

ANTHOCYANINS IN WHEAT SEED – A MINI REVIEW

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Abstract: Improving the micronutrients in food has become an important field of the Second Green Revolution. In recent years, minor bioactive compounds such as polyphenols, pigments and carotenoids, have attracted more and more interest from both researchers and food manufactures as health-promoting and disease-preventing effects in both *in vitro* and *in vivo* studies. One of plant pigments, wheat anthocyanins as plant phenolics are increasingly attractive as natural compounds positively affecting consumer's health and condition moreover wheat is staple food source consumed usually daily. For a purple, blue, or red colour of wheat seed are responsible glycosylated cyanidins, delphinidins, malvinidins, pelargonidins, petunidins, and peonidins located in aleurone layer or pericarp, respectively. Other than white seed colour is not natural for common hexaploid wheat but this trait can be introduced from donors by aimed breeding programs. The way of wheat anthocyanins to provide positive effects for consumer's physiology is limited due to their specific occurrence in seed parts usually removed during grain milling practice and lower stability during processing to foods.

Key words: phenolics, flavonoid, anthocyanins, wheat grain

1. Introduction

Anthocyanins are chemical compounds of plant origin well known as pigments responsible for blue, purple, red, or orange coloration of plant tissues and organs. From the structural point of view anthocyanins are glycosides composed of hydroxylated or methoxylated 2-phenylbenzopyrilium skeleton with hydroxyl and methoxy groups in the B-ring. The structural variation extends bounded sugars (the most frequent are pentoses - xylose, arabinose, rhamnose, fructose and hexoses - galactose, glucose), aliphatic, and aromatic acids.

The consequence of molecular variability is more than six hundreds known natural anthocyanins. Six the most frequent are cyanidins, delphinidins, malvinidins, pelargonidins, petunidins, and peonidins (IWASHINA, 2000). Anthocyanins are phenolic phytochemicals classified within flavonoids together with flavonols, flavones, flavanols, flavanones, and isoflavonoids (LIU, 2004).

Their biosynthesis initializes conjugation of malonyl-CoA and *p*-coumaroyl-CoA derived from phenylpropanoid biosynthetic pathway under operation of seven enzymes - chalcon synthase, chalcon isomerase, flavanone-3-hydroxylase, flavonoid-3-hydroxylase, dihydroflavonol-4-reductase, anthocyanin synthase, and flavonoid-3-O-glucosyltransferase (HOLTON and CORNISH, 1995; SCHIJLEN *et al.*, 2004).

Anthocyanins are generally specified as bioactive, non basicly-nutritional compounds, responsible for antioxidant and UV/photoprotective functions (RYAN *et al.*, 2001), playing also role in plant reproduction (KONG *et al.*, 2003). Anthocyanins participate in the formation of non-specific disease resistance in plants (TREUTTER, 2006) such as pre-harvest sprouting where the red pigment of red-grained wheat is synthesized through the flavonoid biosynthetic pathway, in which the dihydroflavonol-4-reductase gene (DFR) is one of the genes involved in anthocyanin synthesis (BI *et al.*, 2014).

Specific properties make anthocyanins attractive in prophylaxis and therapy (ANONYMUS, 2014) due to their antitumor, cardio- and hepatoprotective, antimutagenic, antiulceral, UV-protective, and other health beneficial effects (KONG *et al.*, 2003; KAY, 2006; PRIOR and WU, 2006; HODGSON and CROFT, 2006). It appears that these phytochemicals are responsible for the reduced risk of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (JACOBS and STEFFEN, 2003). Compared to vitamins C and E which are absorbed in the upper segments of the intestine, anthocyanins are dietary antioxidants presented in the colon in valuable concentrations (MANACH *et al.*, 2004).

Seeds of cereals are not typical sources of anthocyanins nevertheless blue, red, and purple seeds attract consumers, food producers, and plant breeders (GUO *et al.*, 2012), therefore relevant knowledge has been cumulated also in maize, wheat, barley, oat, and rice as the most important world food sources. Coloured-grain wheat varieties with good genetic stability, excellent stress resistance and high yield are still required (GUO *et al.*, 2012).

2. Anthocyanins in wheat seeds

Isolation, identification, and analyses of wheat anthocyanins have begun long time ago. DEDIO *et al.* (1972) detected cyanidine-3-glucoside and peonidine-3-glucoside (Fig. 1) as the main anthocyanins of pericarp in purple wheat seeds. Cyanidine-3-glucoside as the dominant anthocyanin in purple wheat seeds was detected also by HOSSEINIAN *et al.* (2008) and ABDEL-AAL and HUCL (2003) together with cyanidine-3-galactoside (Fig. 1), peonidine-3-glucoside, and others unidentified ones. As the most frequent anthocyanins in blue wheat seeds were declared delphinidine-3-glucoside and delphinidine-3-rutinoside (ABDEL-AAL *et al.*, 2006; Fig. 1), cyanidine-3-glucoside and peonidine-3-glucoside (ABDEL-AAL and HUCL, 2003). The blue wheat seeds contain more anthocyanins in flour than in bran, in comparison with purple seeds (ABDEL-AAL and HUCL, 1999). On the other hand, in Hungarian hard red winter wheat varieties and blue-grained varieties, the anthocyanin content of the grind was 21-157 mg kg⁻¹, while that of the flour was only 5.3-17.4 mg kg⁻¹ (VARGA *et al.*, 2013) so most of the anthocyanin content was in the bran.

HU *et al.* (2007) detected in the wheat cultivar Hedong Wumai also minor anthocyanins - cyanidine-3-galactoside, pelargonidine-3-glucoside (Fig. 1), peonidine 3-glucoside, and ferulic acid (Fig. 1).

Red-coloured wheat seeds contain incomparably lower content of anthocyanins in comparison with blue and purple ones. Relationships between content of catechins,

tannins, and red colour of wheat seeds published MIYAMOTO and EVERSON (1958). WINKEL-SHIRLEY (2001) inclined to the opinion that pigmentation causes proanthocyanins (condensed tannins).

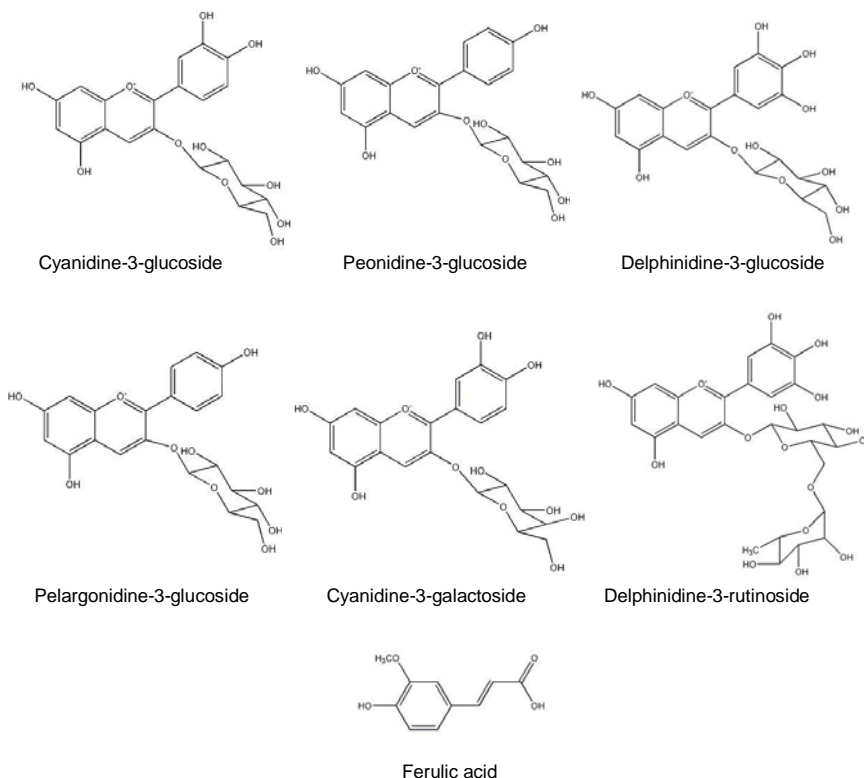


Fig. 1. Main anthocyanins of coloured wheat grains.

It is known that environment affects production of polyphenols (ETICHA *et al.*, 2011; KHLESTKINA, 2013) as well as anthocyanins (VARGA *et al.*, 2013) and abiotic stresses like high light intensity, low temperature, high salinity and/or drought stress, and others increase their amounts (CHALKER-SCOTT, 1999). Cold stressed coloured wheat genotypes show either reduced, similar or increased anthocyanin content compared to unstressed plants caused possibly by the allelic state of the *Rc* genes responsible for the seed colour (GORDEEVA *et al.*, 2013). According to authors, the changes in anthocyanin content correlate negatively with the changes of growth parameters in response to cold stress, suggesting the presence of some stress-dependent regulation of anthocyanin biosynthesis in wheat coleoptiles.

Changes in anthocyanin content are associated with physiological maturity of the plant and grain, respectively. Location of grains in spike affects anthocyanin content by decreasing at distal positions reflecting positive response to higher source-sink ratio

(BUSTOS *et al.*, 2012). In the experiments of ŽOFAJOVÁ *et al.* (2012) genotypes with purple pericarp reached the highest total anthocyanins concentration on the 22nd day post anthesis with increasing and decreasing before and after sampling time, respectively. Pre-anthesis halving increased anthocyanins content by 54 and 37%, while post-anthesis halving increased this trait by 31 and 29%, respectively (BUSTOS *et al.*, 2012). Promising to increase anthocyanins in purple wheat is the combination of early harvesting and magnesium fertilization (BUSTOS *et al.*, 2012). Content of total phenolic compounds in wheat seeds can be also affected by growing condition (VAHER *et al.*, 2010) or by application of selenium into the soil (CHU *et al.*, 2010).

3. Origin and genetic control of the seed colour

The blue and purple colours are not natural for seeds of common wheat. Anthocyanins responsible for blue pigmentation are located in aleurone layer and for purple colour in pericarp, respectively. According to ZEVEN (1991) the blue colour was introduced into wheat from blue coloured diploid wild einkorn wheat *Triticum boeoticum*, *Agropyron tricholporum*, *Agropyron glaucum* or tall wheatgrass *Agropyron elongatum*, and purple colour from tetraploid emmer *Triticum dicoccum*. ZELLER *et al.* (1991) specified that hexaploid wheat received the blue aleurone colour through chromosomal substitutions of 4A and 4B chromosomes with 4A chromosomes of diploid einkorn wheat *Triticum monococcum* or *Triticum boeoticum*, respectively. “Black” (deep purple) hues of wheat kernel colour can be due to the combination of anthocyanin genes for blue pericarp and blue aleurone (LI *et al.*, 2005). Results of GUO *et al.* (2012) showed that the colour inheritance of purple-grained wheat follows a maternal inheritance pattern and that the blue-grained wheat expresses xenia in most cases.

Nevertheless, the genetics of wheat anthocyanins has been unlocking more than fifty years. KNOTT (1958) published that partially dominant gene/genes with different effects were responsible for the blue seed colour. HURD (1959) supported responsibility of two partially dominant genes. Later, KEPPE and BAENZIGER (1990) presented that blue colour of wheat aleurone is controlled by single dominant gene. Nowadays, the *Ba* (blue aleurone) gene originated from the 4E chromosome of *Thinopyrum ponticum* is considering as the incompletely dominant gene responsible for the blue wheat aleurone (DUBCOVSKY *et al.*, 1996). The concept of single dominant gene has been published recently, too (KNIEVEL *et al.*, 2009). Results on mapping of anthocyanins pigmentation genes in wheat along with the observation of previous studies related were presented by KHLESTKINA *et al.* (2008). The purple colour of pericarp originated from purple Ethiopian tetraploid and hexaploid wheat and purple Abyssinian wheat (*Triticum aethiopicum* Jakubz.) was firstly described long ago (WITTMACK, 1906). McINTOSH and BAKER (1967) published hypothesis that the purple colour of wheat pericarp is controlled by two duplicated genes. Two independent responsible genes were located later at the 3A and 7B chromosomes (PIECH and EVANS, 1979), GRIFFIN (1987) defined these genes as dominantly duplicated. ARBUZOVA *et al.* (1998) and ARBUZOVA and MAYSTRENKO (2000) located genes for purple seeds in the *Triticum aestivum* cultivar Purple Feed at

chromosomes 7B (gene *Pp1*) and 6A (*Pp2*), and in the purple varieties at chromosomes 7B (*Pp1*) and 2A (*Pp3*). DOBROVOLSKAYA *et al.* (2006) located three genes - *Pp1*, *Pp2*, and *Pp3* and relabelled at once them as *Pp1* (at the 7BL chromosome), and *Pp3b*, *Pp3a* (chromosome 2A). The work of TERESHCHENKO *et al.* (2012) demonstrated that the D genome of bread wheat Purple carries one of two complementary genes determining purple grain colour and this gene was mapped on the short arm chromosome 7D 2.5 cM distal to the locus *Rc-D1* determining red coleoptile colour. This position is highly comparable with that of the *Pp1* gene mapped earlier in tetraploid *Triticum durum*.

Recently, KNIEVEL *et al.* (2009) concluded that genetic control of purple coloured wheat pericarp is more complicated and authors also presented design of three dominant alleles. Their results also indicate that wheat breeding programmes aimed to blue seed colour should be feasible, moreover supported by available genetic maps of wheat chromosomes 2A and 7B, mapped genes *Pp3* and *Pp1*, and linked molecular markers (KHLESTKINA *et al.*, 2010; TERESHCHENKO *et al.*, 2012). Red pigmentation of wheat seeds is also based on the content of polyphenols. According to HIMI *et al.* (2005), expression of four genes (chalkonsynthase, chalkoniserase, flavanon-3- hydroxylase, and dihydroflavanol-4-reductase) in white coloured wheat seeds was reduced in comparison with red coloured ones. This suggests that three *R* genes (*R1*, *R2*, *R3*) encoding the seed colour and located at the chromosomes 3A, 3B, and 3D (McINTOSH *et al.*, 1998; KHLESTKINA *et al.*, 2011) are transcription activators (*Myb* transcription factors) of genes in flavonoid synthesis (HIMI and NODA, 2005).

4. Coloured wheat seeds for nutrition

Coloured wheat seeds are natural source of pigments as phytochemicals and may impart desirable colour and stability for commercial food products (GIUSTI and WROLSTAD, 2003), especially for some slightly alkaline ones (CABRITA *et al.*, 2000). Results of MAZZARACCHIO *et al.* (2012) indicate a presence of strong interaction between structure of anthocyanins and gluten molecule by playing an important role in pigments adsorption by gluten or its fraction, whereby delphinidine is more adsorbed than cyanidine. Occurrence of anthocyanins in foods and beverages, their transformation during processing, and their bioavailability are described by CLIFFORD (2000). They are beneficial for human health and fitness, possessing several unique traits and functions in organism including anti-obesity effects and influencing of brain activities (PRIOR and WU 2006). Anthocyanins are competent for effective elimination of oxidative stress in human body by balancing between oxidants and antioxidants (TEMPLE, 2000).

Coloured seeds in comparison to colourless equivalent contain higher level of anthocyanins and phenolic compounds and their extracts have stronger antioxidant competence (LIU *et al.*, 2010). This confirmed CHOI *et al.* (2007) in rice and millet, and LIU *et al.* (2005) in wheat where purple and blue coloured seeds had higher capability to scavenge free radicals than white ones.

Total amount of anthocyanins in pigmented wheat seeds is relatively high, ABDEL-AAL and HUCL (1999) quantified their amount on average 15.7 mg kg⁻¹ and

45.8 mg kg⁻¹ in flour and bran of blue coloured wheat, respectively. On the opposite, IQBAL *et al.* (2007) detected in extracts from red wheat's bran much lower content of anthocyanins (3.0-3.8 mg kg⁻¹), nevertheless their occurrence relates to oxygen radical absorbance capacity, radical scavenging activity, and total phenolic content.

There are several specific traits of wheat anthocyanins including the location in seed. Due to occurrence in seed pericarp and aleurone about 80% of phenolic compounds and flavonoids are in bran or wholemeal (LIU, 2007) and the free radical scavenging capacity of endosperm is much lower (LIYANA-PATHIRANA and SHAHIDI, 2007; SIEBENHANDL *et al.*, 2007). Wheat particle size effect the extraction of phytochemicals. The coarse treatment (unmilled whole bran) exhibit significantly higher antioxidant properties compared to fine treatment, but on the other hand, the fine treatment was higher in anthocyanin content (BREWER *et al.*, 2014).

Common disadvantage of anthocyanins in food processing is the sensitivity to different factors such as temperature (IBANOĞLU, 2002), light intensity, storage conditions, pH, metal ions, enzymes, oxygen, sulphur dioxide, ascorbic acid, sugars, and co-pigments (MAZZA and BROUILLARD, 1987; CABRITA *et al.*, 2000; BAŁOWSKA-BARCZAK, 2005), nevertheless their colour stability can be improved by acylation (BAŁOWSKA-BARCZAK, 2005). The antioxidant capacity can be affected during the thermal processing by both, the extraction solvent and matrix composition (LI *et al.*, 2007).

Analysis of nutrient composition of purple wheat shows that the amount of 40 kinds of nutrients is higher than those of the control (GUO *et al.*, 2012; GUO *et al.*, 2013). For example, the amounts of sodium and manganese in purple wheat are higher than the standards by 312 - 2018% and 548 - 733%, respectively. The contents of beta plus gama-vitamin E were higher by 300% and zinc, iron, magnesium, and potassium were higher than the control by 100%. In biofortification breeding and functional food development there are also useful significant positive correlations between anthocyanins and molybdenum and glutamic acid concentration observed. On the other hand, significant negative correlations between anthocyanin's concentration and free tryptophan and calcium have been found, too (GUO *et al.*, 2013).

On the opposite, in comparison with other typical anthocyanin sources (e.g. fruits and vegetables) cereals seeds can be stored easily and treated to stable products (ABDEL-AAL *et al.*, 2006). Improvement of anthocyanin content in wheat relates to available genetic resources and breeding programmes. Desirable sources of seed phenolic compounds could be wheat landraces where were also new unique phenolic substances identified (DINELLI *et al.*, 2009). Coloured wheat has been traditionally used like purple hexaploid wheat for special bread baking in the New Zealand. The wheat cultivar Indigo with purple seeds designed for special food products has been released in Austria in the year 2006 (ETICHA *et al.*, 2011). Indigo possess about 200 mg kg⁻¹ of anthocyanins in grains what is the amount comparable with the red wine. The wheat cultivar PS Karkulka with purple grains has been registered in the year 2014 as results of the breeding program "rainbow wheats" in Slovakia.

More than twenty years running effort in breeding of coloured wheat led to the development of black-grained wheat (BGW) cultivar in China. The BGW possess also high content of proteins, selenium, and amino acids, but has lower bread-making quality (LI *et al.*, 2006).

The number of registered coloured wheat cultivars is very limited and those available ones have low baking quality and agronomic value (VACULOVÁ *et al.*, 2010).

5. Conclusions

Information presented in this mini-review is based on our own experiments as well as published results in topic of wheat anthocyanins. There are both known, the chemical structure of major anthocyanins of the wheat seed as well as their location in specific part of the seed. From the genetic point of view, the final opinion about the number and location of genes is still developing. Different donors of purple, blue, and red colour of the seed have been found and used for incorporation these traits into wheat cultivars. Following challenge for wheat breeders is to create modern wheat cultivars with coloured seeds but also adapted to local growing conditions: materials with accepted agronomic characteristics, technological quality traits, and therefore profitable for producers.

Currently, based on accumulated knowledge also genetic maps and molecular markers linked to relevant loci can effectively support molecular breeding programmes. The interest in creation of new wheat cultivars with seeds coloured by presence of anthocyanins results mainly from their positive effects on health and wellness of the consumer. On the other side, there are also problems to include wheat anthocyanins into food chain due to their unstability during the food processing.

Presented information about wheat anthocyanins can initiate further related challenges e.g. improvement of anthocyanin production in wheat seed, modification of wheat agricultural practice and analyses of environmental influences, redirecting of synthesis or expression of anthocyanin to other seed part that aleurone and pericarp, development of new processing methods more protecting biological properties of anthocyanins.

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