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Effect of lutein esterification on the differential distribution of carotenoids in germ and endosperm fractions from tritordeum grains

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ABSTRACT

The effect of lutein esterification on the carotenoid distribution profile in the different fractions of tritordeum grains was studied. Durum wheat, a cereal lacking of lutein esters, was included for comparison in the study. Although carotenoid contents in endosperm and germ were significantly different for both cereals, the pigment distribution showed a marked dependence on the cereal's genetic background. Thus pigment content in durum wheat was 3 times higher in germ (2.52 μg/g fresh weight (fw)) than the endosperm (0.74 μg/g fw). In contrast, carotenoids in tritordeum were distributed more homogeneously (4.16 and 4.59 μg/g fw for germ and endosperm, respectively). Lutein esters were exclusively present in tritordeum fractions, with a 3-fold higher content in the endosperm, which suggests a preferential esterifying activity in this tissue. The fatty acid profile indicated that the presence of lutein esters could be limited by the existence of specific XAT (xanthophyll acyltransferase) enzymes and not by substrate availability. A positive impact of esterification on the even deposition pattern of pigments throughout the tritordeum grain was observed. These data could be useful for optimizing the retention of lutein through the food chain as well as to direct the breeding of crops enriched in lutein esters.

1. Introduction

A diet rich in whole grains and derived products has been associated with a reduced risk of developing chronic diseases (cardiovascular diseases, type 2 diabetes, some cancers, etc.). This is attributed in part to their antioxidant content; however, more information is required about the composition and distribution of antioxidant phytochemicals (including carotenoids) in cereal grains (Fardet, 2010). Carotenoids are a class of natural pigments that are ubiquitous components of all photosynthetic organisms because they are required for the assembly and function of the photosynthetic apparatus. In wheat grains, the antioxidant activities of carotenoids protect the seed from deterioration and contribute to the successful progress of the germination process (Howitt and Pogson, 2006). Carotenoid functions in humans are no less important: β-carotene, among others, is a precursor of vitamin A, and lutein and zeaxanthin have an important role in the prevention of ocular diseases (Landrum and Bone, 2001). Humans and animals are unable to synthesize carotenoids *de novo* and rely upon dietary sources of these compounds. Fruits and vegetables are generally considered to be the main dietary source of carotenoids. In addition, staple cereals often contain minor amounts of carotenoids in their grains. Consequently, cereals are being bred to boost their levels of these phytochemicals by both conventional and transgenic breeding methods. Certain cereals, such as yellow corn, durum wheat, and specialty primitive wheat (e.g., Einkorn and Khorasan), contain relatively high amounts of carotenoids, mainly lutein (reviewed by Shewry and Hey, 2015). Some synthetic cereals, such as tritordeum (×*Tritordeum* Ascherson et Graebner), offer even better properties than these ancient cereal species. Hexaploid tritordeum is the amphiploid derived from the cross between a wild barley (*Hordeum chilense* Roem. et Schultz.) and durum wheat (*Triticum turgidum* conv. *durum*). Special attention has been focused on this new cereal due to its increased carotenoid content (about 5–8 times higher than durum wheat) and high proportion of lutein esterified with fatty acids (including both lutein monoesters and diesters) (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2012).

Current research has shown that the total phytochemical content and antioxidant activity of whole grains have been commonly underestimated in the literature, so that whole grains contain more

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phytochemicals than previously reported (Hentschel et al., 2002). The carotenoid profile of cereals is mainly composed of xanthophylls, with lutein the most abundant, followed by zeaxanthin and β-cryptoxanthin, as well as small amounts of carotenes such as α- and β-carotene. In general, the bioactive compounds are concentrated in the outer layers (bran) of the cereal grain (Fardet, 2010). The highest concentration of carotenoids can be found in the embryo, although this part of the seed only represents 3–5% of the grain's total weight. However, the endosperm, representing around 80–85% of the grain has the most influential contribution in terms of the total carotenoid content. Moreover, the distribution of the carotenoid content and its profile seems to vary between and within cereal varieties (Ziegler et al., 2016). Most studies of carotenoid pigments in cereals attribute their location to the endosperm, while only a few considered the distribution of these pigments in the different fractions of the grain kernels (Borrelli et al., 2008). One study developed a new chromatographic method to verify the qualitative and quantitative distribution of carotenoids in the kernel fractions and in cereal by-products (Panfili et al., 2004). Later, Adom et al. (2005) published the detailed carotenoid composition of the milled fractions (endosperm and bran/germ) of three different wheat varieties. Significant differences in the carotenoid content (lutein and zeaxanthin) between different millstreams, including bran, of purple wheat, blue wheat, and black barley were reported by Siebenhandl et al. (2007). More recently, Ndolo and Beta (2013) and Masisi et al. (2015) provided data about the carotenoid composition of the aleurone layer of different cereals, a grain fraction with high levels of potentially health-promoting compounds including zeaxanthin and lutein. Although the esterification of xanthophylls has been found to increase the accumulation and stability of carotenoid pigments during grain storage and processing, there is a lack of information regarding their tissue distribution in wheat grains (Ahmad et al., 2015). The aim of this study was to investigate the effect of esterification on the distribution profile of carotenoids in the two major grain fractions, endosperm/bran and germ/embryo. For this purpose, durum wheat and tritordeum grains were selected as none- and high-carotenoid ester containing cereals, respectively. A better understanding of lutein ester formation and distribution within the grain will provide critical information to help optimize the retention of lutein through the food chain.

2. Materials and methods

2.1. Plant material

Grains from a tritordeum line (high-carotenoid advanced line HT621) and a commercial durum wheat variety (Simeto) were used in the present study. Tritordeum HT621 is an advanced line developed by the Cereal Breeding Program of the Institute for Sustainable Agriculture (IAS-CSIC) in Córdoba, Spain. The Simeto variety is one of the most widely cultivated durum wheat varieties in Europe due to its excellent quality parameters: high semolina yield, extraordinary gluten content, and high protein value. Both grain samples are good representatives of the two cereal genotypes and their carotenoid profiles have previously been characterized (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2015, 2017; Mellado-Ortega et al., 2015; Mellado-Ortega and Hornero-Méndez, 2012).

2.2. Sample preparation and isolation of the germ-endosperm grain fractions

For each cereal type, 1000 grains were manually dissected under a magnifying glass to separate the germ from the endosperm using a scalpel. Strictly speaking, the endosperm fraction also contains the bran, which consists of the aleurone and pericarp layers, although in this study, this fraction will simply be referred to as endosperm. Isolated germs and endosperms were individually weighed and subsequently collected into the corresponding fractions. Table 1 shows the average weight (mg) of whole grain and the percentage proportions for the germ and endosperm fractions of both cereals.

2.3. Chemicals and reagents

HPLC-grade deionized water was produced with a Milli-Q Advantage A10 system (Merck Chemicals and Life Science, Madrid, Spain) and HPLC-grade acetone, methanol, toluene, and heptane were supplied by BDH Prolabo (VWR International Eurolab, Barcelona, Spain). Heptadecanoic acid (C17:0), butylated hydroxytoluene (BHT), 2,2-dimethoxypropane, and fatty acid methyl ester (FAME) standard mixtures were purchased from Sigma–Aldrich Química, (Madrid, Spain). Other reagents were all of analytical grade.

2.4. Extraction of carotenoids

Carotenoid pigments were extracted from grain fractions (germ and endosperm) according to the method of Atienza et al. (2007) with some modifications (Mellado-Ortega and Hornero-Méndez, 2012). Briefly, 0.1 g and 1 g of milled grain sample from germ and endosperm, respectively, was placed in a 15 mL round-capped polypropylene tube, and then extracted with 4 mL of acetone (containing 0.1% BHT) for 2 min by vortexing, following sonication for 1 min. The mixture was centrifuged at 4,500 × g for 5 min at 4 °C. The extraction process was repeated three times and the acetone fractions were pooled. The solvent was gently evaporated under a nitrogen stream, and the pigments were dissolved in 0.5 mL of acetone for both grain fractions. Prior to chromatographic analysis, samples were centrifuged at 13,000 × g for 5 min at 4 °C. The analyses were carried out in quadruplicate for each sample. All operations were performed under dimmed light to prevent the isomerization and photo-degradation of carotenoids.

2.5. HPLC analysis of carotenoids

The identification of carotenoid pigments in tritordeum grains and durum wheat has been described in previous works (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2012).

Table 1
Average weight and germ-endosperm distribution (%) in durum wheat (Simeto) and tritordeum (HT621 line) grains.

	Durum wheat (Simeto variety)	Tritordeum (HT621 advanced line)
Average weight of whole grain (mg)[a]	68.1 ± 3.2	34.5 ± 2.6
Germ (%)	2.9 ± 0.4	7.1 ± 0.6
Endosperm (%)	97.1 ± 5.1	92.9 ± 4.7

[a] Data are the mean ± standard deviation (n = 1000).