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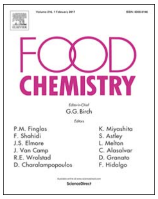

Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains

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ABSTRACT

Carotenoids are important phytonutrients responsible for the yellow endosperm color in cereal grains. Five 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_{40} 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

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

[a] Tschermak's blaukörniger Sommerweizen.
[b] V1-131-15: (RU-440 × V1-702) × (Citrus × Bona Dea).
[c] V1-133-15: RU-440 × UC 66049.
[d] 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 (9*Z*)-lutein (0.13 µg/g DW), α- and β-carotene (0.04 and 0.07 µg/g DW) (Konopka, Czaplicki, & 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 (Masisi et al., 2015; Siebenhandl et al., 2007), and corn (Žilić, Serpen, Akıllıoğlu, 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, luvic 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^2) 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