Ziegler et al. 2015; Giordano et al. 2017). Dle námi naměřených dat představoval celkový podíl luteinu na TCC napříč sledovanými barevnými skupinami od 68,1 % do 79,5 %. Následoval zeaxantin (11,8-19,8 %) a  $\beta$ -karoten (2,2-3,3 %). Tímto se naše výsledky velmi dobře shodují s poznatky dřívější publikace Konopky et al. (2006) zabývajících se tradičně zbarvenými pšenicemi setými, která uvádí podíl luteinu a zeaxantinu na TCC v rozmezí 71,3-83,3 % a 15,0-24,0 %. Mírně vyšší průměrné zastoupení luteinu (84,7 %) plyne z výsledků studie Giordano et al. (2017), kde byly podobně jako v našem experimentu srovnávány různě zbarvené varianty pšenice seté (red 93,6 %; bílé 74,3 %; žluté 91,4 %; purpurové 83,1 % a modré 81,0 %). Obdobné karotenoidní profily byly zjištěny také v dalších druzích pšenice: v pšenicí tvrdé (Adom et al. 2003; Abdel-Aal et al. 2007; Digesù et al. 2009; Mellado-Ortega et al. 2015), jednozrnce (Adom et al. 2003; Abdel-Aal et al. 2007; Hidalgo et al. 2010; Hidalgo & Brandolini 2014) nebo dvouzrnce (Digesù et al. 2009). Naproti tomu Ndolo & Beta (2013) v purpurové pšenicí identifikovali pouze lutein a zeaxantin v zastoupení 52,3 % a 47,7 %.

Z tohoto lze usuzovat, že pšenice různého druhu nebo barvy obilky se sice vzájemně významně liší obsahem karotenoidů, nikoliv však spektrem resp. procentuálním zastoupením jednotlivých karotenoidů na TCC v zrně.

Karotenoidy se v zrně nacházejí ve dvou formách, jako a) volné b) estery s mastnými kyselinami. Většina analyzovaných vzorků zrna obsahovala obě formy. Výjimku tvořily pouze tři pšenice (Bohemia, V1 131-15 a V1 133-15), které obsahovaly pouze volné formy karotenoidů. Estery jsou produkty esterifikačních reakcí hydroxylových skupin xantofylů s karboxylovými funkčními skupinami mastných kyselin prostřednictvím acyl-CoA (Howitt & Pogson 2006), přičemž tato modifikace zvyšuje stabilitu barviva a umožňuje dlouhodobé skladování uvnitř rostlinné buňky (Atienza et al. 2007). Jednoznačně nejvyšší podíl esterů připadal luteinu, avšak ve vzorcích byly zjištěny taktéž estery ostatních přítomných xantofylů: anteraxantinu a zeaxantinu. Jednotlivé genotypy se rovněž vzájemně lišily procentem esterově vázaných karotenoidů z TCC. Největší podíl esterů byl nalezen v pšenicí TA 4024 (Ye; 52,8 %), dále UC 66049 (Ba; 49,8 %) a Purple Feed (Pp; 49,7 %). Ziegler et al. (2015) uvádí, že schopnost rostliny tvořit estery jakož i stupeň esterifikace luteinu jsou podmíněny geneticky. Rostliny se tak v tomto ohledu vzájemně liší mezi druhy ale i v rámci druhu. Mezi- a vnitrodruhovou variabilitu pak Ziegler et al. (2015) podkládají výsledky analýz různých genotypů pšenice seté, tvrdé, špaldy i jednozrnky, kdy v zrně některých pšenic setých nebyly estery detekovány, jiné pak uchovávaly přibližně jednu čtvrtinu TCC v esterované podobě. Podobně pak u pšenic tvrdých, kdy Atienza et al. (2007) zaznamenali 19,4% podíl esterovaného luteinu na jeho celkovém obsahu, zatímco Mellado-Ortega et al. (2015) uvádí, že zrna pšenice tvrdé buď neobsahují esterované karotenoidy, nebo je jejich podíl na TCC pouze minimální.

V zrna tritordea HT 439 pak byla v podobě esterů skladována více než jedna čtvrtina karotenoidů (27,9 %). Výrazně vyšší, až téměř poloviční (49,2 %), podíl esterů na základě svého zkoumání uvádí Atienza et al. (2007). Naopak Mellado-Ortega et al. (2015) nebo Mattera et al. (2017) zjistili, že estery představují pouze 16% a 13% podíl na TCC v tritordeu.

Z hodnot uvedených v publikacích Paznocht et al. (2018) i Zrcková et al. (2018) je patrný významný meziročníkový rozdíl v obsahu karotenoidů. Článek Paznocht et al. (2018) zahrnuje data kultivačních sezón 2013/2014 a 2014/2015, článek Zrcková et al. (2018) se pak opírá o data naměřená v sezónách 2015/2016 a 2016/2017, přičemž zde byl již cíleně sledován vliv ročníku také na řadu dalších látek (fenolické kyseliny, celkové fenolické látky či antokyany jak je diskutováno dále). Článek Paznocht et al. (2018) v podstatě rozděluje zkoumané genotypy do čtyř skupin dle pozorovaných rozdílů mezi sledovanými lety. První, která reagovala velmi výrazným nárůstem TCC v sezóně 2014/2015 oproti 2013/2014 (Xiao Yian, +194,2 %; UC 66049, +102,3 %). Druhá skupina reagovala o něco méně výrazným navýšením TCC (Purple Feed, +81,9 %; Konini, +61,6 %; V1 133-15, +56,1 %). U genotypů třetí skupiny byl naměřen prakticky stejný obsah karotenoidů v obou letech (Bohemia, +4,8 %; V1 131-15, +1,2 %). A poslední, čtvrtá skupina pšenic vykazala mírný pokles v roce 2015 (Purple, -24,3 %; RU 687-12, -12,0 %).

Značné rozdíly jsou rovněž patrné z výsledků publikace Zrcková et al. (2018), kde v rámci sledovaného souboru genotypů byl ve vzorcích ze sklizně 2015/2016 naměřen průměrný TCC 3,00  $\mu\text{g/g}$ , tedy téměř dvojnásobek oproti průměru ze sklizně následující (1,64  $\mu\text{g/g}$ ). Vzhledem k tomu, že vzorky zrna pro naše analýzy pocházely vždy ze dvou po sobě jdoucích sklizní a rostliny byly pěstovány na tomtéž stanovišti, za identických agrotechnických podmínek, usuzujeme na významný vliv počasí v průběhu vegetace na výsledný TCC v zrna. Pravděpodobným vysvětlením rozdílů pozorovaných v obou pokusech (Paznocht et al. 2018 i Zrcková et al. 2018) jsou proto rozdíly v průměrných teplotách a srážkových úhrnech resp. vyšší průměrné teploty současně s výrazně nižšími srážkovými úhrny v období tvorby zrna v sezónách 2014/2015 (oproti 2013/2014) a 2015/2016 (oproti 2016/2017). Obsah karotenoidů v obilkách je dle některých autorů (Lv et al. 2013; Lu et al. 2015) výrazně ovlivněn právě podmínkami prostředí (E; Environment) resp. ročníkem (průběhem počasí) a lokalitou pěstování. Ze statistického hodnocení našich dat (Zrcková et al. 2018) je patrné nejvýznamnější ovlivnění TCC ročníkem (52 %), méně pak genotypem (42 %) (hodnoty představují průměr z celého sledovaného souboru). Lv et al. (2013) při pokusech s pšenicí setou rovněž zjistil, že TCC je nejvíce ovlivněn E (46 %), méně pak vzájemnou interakcí genotypu a prostředí (G×E; 34 %) a pouze z 21 % genotypem (G; Genotype). Lu et al. (2015) vyzdvihují větší důležitost interakce G×E (46 %), méně pak E a G (37 a 17 %). K poněkud odlišným výsledkům však dospěli Martini et al. (2015), kteří, přesto že shledali různé meziročníkové rozdíly, naopak vyzdvihují zásadní vliv G na TCC.

Vliv teploty a vodního režimu v průběhu vegetace rostlin na celkový obsah karotenoidů v obilném zrna se stal předmětem rozsáhlého zkoumání, avšak nebyl doposud zcela uspokojivě objasněn, jelikož dostupné vědecké publikace se ve svých výsledcích rozcházejí (Abdel-Aal et al. 2007; Dias et al. 2011; Fratianni et al. 2013; de Leonardis et al. 2015). Dle našich pokusů vyšší průměrná teplota a nižší množství vodních srážek zvyšuje obsah karotenoidů v zrna pšenice. Podobný je pak závěr dvouletého pěstebního pokusu, jenž provedli Fratianni et al.



(2013), kde byl zaznamenán 20% nárůst obsahu celkových karotenoidů (z 3,6 na 4,3 µg/g) v obilkách pšenice tvrdé (odrůdy Simeto) působením sucha. Autoři dále píší, že vodní deficit měl celkově za následek 15% nárůst obsahu karotenoidů v semolinách vyrobených z vypěstovaného zrna. Avšak publikace de Leonardis et al. (2015) zabývající se rovněž pšenicí tvrdou uvádí, že nebyla zpozorována změna v obsahu karotenoidů v zrně vlivem stresu vysokými teplotami. Další publikované výzkumy (Abdel-Aal et al. 2007; Mattera et al. 2017) pak dokonce dospěly ke zcela odlišným závěrům. Abdel-Aal et al. (2007) zjistili vyšší průměrné obsahy karotenoidů (až o 42 %) v obilkách pšenice jednozrnek naopak v sezóně charakterizované chladnějším a vlhčím průběhem počasí. Podobně pak Mattera et al. (2017) uvádí, že vysoké teploty v průběhu tvorby obilného zrna mají za následek pokles obsahu karotenoidů. Pozorovaný efekt vysvětlují zvýšenou produkcí reaktivních forem kyslíku a následným rozvojem oxidačního stresu v důsledku vysokých teplot. Karotenoidy jsou pak dle Mattery et al. (2017) rostlinou pravděpodobně spotřebovávány za účelem detoxikace kyslíkových radikálů.

Na základě těchto poznatků lze říci, že výsledný obsah karotenoidů v pšeničném zrně je výsledkem interakce mnoha různých faktorů (vnitřních i vnějších). Různé genotypy tak pravděpodobně, i na základě přizpůsobení se podnebným podmínkám místa svého původu, vykazují různé reakce ve smyslu obsahu karotenoidů. Vliv genotypu, ročníku a systému pěstování i na jiné typy nutričně významných látek byl předmětem dalších publikací, které jsou diskutovány dále.

Cílem další z publikovaných prací *Carotenoid changes of colored-grain wheat flours during bun-making* (Paznocht et al. 2019) bylo detailní popsání změn v obsahu karotenoidů vlivem jednotlivých dílčích operací výroby (příprava těsta a kynutí, pečení) a krátkodobého skladování pečiva z celozrnné mouky umleté z pšenice s různou barvou zrna.

V předchozí publikaci (Paznocht et al. 2018) byla velká pozornost věnována především esterovaným formám karotenoidů, jelikož dle literárních zdrojů právě esterifikace xantofylů je efektivním způsobem skladování, resp. umožňuje akumulaci vyššího množství karotenoidů v zrně (Atienza et al. 2007) a patrně zvyšuje stabilitu karotenoidních barviv během technologického zpracování (Atienza et al. 2007; Fu et al. 2010; Mellado-Ortega et al. 2015; Ahmad et al. 2015). Dle Ahmada et al. (2015) a Zieglera et al. (2015) je tvorba esterů podmíněna geneticky, a proto byly na základě předchozího podrobného šetření celkového obsahu karotenoidů a podílu esterovaných xantofylů (dle výsledků publikace Paznocht et al. 2018) vybráni reprezentativní zástupci jednotlivých barevných skupin pšenice pro pokusy technologických úprav zrna. Účelem těchto pokusů bylo prozkoumání různých způsobů možného potravinářského využití pigmentovaného zrna, především pro pečení, extruzi či pufování. Klíčem k výběru byla jednak barva zrna a rovněž schopnost skladovat karotenoidy, resp. xantofyly v podobě esterů s mastnými kyselinami. Do pekařského pokusu tak byly zařazeny vždy dva genotypy pšenice od každé barvy zrna (red, Ye, Ba) a tři pšenice s Pp. Dvojice, resp. trojice byly vybírány vždy tak, aby byly zastoupeny jak pšenice obsahující výhradně volné formy karotenoidů, tak i pšenice s určitým podílem esterově vázaných karotenoidů.

Celý proces výroby celozrnného pečiva způsobil průměrně za všechny sledované vzorky pokles TCC o 75,1 %, tedy na pouhou jednu čtvrtinu původní hodnoty (p.h.) v celozrnné mouce. Ještě výraznější míru degradace (92 %) vlivem pečení zaznamenali Leenhardt et al. (2006). Naproti tomu podstatně nižší ztráty karotenoidů během pečení chleba uvádí Hidalgo et al. (2010), kteří stanovovali TCC zvlášť v chlebové kůrce (55 %) a chlebové střídě (24 %). Příčinou rozdílností výsledků zmíněných experimentů je pravděpodobně odrůdová variabilita použitých mouk (s ní související různá enzymatická aktivita – viz dále) a odlišnosti ve výrobních metodách (velikosti pekařských výrobků, doby kynutí, teploty i časy pečení).

Z jednotlivých sledovaných operací mělo na obsah karotenoidů ve finálním výrobku největší vliv hnětení těsta společně s kynutím bulek. TCC během této pracovní operace klesl průměrně na 38,5 % p.h. v celozrnné mouce. Pečení mělo za následek podstatně nižší úbytek celkových karotenoidů (průměrně o 11,1 %; tedy na 27,4 % p.h.). A dále pak v průběhu krátkodobého skladování za pokojové teploty došlo pouze k minimálnímu poklesu TCC (o 2,5 %; na 24,9 % p.h. což představuje 0,266 µg/g). Přípravu těsta tak lze označit, z pohledu degradace karotenoidních barviv, za jednoznačně nejvíce destruktivní krok výroby celozrnného pečiva. Z jednotlivých sledovaných karotenoidů se jako nejméně stabilní jeví β-karoten, anteraxantin a také esterované formy, z nichž největší podíl připadá esterům luteinu, jak plyne z našich dříve publikovaných výsledků (Paznocht et al. 2018). Nebyl tak pozorován žádný výrazný ochranný efekt esterifikace karotenoidů obecně vůči degradaci, ani před působením vysokých teplot, který uvádí jiné publikace (Fu et al. 2010; Ahmad et al. 2015; Mellado-Ortega et al. 2015; Mattera et al. 2017).

Významný vliv hnětení těsta na výsledný TCC (pokles o 66 %) zaznamenali ve svém výzkumném pokusu rovněž Leenhardt et al. (2006). Markantní míra degradace karotenoidů při přípravě těsta je přičítána především působení oxidativních enzymů jako je lipoxygenáza (LOX), peroxidáza či polyfenoloxidáza, k jejichž aktivaci dochází přidávkem vody a zapracováním vzdušného kyslíku do hmoty těsta (Leenhardt et al. 2006; Luthria et al. 2015; Ficco et al. 2016). Významný vliv enzymatických pochodů potvrzují svou studií Leenhardt et al. (2006), kteří uvádí těsnou korelaci aktivity LOX a úbytku karotenoidů během kynutí chlebového těsta. Hidalgo & Brandolini (2012) doplňují, že prostřednictvím oxidace nenasycených mastných kyselin katalyzované LOX dochází k následné oxidaci karotenoidů. Dále potom Hidalgo et al. (2010) poukazují na různou aktivitu LOX v závislosti na druhu pšenice (setá, tvrdá, jednozrnka). Z výsledků našeho pokusu, kde všechny použité pšenice patřily k druhu *T. aestivum*, je pak patrná rovněž značná vnitrodruhová variabilita, ačkoliv přímo aktivita LOX měřena nebyla.

Vysokou teplotou v průběhu samotného pečení došlo k průměrnému poklesu o 11,1 % p.h. TCC, což je výrazně méně v porovnání s předchozím hnětením a kynutím. Hidalgo et al. (2010) pečení naopak označili za operaci nejvíce destruktivní z pohledu obsahu karotenoidů. Nicméně jejich výsledky jsou s našimi jen stěží přímo srovnatelné, jelikož u finálního pekařského výrobku rozlišují ztráty TCC zvlášť v kůrce a střídě (29 % a 3 %). Leenhardt et al. (2006) uvádí pokles TCC během pečení o 36 % (tato hodnota je vztažena k obsahu karotenoidů po fázi kynutí). V případě našeho experimentu byl tak zaznamenán o něco nižší pokles mezi kynutým těstem a pečenou bulkou (o 29 %).

Výsledky vlivu dalších technologických úprav zrna na obsah karotenoidů nejsou v disertační práci uvedeny, jelikož nebyly doposud publikovány.

Publikace *Total phenolic content and antioxidant activity of tritordeum wheat and barley* (Eliášová & Paznocht 2017) se zabývala celkovým obsahem fenolických látek a *in vitro* antioxidační aktivitou různých druhů obilovin. Právě tyto sloučeniny jsou dle mnohých autorů nejvíce zodpovědné za antioxidační aktivitu obilovin (Li et al. 2005; Dykes & Rooney 2007; Dinelli et al. 2009; Fogarasi et al. 2015). Lachman et al. (2012) a Hejtmánková et al. (2010) uvádí ale i další významné antioxidanty: fytosteroly, karotenoidy, tokoly či selen. Celkový obsah fenolických látek (TPC; Total Phenolics Content) byl stanoven za použití Folin-Ciocalteova činidla a pro spektrofotometrickou metodu detekce a značnou kvalitativní variabilitu této skupiny sloučenin byl výsledek vyjadřován jako ekvivalent gallové kyseliny (GAE; Gallic Acid Equivalent). Antioxidační aktivita vzorků (TAA; Total Antioxidant Activity) měřená jako barevná, spektrofotometricky detekovaná změna v důsledku reakce extrahovaných antioxidantů se syntetickým radikálem DPPH (2,2-difenyl-1-pikrylhydrazyl) je pak kvantifikována jako ekvivalent aktivity Troloxu (TEAC; Trolox Equivalent Antioxidant Activity). Pro analýzy byla použita tři tritordea, tři ječmeny (dva tradiční barvy a jeden s černou barvou zrna) a devět pšenic různého zbarvení (čtyři Ba, tři Pp a dvě tradiční barvy – red).

Nejnižší TPC byl naměřen u tritordeí (65,9 mg GAE/100 g). Významný nutriční potenciál tritordea je tak dán především vysokým obsahem karotenoidů (Mellado-Ortega & Hornero-Méndez 2016). Naopak nejvyšší TPC byl naměřen u ječmenů (průměrně 129 mg GAE/100 g). Pšenice (napříč různými barvami zrna) obsahovaly průměrně 73,7 mg GAE/100 g. V rámci pšenic pak nebyly pozorovány významné rozdíly mezi barevnými skupinami (red 81,8; Pp 78,5; Ba 66,1 mg GAE/100 g). Tyto hodnoty jsou mírně vyšší v porovnání s výsledky Moora et al. (2005) i Lachmana et al. (2011), kteří naměřili průměrně 60,0 a 55,2 mg GAE/100 g. Naproti tomu k vyšším hodnotám 136,0 a 112,0 mg GAE/100 g dospěli Adom & Liu (2002) a Abozed et al. (2014). Vliv na stanovený obsah fenolických látek má celá řada vnitřních i vnějších faktorů, v neposlední řadě také analytická metoda, resp. použité extrakční činidlo, a tedy účinnost extrakce v závislosti na polaritě použitého rozpouštědla (Abozed et al. 2014).

Zjištěné hodnoty TAA pak vykazovaly velmi těsnou pozitivní korelaci s TPC ( $R^2 = 0,93$ ). Což velmi dobře odpovídá výsledkům analýz vzorků purpurových pšenic ( $R^2 = 0,87$ ; Ivanišová et al. 2014) nebo ječmene ( $R^2 = 0,94$ ; Zhao et al. 2006). Naproti tomu Heimler et al. (2010) neshledala žádnou korelaci mezi TAA a TPC. Aplikované metody přípravy vzorků, pro stanovení TAA i TPC, využívají polárních rozpouštědel (pro TAA methanol a pro TPC methanol + 0,1 % HCl) a v obou postupech tak dochází k vyextrahování polárních molekul s antioxidačními vlastnostmi. V obilovinách jsou to právě především fenolické látky jako fenolické kyseliny, flavonoidy či lignany (Dykes & Rooney 2007; Dinelli et al. 2009; Lachman et al. 2017; Luthria et al. 2015).

Cílem publikace *The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain* (Zrcková et al. 2018) bylo stanovit vliv genotypu, přírodních podmínek a systému pěstování na antioxidační aktivitu (TAA) a obsah významných antioxidačních látek:

karotenoidů (TCC), fenolických látek obecně (TPC), fenolických kyselin (PAs; Phenolic Acids) či antokyanů (TAC; Total Anthocyanins Content) v pšenících s barevným zrnem. K dvouletému polnímu pokusu (sezóny 2015/2016 a 2016/2017) s ozimými formami pšenice byli vybráni vždy dva zástupci s Ba a Pp, jeden tradiční a jeden s Ye. Následující publikace s názvem *Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year* (Zrcková et al. 2019) úzce navazovala na předchozí pěstební pokus s pšenicemi s barevným zrnem. V letech 2017 a 2018 byl sledován vliv druhu, genotypu a ročníku na celkový obsah polyfenolických látek (TPC) a fenolických kyselin v zrnu různých druhů jarních forem pšenice. Předmětem zkoumání byla tradiční pšenice setá (*Triticum aestivum* L.) a další, méně běžné druhy jako špalda (*T. spelta* L.), jednozrnka (*T. monococcum* L.) a dvouzrnka (*T. dicoccum* Schuebl), které jsou charakteristické pluchatými obilkami a zejména v systému ekologického zemědělství představují vhodnou alternativu k tradiční pšenici seté, oproti níž se vyznačují vyšší odolností vůči nepříznivým vlivům prostředí, nižšími nároky na kvalitu půdy a úroveň hnojení (Konvalina et al. 2012).

Všechny stanovované parametry v obou zrealizovaných experimentech byly významně ovlivněny jak genetickými faktory, tak podmínkami prostředí. TCC, PAs a TPC v prvním pokusu byly nejvíce ovlivněny ročníkem, méně potom genotypem, systémem pěstování a jejich vzájemnými interakcemi. Výsledky druhého experimentu pak potvrdily a doplnily předchozí poznatky o pšenici seté, tradiční i netradiční barvy zrna, totiž že ročník sklizně má výrazný vliv i na TPC a obsah PAs v obilkách pšenice jedno- a dvouzrnky či špaldy.

V prvním pokusu s barevnými pšenicemi byly zjištěny statisticky prokazatelně vyšší hodnoty TPC i PAs v sezóně 2015/2016 oproti 2016/2017. Průměrný celkový obsah PAs dosáhl 811 vs. 731  $\mu\text{g/g}$ , což představuje rozdíl mezi sklizněmi téměř 11 %. Podobný trend byl pozorován také v TPC, kde rozdíl mezi roky činil cca 17 % (702 vs. 599  $\mu\text{g GAE/g}$ ). Sezóna 2015/2016 se oproti 2016/2017 vyznačovala vyššími průměrnými teplotami a zároveň celkově nižšími srážkovými úhrny v období od počátku kvetení po úplnou zralost zrna. Následující experiment zahrnující jarní pšenice (seté, jednozrnky, dvouzrnky a špaldy) taktéž zaznamenal rozdíl mezi sklizněmi. V roce 2018 byly oproti produkci 2017 naměřeny mírně vyšší hodnoty TPC (731 vs. 693  $\mu\text{g GAE/g}$ ) i PAs (834 vs. 735  $\mu\text{g/g}$ ), což představovalo průměrný nárůst o 5,4 % a 13,5 %. Oba tyto roky byly velmi podobné z pohledu průměrných teplot, avšak rok 2018 se v období od metání po dozrání zrna vyznačoval výrazně menším množstvím dešťových srážek. Rozdíly v TPC a obsahu PAs mezi lety zaznamenané v obou experimentech (Zrcková et al. 2018; Zrcková et al. 2019) byly tedy opět zřejmě projevem zvýšené syntézy antioxidantních látek v důsledku různé úrovně působení abiotického stresu. Z publikace Zrcková et al. (2019) plyne, že výsledný TPC a obsah PAs v pšeničném zrně je z 58 % a 69 % ovlivněn ročníkem. Tomuto nasvědčují i výsledky, jež publikovali Lu et al. (2015), z kterých je zřejmé, že TPC v zrně pšenice je nejvíce ovlivněn E (43 %), méně pak G (37 %). I další studie potvrzují významný vliv ročníku na obsah polyfenolů (Heimler et al. 2010; Menga et al. 2010; Martini et al. 2015) a PAs (Mpofu et al. 2006; Fernandez-Orozco et al. 2010; Martini et al. 2015). Výsledky experimentů, které provedli Heimler et al. (2010) a Martini et al. (2015) však ukazují na vyšší TPC v sezóně vyznačující se nižší teplotou a zároveň vyšším množstvím srážek v období dozrávání obilky a to až o 29 % jak uvádí Martini et al. (2015). Výsledky

tříletého pokusu, které publikovala Martini et al. (2015) rovněž označují podmínky prostředí za nejvýznamnější faktor ovlivňující konečné obsahy PAs a TPC, menší vliv pak přisuzují genotypu.

TAC stanovený v zrně Ba a Pp pšenice byl nejvíce ovlivněn genotypem (49 %; což poukazuje na vysokou heritabilitu tohoto znaku), méně pak ročníkem (37 %) a systémem pěstování (13 %). V Ba pšenicích byl naměřen podstatně vyšší průměrný TAC (60,2 µg/g) oproti Pp pšenicím (16,6 µg/g). V zrně pšenice Annie (red) ani Citrus (Ye) nebyly antokyany detekovány. Významný vliv G na obsah antokyanů potvrzuje rovněž Knievel et al. (2009) a Garg et al. (2016), dle jejichž výsledků se nejvyšším obsahem antokyanů obvykle vyznačují pšenice s černým zrnem, méně obsahují pšenice s Ba a nejméně zrna pšenice s Pp.

Rostlinný druh pak mnohem menší měrou ovlivnil TPC a PAs (21 % a 29 %). Toto zjištění plyne ze statisticky průkazně rozdílných výsledků průměrného obsahu PAs v jednotlivých druzích pšenice: jednozrnka (827 µg/g) > špalda (804 µg/g) > dvouzrnka (790 µg/g) > setá (716 µg/g). Výrazně nižší průměrné hodnoty obsahu PAs (v porovnání s našimi až o 28 %) i odlišné pořadí pšenice publikovala Brandolini et al. (2013): setá > špalda > jednozrnka > dvouzrnka (673 > 626 > 612 > 568 µg/g).

Z hlediska TPC byly pšenice seřazeny sestupně jednozrnka > dvouzrnka > setá > špalda (745; 705; 702; 695 µg GAE/g), dle statistického šetření však byla jedinou od ostatních významně odlišnou skupinou pšenice jednozrnka. Výraznější rozdíly v TPC mezi pšenicemi zjistili Lachman et al. (2011): dvouzrnka > jednozrnka > pšenice setá (761; 618; 562 µg GAE/g). Naopak Brandolini et al. (2013) ve své studii našli podstatně méně výrazné rozdíly v TPC mezi skupinami pšenice, přičemž pořadí zůstalo stejné jako dle PAs (setá > špalda > jednozrnka > dvouzrnka). Naměřené hodnoty vyjádřené v ekvivalentu ferulové kyseliny (FAE; Ferulic Acid Equivalent) přitom naopak výrazně (o 50-80 %) převyšují naše výsledky (1294; 1149; 1121 a 1115 µg FAE/g). Tyto značné odlišnosti jsou velmi pravděpodobně způsobeny vyjádřením celkových hodnot v jiných jednotkách (FAE vs. GAE) i faktory prostředí, jak bylo uvedeno v přechozích odstavcích.

Systém pěstování ovlivnil obsahy sledovaných látek oproti ostatním faktorům podstatně méně (TAC 13 %; TPC 8 %; PAs 6 % a TCC pouze z 0,2 %). Přesto byly v zrně původem z ekologické produkce zjištěny průměrně mírně vyšší obsahy všech sledovaných analytů PAs, TAC, TPC i TCC oproti konvenční produkci (+7 %; +6 %; +6 %; +4 %). Rozdíly pak byly patrnější u TPC (+9 %) a TCC (+12 %) v Pp pšenicích. Ačkoliv některé publikace zabývající se vlivem systému pěstování na nutriční kvalitu produktů uvádí vyšší obsahy sekundárních metabolitů (fenolických látek) v ovoci či zelenině pocházející z produkce ekologického režimu (Rembiałkowska 2007; Mitchell et al. 2007; Lairon 2010), výsledky pokusů s pšenicí významné rozdíly nepotvrdily. Například Stracke et al. (2009a), Gasztonyi et al. (2011) či Mazzoncini et al. (2015) neshledali výrazný rozdíl v obsahu fenolických kyselin mezi ekologickou a konvenční produkcí a to ani v rámci několikaletých polních pokusů. Dle Gasztonyi et al. (2011) a Strackeho et al. (2009a) je obsah fytochemikálií (karotenoidů a fenolických kyselin) v pšeničném zrně podstatně více ovlivněn klimatickými faktory než systémem produkce, což v podstatě dokládají i naše výsledky.

Z pokusů s pšenicí setou (tradiční barvy zrna) provedených Zuchowskim et al. (2011) vychází pouze minimálně vyšší obsahy PAs v ekologické oproti konvenční produkci. V případě sledovaných jarních a ozimých forem činí rozdíl 5,2 % (612 vs. 582  $\mu\text{g/g}$ ) a 3,2 % (565 vs. 547  $\mu\text{g/g}$ ). Zuchowski et al. (2011) dále konstatují, že na základě jimi publikovaných výsledků nelze jednoznačně potvrdit hypotézu o vyšším obsahu sekundárních metabolitů v produktech ekologického systému pěstování. Tato hypotéza je založena na předpokladu intenzivnější syntézy sekundárních metabolitů včetně fenolických sloučenin, jakožto obranných látek, v metabolismu ekologicky pěstovaných rostlin, kde je obvykle vyšší míra působení rozličných biotických či abiotických stresových faktorů (Rembiałkowska 2007; Zuchowski et al. 2011). Plodiny konvenčního systému zemědělství jsou naproti tomu podporovány aplikací řady průmyslových hnojiv nebo pesticidních či fungicidních přípravků (Zuchowski et al. 2011; Gasztonyi et al. 2011).

## 6. Závěr

Pšenice představuje celosvětově velmi důležitou zemědělskou komoditu, která se značnou měrou podílí na potravinovém zabezpečení lidské populace. Tato plodina byla, je a pravděpodobně i nadále bude předmětem zájmu mnohých šlechtitelů a dalších výzkumníků. Zejména v posledních dvou dekadách nabývají na významu méně obvyklé druhy a odrůdy pšenice. Prostor tak dostávají jednozrnky či dvouzrnky nebo tzv. pšenice s barevným zrnem. Pro efektivní funkci šlechtitelských programů, zabývajících se právě přírodními pigmenty v pšeničném zrně, je nezbytná přímá spolupráce s chemickými laboratoři, jež jsou schopné detailně zanalyzovat produkovaný šlechtitelský materiál.

V rámci této práce byly vyvinuty či optimalizovány analytické metody stanovení karotenoidů a fenolických kyselin v obilovinách, od přípravy vzorku (extrakce, různé typy hydrolyzy apod.), přes metody separace až po samotnou identifikaci obsažených analytů (použitá HPLC-DAD sestava viz příloha č. 1).

Z výsledků publikovaných prací pak vyplývá, že se jednotlivé genotypy pšenice vzájemně liší také schopností syntetizovat a skladovat karotenoidy v esterované formě, jelikož jde o geneticky podmíněný znak. Některé (např. Bohemia, V1 131-15) obsahují karotenoidy pouze ve volné formě, jiné (Konini, TA 4024) pak ukládají až polovinu karotenoidů v podobě esterů s mastnými kyselinami. Celkově vysokým obsahem karotenoidů se, oproti tradičním pšenicím (Bohemia; 3,56 µg/g), vyznačuje zejména zrna pšenice se žlutým endospermem (TA 4024; 7,04 µg/g). Tyto pšenice se od ostatních liší, jak už samotné označení napovídá, zvýšenou kumulací karotenoidů v samotném endospermu zrna, tedy v části představující největší podíl z celé obilky. Z pohledu obsahu karotenoidů jsou rovněž zajímavé pšenice s purpurovým perikarpem (3,60 µg/g), naproti tomu genotypy s modrým aleuronem se obecně vyznačují obsahy relativně nejnižšími (2,41 µg/g). Výjimkou byla pšenice s prozatímním pracovním označením V1 131-15 (pravděpodobně kombinace Ba a Ye) obsahující průměrně 7,46 µg karotenoidů v gramu zrna.

Za účelem nalezení vhodného technologického zpracování zrna šetrného k obsaženým barvivům byl proveden nejprve pokus pečení. V průběhu celého procesu výroby pečiva došlo k velmi zásadnímu poklesu obsahu karotenoidů na 63,9 % až 12,9 % p.h. v mouce. Překvapivě nejvíce destruktivním krokem se ukázalo být kynutí (snížení TCC průměrně na 38,5 % p.h.), resp. enzymatická činnost spojená se zpracováním vody a vzdušného kyslíku. Z tohoto důvodu pak byly zkoumány další potenciálně využitelné úpravy, kde by byla činnost oxidativních enzymů omezena: vaření, extruze a pufování (produkty úprav viz příloha č. 2). Naměřená data jsou v současné době zpracovávána do podoby publikačních výstupů.

Z dalších publikovaných výsledků bylo patrné, že obsah karotenoidů a fenolických kyselin byl značně proměnlivým parametrem, výrazně ovlivněným rostlinným druhem a odrůdou (resp. genotypem). Projevil se rovněž velký vliv přírodních podmínek pěstování. Konkrétně působením vyšších teplot (popřípadě kombinace vysokých teplot a slabšího zásobení vodou) došlo u mnohých sledovaných pšenic k markantnímu zvýšení obsahu karotenoidů v zrně. Menší, avšak nezanedbatelný, vliv na zvýšení množství nutričně žádoucích fotochemikálií vykázal rovněž systém kultivace. U ekologicky pěstovaných rostlin (oproti

konvenční produkci) pak lze očekávat intenzivnější syntézu fenolických sloučenin, jakožto obranného mechanismu, hlavně v důsledku působení různých abiotických či biotických stresových faktorů.

Pšenice se žlutým endospermem či purpurovým perikarpem zrna nebo tritordea, vyznačující se vysokým obsahem karotenoidních barviv v obilce, tak do budoucna jistě představují vhodnou možnost rozšíření sortimentu odrůd potravinářských pšenic, využitelných jakožto výchozích surovin pro výrobu určitých typů tzv. funkčních potravin. „Na poli barevných pšenic“ je však zapotřebí dalšího šlechtitelského úsilí, podporovaného přesnými chemickými analýzami pro získání nových perspektivních odrůd.

Shrnutí stanovených cílů a hypotéz:

**Cíl 1.** Stanovit obsah celkových polyfenolů v obilkách vybraných odrůd barevných pšenic spektrofotometricky. Cíl byl splněn, viz publikace Eliášová & Paznocht (2017), Zrcková et al. (2018), Zrcková et al. (2019).

**Cíl 2.** Identifikovat chromatograficky jednotlivé fenolické kyseliny charakteristické pro barevné pšenice a jejich zastoupení v jednotlivých odrůdách. Cíl byl splněn, viz publikace (obsahy vyjádřeny jako sumy fenolických kyselin) Zrcková et al. (2018), Zrcková et al. (2019). Článek s názvem *Phenolic acids in kernels of different coloured-grain wheat genotypes* zabývající se podrobněji jednotlivými fenolickými kyselinami je však ještě v recenzním řízení.

**Cíl 3.** Charakterizovat chromatograficky jednotlivé karotenoidy a stanovit jejich obsah v barevných odrůdách pšenic. Cíl byl splněn, viz publikace Paznocht et al. (2018).

**Cíl 4.** Stanovit antioxidační aktivitu v nových odrůdách pšenice a vyhodnotit vztah mezi antioxidační aktivitou a zastoupením jednotlivých antioxidantů v barevných pšenicích. Cíl byl splněn, viz publikace Eliášová & Paznocht (2017), Zrcková et al. (2018).

**Cíl 5.** Vytipovat vhodné kultivary barevných pšenic s vysokým obsahem antioxidantů a antioxidační aktivitou pro další šlechtění a vhodnosti pro potravinářské účely. Cíl byl splněn, viz publikace Paznocht et al. (2019).

**Hypotéza 1.** Vyvinuté HPLC-DAD metody budou vhodné pro analýzu karotenoidů a fenolických sloučenin v obilkách barevných odrůd pšenice.

Metody stanovení karotenoidů a celkových fenolických látek byly detailně popsány v publikacích Paznocht et al. (2018) a Eliášová & Paznocht (2017). Metoda pro stanovení fenolických kyselin, včetně jednotlivých frakcí (volné, konjugované a vázané) i *cis*-izomerů je součástí připravované publikace *Phenolic acids in kernels of different coloured-grain wheat genotypes*.



**Hypotéza 2.** Obilky barevných odrůd pšenice obsahují karotenoidy (uložené především v endospermu) a fenolické sloučeniny, které se nacházejí hlavně v otrubách a svrchních částech zrna.

Hypotéza o přítomnosti sledovaných analytů v zrně pšenice byla potvrzena, viz publikace Eliášová & Paznocht (2017), Paznocht et al. (2018), Paznocht et al. (2019), Zrcková et al. (2018), Zrcková et al. (2019). Distribuce karotenoidů a fenolických sloučenin uvnitř pšeničného zrna však nebyla analyzována ani zpracována do odborných publikací.

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## 8. Seznam použitých zkratek

- AACT – acetoacetyl-CoA thioláza (EC 2.3.1.9)
- ABA – abscisová kyselina (z angl. Abscisic Acid)
- ADT – arogenát dehydratáza (EC 4.2.1.91)
- ATP – adenosintrifosfát
- Ba – modrý aleuron (z angl. Blue aleurone)
- BPA – nerozpustné vázané fenolické kyseliny (z angl. Bound Phenolic Acids)
- C3H – *p*-kumarát 3-hydroxyláza (EC 1.14.18.1)
- C4H – *trans*-cinamát monooxygenáza (EC 1.14.13.11)
- CCOs – karotenoidy štěpící oxygenázy (z angl. Carotenoid Cleavage Oxygenases; EC 1.13.11.\*)
- CCS – kapsantin/kapsorubin syntáza (EC 5.3.99.8)
- CDP-ME – 4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol
- CDP-ME-2P – 4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol-2-fosfát
- CM – chorismát mutáza (EC 5.4.99.5)
- CMK – 4-(cytidin 5'-difosfo)-2-C-methyl-D-erythritol kináza (EC 2.7.1.148)
- CoA – koenzym A
- CPAs – rozpustné konjugované fenolické kyseliny (z angl. Conjugated Phenolic Acids)
- CRTI – fytoen desaturáza bakteriálního typu (EC 1.3.99.31)
- CRTISO – karotenoid izomeráza (EC 2.5.1.13)
- CrtO/CrtW – karotenoidní ketolázy (EC 1.3.5.B4)
- CS – chorismát syntáza (EC 4.2.3.5)
- DAHP – 3-deoxy-D-arabino-heptulosonát-7-fosfát
- DAHPS – 3-deoxy-D-arabino-heptulosonát-7-fosfát syntáza (EC 2.5.1.54)
- DHD/SDH – 3-dehydrochinát dehydratáza/šikimát 5-dehydrogenáza (EC 4.2.1.10/EC 1.1.1.25)
- DHQS – 3-dehydrochinát syntáza (EC 4.2.3.4)
- DMAPP – dimethylallyldifosfát
- DPPH – 2,2-difenyl-1-pikrylhydrazyl
- DXP – 1-deoxy-D-xylulóza-5-fosfát
- DXR – 1-deoxy-D-xylulózo-5-fosfát reduktoizomeráza (EC 1.1.1.267)
- DXS – 1-deoxy-D-xylulózo-5-fosfát syntáza (EC 2.2.1.7)

E – podmínky prostředí (z angl. Environment)

E4P – erytróza-4-fosfát

EPSP – 5-enolpyruvylšikimát-3-fosfát

EPSPS – 5-enolpyruvylšikimát-3-fosfát syntáza (EC 2.5.1.29)

FAE – ekvivalent ferulové kyseliny (z angl. Ferulic Acid Ekvivalent)

FPAs – rozpustné volné fenolické kyseliny (z angl. Free Phenolic Acids)

F5H – ferulát 5-hydroxyláza (EC 1.14.13.B32)

G – genotyp (z angl. Genotype)

GAE – ekvivalent gallové kyseliny (z angl. Gallic Acid Equivalent)

GGPP – geranylgeranyldifosfát

GGPPS – geranylgeranyldifosfát syntáza (EC 2.5.1.29)

HDR – 1-hydroxy-2-methyl-2-(E)-butenyl-4-difosfát reduktáza (EC 1.17.7.4)

HDS – 1-hydroxy-2-methyl-2-(E)-butenyl-4-difosfát syntáza (EC 1.17.7.3)

HMBPP – 1-hydroxy-2-methyl-2-(E)-butenyl-4-difosfát

HMG-CoA – 3-hydroxy-3-methylglutaryl-CoA

HMGR – 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reduktáza (EC 1.1.1.34)

HMGS – 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) syntáza (EC 2.3.3.10)

HPLC-DAD – vysokoúčinná kapalinová chromatografie s detektorem diodového pole

HYD B –  $\beta$ -karoten hydroxyláza (EC 1.14.15.24)

HYD E –  $\epsilon$ -karoten hydroxyláza (EC 1.14.14.158)

IPP – izopentenyldifosfát

IPPI – izopentenyldifosfát izomeráza (EC 5.3.3.2)

LCY – lykopen cyklázy (EC 5.5.1.\*)

LCY B – lykopen cykláza tvořící  $\beta$ -jononové cykly (EC 5.5.1.19)

LCY B/E – lykopen cykláza tvořící  $\beta$ - nebo  $\epsilon$ -jononové cykly

LCY E – lykopen cykláza tvořící  $\epsilon$ -jononové cykly (EC 5.5.1.18)

LOX – lipoxygenáza (EC 1.13.11.\*)

MCT – 2-C-methyl-D-erythritol-4-fosfát cytidyltransferáza (EC 2.7.7.60)

MDS – 2-C-methyl-D-erythritol-2,4-cyklodifosfát (MECDP) syntáza (EC 4.6.1.12)

MECDP – 2-C-methyl-D-erythritol-2,4-cyklodifosfát

MEP – 2-C-methyl-D-erythritol-4-fosfát

MK – mevalonát kináza (EC 2.7.1.36)

MVA – mevalonová kyselina  
MVA-P – mevalonát-5-fosfát  
MVA-PP – mevalonát-5-difosfát  
MVD – difosfomevalonát dekarboxyláza (EC 4.1.1.33)  
NADPH – redukovaný nikotinamidadeninukleotidfosfát  
NCED – 9-*cis*-epoxykarotenoid dioxygenáza (EC 1.13.11.51)  
NSY – neoxantin syntáza (EC EC 5.3.99.9)  
OMT – *o*-methyl transferáza (EC 2.1.1.68)  
PAs – fenolické kyseliny (z angl. Phenolic Acids)  
p.h. – původní hodnota  
PAL – fenylalanin amoniaklyáza (EC 4.3.1.5)  
PDS – fytoen desaturáza (EC 1.3.5.5)  
PDT – prefenát dehydratáza (EC 4.2.1.51)  
PEP – fosfoenolpyruvát  
PMK – fosfomevalonát kináza (EC 2.7.4.2)  
Pp – purpurový perikarp (z angl. Purple pericarp)  
PPA-AT – prefenát aminotransferáza (EC 2.6.1.78)  
PPY-AT – fenylpyruvát aminotransferáza (EC 2.6.1.5)  
PSY – fytoen syntáza (EC 2.5.1.32)  
S3P – šikimát 3-fosfát  
SK – šikimát kináza (EC 2.7.1.71)  
TAA – celková antioxidační aktivita (z angl. Total Antioxidant Activity)  
TAC – celkový obsah antokyanů (z angl. Total Anthocyanins Content)  
TCC – celkový obsah karotenoidů (z angl. Total Carotenoids Content)  
TEAC – ekvivalent antioxidační aktivity troloxu (z angl. Trolox Equivalent Antioxidant Activity)  
TPAs – celkové fenolické kyseliny (z angl. Total Phenolic Acids)  
TPC – celkový obsah fenolických látek (z angl. Total Phenolics Content)  
VDE – violaxantin deepoxidáza (EC 1.23.5.1)  
Ye – žlutý endosperm (z angl. Yellow endosperm)  
ZDS – ζ-karoten desaturáza (EC 1.3.5.6)  
ZEP – zeaxantin epoxidáza (EC 1.14.15.21)

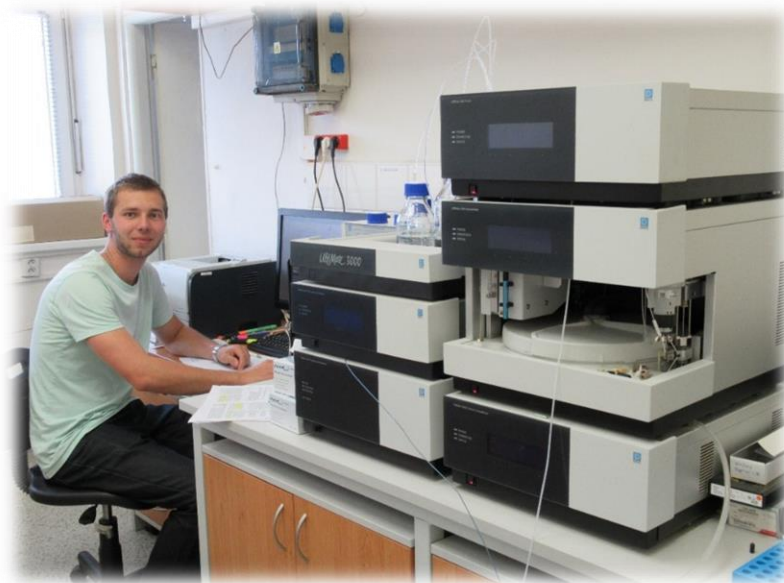
ZISO – ζ-karoten izomeráza (EC 5.2.1.12)

## 9. Seznam obrázků

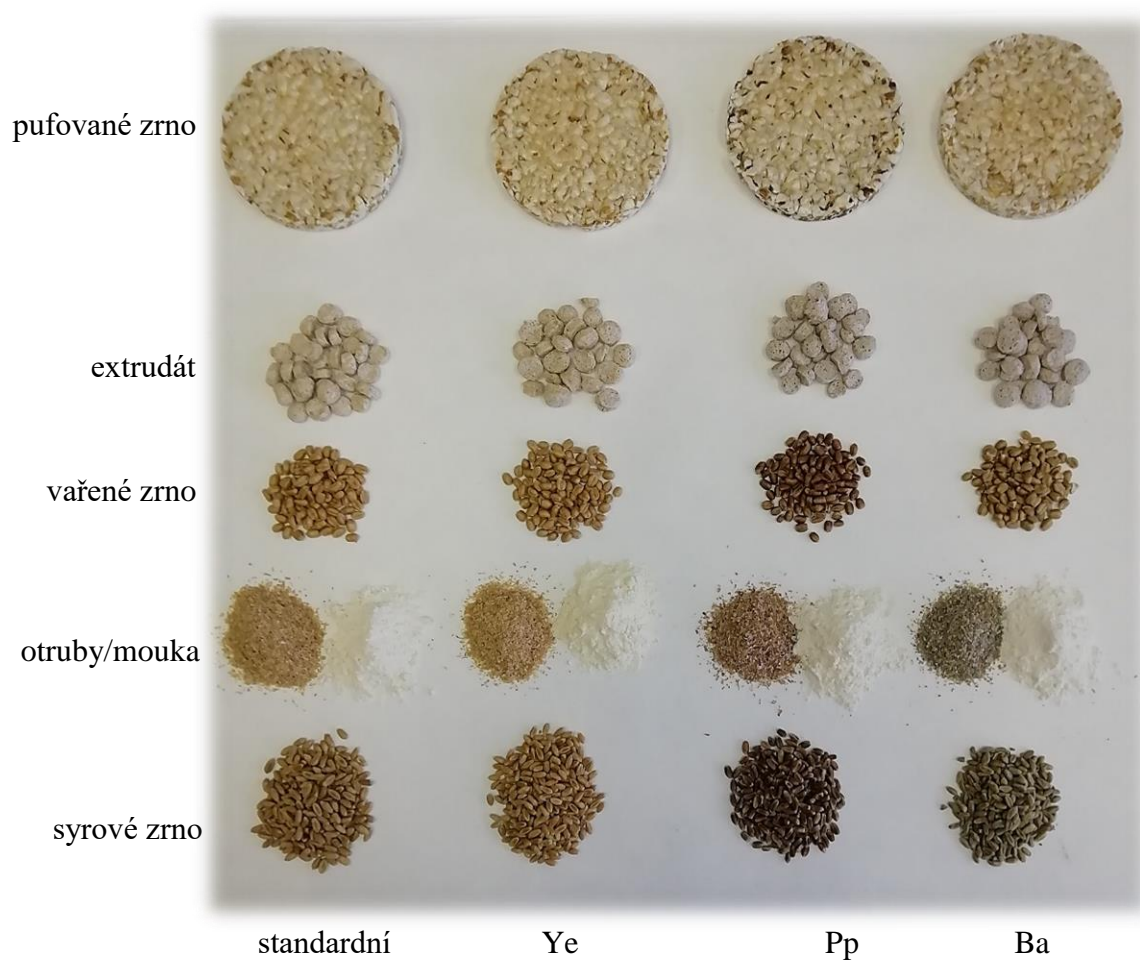
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## 10. Přílohy



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Příloha č. 2 Vzorčky barevných pšeníc před a po různých technologických úpravách