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Carotenoid evolution during postharvest storage of durum wheat (*Triticum turgidum* conv. *durum*) and tritordeum (×*Tritordeum* Ascherson et Graebner) grains.

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Chemical compounds studied in this article:

β-Carotene (PubChem CID: 5280489); Zeaxanthin (PubChem CID: 5280899); Lutein (PubChem CID: 5368396)

Abstract

The process of *in vivo* esterification of xanthophylls has proven to be an important part of the post-carotenogenesis metabolism which mediates their accumulation in plants. The biochemical characterization of this process is therefore necessary for obtaining new and improved crop varieties with higher carotenoid contents. This study investigates the impact of postharvest storage conditions on carotenoid composition, with special attention to the esterified pigments (monoesters, diesters and their regioisomers), in durum wheat and tritordeum, a novel cereal with remarkable carotenoid content. For tritordeum grains, the total carotenoid content decreased during the storage period in a clear temperature-dependent manner. On the contrary, carotenoid metabolism in durum wheat was very much dependent on the physiological adaptation of the grains to the imposed conditions. Interestingly, when thermal conditions were more intense (37 ºC), a higher carotenoid retention was observed for tritordeum, and was directly related to the *de novo* esterification of the lutein induced by temperature. The profile of lutein monoester regioisomers was constant during storage, indicating that the regioisomeric selectivity of the XAT enzymes was not altered by temperature. These data can be useful for optimizing the storage conditions of grains favoring a greater contribution of carotenoids from these staple foods.

1. Introduction

Wheat is the most widespread cultivated cereal in the world (FAO, 2014) and, along with rice, constitutes the main source of carbohydrates for human consumption. However, these staple foods not only represent an important dietary source of carbohydrates and proteins, but also provide minerals, fiber, vitamins and phytochemicals, including carotenoids, phenols, tocopherols, sterols and phytates (Liu, 2007). Carotenoids are an important group of natural pigments responsible for the coloration of most fruits and vegetables and are present in many parts of the plant: fruits, flowers, roots, leaves, and seeds (Britton and Hornero-Méndez, 1997). Plant carotenoids are C_{40} isoprenoids (tetraterpenoids) with a polyene skeleton consisting of a long conjugated double bond system, which constitutes the chromophore responsible for the color that these pigments confer to most fruits and vegetables, and play an important role in attracting animals to act as pollinators and seed dispersion vehicles, including the consumption of food by humans (Howitt and Pogson, 2006). The known number of naturally occurring carotenoids is about 750 and continues to rise (Britton et al., 2004). Carotenoids can only be synthesized *de novo* by plants, certain bacteria, and fungi, whereas animals are unable to synthesize carotenoids, so they need to obtain them from the diet. Carotenoids are essential components of the photosynthetic apparatus and are involved in the light harvesting process, as well as in the photo-protection mechanisms of plants (Cuttriss et al., 2011). When carotenoids are ingested, they show important biological activities: antioxidant, inhibition of carcinogenesis, enhancement of the immune response and cell defense against reactive oxygen species (ROS) and free radicals, and the reduction in the risk for developing cardiovascular and other degenerative diseases (reviewed by Britton et al., 2009). In addition, some carotenoids (β-carotene, α-carotene, β-cryptoxanthin, etc) have provitamin A activity (Olson, 1989).

Of particular interest are the epidemiological studies showing an inverse correlation between the progression of age-related macular degeneration (AMD) and cataracts and the high intake of lutein and zeaxanthin rich-vegetables, and both pigments are present in high concentrations at the macula in the retina of humans and primates (Landrum and Bone, 2004).

Despite having a low carotenoid content when compared with the majority of fruits and vegetables, the consistent daily intake of cereals and other staple foods may have an important impact on the nutritional status of consumers, which can be especially significant in developing countries, where cereals and cereal-based foods are the main constituents of the diet. Thus, there is great potential for providing health benefits to consumers without significantly altering their dietary habits by manipulating the carotenoid content of foods such as cereals **(**Howitt and Pogson, 2006). The endosperm color of cereal grains, which is mainly due to carotenoid accumulation, is an important quality criterion in wheat breeding programs. Common wheat (*Triticum aestivum* L.) varieties have been traditionally selected for their white color since consumers prefer white flours for bread making. In contrast, durum wheat (*Triticum turgidum* ssp. *durum*) is selected for high yellow pigment content (YPC) as it is a desirable property for pasta products (Troccoli et al., 2000). In previous works (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2012), we have characterized the carotenoid composition of tritordeum (×*Tritordeum* Ascherson et Graebner), a novel cereal obtained as an amphiploid (2n=6x=42, AABBH^{ch}H^{ch}) resulting from the cross between a wild barley (*Hordeum chilense* Roem. & Schult.) and durum wheat (Martín and Sanchez-Monge Laguna, 1982). As observed in most species of the *Triticum* genus, lutein (*3R,3'R,6'R*-β,ε-carotene-3,3'-diol) is the main carotenoid present in tritordeum, showing a lutein content 5-8 times higher than durum wheat (Atienza et al., 2007;

Mellado-Ortega and Hornero-Méndez, 2012). Moreover, it has been found that lutein in tritordeum grains is characterized by presenting a distinctive profile of esterification with specific fatty acids (palmitic and linoleic acids), whereas the esters are absent or at very low concentration levels in durum wheat grains. For the first time in a cereal, the analysis of the mass spectrometry fragmentation pattern of lutein has recently allowed for the unambiguous structural identification of the lutein esters present in tritordeum, which consisted of four monoesters (lutein 3'-*O*-linoleate, lutein 3-*O*-linoleate, lutein 3'-*O*-palmitate, lutein 3-*O*-palmitate) and four diesters (lutein dilinoleate, lutein 3'-*O*-linoleate-3-*O*-palmitate, lutein 3'-*O*-palmitate-3-*O*-linoleate, lutein dipalmitate) (Mellado-Ortega and Hornero-Méndez, 2012). Tritordeum exhibits agronomic, morphological, chemical, physico-chemical and rheological characteristics similar to bread wheat (Martín et al., 1999). These properties, together with the enormous genetic variability potentially available for breeding this new crop, make tritordeum a promising cereal for agriculture and food processing. The xanthophyll esterification process in tritordeum, which seems to be a key mechanism for the carotenoid accumulation in the endosperm, has already been characterized as a post-developmental grain process (Rodríguez-Suárez et al., 2014). The high degree of lutein esterification in tritordeum grains at harvest may reveal the activation of a carotenoid sequestering mechanism probably leading to the absence of metabolic feedback to inhibit the carotenoid biosynthetic pathway. So the complete biochemical characterization of the molecular mechanism underlying the formation of carotenoid esters is important for the improvement of cereals and other vegetables with higher carotenoid content.

Reports about the occurrence of lutein acylesters in wheat and other cereals, as well as the studies of their metabolism changes during the storage of grains are very scarce (Kaneko et al., 1995; Kaneko and Oyanagi, 1995). Recently, a more complete

119 study conducted by Ahmad et al. (2013) has reported the formation of lutein esters
120 during storage under a wide range of temperatures in a high lutein wheat developed at
121 The Waite Campus, The University of Adelaide. Cereal grains are stored for long
122 periods and consequently they may undergo important physical, chemical and
123 physiological modifications promoted by the storage conditions, i.e. temperature,
124 moisture content, oxygen content, light and microbial activity. Seeds deteriorate
125 following a time dependent process termed “aging”, which has led some researchers to
126 investigate these changes in seeds during natural (Pinzino et al., 1999) or accelerated
127 aging (Galleschi et al., 2002), the last one quickly mimicking the long-term storage
128 effects which are observed under industrial conditions and the impact on the viability of
129 seeds. After harvesting, the kernels are stored in silos, where they are maintained at
130 15.5% moisture or less in order to minimize microbial growth and to favor conservation
131 over time (Galleschi et al., 2002). In spite of this, pronounced chemical changes take
132 place, and some antioxidant compounds such as carotenoids are degraded by both direct
133 and lipoxygenase (EC 1.13.11.12) mediated oxidation (Doblado-Maldonado et al.,
134 2012). Limited information is available in the literature on the carotenoid pigment
135 metabolism in cereal grains during the immediate postharvest storage (Burt et al., 2010).
136 The exploration of this period may shed light on the carotenoid metabolism in grains in
137 which perhaps physiological maturity has still not been reached. Thus, in this study we
138 have investigated the influence of postharvest storage conditions (temperature and time)
139 on the carotenoid stability and metabolism in tritordeum and durum wheat grains. The
140 role of esterification on the stability of lutein is also discussed. With this aim, the
141 carotenoid content and profile of the grains of three durum wheat varieties and three
142 advanced tritordeum lines were measured during their postharvest storage for up to 90
143 days at three different temperatures (4, 20 and 37 °C).

2. Materials and methods

2.1. Plant material, storage conditions and sample preparation

Three advanced tritordeum lines (HT630, HT621 (Ballesteros et al., 2005) and HT609), developed in the Cereal Breeding Program of the Institute for Sustainable Agriculture (IAS-CSIC, Córdoba, Spain), characterized by a high carotenoid content in the endosperm, and three durum wheat varieties (Don Pedro, Simeto and Claudio) were used for the present study. Plants were first grown in a climate chamber under controlled conditions (at 22/16 ºC day/night with 12/12 h Light/darkness) and then transplanted to field conditions, following a completely randomized block design with 3 replications. The harvested grains were subsequently used for the storage experiments as follows. Three separated batches (300 g) of grains from each field replicate were placed in open containers under controlled temperature conditions (4, 20 and 37 °C), maintaining low relative humidity for a period of 90 days. Samples were taken at monthly intervals. A control sample (t=0 days), consisting of 6 subsamples (three batches by duplicate), was taken for each line or variety and stored at -30 ºC until analysis. Grains were milled by using a spice hand mill, and the resulting whole flour was used for carotenoid extraction.

2.2. Chemicals and reagents

HPLC-grade acetone was supplied by BDH Prolabo (VWR International Eurolab, S.L., Barcelona, Spain), and HPLC-grade deionized water was produced with a Milli-Q 50 system (Millipore Iberica S.A., Madrid, Spain). The rest of reagents were all of analytical grade.

2.3. Extraction of carotenoids

The extraction of carotenoids was carried out according to the method of Atienza et al. (2007) with some modifications (Mellado-Ortega and Hornero-Méndez, 2012). Briefly, 1 g of milled grain sample was placed in a round capped polypropylene 15-mL centrifuge tube, and 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 operation 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 1.0 or 0.5 mL of acetone for durum wheat and tritordeum samples, respectively. Prior to the chromatographic analysis, samples were centrifuged at 13,000×*g* for 5 min at 4 ºC. The analyses were carried out in duplicate for each sample. All operations were performed under dimmed light to prevent isomerization and photo-degradation of carotenoids.

2.4. Pigment identification

The procedures for the isolation and identification of carotenoid pigments and its esters have already been described in previous works (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2012).

2.5. HPLC analysis of carotenoids

HPLC quantitative analysis of carotenoids was carried out according to the method of Mínguez-Mosquera and Hornero-Méndez (1993) with some modifications (Atienza et al., 2007). The HPLC system consisted of a Waters 2695 Alliance chromatograph fitted with a Waters 2998 photodiode array detector, and controlled with Empower2 software (Waters Cromatografía, S.A., Barcelona, Spain). A reversed-phase column (Mediterranea SEA18, 3 μm, 20×0.46 cm; Teknokroma, Barcelona, Spain) was

used. Separation was achieved by a binary-gradient elution using an initial composition of 75% acetone and 25% deionized water, which was increased linearly to 95% acetone in 10 min, then raised to 100% in 2 min, and maintained constant for 10 min. Initial conditions were reached in 5 min. An injection volume of 10 μL and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and the online spectra were acquired in the 350-700 nm wavelength range. Quantification was carried out using calibration curves prepared with lutein, α- and β-carotene and zeaxanthin standards isolated and purified from natural sources (Mínguez-Mosquera and Hornero-Méndez, 1993). Calibration curves were prepared in the pigment concentration range of 0.5-45 μg/ml. Lutein esters contents were estimated by using the calibration curve for free lutein, since the esterification of xanthophylls with fatty acids does not modify the chromophore properties. The calibration curve of free lutein was also used to determine the concentration of the *Z*-isomers of lutein. Data were expressed as μg/g fresh weight.

2.6. Statistical analysis

The compositional data of total and individual pigments are expressed as mean and standard error of the mean (SEM). The existence of significant differences between means was determined by one-way ANOVA, followed by a post-hoc test of mean comparison using the Duncan test for a confidence level of 95% ($p < 0.05$) utilizing the STATISTICA 6.0 software (StatSoft Inc.).

3. Results and discussion

3.1. Carotenoid composition

In the present study, lutein was confirmed as the main carotenoid pigment (>85%) found in both tritordeum and durum wheat grains (**Tables 1** and **2**). However,

as shown in the respective HPLC chromatograms (see **Figure 1** at t=0 days), the carotenoid profiles were clearly different for both types of samples, in agreement with our previous studies (Atienza et al., 2007). In addition to lutein (86.1%, sum of *E*- and *Z*- isomers), durum wheat grains also contained zeaxanthin (10.7%) and lower amounts of β-carotene (1.8%) and α-carotene (1.4%), the latter being absent in tritordeum. In tritordeum grains, lutein showed the characteristic esterification pattern described in previous works, and total lutein (sum of free and esterified forms) accounted for up to 98.9% of the total carotenoid composition, the rest (1.1%) pertained to β-carotene. The structural assignment of the lutein esters in tritordeum, including their regioisomeric forms, has been recently investigated in our laboratory, and consisted of monoesters and diesters (homodiesters and heterodiesters) with palmitic (C16:0) and linoleic (C18:2) acids (Mellado-Ortega and Hornero-Méndez, 2012). On average, the total carotenoid content of the tritordeum lines (6.5 μg/g fw) was significantly higher, about 8 times, compared to durum wheat varieties (0.7 μg/g fw), which is in accordance with previous results (Atienza et al., 2007). The initial carotenoid contents at the harvest stage (t=0 days) are summarized in **Tables 1** and **2** for durum wheat and tritordeum, respectively. We have recently characterized (Mellado-Ortega and Hornero-Méndez, 2015) the carotenoid profile of *H. chilense*, the other parent of tritordeum, confirming that the high level of carotenoids and the esterification pattern of tritordeum is a genetic trait derived from this parent. Strikingly, the carotenoid profile of both parents (*H. chilense* and durum wheat) included zeaxanthin, thus indicating that the absence of this pigment in the amphiploid could be due to the over-activation of the β,ε- branch of the biosynthetic pathway, which leads to the formation of lutein, at the expense of the β,β-branch, leading to the formation of zeaxanthin. This possibility seems more likely than the amphiploid inability to synthesize zeaxanthin, since tritordeum has detectable levels

of β-carotene, its precursor. Besides, zeaxanthin is detected in tritordeum during grain development (Rodríguez-Suárez et al. 2014). The overactivation of β,ε- branch along with the existence of active sequestering mechanisms from the high degree of esterification may result in the fact that the hydroxylation step for the formation of lutein is very active. This could explain the absence of α-carotene in tritordeum as well.

Regarding the esterified fraction in tritordeum, this represented about 16% of the total lutein with a greater contribution from the monoesters. The relative abundance of the individual esterified xanthophylls with respect to the total carotenoids was: lutein monopalmitate (10.1%), lutein monolinoleate (4.8%), lutein dipalmitate (0.5%), lutein linoleate palmitate (0.4%) and lutein dilinoleate (0.2%). Regioisomers of lutein monoesters at position 3 (lutein-3-*O*-linoleate and lutein-3-*O*-palmitate) were found at higher concentration levels than the monoesters at position 3' (lutein-3'-*O*-linoleate and lutein-3'-*O*-palmitate), which is consistent with the regioisiomer profile described for lutein monoesters in advanced tritordeum lines (Mellado-Ortega and Hornero-Méndez, 2012). The analysis of the diester fraction shows a higher presence of the homodiester with palmitic acid (lutein dipalmitate, ~ 50% of total diesters) and much lower for its counterpart with linoleic acid (lutein dilinoleate, ~ 14% of total diesters), suggesting a greater affinity for the esterification with palmitic acid. As proposed in previous works (Mellado-Ortega and Hornero-Méndez, 2012), these results indicate the preferential acylating action of the responsible enzymes (XAT: xanthophyll acyltransferase) over the β-end ring of lutein compared to the ε-end ring, as well as a higher selectivity for palmitic acid. The average ratios for the regioisomers of monoesters at positions 3 and 3' reached values of 4.3 and 2.2 for lutein monolinoleate and lutein monopalmitate, respectively. This suggests that while the esters with palmitic acid are always more abundant than those with linoleic acid, the relative affinity of XAT enzymes between

positions 3 and 3' of lutein molecules is more pronounced for the monoesters with linoleic acid.

It is important to note that the chromatographic peak assigned as the heterodiester lutein linoleate-palmitate consisted of two regioisomers, lutein-3'-*O*-linoleate-3-*O*-palmitate and lutein-3'-*O*-palmitate-3-*O*-linoleate (38% of total diesters). The use of the C18 column for the chromatographic analysis did not allow for the resolution of these two regioisomers, and therefore we cannot establish whether there are differences in their relative abundance.

3.2. Changes in the carotenoid content during the postharvest storage of durum wheat and tritordeum grains

The evolution of the total carotenoid content (**Figure 2**), resulting from the balance between the carotenogenic and catabolic processes, was markedly different between the two groups of cereals. For the tritordeum lines, the carotenoid content decreased progressively throughout the storage period, showing some dependence on the applied temperature. Thus, the average decrease in the carotenoid content for the tritordeum lines reached a maximum value of 24% at the end of the storage period (90 days) at 37 °C. In the case of durum wheat cultivars, it was noticed that such a biosynthetic/catabolic balance was displaced, favoring the anabolic ones, and the degradation of carotenoids was compensated by the carotenogenesis activated during the adaptation of the grains to the storage conditions, possibly due to a certain degree of immaturity of the harvested durum wheat grains. These results are consistent with other studies on durum wheat (Ramachandran et al., 2010). This phenomenon was very evident at the lower storage temperature (4 °C) with a net increase in the carotenoid content after the first 30 days of storage, of 65% and 31% for Claudio and Simeto,

294 respectively. These changes might be the adaptation response of the grains to the newly
295 imposed storage conditions, especially for grains stored at 4 °C (a temperature very
296 different from the harvest temperature), suggesting a metabolic activation or dormancy
297 breakage of grains, a process during which there is evidence of general increases in
298 antioxidant contents (Howitt and Pogson, 2006). Carotenoids play a protective role
299 against the action of free radicals and prevent the aging of the seeds, in this case
300 contributing to their germination success. Several studies have shown a direct
301 correlation between antioxidant contents, including carotenoids, and aging or vegetative
302 state of the seed (Galleschi et al., 2002; Pinzino et al., 1999). An average decrease of
303 30% was observed at the end of the storage period at 37 °C in durum wheat compared
304 with 24% in tritordeum. In any case, the observed higher retention of carotenoid in
305 tritordeum grains at the end of the storage period at 37 °C seems to be more directly
306 related to the esterification of lutein, rather than the differences at pigment level for both
307 cereals, an aspect which is discussed below.

308 The evolution of individual carotenoid pigments present in the grains of the
309 durum wheat varieties and tritordeum lines are shown in **Tables 1** and **2**. The behavior
310 of the three durum wheat varieties was consistent with the changes in the total
311 carotenoid content. As a general trend, durum wheat varieties showed a net increase in
312 the concentration of all pigments at 4 °C in agreement with the observation for total
313 carotenoid content, suggesting a general activation of the carbon flux through the
314 carotenoid pathway. Likewise, the decrease in the concentration of pigments was more
315 evident at 37 °C, so that, at the end of the storage period (90 days), the net loss for all-
316 *E*-lutein amounted to 37% in the Don Pedro and Simeto varieties and 25% for the
317 Claudio variety. With respect to all-*E*-zeaxanthin a decline of around 23% in the Don
318 Pedro and Simeto varieties and 12% in Claudio was observed. In contrast, *Z*-lutein

isomers experienced a smaller drop in all three varieties than *E*-lutein and the rest of the pigments. This is consistent with the fact that the *E* to *Z* isomerization of carotenoids is a frequent transformation taking place during the storage and processing of fruits and vegetables (Liaaen-Jensen and Lutnaes, 2008). For the case of carotenes, storage at 37 °C for three months resulted in greater changes in concentration, so that α- and β-carotene registered losses of around 40% in Simeto and Don Pedro varieties, and even 64% in Claudio. These results indicate a greater instability of carotenes compared to xanthophylls. For tritordeum grains, the most significant changes were observed in the contents of free lutein (including all-*E*-lutein and *Z*-lutein) and lutein esters (**Table 2**). All tritordeum lines experienced an increase in the mono- and diesterified lutein fractions with a concomitant decrease in the levels of free lutein (**Figure 3**). For both fractions, monoesters and diesters, their relative contents increased following a temperature-dependent manner, thus their increases were more pronounced at 37 °C, coinciding with a decrease in all-*E*-lutein levels in the range of 40-60% compared to the levels observed at 4 and 20 °C (**Table 2**). This finding indicates a positive and modulating effect of temperature on the *in vivo* process of the esterification of xanthophylls. Recently, Ahmad et al. (2013), in a study assessing lutein ester synthesis over a wide temperature range in bread wheat and durum wheat grains, concluded that the optimum temperature for lutein esterification with minimum loss was in the range 30 to 60 °C. In addition, these authors reported that storage at 37 °C for 8 weeks significantly promoted the esterification of lutein. Our results clearly showed that the diester fraction experienced higher increases than the monoester fraction, which was at its maximum at the end of the storage period at 37 °C, at 6.99, 9.87 and 9.31 times higher compared to the initial values for HT630, HT621 and HT609, while the monoesterified fraction increased its concentration by 1.58, 2.33 and 2.09 times,

respectively (**Figure 3**). As observed for durum wheat, *Z*-lutein isomers were characterized by a smooth rate of degradation, probably due to the compensation of catabolism by the *E* to *Z* isomerization that compensates net degradation. Finally, note that β-carotene showed a general trend towards degradation, according to a free pigment (**Table 2**).

3.3. Effect of postharvest storage on the esterified lutein fractions in tritordeum.

Based on the above, it is worthwhile to analyze the evolution of the different lutein monoesters and diesters identified in the tritordeum lines in more detail, in order to distinguish the corresponding regioisomers of the monoesters (**Table 2**). The evolution of the esterified lutein fractions at different temperatures coincided with a gradual rise, with a very marked increase at 37 °C in all cases at the end of the storage period (90 days). In addition, the lutein monopalmitate content was higher than lutein monolinoleate, and experienced a higher increase throughout storage (1.4, 1.3 and 1.6 times for HT621, HT609 and HT630, respectively). These data again confirm a higher affinity of the involved enzyme systems (XAT) for palmitic acid versus linoleic acid, although the latter is the most abundant fatty acid in the lipid pool of cereals (Mellado-Ortega and Hornero-Méndez, 2012). Moreover, another factor to be considered for influencing in the monoester levels, with one or another fatty acid, is the involvement of lipid peroxidation reactions during the storage period (Hildebrand, 1989). Thus, polyunsaturated fatty acids such as linoleic acid are more prone to oxidation than saturated fatty acids, e.g. palmitic acid. The ratios between the regioisomers (**Table 2**), lutein 3-*O*-linoleate/lutein 3'-*O*-linoleate and lutein 3-*O*-palmitate/lutein 3'-*O*-palmitate, showed constant values for each monoester during the postharvest storage period, indicating that the regioisomeric selectivity of the XAT enzymes is not altered by the

369 temperature with respect to the preferential position of esterification in the lutein
370 molecule (position 3 at the β-end ring).

371 As described for monoesters, lutein dipalmitate turned out to be the most
372 abundant of diesters in all tritordeum lines, followed by the heterodiester lutein
373 linoleatepalmitate, and finally by trace amounts of lutein dilinoleate. This is consistent
374 with the specificity for palmitic acid as previously indicated and with a plausible
375 negative effect of oxidation over linoleic acid. The increases observed at the end of the
376 storage period (90 days) at 37 °C were very pronounced (**Table 2**). These results are
377 consistent with the earlier studies of Kaneko et al. (1995) and Kaneko and Oyanagi
378 (1995), who evaluated the effect of relative humidity on the promotion of the
379 esterification reaction of lutein during the storage of wheat seeds at 30 °C. These works
380 also showed a greater increase in the fraction of diesters versus monoesters. The authors
381 reported that the esterification of lutein was highly influenced by the cereal genome.

382 As deducted from the lower loss values for the total carotenoid content in
383 tritordeum versus durum wheat, the progressive increase in the esterification of lutein
384 provides greater stability. The greater stability of esterified carotenoids compared to the
385 free forms has been demonstrated in various studies (Khachik and Beecher, 1988;
386 Schweiggert et al., 2007; Subagio et al., 1999). The esterification increases the apolar
387 nature of these molecules, facilitating their accumulation and storage in lipophilic
388 membrane structures or bodies that enable greater protection against degradative
389 enzyme systems. Therefore, the ability of tritordeum grains to produce lutein esters and
390 the possibility to modulate their content by means of postharvest storage conditions
391 (specially the temperature) must be exploited in order to optimize their use as a
392 functional cereal.

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Figure captions

Figure 1. HPLC chromatograms obtained during postharvest storage (90 days) at three different temperatures (4, 20 and 37 °C) of durum wheat (Don Pedro) and tritordeum (HT621) grains. Peak identities are: 1, all-*E*-zeaxanthin; 2, all-*E*-lutein; 3, 9-Z and 13-Z isomers of lutein; 4, lutein 3'-*O*-linoleate; 5, lutein 3-*O*-linoleate; 6, lutein 3'-*O*-palmitate; 7, lutein 3-*O*-palmitate; 8, all-*E*-α-carotene; 9, all-*E*-β-carotene; 10, lutein dilinoleate; 11, lutein 3'-*O*-linoleate-3-*O*-palmitate and lutein 3'-*O*-palmitate-3-*O*-linoleate; 12, lutein dipalmitate.

Figure 2. Total carotenoid content (μg/g fresh weight) evolution in wheat varieties (Don Pedro, Simeto and Claudio) and advanced tritordeum lines (HT630, HT621 and HT609) during the postharvest storage of seeds at 4, 20 and 37 °C. The values shown are the mean and standard error of six analyses (n=6, three blocks x 2). Different letters within the same line (temperature effect) indicate significant differences ($p<0.05$) determined by the Duncan test.

Figure 3. Effect of temperature (4, 20 and 37 °C) on the degree of esterification of lutein during the storage of tritordeum seeds (lines HT630, HT621 and HT609). Data represented the relative contribution of each fraction (free, monoesterified and diesterified) in relation to temperature and storage time. The values shown are the mean and standard error (n=6, three blocks x 2).

Table 1. Carotenoid composition evolution (μg/g fresh weight)[1] during postharvest storage of durum wheat grains (Don Pedro, Simeto and Claudio varieties).

		Don Pedro				Simeto				Claudio			
		Time (days)											
Carotenoid	**Temp (°C)**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**
all-*E*-Lutein	4	0.62 ± 0.04^{a}	0.72 ± 0.06^{a}	0.75 ± 0.06^{a}	0.74 ± 0.09^{a}	0.54 ± 0.04^{a}	0.78 ± 0.06^{b}	0.78 ± 0.05^{b}	0.73 ± 0.06^{b}	0.43 ± 0.01^{a}	0.68 ± 0.04^{b}	0.72 ± 0.03^{b}	0.71 ± 0.03^{b}
	20		0.65 ± 0.04^{a}	0.59 ± 0.03^{a}	0.58 ± 0.05^{a}		0.57 ± 0.06^{a}	0.56 ± 0.04^{a}	0.55 ± 0.03^{a}		0.55 ± 0.06^{a}	0.54 ± 0.05^{a}	0.52 ± 0.02^{a}
	37		0.42 ± 0.02^{b}	0.40 ± 0.01^{b}	0.39 ± 0.01^{b}		0.40 ± 0.02^{b}	0.34 ± 0.01^{b}	0.34 ± 0.02^{b}		0.37 ± 0.02^{b}	0.34 ± 0.02^{bc}	0.33 ± 0.01^{c}
9*Z*- and 13*Z*-Lutein	4	0.12 ± 0.00^{a}	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.02^{a}	0.11 ± 0.01^{a}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.14 ± 0.01^{b}	0.09 ± 0.00^{a}	0.13 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}
	20		0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}		0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}		0.11 ± 0.01^{b}	0.11 ± 0.01^{b}	0.11 ± 0.01^{ab}
	37		0.11 ± 0.00^{b}	0.10 ± 0.00^{b}	0.10 ± 0.00^{b}		0.10 ± 0.00^{a}	0.09 ± 0.00^{b}	0.08 ± 0.00^{b}		0.09 ± 0.00^{ab}	0.10 ± 0.00^{b}	0.08 ± 0.00^{a}
all-*E*-Zeaxanthin	4	0.10 ± 0.00^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.08 ± 0.00^{a}	0.10 ± 0.01^{b}	0.10 ± 0.00^{b}	0.09 ± 0.00^{b}	0.06 ± 0.00^{a}	0.09 ± 0.00^{b}	0.10 ± 0.00^{b}	0.09 ± 0.00^{b}
	20		0.10 ± 0.01^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}		0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.08 ± 0.00^{a}		0.07 ± 0.01^{b}	0.07 ± 0.00^{b}	0.07 ± 0.00^{ab}
	37		0.08 ± 0.00^{b}	0.07 ± 0.00^{b}	0.08 ± 0.00^{b}		0.06 ± 0.00^{b}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}		0.06 ± 0.00^{ab}	0.05 ± 0.00^{bc}	0.05 ± 0.00^{c}
all-*E*-α-Carotene	4	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{b}
	20		0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}		0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
	37		0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}		0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}
all-*E*-β-Carotene	4	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}
	20		0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}		0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}
	37		0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}		0.01 ± 0.00^{b}	0.01 ± 0.00^{ac}	0.01 ± 0.00^{c}	^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}

[1]Data are the mean ± standard error (n=6, three batches x 2). Different letters within the same line (for each pigment and variety of cereal) indicate significant differences ($p<0.05$) determined by the Duncan test.

Table 2. Carotenoid composition evolution (μg/g fresh weight)[1] during postharvest storage of tritordeum grains (HT630, HT621 and HT609 lines).

		HT630				HT621				HT609			
		Time (days)											
Carotenoid	**Temp (°C)**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**
all-*E*-Lutein	4	5.12 ± 0.10^{a}	4.88 ± 0.06^{a}	4.77 ± 0.12^{a}	4.75 ± 0.26^{a}	4.72 ± 0.17^{a}	4.08 ± 0.07^{a}	4.13 ± 0.44^{a}	4.09 ± 0.20^{a}	4.06 ± 0.19^{a}	3.95 ± 0.15^{a}	3.97 ± 0.20^{a}	3.46 ± 0.30^{a}
	20		4.39 ± 0.07^{b}	3.75 ± 0.08^{c}	3.91 ± 0.33^{bc}		4.20 ± 0.13^{ab}	3.56 ± 0.27^{b}	3.58 ± 0.32^{b}		3.71 ± 0.18^{ab}	3.25 ± 0.10^{b}	3.42 ± 0.24^{b}
	37		2.92 ± 0.08^{b}	2.17 ± 0.09^{c}	1.95 ± 0.06^{c}		3.08 ± 0.06^{b}	2.46 ± 0.08^{c}	2.18 ± 0.04^{c}		2.70 ± 0.07^{b}	2.02 ± 0.04^{c}	1.77 ± 0.02^{c}
9*Z*- and 13*Z*-Lutein	4	0.69 ± 0.01^{a}	0.68 ± 0.01^{a}	0.64 ± 0.02^{a}	0.69 ± 0.03^{a}	0.64 ± 0.02^{a}	0.59 ± 0.02^{a}	0.55 ± 0.05^{a}	0.57 ± 0.03^{a}	0.54 ± 0.02^{a}	0.54 ± 0.03^{a}	0.54 ± 0.03^{a}	0.45 ± 0.04^{a}
	20		0.64 ± 0.00^{ab}	0.56 ± 0.02^{c}	0.59 ± 0.04^{bc}		0.61 ± 0.01^{a}	0.52 ± 0.03^{b}	0.54 ± 0.02^{b}		0.52 ± 0.02^{ab}	0.46 ± 0.01^{c}	0.47 ± 0.02^{bc}
	37		0.59 ± 0.02^{b}	0.50 ± 0.02^{c}	0.47 ± 0.01^{c}		0.63 ± 0.02^{b}	0.55 ± 0.01^{c}	0.51 ± 0.01^{c}		0.53 ± 0.01^{a}	0.45 ± 0.01^{b}	0.41 ± 0.00^{c}
Lutein monolinoleate	4	0.49 ± 0.01^{ab}	0.47 ± 0.01^{a}	0.52 ± 0.02^{ab}	0.53 ± 0.03^{b}	0.20 ± 0.01^{a}	0.18 ± 0.00^{a}	0.21 ± 0.03^{a}	0.21 ± 0.02^{a}	0.26 ± 0.01^{a}	0.26 ± 0.01^{a}	0.27 ± 0.01^{a}	0.26 ± 0.01^{a}
	20	^{a}	0.49 ± 0.02^{a}	0.52 ± 0.03^{a}	0.62 ± 0.01^{b}		0.21 ± 0.01^{a}	0.24 ± 0.02^{ab}	0.28 ± 0.03^{b}		0.26 ± 0.01^{a}	0.31 ± 0.02^{b}	0.36 ± 0.01^{c}
	37	^{a}	0.66 ± 0.03^{b}	0.78 ± 0.03^{c}	0.86 ± 0.04^{c}		0.32 ± 0.00^{b}	0.45 ± 0.02^{c}	0.52 ± 0.02^{d}		0.41 ± 0.01^{a}	0.53 ± 0.01^{c}	0.57 ± 0.01^{d}
Lutein 3'-*O*-linoleate	4	0.09 ± 0.00^{a}	0.08 ± 0.00^{a}	0.09 ± 0.00^{a}	0.09 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
	20		0.09 ± 0.01^{a}	0.09 ± 0.00^{a}	0.11 ± 0.00^{b}		0.04 ± 0.00^{a}	0.05 ± 0.00^{ab}	0.06 ± 0.01^{b}		0.05 ± 0.00^{a}	0.06 ± 0.00^{b}	0.07 ± 0.00^{c}
	37		0.12 ± 0.01^{b}	0.15 ± 0.01^{c}	0.17 ± 0.01^{d}		0.07 ± 0.00^{b}	0.10 ± 0.00^{c}	0.11 ± 0.00^{d}		0.08 ± 0.00^{b}	0.10 ± 0.00^{c}	0.12 ± 0.00^{d}
Lutein 3-*O*-linoleate	4	0.40 ± 0.01^{ab}	0.39 ± 0.01^{a}	0.43 ± 0.02^{ab}	0.44 ± 0.02^{b}	0.16 ± 0.01^{a}	0.14 ± 0.00^{a}	0.17 ± 0.03^{a}	0.17 ± 0.02^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.22 ± 0.01^{a}	0.21 ± 0.01^{a}
	20	^{a}	0.40 ± 0.02^{a}	0.43 ± 0.02^{a}	0.51 ± 0.01^{b}		0.17 ± 0.01^{a}	0.19 ± 0.01^{ab}	0.22 ± 0.02^{b}		0.20 ± 0.01^{a}	0.25 ± 0.01^{b}	0.29 ± 0.01^{c}
	37	^{a}	0.54 ± 0.02^{b}	0.63 ± 0.02^{c}	0.69 ± 0.03^{c}		0.26 ± 0.00^{b}	0.36 ± 0.01^{c}	0.40 ± 0.02^{d}		0.33 ± 0.01^{b}	0.42 ± 0.01^{c}	0.46 ± 0.01^{d}
Luteín monopalmitate	4	0.81 ± 0.01^{a}	0.76 ± 0.03^{a}	0.84 ± 0.04^{a}	0.86 ± 0.05^{a}	0.49 ± 0.02^{a}	0.42 ± 0.01^{a}	0.50 ± 0.08^{a}	0.50 ± 0.06^{a}	0.62 ± 0.02^{a}	0.61 ± 0.04^{a}	0.63 ± 0.03^{a}	0.61 ± 0.03^{a}
	20		0.77 ± 0.03^{a}	0.79 ± 0.04^{a}	0.93 ± 0.01^{b}		0.50 ± 0.02^{a}	0.55 ± 0.03^{ab}	0.63 ± 0.05^{b}	^{ab}	0.59 ± 0.02^{a}	0.68 ± 0.04^{b}	0.78 ± 0.02^{c}
	37		0.97 ± 0.03^{b}	1.05 ± 0.04^{bc}	1.12 ± 0.05^{c}		0.73 ± 0.01^{b}	0.93 ± 0.03^{c}	0.99 ± 0.04^{c}	^{a}	0.88 ± 0.03^{b}	1.04 ± 0.01^{c}	1.09 ± 0.02^{c}
Lutein 3'-*O*-palmitate	4	0.24 ± 0.00^{ab}	0.23 ± 0.01^{a}	0.26 ± 0.01^{ab}	0.26 ± 0.01^{b}	0.15 ± 0.01^{a}	0.13 ± 0.00^{a}	0.16 ± 0.03^{a}	0.16 ± 0.02^{a}	0.18 ± 0.01^{a}	0.18 ± 0.01^{a}	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}
	20	^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.29 ± 0.01^{b}		0.16 ± 0.01^{a}	0.18 ± 0.01^{ab}	0.21 ± 0.02^{b}		0.18 ± 0.01^{a}	0.21 ± 0.01^{b}	0.24 ± 0.01^{c}
	37	^{a}	0.30 ± 0.01^{b}	0.33 ± 0.01^{c}	0.35 ± 0.01^{c}		0.24 ± 0.00^{b}	0.32 ± 0.01^{c}	0.34 ± 0.01^{c}		0.27 ± 0.01^{b}	0.33 ± 0.00^{c}	0.35 ± 0.01^{c}
Lutein 3-*O*-palmitate	4	0.57 ± 0.01^{a}	0.53 ± 0.02^{a}	0.58 ± 0.03^{a}	0.60 ± 0.03^{a}	0.34 ± 0.01^{a}	0.29 ± 0.01^{a}	0.34 ± 0.05^{a}	0.34 ± 0.04^{a}	0.44 ± 0.01^{a}	0.43 ± 0.02^{a}	0.44 ± 0.02^{a}	0.42 ± 0.02^{a}
	20		0.53 ± 0.02^{a}	0.54 ± 0.03^{a}	0.64 ± 0.01^{b}		0.34 ± 0.01^{a}	0.37 ± 0.02^{ab}	0.42 ± 0.03^{b}		0.41 ± 0.01^{a}	0.47 ± 0.03^{b}	0.54 ± 0.01^{c}
	37		0.67 ± 0.02^{b}	0.72 ± 0.03^{bc}	0.77 ± 0.04^{c}		0.49 ± 0.01^{b}	0.61 ± 0.02^{c}	0.65 ± 0.02^{c}		0.61 ± 0.02^{b}	0.71 ± 0.01^{c}	0.74 ± 0.02^{c}
Lutein dilinoleate	4	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
	20		0.02 ± 0.00^{ab}	0.03 ± 0.01^{b}	0.05 ± 0.00^{c}		0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}		0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{c}
	37		0.05 ± 0.00^{b}	0.10 ± 0.00^{c}	0.14 ± 0.01^{d}		0.01 ± 0.00^{b}	0.02 ± 0.00^{c}	0.02 ± 0.00^{d}		0.01 ± 0.00^{b}	0.03 ± 0.00^{c}	0.04 ± 0.00^{d}
Lutein dipalmitate	4	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}	0.07 ± 0.00^{a}	0.08 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{ab}	0.03 ± 0.00^{b}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{b}	0.05 ± 0.00^{b}

	20		0.07 ± 0.00^{a}	0.08 ± 0.02^{a}	0.13 ± 0.00^{b}		0.02 ± 0.00^{a}	0.04 ± 0.00^{b}	0.05 ± 0.01^{c}		0.04 ± 0.00^{ab}	0.06 ± 0.01^{b}	0.09 ± 0.00^{c}
	37		0.12 ± 0.00^{b}	0.22 ± 0.00^{c}	0.28 ± 0.01^{d}		0.06 ± 0.00^{b}	0.11 ± 0.01^{c}	0.14 ± 0.01^{d}		0.10 ± 0.01^{b}	0.18 ± 0.00^{c}	0.23 ± 0.00^{d}
Lutein linoleate palmitate	4	0.05 ± 0.00^{a}	0.06 ± 0.00^{ab}	0.07 ± 0.00^{b}	0.08 ± 0.00^{c}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
	20		0.07 ± 0.00^{ab}	0.09 ± 0.02^{b}	0.15 ± 0.00^{c}		0.02 ± 0.00^{a}	0.03 ± 0.00^{b}	0.04 ± 0.00^{c}		0.03 ± 0.00^{a}	0.04 ± 0.01^{b}	0.06 ± 0.00^{c}
	37		0.15 ± 0.00^{b}	0.28 ± 0.00^{c}	0.38 ± 0.02^{d}		0.04 ± 0.00^{b}	0.09 ± 0.00^{c}	0.13 ± 0.01^{d}		0.07 ± 0.00^{b}	0.16 ± 0.00^{c}	0.21 ± 0.00^{d}
all-*E*-β-Carotene	4	0.08 ± 0.00^{a}	0.07 ± 0.00^{a}	0.07 ± 0.00^{a}	0.07 ± 0.01^{a}	0.06 ± 0.00^{a}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	0.04 ± 0.01^{b}	0.07 ± 0.00^{a}	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}	0.06 ± 0.01^{a}
	20		0.06 ± 0.00^{b}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}		0.05 ± 0.00^{b}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}		0.05 ± 0.00^{ab}	0.05 ± 0.00^{b}	0.06 ± 0.00^{b}
	37		0.06 ± 0.01^{b}	0.05 ± 0.00^{b}	0.06 ± 0.01^{b}		0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}		0.06 ± 0.00^{b}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}
Regioisomers ratios													
Lutein 3-*O*-linoleate / Lutein 3'-*O*-linoleate	4	4.5	4.7	4.7	4.9	3.8	4.0	4.1	4.1	4.0	4.1	4.1	4.2
	20		4.6	4.7	4.7		3.9	4.0	4.0		4.0	4.1	4.2
	37		4.6	4.3	4.2		3.8	3.6	3.7		4.2	4.0	3.9
Lutein 3-*O*-palmitate / Lutein 3'-*O*-palmitate	4	2.3	2.3	2.3	2.2	2.3	2.3	2.2	2.1	2.4	2.4	2.3	2.3
	20		2.3	2.2	2.2		2.2	2.1	2.0		2.3	2.3	2.2
	37		2.2	2.2	2.2		2.0	1.9	1.9		2.3	2.2	2.1

[1]Data are the mean ± standard error (n=6, three batches x 2). Different letters within the same line (for each pigment and variety of cereal) indicate significant differences ($p<0.05$) determined by the Duncan test.

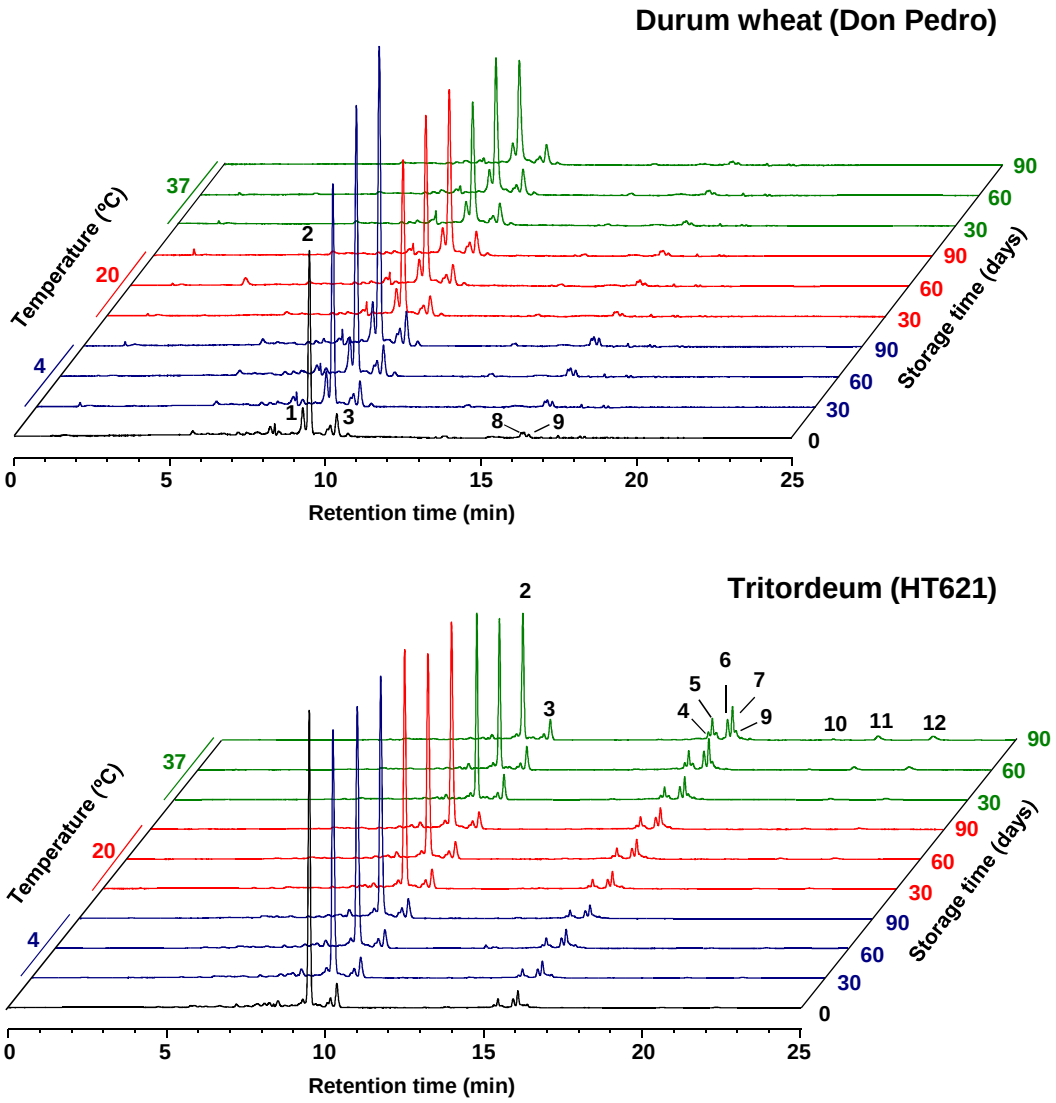
Durum wheat (Don Pedro)
Temperature (°C)
37
20
4
Storage time (days)
90
60
30
90
60
30
90
60
30
0
1
2
3
8
9
0
5
10
15
20
25
Retention time (min)
Tritordeum (HT621)
Temperature (°C)
37
20
4
Storage time (days)
90
60
30
90
60
30
90
60
30
0
2
3
4
5
6
7
9
10
11
12
0
5
10
15
20
25
Retention time (min)

Figure 1.

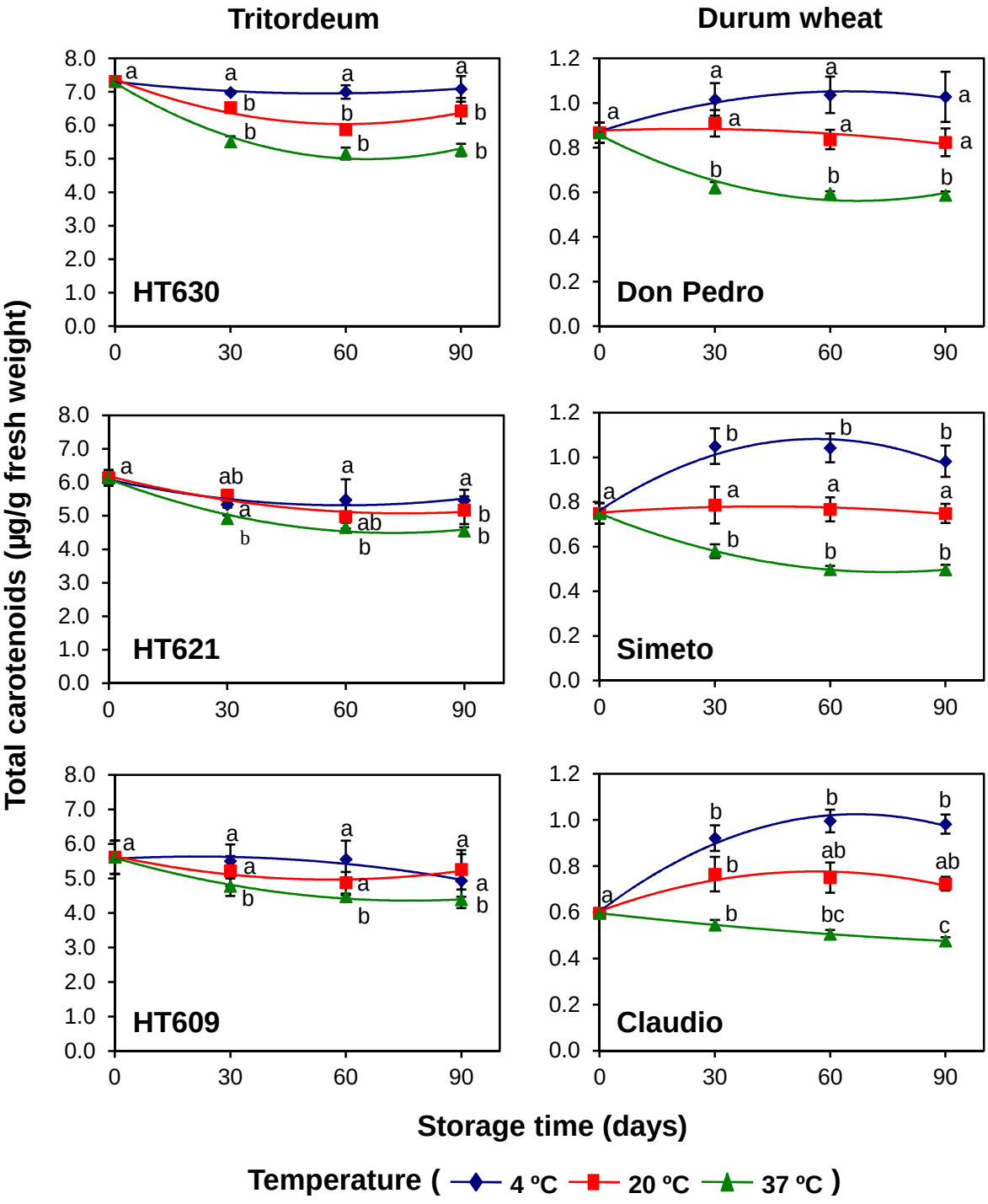
Tritordeum
Durum wheat
HT630
Don Pedro
HT621
Simeto
HT609
Claudio
Total carotenoids (µg/g fresh weight)
Storage time (days)
Temperature (4 ºC 20 ºC 37 ºC)

Figure 2

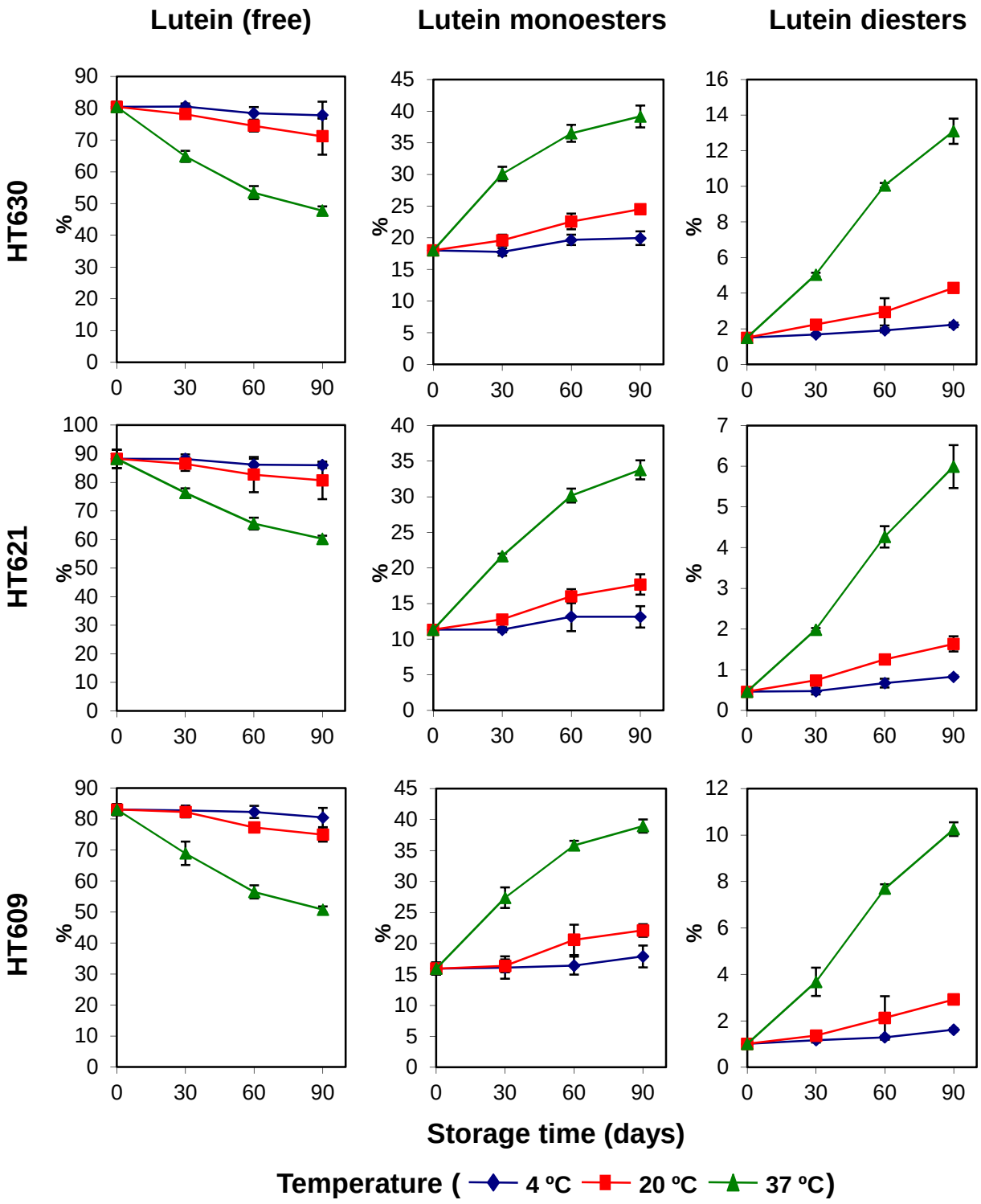
Lutein (free)
Lutein monoesters
Lutein diesters
HT630
HT621
HT609
%
Storage time (days)
Temperature (4 ºC 20 ºC 37 ºC)

Figure 3