Carotenoids in tritordeum (*Tritordeum Ascherson et Graebner*). Effect of storage conditions on their content.

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Introduction

The hexaploid tritordeum (*Tritordeum* Ascherson et Graebner) (**Figure 1**), is the fertile amphiploid (2n=6x=42, AABBH^{ch}H^{ch}) resulting from the cross between a wild barley (*Hordeum chilense*) and durum wheat (*Triticum turgidum* Desf) (1). The agronomic, morphological, chemical, physico-chemical and rheological characteristics of this new crop, similar to bread wheat, indicate that tritordeum is a promising cereal for agriculture and food processing (2). Moreover, there is a huge genetic variability available for breeding this new crop as a consequence of the synthesis of hundreds of different amphiploids, becoming tritordeum a potentially donor of useful traits to wheat. Previous studies (3), carried out in our laboratories, have demonstrated that lutein is the major carotenoide present in tritordeum lines, and at much higher concentration (more than 5 times) than in durum wheat, which suggests a high potential of this crop to become a functional food. In addition, it was found that in the case of tritordeum, lutein shows a characteristic esterification with fatty acids, which is absent or at very low levels in durum wheat grains. In the present study, the effect of storage of grains (temperature and time) on the carotenoid content of three advanced tritordeum lines and three commercial durum wheat varieties has been investigated. The role of esterification on the stability of lutein is also discussed.

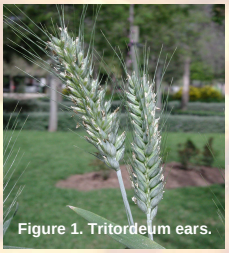

Figure 1. Tritordeum ears.

Materials and Methods

Raw Material

Three advanced tritordeum lines (HT630, HT621 and HT609), developed in the Cereal Breeding Program of the Institute for Sustainable Agriculture (IAS-CSIC, Córdoba, Spain), and three commercial durum wheat varieties (Don Pedro, Claudio and Simeto) were grown in greenhouse conditions. Grains (**Figure 2**) were harvested and subsequently used for the storage experiments.

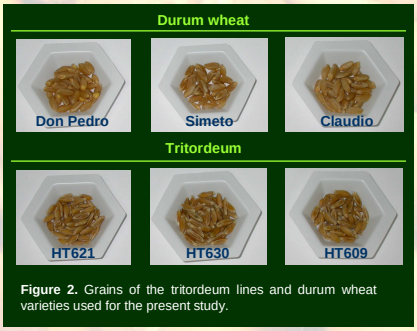

Figure 2. Grains of the tritordeum lines and durum wheat varieties used for the present study.

Storage conditions

Three separated batches of grains for each line or variety were placed in open containers under controlled temperature conditions (4, 20 and 37ºC) and kept for a period of 90 days. Sampling (15 grams from each batch) was performed every 30 days. A control sample (t=0 days), consisting of 6 subsamples, was taken for each line or variety and stored at -30ºC until analysis.

Carotenoid extraction

Grains were milled with a spice hand mill, and 1 g of the resulting flour was used for carotenoid extraction by using the protocol proposed by Atienza *et al.* (3) with minor modifications. Following the extraction, the solvent was evaporated under a nitrogen stream and the pigments were dissolved in 1.0 or 0.5 mL of acetone for durum wheat and tritordeum samples, respectively. All operations were carried out under dimmed light to prevent isomerisation and photodegradation of carotenoids. The analyses were carried out performed in quadruplicate for each grain batch.

HPLC analysis of carotenoids

Carotenoids were analyzed by HPLC according to the method of Mínguez-Mosquera and Hornero-Méndez (4) with some modifications of the elution gradient (3). The HPLC system consisted of a Waters 2690 Alliance together with a Waters 2990 photodiode array detector, and controlled with Millennium32 software. A reverse phase column (Mediterranea SEA18, 3µm, 20 cm×0.46 cm; Teknokroma, Barcelona, Spain) was used. An injection volume of 10 µL and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and online spectra were acquired in the 350-600 nm wavelength range. Quantification was carried out using a calibration curve obtained with lutein standard. This calibration curve was used to quantify both free and esterified lutein. According to our previous work (3), these chromatographic conditions allowed distinguishing three different lutein fractions regarding to the degree of esterification, that is free (F), monoesterified (ME) and diesterified (DE) lutein.

Results and Discussion

In the present study, it was confirmed that lutein is the main carotenoid pigment found in both tritordeum and durum wheat grains. However, as shown in the respective HPLC chromatograms (**Figure 3**), the carotenoid profiles for both types of samples were clearly different. Apart from lutein and β-carotene, durum wheat grains contained zeaxanthin and small amounts of α-carotene, which were absent in tritordeum. On the contrary, lutein showed a characteristic esterification pattern in tritordeum grains. The structural assignment of the lutein esters in tritordeum have been recently investigated in our laboratory (5), consisting on monoesters and diesters (homodiesters and heterodiesters) with palmitic and linoleic acid.

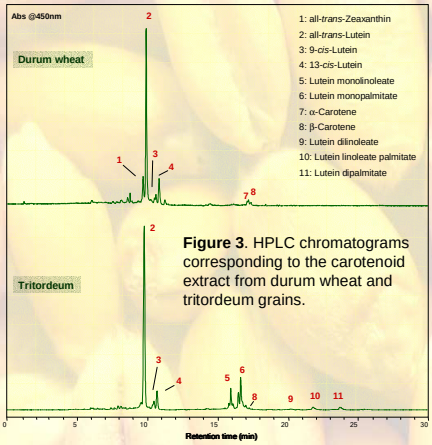

Figure 3. HPLC chromatograms corresponding to the carotenoid extract from durum wheat and tritordeum grains.

In general terms, the carotenoid contents of the tritordeum lines were about 8 times higher compared to durum wheat (**Figure 4**). When the grains were stored, at 4, 20 and 37ºC for up to 90 days, the evolution of the carotenoid content, resulting from the balance between carotenogenic and catabolic processes, was markedly different in both cases. For the tritordeum lines, the carotenoid content decreased during the storage period, being very much influenced by the temperature. The average decrease of the carotenoid content for tritordeum lines at 37ºC was 24%. In the case of durum wheat, it was noticed that grains were not fully ripe, so that the biosynthetic/catabolic balance was displaced favoring to the anabolic ones, being the degradation of carotenoids compensated by the carotenogenesis during the adaptation of the grains to the storage conditions. This fact was very evident at 4ºC, with a net increase on the carotenoid content, whereas an average decrease of 30% was observed at the end of the storage period at 37ºC. It might be logical to suppose that degradation of carotenoids would be higher in durum wheat when using fully ripe grains. In any case, the observed higher retention of carotenoid in tritordeum grains seems to be directly related with the esterification of lutein, which results in a higher stability and better structural ability to accumulate in lipophilic bodies within the germ cells. **Figure 5** shows the evolution of free, monoesterified and diesterified lutein under the assayed storage conditions for HT630 tritordeum line (the same results were observed for the other lines, HT621 and HT609). It is evident that the increasing storage temperature has an stimulating effect on the esterification of lutein. As illustrated in **Figure 6**, the relative amount of lutein monoesters and lutein diesters changes from 18 to 39% and from 2 to 15%, respectively, during the storage at 37ºC.

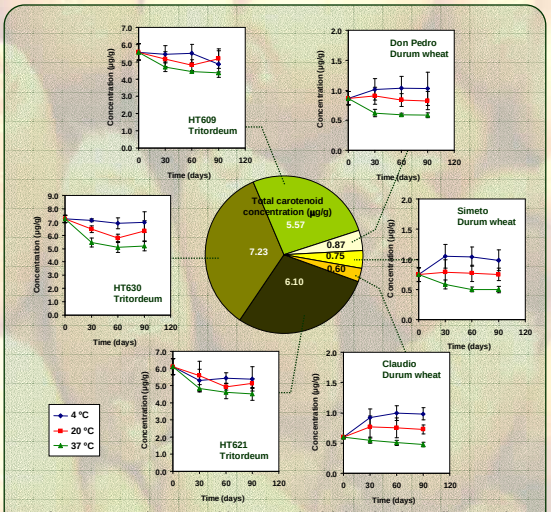

Figure 4. Total carotenoid content (µg/g) in tritordeum lines and durum wheat varieties. Evolution during the grain storage at different temperatures (4, 20 and 37ºC).

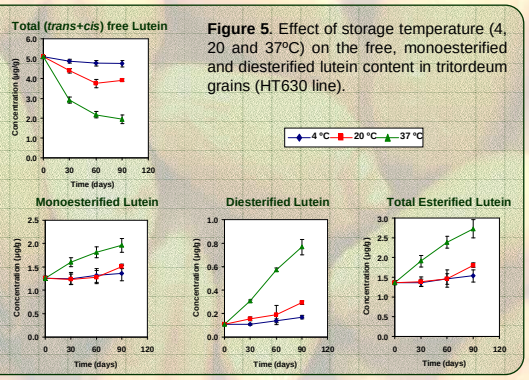

Figure 5. Effect of storage temperature (4, 20 and 37ºC) on the free, monoesterified and diesterified lutein content in tritordeum grains (HT630 line).

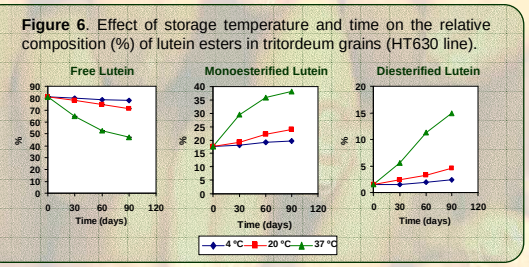

Figure 6. Effect of storage temperature and time on the relative composition (%) of lutein esters in tritordeum grains (HT630 line).

Literature cited

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Conclusions

In the sight of the present results, it can be concluded that the storage temperature of tritordeum grains is an important technological factor that may modulate the esterification of lutein with fatty acids, increasing the retention of carotenoids in the grains, and possibly in the resulting processed products, preserving the beneficial health promoting properties of these phytochemicals.

Acknowledgements

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