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Influence of baking on anthocyanin content in coloured-grain wheat bread

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Abstract: Composition and degradation of anthocyanins in blue and purple grain wheat during bread production were investigated using the HPLC-MS/MS method. The most abundant anthocyanins were delphinidin-3-rutinoside (blue grain wheat), cyanidin-3-glucoside and peonidin-3-glucoside (purple grain wheat). Peonidin-3-glucoside was also the most stable during grain treatment while delphinidin-3-glucoside had the greatest loss. Both blue and purple grain anthocyanins decreased significantly during bread production to 41.81% and 70.10% after baking, respectively, and to 24.21% and 60.00% after short-term storage, respectively. The blue grain wheat anthocyanins were lost mostly during baking, but in the purple grain wheat, the greatest decrease occurred during dough production. Despite the higher degradation, the blue grain wheat still showed higher anthocyanins content.

Keywords: antioxidants; cereals; high temperature; *Triticum aestivum* L.; vacuolar pigments; thermal treatment

The coloured-grain cereals with anthocyanin content are an interesting alternative to conventional cereals thanks to the added nutritional value. Anthocyanins are natural antioxidants with many health effects and are therefore valuable components of the human diet (Garg et al. 2016). Synthesis of coloured substances in wheat grain developed as adaptation to stress conditions of the external environment. This ability has been lost due to breeding, but is retained in the original and wild cereal species. Today's breeding work uses original gene resources to restore this feature. The coloured-grain wheat with a content of anthocyanins forms two basic colour variants – purple (Pp – purple pericarp) and blue (Ba – blue aleurone). The genotype determines a level of anthocyanin synthesis, but the final anthocyanin content depends on many other parameters: external temperature, intensity of exposure to light, harvest time or magnesium fertilisation (Adbel-Aal et al. 2006, Garg et al. 2016). Anthocyanins are known for their low stability when exposed to high temperature, oxygen or light. Since a heat treatment before consumption is required for cereals, the thermal lability of anthocyanins should be taken into account (Cavalcanti et al. 2011). The knowledge of anthocyanin degradation could help to choose wheat cultivars suitable for commercial production of products rich in health beneficial compounds.

The aim of this study was therefore to describe the effect of baking on anthocyanin degradation in blue and purple grain wheat. To our best knowledge, this is the first study to describe a decrease in individual anthocyanins during baking, and one of very few to describe the anthocyanin composition in specific wheat cultivars.

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MATERIAL AND METHODS

Plant material. Wheat genotypes and breeding lines (*Triticum aestivum* L.) were harvested in 2016/2017 at the Agricultural Research Institute (Agrotest Fyto, Ltd.) in Kroměříž, Czech Republic. The genotypes and breeding lines were AF Jumiko, Konini, ANK-28A, UC66049 and V1 131-15. Plants were grown on the experimental plots (10 m^2) using conventional growing technology. Each wheat genotype was grown on 5 independent plots, which were harvested separately and 1 kg of grain from each experimental plot was used to create a sample. Prior to analysis, the samples were stored in paper bags in the dark at a temperature of 25 °C after being harvested.

Bread baking procedure. The wholegrain flour was prepared on an YM1 Wet Wheat Grinding Machine Y-10 (Yücebaş Machine Analytical Equipment Industry, İzmir, Turkey) 21 days prior to the bread baking procedure. The dough was prepared using aged flour (300 g), 6 g of yeast, 4.8 g of salt and 180 mL of water (30 °C). The mixture was kneaded for 30 min at 30 °C and was left to rise 60 min at 30 °C. The dough was then divided into 3 pieces of which the first was frozen immediately for further analysis and the other two were baked for 14 min at 240 °C. The second piece was frozen right after the baking procedure and the third piece was stored for 24 h at room temperature and then frozen. All three frozen pieces were lyophilised using a Lyovac GT2 (Steris, Derby, UK).

Extraction of anthocyanins. Anthocyanins were extracted according to the method based on Jaafar et al. (2013) with minor modifications. 800 mg were finely ground and internal standard (cyanidin-3-galactoside) in the final concentration 1 µg/mL was added to the sample prior to extraction. The samples were extracted by 10 mL methanol/1 mol/L HCl (85:15, *v*/*v*), sonicated for 30 min in an ultrasonic bath (PS 04, Powersonic-Notus Ltd., Vráble, Slovak Republic) and shook at 150 rpm in the dark for 18 h. The sample was then centrifuged at 3 500 rcf for 5 min (5810 R, Eppendorf, Hamburg, Germany) and the recovered supernatant was transferred to a 10-mL volumetric flask and adjusted to the mark. Samples were filtered through PTFE syringe filter and analysed. All determinations were carried out in triplicate.

HPLC-MS/MS analysis. The analysis was performed using the UltiMate 3000 RS HPLC (Thermo Fisher Scientific, Waltham, USA) coupled with the 3200 QTRAP triple quadrupole mass spectrometer (AB Sciex, Framingham, USA). The analytical column was Zorbax SB-C18 (150 mm × 3.0 mm, particle size 5 µm, Agilent, Santa Clara, USA). Chromatographic conditions were: flow rate 0.5 mL/min, column temperature 35 °C, autosampler temperature 10 °C, injection volume 2 µL, total run time 27 min. The mobile phase consisted of 1% formic acid in water (A) and 1% formic acid in water, 22.5% methanol and 22.5% acetonitrile (B). The gradient for solvent A was: 0–7 min 85%, 7–19 min 85% to 50%, 19–21 min 50%, 21–23 min 50% to 85%, 23–27 min 85%. The mass analysis conditions were: electrospray ionisation in positive mode, ion spray voltage 1.5 kV, source temperature 600 °C, curtain gas 20 psi, nebuliser gas 40 psi, turbo gas 40 psi.

The analytes were identified using mass analysis based on multiple reaction monitoring (MRM) in comparison with analytical standards, using cyanidin-3-galactoside as internal standard. Compound parameters are shown in Table 1 and typical chromatogram is represented in Figure 1. Prior to use of cyanidin-3-galactoside as the internal standard, all the wheat genotypes and breeding lines were analysed to prove total absence of natural cyanidin-3-galactoside. The amount of anthocyanins was expressed in dry

Table 1. Compound parameters of mass detection

Identity	Retention time (min)	$[M]^+$ (m/z)	MS/MS (m/z)	Limit of detection (µg/g)
Cyanidin-3-glucoside	12.55	449.4	287.3*, 241.3	0.001
Cyanidin-3-rutinoside	13.41	595.3	287.3*, 449.3	0.002
Delphinidin-3-glucoside	10.65	465.2	303.2*, 257.3	0.005
Delphinidin-3-rutinoside	11.45	611.3	303.3*, 229.4	0.001
Peonidin-3-glucoside	15.55	463.3	301.1*, 286.1	0.002
Petunidin-3-glucoside	13.63	479.2	317.2*, 302.2	0.001
Cyanidin-3-galactoside	11.63	449.4	287.2*, 213.2	0.001

*ion used for the quantification

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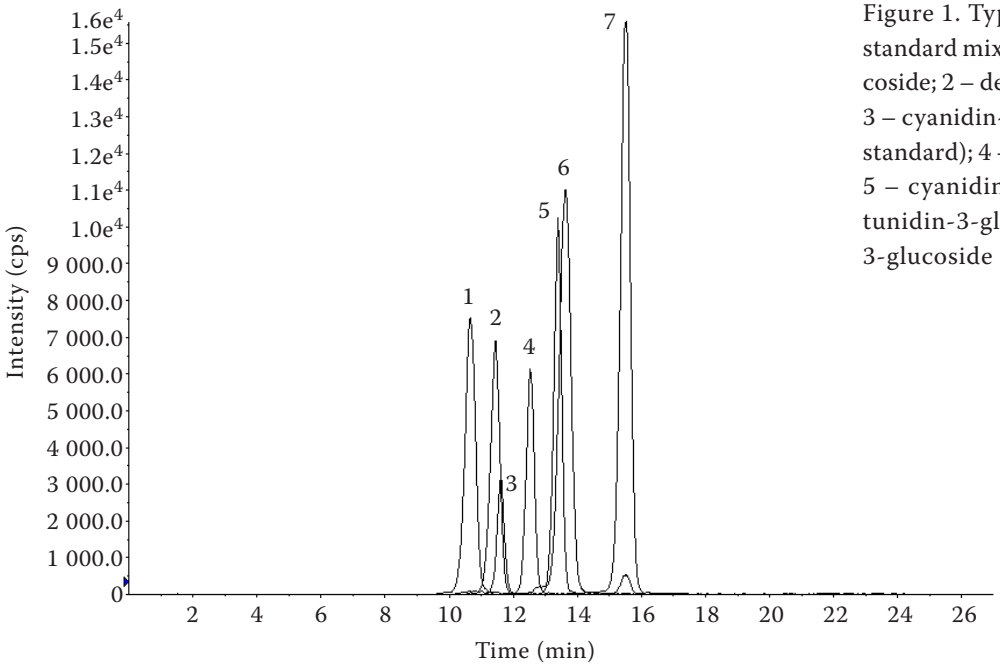

Figure 1. Typical chromatogram of standard mix. 1 – delphinidin-3-glucoside; 2 – delphinidin-3-rutinoside; 3 – cyanidin-3-galactoside (internal standard); 4 – cyanidin-3-glucoside; 5 – cyanidin-3-rutinoside; 6 – petunidin-3-glucoside; 7 – peonidin-3-glucoside

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

Statistical analysis. The data were processed in Analyst 1.4 (AB Sciex, Framingham, USA), Statistica 12 (StatSoft Inc., Tulsa, USA) and Excel (Microsoft, Redmont, USA). The anthocyanin decrease was evaluated by the one-way ANOVA ($P < 0.05$) and Fisher's *LSD* (least significant differences) test in detail.

RESULTS AND DISCUSSION

Anthocyanin content and profile in wholemeal flour. The identified analytes were quantified and

Table 2. Content and composition of anthocyanins in purple (Pp) and blue (Ba) grain wheat

	AF Jumiko (Pp)		ANK 28-A (Pp)		Konini (Pp)		V1 131-15 (Ba)		UC 66049 (Ba)	
	(µg/g DW)	(%)	(µg/g DW)	(%)	(µg/g DW)	(%)	(µg/g DW)	(%)	(µg/g DW)	(%)
cya-3-glu	0.039 ± 0.003^{a}	18.88	0.741 ± 0.108^{k}	35.07	0.526 ± 0.030^{j}	34.85	10.22 ± 0.419^{p}	14.97	3.008 ± 0.192^{n}	7.25
cya-3-rut	nd	–	0.096 ± 0.003^{b}	4.52	0.187 ± 0.053^{e}	12.39	12.89 ± 0.542^{q}	18.88	8.500 ± 0.363^{o}	20.48
peo-3-glu	0.170 ± 0.011^{d}	81.12	1.231 ± 0.175^{m}	58.28	0.359 ± 0.032^{h}	23.77	0.236 ± 0.010^{f}	0.35	0.126 ± 0.025^{c}	0.30
del-3-glu	nd	–	nd	–	0.184 ± 0.077^{e}	12.16	18.44 ± 0.828^{r}	27.02	10.57 ± 0.747^{p}	25.43
del-3-rut	nd	–	nd	–	0.254 ± 0.120^{g}	16.82	25.42 ± 1.460^{s}	37.23	18.89 ± 1.354^{r}	45.50
pet-3-glu	nd	–	0.045 ± 0.001^{a}	2.12	nd	–	1.058 ± 0.058^{l}	1.55	0.431 ± 0.112^{i}	1.04
Total sum	0.209 ± 0.011	100.0	2.113 ± 0.287	100.0	1.510 ± 0.298	100.0	68.27 ± 3.263		41.51 ± 2.650	

Values marked with different letters are statistically different ($P < 0.05$). DW – dry weight; nd – not detectable; % – % of the sum. cya-3-glu – cyanidin-3-glucoside; cya-3-rut – cyanidin-3-rutinoside; del-3-glu – delphinidin-3-glucoside; del-3-rut – delphinidin-3-rutinoside; peo-3-glu – peonidin-3-glucoside; pet-3-glu – petunidin-3-glucoside; cya-3-gal – cyanidin-3-galactoside; Pp – purple grain wheat; Ba – blue grain wheat

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the content of anthocyanins was expressed as their sum (Table 2). The lowest content of anthocyanins was found in AF Jumiko genotype and the highest content in V1 131-15 genotype. While both blue grain wheats had an identical anthocyanin profile, the profiles of purple grain wheats were more variable. Peonidin-3-glucoside and cyanidin-3-glucoside were the most abundant anthocyanins, while delphinidin-3-rutinoside and delphinidin-3-glucoside were identified only in Konini genotype. Obviously, the profile of anthocyanins in the purple grain genotypes is very diverse and may vary significantly from one genotype to another (Knievel et al. 2009). This was also confirmed by Ficco et al. (2014), who believe that this difference is due to differences in genetic control of colour for purple and blue grain wheat. In contrast, the profile of blue wheat is less variable. In this study delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside were identified as major blue wheat anthocyanins. The presence of all these anthocyanins in blue genotypes was also confirmed by other authors (Abdel-Aal et al. 2006, Knievel et al. 2009, Ficco et al. 2014).

Degradation of individual anthocyanins during bread production. The individual anthocyanins degradation expressed as average of all genotypes are shown in Figure 2A. Analytes (along with percentage of loss) ranging from least to most degrading were: peonidin-3-glucoside (44%) < cyanidin-3-glucoside (52%) < cyanidin-3-rutinoside (61%) < petunidin-3-glucoside (65%) < delphinidin-3-rutinoside (80%) < delphinidin-3-glucoside (82%). Overall, peonidin,

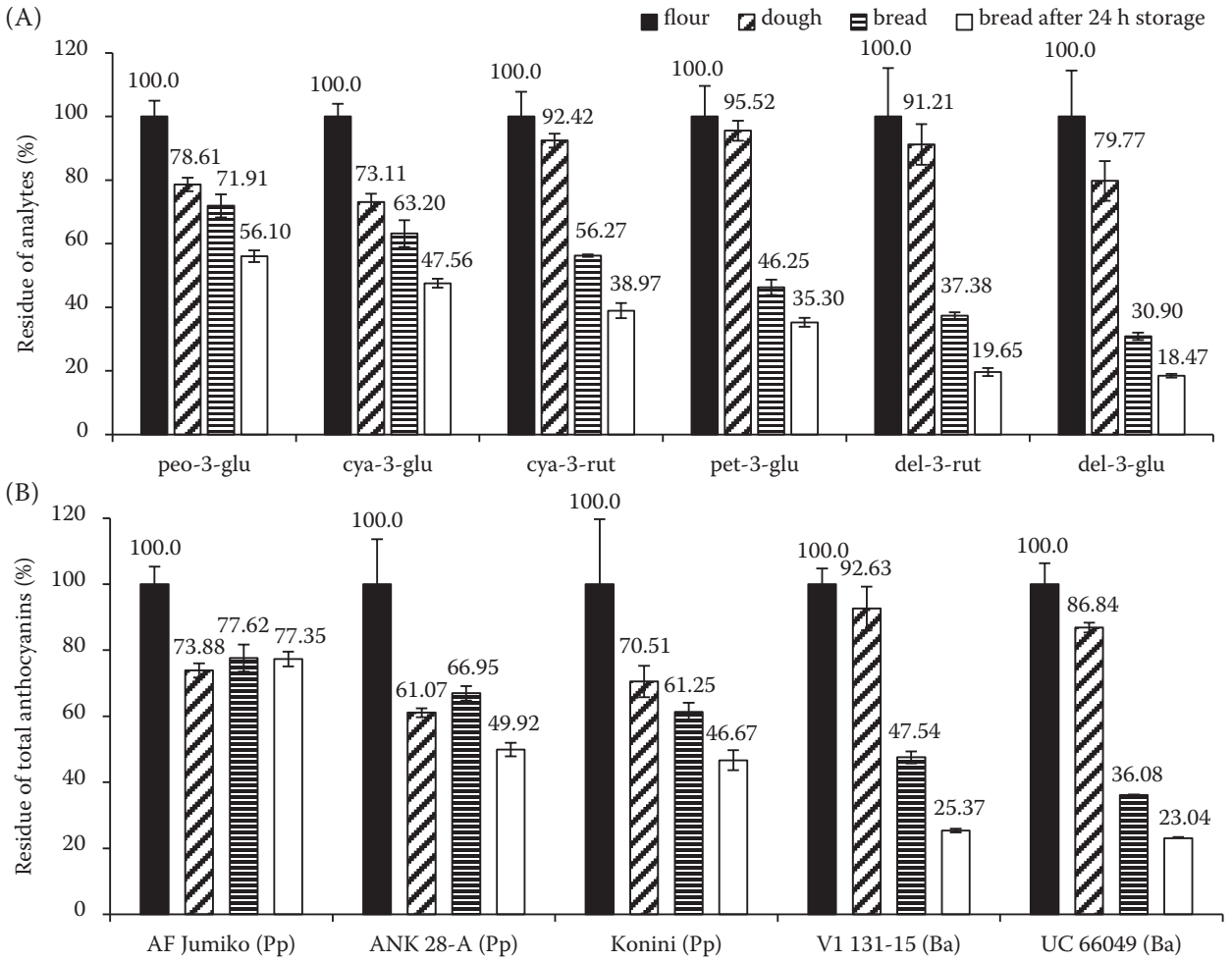

Figure 2. Loss of (A) individual and (B) total anthocyanins amount during bread production – expressed as percentage residue of the original amount of anthocyanins in flour. cya-3-glu – cyanidin-3-glucoside; cya-3-rut – cyanidin-3-rutinoside; del-3-glu – delphinidin-3-glucoside; del-3-rut – delphinidin-3-rutinoside; peo-3-glu – peonidin-3-glucoside; pet-3-glu – petunidin-3-glucoside; cya-3-gal – cyanidin-3-galactoside; Pp – purple grain wheat; Ba – blue grain wheat

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cyanidin and petunidin glycosides were more stable, while delphinidin glycosides showed the highest degradation. This was also confirmed by Hou et al. (2013), who determined the stability of anthocyanins to be: peonidin-3-glucoside > cyanidin-3-glucoside > cyanidin-3-rutinoside, which corresponds to our results. The dependence of anthocyanins' stability on their chemical structure was also established by Fleschhut et al. (2006), who reported that the stability of anthocyanins decreases with a higher degree of hydroxylation and methoxylation of aglycone. This is also reflected in the results of our study, where the stability of peonidin > cyanidin > petunidin > delphinidin decreases with an increasing number of substituents. Therefore, the inter-genotype differences in anthocyanins are supposed to be a significant factor in their thermal degradation (Szalóki-Dorkó et al. 2015).

Total anthocyanin degradation during bread production. Changes of the anthocyanins content during bread production in particular technological steps production were expressed as total sum of anthocyanins (Table 3) and as a percentage decrease (Figure 2B). In the blue grain wheat samples, the largest decrease was observed in the baking stage (45% and 51%). The total loss of anthocyanins in the blue genotypes was therefore 75% and 77%, and all decreases in the individual technological steps were statistically significant. On the other hand, the purple grain genotypes showed lower total loss of anthocyanins (17, 50 and 53%) and the greatest decline was observed during dough production (26, 29 and 39%).

The AF Jumiko and ANK28-A genotypes showed a typical slight increase during the baking stage which was not significant. The modest rise in anthocyanin content after baking could be due to greater analytes extractability. Anthocyanins can evidently bind to plant cell walls and interact with various flour components (cellulose, proteins) (Sivam et al. 2012). If solubility of these components was increased by baking, extractability of anthocyanins could be positively affected too. This could be corroborated by the fact that thermal treatment causes higher solubility of cell components of grape skin and is therefore used to increase extractability of anthocyanins from this material (Lambri et al. 2015). However, it can be assumed that this effect would only have little significance in wheat grain as indicated by the fact that this effect was only found in wheat cultivars with the lowest anthocyanin content and was not statistically significant.

During dough preparation, the content of anthocyanins is reduced by addition of other dough components. At the same time, air is incorporated into the dough and enzymes causing degradation of anthocyanins, especially polyphenol oxidase and peroxidase, are activated (Nayak et al. 2015). Diverse levels of degradation in particular genotypes are possibly caused by different activity of oxidative enzymes since polyphenol oxidase and peroxidase activities are very different in the various wheat genotypes (Geng et al. 2019).

High temperature, which is a major factor of anthocyanin degradation (Cavalcanti et al. 2011), caused a decrease in anthocyanin amount in most of genotypes. Similar degradation in purple grain wheat bread was described by Yu and Beta (2015). However, Bartl et al. (2015) reported greater decline of anthocyanins in purple grain bread than in blue grain bread, which does not correspond to our results. This could be probably caused by diverse wheat genotypes use. As already mentioned, inter-genotype anthocyanin content differences could be an important factor of their thermal degradation. It can be assumed that the degradation will be more significant in wheat genotypes with a high amount of less stable delphinidin-3-rutinoside and delphinidin-3-glucoside. In contrast, wheat genotypes containing mainly peonidin-3-glucoside, cyanidin-3-rutinoside

Table 3. The average total amount of anthocyanins (μg/g DW) in particular production stages

	AF Jumiko (Pp)	ANK 28-A (Pp)	Konini (Pp)	V1 131-15 (Ba)	UC 66049 (Ba)
Wholemeal flour	0.209 ± 0.010^{a}	2.113 ± 0.287^{a}	1.510 ± 0.298^{a}	68.27 ± 3.263^{a}	41.51 ± 2.650^{a}
Dough	0.154 ± 0.004^{b}	1.304 ± 0.040^{bc}	1.065 ± 0.072^{b}	63.29 ± 4.492^{b}	36.05 ± 0.611^{b}
Bread	0.163 ± 0.006^{b}	1.414 ± 0.046^{c}	0.925 ± 0.042^{bc}	32.46 ± 1.265^{c}	14.99 ± 0.084^{c}
Bread after 24 h	0.162 ± 0.004^{b}	1.055 ± 0.044^{d}	0.705 ± 0.046^{c}	17.32 ± 0.398^{d}	9.564 ± 0.166^{d}

Values within columns marked with different letters are statistically different ($P < 0.05$). DW – dry weight; Pp – purple grain wheat; Ba – blue grain wheat

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and cyanidin-3-glucoside will have presumably more stable anthocyanin content and its degradation will be minor.

Although the purple grain genotypes used in this study showed lower anthocyanin degradation, the total anthocyanin amount after baking and short-term storage was still higher in blue genotypes. Hence, the coloured-grain wheat cultivars with high anthocyanin content are particularly suitable for bread production due to their higher remaining anthocyanin amount after baking. For this reason, UC 66049 and V1 131-15 breeding lines are recommended in this study for cultivation and commercial production of anthocyanin-rich products.

Conflict of interest. The authors declare no conflict of interest regarding this work.

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