Accepted Manuscript

Carotenoid evolution during short-storage period of durum wheat (*Triticum turgidum* conv. *durum*) and tritordeum ($\times$ *Tritordeum* Ascherson et Graebner) whole-grain flours

Elena Mellado-Ortega, Dámaso Hornero-Méndez

PII: S0308-8146(15)01068-7

DOI: <http://dx.doi.org/10.1016/j.foodchem.2015.07.057>

Reference: FOCH 17853

To appear in: *Food Chemistry*

Received Date: 1 May 2015

Revised Date: 9 July 2015

Accepted Date: 13 July 2015

Please cite this article as: Mellado-Ortega, E., Hornero-Méndez, D., Carotenoid evolution during short-storage period of durum wheat (*Triticum turgidum* conv. *durum*) and tritordeum ($\times$ *Tritordeum* Ascherson et Graebner) whole-grain flours, *Food Chemistry* (2015), doi: <http://dx.doi.org/10.1016/j.foodchem.2015.07.057>

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1 **Carotenoid evolution during short-storage period of durum wheat (*Triticum***
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3 **whole-grain flours.**

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5 Elena Mellado-Ortega and Dámaso Hornero-Méndez*

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7 Group of Chemistry and Biochemistry of Pigments. Food Phytochemistry Department.
8 Instituto de la Grasa (CSIC). Campus Universidad Pablo de Olavide, Ctra. de Utrera
9 km. 1. 41013 - Seville (Spain).

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12
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16
17 * Corresponding author.

18 Telephone: +34 954611550; Fax: +34 954616790; e-mail: hornero@ig.csic.es
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ABSTRACT

This study investigates the effect of storage temperature on carotenoid composition in durum wheat and tritordeum whole-grain flours. For both cereal genotypes, total carotenoid content significantly decreased during storage, following a temperature dependent first-order kinetic model. Individual and total carotenoid content decay were similar for durum wheat, with a maximum at 50 °C at the end of the storage period (94%). In contrast, the evolution of lutein ester fractions in tritordeum showed lower losses than for free lutein (~50%), and consequently the total carotenoid content was less affected (83%). A decrease in the lutein monoesters fraction was observed, coinciding with an increase in the diesterified forms, especially for lutein dilinoleate. These data suggest an esterifying activity in flours different from the enzyme systems operating *in vivo* (xanthophyll acyl transferase). The formation of lutein diesters, with greater stability, explains the slower carotenoid degradation in tritordeum whole-grain flours.

KEYWORDS

Carotenoids; lutein esters; tritordeum; *Triticum turgidum* conv. *durum*; storage; whole-grain flour; kinetics.

Chemical compounds studied in this article:

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

1. Introduction

Carotenoids are a major class of natural pigments produced *de novo* by plants and microbes. In plants, carotenoids can be found in fruits, flowers, roots, leaves, and seeds, and in most cases are responsible for the red, orange and yellow colorations (Britton & Hornero-Méndez, 1997). In contrast, animals are unable to synthesize carotenoids, so they need to incorporate them from the diet. Carotenoid pigments are a class of isoprenoid compounds (mostly C₄₀) that contain up to 15 conjugated double bonds, and can be classified into two major groups according to their chemical structures; carotenes, which are hydrocarbons (i.e. β -carotene and lycopene), and their oxygenated derivatives denoted as xanthophylls (i.e. lutein and zeaxanthin) (Britton et al., 2004). To date, over 700 carotenoids have already been identified. In plants, the carotenoids are synthesized and accumulated in plastids, fulfilling two essential functions during photosynthesis, light harvesting and protecting the photosynthetic apparatus from photo-oxidation. They are also the precursors to signaling molecules which influence the development and biotic/abiotic stress responses and attract animals as pollinators and for seed dispersion. Carotenoid production in the seeds is a determinant for ABA production and seed dormancy (Taylor & Ramsay, 2005). In wheat grains, carotenoids are important antioxidants that limit the levels of free radicals and reduce the activities of degradative enzymes, thus avoiding the ageing of seeds (Pinzino et al., 1999). Carotenoids protect the seed from deterioration and thereby contribute to a successful germination process.

Likewise, carotenoids are a vital part of the human diet as antioxidants and some of them as precursors to vitamin A, which is required and involved in a wide range of biological processes, including reproduction, embryonic development, cell differentiation, immunity, and metabolic control. As a result of their antioxidant activity

important biological functions are derived. Some of these are related to their role in the prevention of certain chronic disorders such as cancer, cardiovascular disease and osteoporosis, among others (reviewed by Rao & Rao, 2007). Certain carotenoids such as lutein and zeaxanthin are involved in the prevention of ocular diseases, including cataracts and age-related macular degeneration (AMD), and are almost exclusively the only carotenoids found in human macula (Landrum & Bone, 2001).

Cereals, although having a low carotenoid content when compared with the majority of fruits and vegetables, may have an important impact in the nutritional status of consumers, which is especially significant in developing countries, where cereals (especially wheat, rice and maize) are staple foods in the diet. Thus, significant progress has been made towards producing staple food and/or crops with higher carotenoid contents, as an important first step in alleviating worldwide vitamin A deficiency as well as other nutritional and health problems. Since cereals show significant natural variation in carotenoid levels, breeding or genetic modifications have made carotenoid-biofortification possible in these staple foods (Farré et al., 2010). This variation depends on three factors: a) the selection and domestication of wild varieties: for example the common wheat (*Triticum aestivum* L.) varieties, which have been traditionally selected for their white color since consumers prefer white flour for bread making. In contrast, durum wheat (*Triticum turgidum* ssp. *durum*) has been selected for its high yellow pigment content (YPC) as it is a desirable property in pasta products (Troccoli, Borelli, De Vita, Fares, & Di Fonzo, 2000); b) Genotype and cereal developmental stage; c) Growing environment, storage and milling and processing techniques (Lv et al., 2013).

The AACC (American Association of Cereal Chemists) defined whole-grain flour as being prepared from wheat such that the proportions of the intact grain, the bran, germ, and endosperm, remain unaltered (AACC International, 1999). Whole-grain

95 wheat is composed of 10–14% bran, 2.5–3.0% germ and 80–85% endosperm. The
 96 bioactive compounds are unevenly distributed within these parts and concentrated in the
 97 outer portions of the grain (Ndolo & Beta, 2013). Thus, whole-grain wheat flour
 98 contains substantially more vitamins, minerals, antioxidants and other nutrients,
 99 including carotenoids, than refined wheat flour (Fardet, 2010). It is therefore easy to
 100 understand the relevant influence of processing techniques and milling on the
 101 phytonutrients composition and bioactive compounds of these staple foods. This fact
 102 has led to the modification and development of new technological treatments in order to
 103 preserve and enhance the contents of carotenoids and other phytonutrients of nutritional
 104 relevance. Examples of these include: the different pretreatments such as physical
 105 (exposure to ultraviolet radiation), biochemical (enzymes), and chemical (CaCl_2 , ozone
 106 treatments); pre-processing prior to milling such as degerming, debranning (which can
 107 be carried out by friction (peeling), by abrasion (pearling), or by a combination of both
 108 processes); and the bran separation through histological fractionation (electrostatic
 109 separation) and macromolecular fractionation using superfine grinding (jet-milling and
 110 cryogenic grinding techniques) (reviewed by Hemery, Rouau, Lullien-Pellerin, Barron,
 111 & Abecassis, 2007).

112 Tritordeum is an excellent example of the efforts made for selecting new cereal
 113 varieties with higher carotenoid contents, since its pigment content is 5 to 8 times higher
 114 than durum wheat (Atienza, Ballesteros, Martín, & Hornero-Méndez, 2007). Tritordeum
 115 ($\times$ *Tritordeum* Ascherson et Graebner) is a novel cereal obtained as an amphiploid
 116 ($2n=6x=42$, $\text{AABBH}^{\text{ch}}\text{H}^{\text{ch}}$) resulting from the cross between a wild barley (*Hordeum*
 117 *chilense* Roem. & Schult.) and durum wheat. In previous works, we have completely
 118 characterized the carotenoid composition of tritordeum (Atienza et al. 2007; Mellado-
 119 Ortega & Hornero-Méndez, 2012). It is mainly composed of lutein (3R,3'R,6'R- β , ϵ -

carotene-3,3'-diol) and its esters (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)). This esterification profile of lutein in cereals involves only two fatty acids, palmitic (C16:0) and linoleic acids (C18:2), indicating that it is a highly specific process. The *in vivo* esterification of xanthophylls is directly related to the stability and abundance of carotenoid pigments in the different plant tissues (Hornero-Méndez & Mínguez-Mosquera, 2000). However, this process is not distributed evenly among the plants tissues. In cereals, such as durum wheat, the xanthophyll esters seem to be absent or at very low concentration levels (Mellado-Ortega, Atienza, & Hornero-Méndez, 2015). However, in *ex vivo* conditions, it is known that lutein is susceptible to esterification due to imposed storage conditions, which is a feature applicable to any cereal (Farrington, Warwick, & Shearer, 1981). More recently, we have characterized the carotenoid profile of *H. chilense* grains for the first time and provide additional biochemical evidence that demonstrates this parental as the origin of the high carotenoid content and esterification pattern of tritordeum (Mellado-Ortega & Hornero-Méndez, 2015).

Flours are undoubtedly the product derived from cereals with the widest applications. Flours are stored, for varying periods of time, preferably in stockpiled sacks in cool dark rooms. Flour millers indicate a product viability of 3 to 9 months after milling on whole-grain wheat flour packages, whereas in regular wheat flour, this range is extended 9 to 15 months after milling. Although these dates can be helpful, actual shelf-life could be shorter or longer depending on temperature and humidity during storage and the features required of the final product by the flour industry (Doblado-Maldonado, Pike, Sweley, & Rose, 2012). The storage of fresh and/or

processes food leads to carotenoid losses driven mostly by oxidation processes, both enzymatic and non-enzymatic. In cereal flours, the disintegration of the grain matrix tissue facilitates the contact between the degradative enzymes and the pigments. The oxygen content of the medium is considered as the most relevant factor in limiting the stability of carotenoids (Britton & Khachik, 2009). In addition, well-known modifications of these molecules are the *cis-trans* (*E/Z*) geometric isomerization promoted by the temperature and/or light, which are characterized by generating alterations in the biological activity of the pigments (Liaaen-Jensen & Lutnaes, 2008). The analysis of the composition and distribution of carotenoids in cereal grains are increasingly accurate (Ndolo & Beta, 2013), being a basic tool for carotenoid biofortification of cereals and for the intelligent design of new processing techniques (recently reviewed by Ficco et al., 2014). However, studies that evaluate the behavior of carotenoids, including their esters, during the storage of cereal flours are very limited despite being the most common cereal-derived product, with a demonstrated activity against many chronic diseases due to the protective role of fiber.

Thus, the objective of this study was to investigate the influence of storage conditions (temperature and time) on the carotenoid stability and metabolism in tritordeum and durum wheat whole-grain flours. Special attention is made on the stability of the esterified pigment and on *de novo* esterification promoted by the imposed storage conditions. With this aim, the carotenoid content and profile of flour from three durum wheat varieties and three advanced tritordeum lines were measured during their storage for up to 90 days at four different temperatures (4, 20, 37 and 50 °C).

168

169 2. Experimental

170

171 *2.1. Plant material, sample preparation and storage conditions*

172 Three advanced tritordeum lines (HT630, HT621 and HT609), characterized by
173 a high carotenoid content in the endosperm, and developed in the Cereal Breeding
174 Program of the Institute for Sustainable Agriculture (IAS-CSIC, Córdoba, Spain), and
175 three commercial durum wheat varieties (Don Pedro, Simeto and Claudio) were used
176 for the present study. Whole-grain flours (containing the intact grain layers bran, germ,
177 and endosperm) were obtained from 250 grams of recently harvested grains of each of
178 the different varieties of cereals. Grains were milled using a hammer-mill Retsch Model
179 SK100 (Retsch, Haan, Germany) with a 2 mm output sieve. The flours were distributed
180 in aliquots of approximately one gram in round-capped polypropylene 15-mL centrifuge
181 tubes, which were placed in containers under controlled temperature conditions (4, 20,
182 37 and 50 °C) for a period of 90 days. Triplicate samples (three sample tubes) were
183 taken at monthly intervals (a total of n=36 for each cereal variety). A control sample
184 (t=0 days), consisting of 4 subsamples (n=4), was taken for each line or variety and
185 stored at -30 °C until analysis. The dry matter content (%) in the samples at each
186 sampling date was measured in triplicate by using an Ohaus moisture balance model
187 MB35 (Ohaus, Switzerland).

188

189 *2.2. Chemicals and reagents*

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

194

195 2.3. Extraction of carotenoids

196 The extraction of carotenoids was carried out according to the method of
197 Atienza et al. (2007) with some modifications (Mellado-Ortega & Hornero-Méndez,
198 2012). Briefly, 1 g of flour sample was placed in a round-capped polypropylene 15-mL
199 centrifuge tube and extracted with 4 mL of acetone (containing 0.1% BHT) for 2 min by
200 vigorous vortexing, following sonication for 1 min. The mixture was centrifuged at
201 4,500×g for 5 min at 4 °C. The extraction operation was repeated three times, and the
202 acetone fractions were pooled. The solvent was gently evaporated under nitrogen
203 stream, and the pigments were dissolved in 0.5 or 1.0 mL of acetone for durum wheat
204 and tritordeum samples, respectively. Prior to the chromatographic analysis, samples
205 were centrifuged at 13,000×g for 5 min at 4 °C. Three independent analyses were
206 carried out for each sample. All operations were performed under dimmed light to
207 prevent isomerization and photo-degradation of carotenoids.

209 2.4. HPLC analysis of carotenoids

210 The procedures for the isolation and identification of carotenoid pigments and its
211 esters have already been described in previous works (Atienza et al., 2007; Mellado-
212 Ortega & Hornero-Méndez, 2012). Quantitative analysis of carotenoids was carried out
213 by HPLC according to the method of Mínguez-Mosquera and Hornero-Méndez (1993)
214 with some modifications (Atienza et al., 2007). The HPLC system consisted of a Waters
215 2695 Alliance chromatograph fitted with a Waters 2998 photodiode array detector, and
216 controlled with Empower2 software (Waters Cromatografía, S.A., Barcelona, Spain). A
217 reversed-phase column (Mediterranea SEA18, 3 µm, 20×0.46 cm; Teknokroma,
218 Barcelona, Spain) was used. Column compartment was thermostated at 25 °C.
219 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 μ L and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and the online spectra were acquired (350-700 nm wavelength range). Quantification was carried out using calibration curves prepared with lutein, zeaxanthin, α -carotene and β -carotene standards isolated and purified from natural sources (Mínguez-Mosquera & Hornero-Méndez, 1993). Calibration curves were prepared in the range of 0.5-50 μ g/ml. The concentrations of lutein esters were estimated by using the calibration curve for free lutein, since the esterification of xanthophylls with fatty acids does not modify the chromophore properties (Britton, 1995). The calibration curve of free lutein was also used to determine the concentration of the (Z)-isomers of lutein. Data were expressed as μ g/g dry weight (μ g/g dw).

233

234 2.5. Degradation kinetics modeling

235 To determine the degradation reaction order of the total carotenoid content, zero-
 236 and first-order kinetics were hypothesized by applying the general reaction rate
 237 expression $-dC/dt = kC^n$, where C is the concentration of the compound (μ g/g dw), C_0 is
 238 the initial concentration, k is the reaction rate constant (days^{-1}), t is the reaction time
 239 (days), and n is the order of the reaction. The order of the reaction with the best
 240 correlation (r^2) and the best correspondence among the experimental values and the
 241 half-life of the compound ($t_{1/2}$) [time needed for the concentration of a reactant to fall to
 242 half its initial value, where $t_{1/2} = C_0/2k$ for zero-order and $t_{1/2} = \ln 2/k$ for first-order]
 243 were selected.

244

245 2.6. Statistical analysis

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

251

252 3. Results and discussion

253 3.1. Changes in the carotenoid content during the short storage period of durum wheat 254 and tritordeum whole-grain flours

255 **Tables 1** and **2** show the carotenoid composition of durum wheat and tritordeum
 256 whole-grain flours under the assayed storage conditions. The carotenoid profiles for
 257 both types of cereals were clearly different (**Figure 1** at $t=0$ days), with a significantly
 258 higher, about 8 times, mean total carotenoid content in tritordeum lines ($10.8 \mu\text{g/g dw}$),
 259 compared to durum wheat ($1.3 \mu\text{g/g dw}$). This pigment composition was similar to those
 260 previously reported (Atienza et al., 2007; Mellado-Ortega & Hornero-Méndez, 2012).
 261 The total carotenoid content changed significantly during the storage period under the
 262 four different temperatures ($p < 0.05$) (**Figure 2**). The results revealed significant losses
 263 due to pigment degradation which followed a temperature dependent trend.
 264 Consequently, the lower total carotenoid content was found at the end of the storage
 265 period (90 days) at 50°C . The average values of reduction in the carotenoid content
 266 were 83% and 94% for tritordeum and durum wheat flours, respectively. The flour is
 267 more vulnerable than the seeds with respect to the storage conditions, due to the loss of
 268 cell integrity and increase in specific surface area. The carotenoids in plant tissues are
 269 stabilized by association with proteins, membrane structures, etc. Tissue disruption may

270 cause substantial reductions in carotenoids, due to the effect of temperature and
271 oxidation processes because of the high degree of unsaturation present in their structure,
272 which makes them susceptible to oxidation. Moreover, it is well accepted that the shelf-
273 life of whole-grain wheat flour is considerably shorter than refined flour, which is
274 associated with the instability of the lipids in the germ (Tait & Galliard, 1988).

275 Thus the oxidative stress generated by the conditions of storage causes an
276 increase in free radicals, which are also generated by enzymatic systems such as
277 lipoxygenase (linoleate oxygen oxide reductase, EC 1.13.11.12). Lipoxygenases (LOX)
278 are a class of non-haem iron-containing dioxygenases that attack the methylene group
279 between two double bonds in polyunsaturated fatty acids, preferentially non-esterified
280 polyunsaturated fatty acids, to form conjugated diene hydroperoxides (Loiseau, Ly Vu,
281 Macherel, & Le Deunff, 2001). These hydroperoxides react with the pigments present in
282 the flour, breaking down the backbone of carbon into smaller compounds, including
283 volatile compounds and apocarotenoids (e.g., epoxyaldehydes, ketones). Although the
284 activity of LOX has been typically linked to kneading and breadmaking, where the
285 moisture contents are higher than those found during storage, the involvement of this
286 enzyme is predictable (Morrison & Panapirai, 1975). LOX is widely distributed in
287 cereals and located in the germ and bran of the grain (Loiseau et al., 2001). Linoleic
288 acid, which is its main substrate, is also the most abundant fatty acid in the lipid
289 composition of the seeds under study (Mellado-Ortega & Hornero-Méndez, 2012).

290 As deduced from **Figure 2**, at the temperatures of 4, 20 and 37 °C, the
291 carotenoids from tritordeum flours showed higher stability than from durum wheat
292 flours. Thus, the increase in temperature in durum wheat flours led to a more noticeable
293 degradation of the total carotenoids. At 37 °C the average losses at the end of the
294 storage period were 78% in durum wheat versus 57% in tritordeum. This marked

295 difference can be attributed not only to the higher initial carotenoid content of
 296 tritordeum, but rather to the esterification of lutein with fatty acids, which has been
 297 demonstrated to increase the carotenoid stability (Subagio, Wakaki, & Morita, 1999).

298 The same trend was observed among the individual carotenoids. For the durum
 299 wheat varieties (**Table 1**) the flours prepared from the Don Pedro variety showed a
 300 more progressive degradation for each of the carotenoids according to the temperature
 301 and the time. Claudio and Simeto flours displayed slightly lower losses for the different
 302 individual carotenoids at 4 and 20 °C. The losses at the end of the storage period (90
 303 days) at 50 °C were pronounced, and even led to the total disappearance of the α - and β -
 304 carotene. These data suggest that the carotenes are more susceptible to oxidation than
 305 the xanthophylls (Sandmann, Römer, & Fraser, 2006). It is noteworthy that the effect of
 306 the temperature at 4 °C during the three months of storage led to a carotenoid retention
 307 of 50% for all durum wheat varieties. These results contrast with those obtained by
 308 Hidalgo and Brandolini (2008) who indicated losses of 23% for Einkorn (*Triticum*
 309 *monococcum* L. ssp. *monococcum*) and bread wheat (*Triticum aestivum* L. ssp.
 310 *aestivum*) flours during storage at 5 °C for up to 239 days.

311 Regarding the individual carotenoids of tritordeum (**Table 2**), the evolution of
 312 (all-*E*)-lutein, (*Z*)-lutein and (all-*E*)- β -carotene indicated a clear pattern of a
 313 temperature-dependent degradation with reductions in the pigment content of 93% at 50
 314 °C at the end of the storage period. In contrast, the evolution pattern of the lutein ester
 315 fractions (monoesters and diesters) was more conservative, showing lower losses than
 316 for free lutein (50% average). These data suggest greater stability of the esterified
 317 xanthophylls under the storage conditions tested in this study. This conclusion can be
 318 clearly deduced when the relative changes (%) of the free, monoesterified and
 319 diesterified lutein fractions are represented (**Figure 3**). Thus the free lutein decrease was

compensated by the increase in esterified forms. The diester fractions showed greater increases than the monoesters for all the tritordeum lines, indicating greater stability, which ultimately results in lower carotenoid losses in tritordeum flours.

323

324 *3.2. Formation of diesterified xanthophylls during the short storage period of* 325 *tritordeum whole-grain flours*

For tritordeum flours, a more marked tendency toward degradation was noted for monoesters with respect to diesters (**Table 2**), which sometimes even showed increases in their concentration. Similarly, at 50 °C, a decrease in the monoester fraction was observed, which coincided with a concomitant increase in the diesterified fraction. This fact indicates that the increase in lutein diesters occurs at the expense of the lutein monoesters. This could suggest the involvement of enzymes like lipases, induced by the storage conditions, which would catalyze the formation of these esters by a different metabolic pathway from that which operates *in vivo*. Lipase (EC 3.1.1.3) usually hydrolyzes triacylglycerols to non-esterified fatty acids and diglycerides, monoglycerides, and eventually glycerol (hydrolytic rancidity). The activity of this enzyme is mostly located in the bran fraction of the cereal grain (Urquhart, Altosaar, Matlashewski, & Sahasrabudhe, 1983). Wheat lipase exhibits maximum activity at 40 °C and 17% moisture content, whereas at the moisture contents commonly observed in flour during storage (10-14%) lipase activity retained 50% of its activity (Rose & Pike, 2006). Interestingly, if the environment becomes even dryer, for example due to a temperature increase up to 50 °C, these enzymes have been shown to catalyze the reverse reaction (esterification) or various transesterification reactions which may involve acids, alcohols and esters (reviewed by Barros, Fleuri, & Macedo, 2010). In any case, this assumption requires further studies to be confirmed.

345 With regard to the diester fractions, lutein dipalmitate was the most abundant
346 except in the HT630 line in which the content of lutein linoleatepalmitate was
347 approximately 4.8% higher than the lutein dipalmitate content. Trace amounts of lutein
348 dilinoleate were detected in the three tritordeum lines. However, the increases at 37 and
349 50 °C at the end of storage period were more pronounced in the case of lutein
350 dilinoleate, at 7 times higher, compared to the initial values, for HT621, 3 times higher
351 for HT630 for both temperatures, and 7 and 9 times for 37 and 50 °C, respectively, in
352 the HT609 line.

353 Similarly, when the evolution of the regioisomers of lutein monoesters (lutein
354 monolinoleate and lutein monopalmitate) were analyzed (**Table 2**), increases in the
355 concentration of the regioisomers of lutein monolinoleate during the first half of the
356 storage at the higher temperatures (37 and 50 °C) were observed, especially for the
357 regioisomer at the position 3' (lutein-3'-*O*-linoleate). Note that the latter is the
358 monoester found in a lower concentration in the grains of tritordeum, supporting the
359 idea that the esterifying activity taking place in flour is due to the activity of enzymes
360 different to those naturally operating in the grains (XAT: Xanthophyll Acyl
361 Transferase) (Mellado-Ortega & Hornero-Méndez, 2012). An interesting observation
362 was the detection of lutein esters in durum wheat flours, especially at higher
363 temperatures (**Figure 1**). Although the very low concentration levels did not allow for
364 their quantification, their presence indicated that the process of esterification of lutein in
365 flours occurs in both types of cereal.

366 The relationship between fatty acids and pigments could be considered not only
367 as a protection for the carotenoid themselves, but also for the fatty acids. Some
368 lipoxygenases, such as type 1 (LOX-1), are specific for non-esterified fatty acids
369 (Trono, Pastore, & Di-Fonzo, 1999). Thus, the carotenoid content in cereals and derived

products could also protect these crops from lipid oxidation because of the hydroperoxides arising from lipoxygenase activity. This contributes to the formation of various volatile compounds which are responsible for both the desirable flavor of vegetables, as well as the undesirable flavor ("off-flavor") that occurs after harvest and during the storage or processing of vegetables (Rodríguez-Amaya, 2003). Also, the presence of other antioxidants, such as tocopherols, may have a protective or synergistic effect and contribute ultimately to a greater stability and self-life of cereal grains and flours under storage conditions (Pastore et al., 2000).

378

3.3. Degradation kinetics of carotenoids

Since the storage and processing conditions may induce important changes in the food components, some qualities attributes may be compromised as a result. The rate at which these alterations occur has been a very active research topic in food technology. **Table 3** summarizes the data on reaction rate constant (k ; days⁻¹) and half-life time ($t_{1/2}$; days) for the total carotenoid content obtained after adjusting to a first-order kinetic model, since this is the most appropriate according to the exponential profile observed for the variations in concentration (**Figure 2**). The data were also adjusted to a zero-order kinetic model (data not shown), which obtained worst adjustments. Usually carotenoid degradation in food matrices which have low water content, such as cereal grains and derived products, follows a first-order degradation reaction. The reaction rate constant k (days⁻¹) increased with an increase in temperature, indicating a faster degradation of the pigments at higher temperatures. The k values were similar among the flours of the different varieties for the same cereal but different between both cereal species depending on temperature. At low or moderate temperatures (4 and 20 °C), the pigment degradation rates were very similar with average values of 7.3×10^{-3} (days⁻¹)

395 and 7.7×10^{-3} (days⁻¹) at 4 °C, and of 8.0×10^{-3} (days⁻¹) and 7.3×10^{-3} (days⁻¹) at 20 °C for
396 durum wheat and tritordeum, respectively. At higher temperatures the differences were
397 very marked. The mean values at 50 °C were of 34×10^{-3} (days⁻¹) for durum wheat versus
398 17×10^{-3} (days⁻¹) for tritordeum. These differences could be attributed to genotype
399 effects. Durum wheat flours showed *k* values of approximately double compared with
400 tritordeum flours, resulting in half-life times ($t_{1/2}$) which are significantly lower for the
401 carotenoids in durum wheat flours. Thus, at 50 °C, the average $t_{1/2}$ values were around
402 21 and 40 days for durum wheat and tritordeum, respectively. Undoubtedly, the strong
403 presence of lutein esters in tritordeum must play an important role in the increased
404 stability of the pigments in this cereal.

405 As deduced from the data, the storage conditions clearly affect the stability and
406 hence the carotenoid content in both cereals, with the increase in temperature being one
407 of the most decisive factors together with oxidative stress. There were important losses,
408 which were higher and faster for durum wheat than for tritordeum. To our knowledge,
409 the only similar study in cereals available in the literature is that of Hidalgo and
410 Brandolini (2008). These authors analyzed the carotenoid content of whole-grain and
411 white flour from einkorn (Monlis cultivar), and bread wheat (Serio cultivar) during
412 eight months of storage at five different temperatures (-20, 5, 20, 30 and 38 °C).
413 Although the storage conditions were not exactly the same, this study derived similar
414 conclusions to ours, obtaining better results for flours from einkorn, which is a cereal
415 with a comparable level of pigments to tritordeum (Abdel-Aal et al., 2002). However,
416 the degradation rates were lower, and consequently the shelf life longer than in our case
417 if we compare the temperatures of 5, 20 and 38 °C with the present study. These
418 differences can be attributed to the influence of the storage environment (oxygen, light,
419 etc) together with the cereal genotype as well as the content in other pro-oxidants and/or

420 antioxidant molecules that may affect the final results. Therefore comparisons with
421 previous studies are not straightforward. It should be mentioned that these authors did
422 not detect lutein esters synthesis.

423 What emerges from this kinetic study is the fact that the best temperature for
424 storing cereal flours should involve a compromise between lower pigment degradation
425 due to thermo-oxidative phenomena and higher esterified pigment proportions in the
426 flours. We therefore propose a temperature of between 25-35 °C as optimal for this
427 purpose.

428

429 **4. Conclusions**

430 Apart from the pioneer work carried out by Farrington et al. (1981) there are few
431 studies on the evolution of lutein esters in stored wheat flours. Therefore, there is little
432 knowledge about the carotenoid metabolism (anabolic and/or catabolic) during the
433 storage of cereal flours, which has important implications for the retention and stability
434 of pigments in these staple foods. In the present study, the role of esterification has been
435 confirmed as a mechanism that provides extended stability to xanthophylls, whose
436 formation is induced by the conditions developed during storage, regardless of the
437 genotype of the cereal. Nevertheless, the advantage of tritordeum characterized by a
438 high pattern of *in vivo* esterification, and how this feature mediates the stability of its
439 carotenoid content *ex vivo* has been demonstrated. In either case, the results obtained in
440 this study indicate the need for carrying out experiments using longer storage times that
441 allow us to conduct a detailed study of the effects on the pigments and to determine the
442 kinetic parameters that characterize the degradation process, thus enabling the
443 estimation of the useful life of the derived products.

444

445 Acknowledgements

446 We are grateful to Dr. Sergio G. Atienza for providing the plant material. This
447 work was supported by funding from the Ministerio de Ciencia e Innovación (Spanish
448 Government, Project AGL2010-14850/ALI) and the Consejería de Economía,
449 Innovación, Ciencia y Empleo (Junta de Andalucía, Project P08-AGR-03477). EMO
450 was the recipient of a JAE-Predocctoral grant (CSIC) cofinanced by the ESF. Authors
451 are members of the IBERCAROT Network, funded by CYTED (ref. 112RT0445).

452

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- 571

572 **FIGURE CAPTIONS**

573

574 **Figure 1.** HPLC chromatograms obtained for carotenoid extracts prepared from durum
575 wheat (Don Pedro) and tritordeum (HT621) whole-grain flours submitted to short
576 storage (90 days) at four different temperatures (4, 20, 37 and 50 °C). Peak identities
577 are: 1, (all-*E*)-zeaxanthin; 2, (all-*E*)-lutein; 3, (9*Z*)- and (13*Z*)- isomers of lutein; 4,
578 lutein 3'-*O*-linoleate; 5, lutein 3-*O*-linoleate; 6, lutein 3'-*O*-palmitate; 7, lutein 3-*O*-
579 palmitate; 8, (all-*E*)- α -carotene; 9, (all-*E*)- β -carotene; 10, lutein dilinoleate; 11, lutein
580 3'-*O*-linoleate-3-*O*-palmitate and lutein 3'-*O*-palmitate-3-*O*-linoleate; 12, lutein
581 dipalmitate.

582

583 **Figure 2.** Changes in the total carotenoid content ($\mu\text{g/g}$ dry weight) in durum wheat
584 varieties (Don Pedro, Simeto and Claudio) and advanced tritordeum lines (HT630,
585 HT621 and HT609) during a short storage (90 days) of whole-grain flours at 4, 20, 37
586 and 50 °C. The values shown are the mean and standard error ($n=4$ for the starting
587 samples $t=0$; $n=3$ for the rest of samples). Different letters within the same line
588 (temperature effect) indicate significant differences ($p<0.05$) determined by the Duncan
589 test.

590

591 **Figure 3.** Effect of temperature (4, 20, 37 and 50 °C) on the degree of esterification of
592 lutein during a short storage (90 days) of tritordeum whole-grain flours (lines HT630,
593 HT621 and HT609). Data represented the relative contribution (%) of each fraction
594 (free, monoesterified and diesterified) in relation to temperature and storage time. The
595 values shown are the mean and standard error ($n=4$ for the starting samples $t=0$; $n=3$ for
596 the rest of samples).

Table 1. Changes in the carotenoid composition ($\mu\text{g/g}$ dry weight)¹ during short storage period (90 days) of durum wheat whole-grain flour (Don Pedro, Simeto and Claudio varieties).

Carotenoid	Temp (°C)	Don Pedro				Simeto				Claudio			
						Time (days)							
		0	30	60	90	0	30	60	90	0	30	60	90
(all-E)-Lutein	4	1.00 ± 0.05 ^a	0.82 ± 0.02 ^b	0.65 ± 0.04 ^{bc}	0.51 ± 0.01 ^c	0.78 ± 0.05 ^a	0.72 ± 0.01 ^a	0.50 ± 0.01 ^b	0.43 ± 0.03 ^b	0.89 ± 0.02 ^a	0.71 ± 0.01 ^b	0.52 ± 0.02 ^c	0.44 ± 0.01 ^d
	20		0.45 ± 0.09 ^b	0.47 ± 0.01 ^b	0.43 ± 0.03 ^b		0.37 ± 0.03 ^b	0.34 ± 0.03 ^b	0.34 ± 0.01 ^b		0.67 ± 0.08 ^b	0.45 ± 0.02 ^c	0.45 ± 0.01 ^c
	37		0.45 ± 0.04 ^b	0.30 ± 0.02 ^{bc}	0.22 ± 0.02 ^c		0.33 ± 0.01 ^b	0.21 ± 0.00 ^{bc}	0.16 ± 0.01 ^c		0.45 ± 0.02 ^b	0.26 ± 0.01 ^c	0.18 ± 0.01 ^d
	50		0.27 ± 0.01 ^b	0.10 ± 0.01 ^c	0.07 ± 0.00 ^c		0.15 ± 0.01 ^b	0.06 ± 0.00 ^b	0.02 ± 0.00 ^b		0.23 ± 0.01 ^b	0.10 ± 0.00 ^c	0.03 ± 0.00 ^c
(9Z)- and (13Z)-Lutein	4	0.20 ± 0.01 ^a	0.17 ± 0.00 ^a	0.13 ± 0.01 ^b	0.11 ± 0.00 ^b	0.14 ± 0.01 ^a	0.14 ± 0.01 ^a	0.09 ± 0.0 ^b	0.08 ± 0.00 ^b	0.17 ± 0.00 ^a	0.15 ± 0.00 ^b	0.10 ± 0.00 ^c	0.09 ± 0.00 ^d
	20		0.10 ± 0.02 ^b	0.12 ± 0.00 ^b	0.11 ± 0.01 ^b		0.07 ± 0.01 ^b	0.07 ± 0.01 ^b	0.07 ± 0.00 ^b		0.14 ± 0.02 ^a	0.10 ± 0.00 ^b	0.10 ± 0.00 ^b
	37		0.15 ± 0.02 ^b	0.10 ± 0.01 ^c	0.08 ± 0.01 ^c		0.10 ± 0.00 ^b	0.06 ± 0.00 ^c	0.05 ± 0.00 ^c		0.14 ± 0.00 ^b	0.09 ± 0.00 ^c	0.07 ± 0.00 ^d
	50		0.13 ± 0.00 ^b	0.04 ± 0.00 ^c	0.03 ± 0.00 ^c		0.06 ± 0.00 ^b	0.02 ± 0.00 ^c	0.01 ± 0.00 ^c		0.10 ± 0.01 ^b	0.04 ± 0.00 ^c	0.01 ± 0.00 ^d
(all-E)-Zeaxanthin	4	0.20 ± 0.01 ^a	0.18 ± 0.00 ^a	0.13 ± 0.01 ^b	0.10 ± 0.00 ^c	0.14 ± 0.01 ^a	0.14 ± 0.00 ^a	0.08 ± 0.00 ^b	0.07 ± 0.00 ^b	0.17 ± 0.00 ^a	0.14 ± 0.00 ^b	0.09 ± 0.00 ^c	0.08 ± 0.00 ^d
	20		0.08 ± 0.02 ^b	0.08 ± 0.00 ^b	0.07 ± 0.01 ^b		0.06 ± 0.00 ^b	0.05 ± 0.01 ^b	0.05 ± 0.00 ^b		0.12 ± 0.01 ^b	0.08 ± 0.01 ^c	0.08 ± 0.00 ^c
	37		0.08 ± 0.01 ^b	0.05 ± 0.01 ^c	0.03 ± 0.01 ^c		0.06 ± 0.00 ^b	0.03 ± 0.00 ^b	0.02 ± 0.00 ^b		0.07 ± 0.00 ^b	0.04 ± 0.00 ^c	0.03 ± 0.00 ^d
	50		0.05 ± 0.00 ^b	0.02 ± 0.00 ^c	0.01 ± 0.00 ^c		0.02 ± 0.00 ^b	0.01 ± 0.00 ^b	0.00 ± 0.00 ^b		0.04 ± 0.00 ^b	0.01 ± 0.00 ^c	0.00 ± 0.00 ^c
(all-E)-α-Carotene	4	0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^c	0.01 ± 0.00 ^d	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.00 ± 0.00 ^b	0.02 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^c	0.01 ± 0.00 ^d
	20		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		0.02 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b
	37		0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^c		0.00 ± 0.00 ^b	0.01 ± 0.00 ^{ab}	0.00 ± 0.00 ^b		0.02 ± 0.00 ^b	0.01 ± 0.00 ^c	0.01 ± 0.00 ^d
	50		0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.00 ± 0.00 ^c		0.01 ± 0.00 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^d		0.01 ± 0.00 ^b	0.01 ± 0.00 ^c	0.00 ± 0.00 ^d
(all-E)-β-Carotene	4	0.03 ± 0.00 ^a	0.04 ± 0.00 ^b	0.03 ± 0.00 ^a	0.02 ± 0.00 ^c	0.02 ± 0.00 ^a	0.03 ± 0.00 ^b	0.02 ± 0.00 ^a	0.01 ± 0.00 ^c	0.03 ± 0.00 ^a	0.03 ± 0.00 ^b	0.02 ± 0.00 ^c	0.01 ± 0.00 ^d
	20		0.02 ± 0.00 ^{bc}	0.02 ± 0.00 ^b	0.01 ± 0.00 ^c		0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b		0.03 ± 0.00 ^a	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b
	37		0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.01 ± 0.00 ^c		0.01 ± 0.00 ^b	0.01 ± 0.00 ^{bc}	0.01 ± 0.00 ^c		0.02 ± 0.00 ^b	0.01 ± 0.00 ^c	0.01 ± 0.00 ^d
	50		0.02 ± 0.00 ^b	0.01 ± 0.00 ^c	0.00 ± 0.00 ^d		0.01 ± 0.00 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c		0.01 ± 0.00 ^b	0.01 ± 0.00 ^c	0.00 ± 0.00 ^d

¹Data are the mean ± standard error (n=4 for the starting sample t= 0 days; n=3 for the rest of samples). Different letters within the same line, for each pigment and cereal variety, indicate significant differences (p<0.05) determined by the Duncan test.

Table 2. Changes in the carotenoid composition ($\mu\text{g/g}$ dry weight)¹ during short storage period (90 days) of tritordeum whole-grain flour (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- <i>E</i>)-Lutein	4	7.98 ± 0.09 ^a	6.04 ± 0.22 ^b	4.33 ± 0.06 ^c	4.03 ± 0.07 ^c	7.60 ± 0.22 ^a	6.10 ± 0.56 ^b	4.09 ± 0.06 ^c	3.47 ± 0.18 ^c	7.00 ± 0.20 ^a	5.29 ± 0.07 ^b	3.91 ± 0.18 ^c	3.52 ± 0.06 ^c
	20		4.78 ± 0.03 ^b	3.51 ± 0.05 ^c	3.57 ± 0.19 ^c		4.60 ± 0.01 ^b	3.23 ± 0.00 ^c	3.13 ± 0.01 ^c		4.18 ± 0.12 ^b	3.40 ± 0.04 ^c	3.12 ± 0.13 ^c
	37		3.67 ± 0.01 ^b	2.29 ± 0.01 ^c	1.86 ± 0.08 ^d		3.67 ± 0.11 ^b	2.29 ± 0.05 ^c	1.98 ± 0.00 ^c		3.37 ± 0.06 ^b	2.12 ± 0.08 ^c	1.87 ± 0.08 ^c
	50		1.93 ± 0.08 ^b	0.87 ± 0.02 ^c	0.45 ± 0.01 ^d		2.21 ± 0.03 ^b	0.87 ± 0.00 ^c	0.82 ± 0.04 ^c		2.53 ± 0.35 ^b	1.08 ± 0.03 ^c	0.63 ± 0.08 ^c
(9 <i>Z</i>)- and (13 <i>Z</i>)-Lutein	4	1.03 ± 0.01 ^a	0.84 ± 0.03 ^b	0.60 ± 0.01 ^c	0.53 ± 0.00 ^d	0.97 ± 0.02 ^a	0.84 ± 0.07 ^b	0.54 ± 0.00 ^c	0.47 ± 0.03 ^c	0.89 ± 0.02 ^a	0.73 ± 0.01 ^b	0.53 ± 0.01 ^c	0.45 ± 0.00 ^d
	20		0.68 ± 0.01 ^b	0.56 ± 0.00 ^c	0.60 ± 0.00 ^d		0.66 ± 0.01 ^b	0.53 ± 0.03 ^c	0.53 ± 0.00 ^c		0.55 ± 0.02 ^b	0.51 ± 0.00 ^b	0.52 ± 0.00 ^b
	37		0.90 ± 0.01 ^b	0.67 ± 0.00 ^c	0.58 ± 0.02 ^d		0.97 ± 0.01 ^a	0.71 ± 0.00 ^b	0.62 ± 0.01 ^c		0.83 ± 0.03 ^a	0.63 ± 0.04 ^b	0.59 ± 0.02 ^b
	50		0.71 ± 0.05 ^b	0.33 ± 0.01 ^c	0.17 ± 0.00 ^d		0.84 ± 0.02 ^b	0.35 ± 0.01 ^c	0.34 ± 0.01 ^c		0.90 ± 0.09 ^a	0.46 ± 0.00 ^b	0.25 ± 0.04 ^c
Lutein monolinoleate	4	1.02 ± 0.01 ^a	0.85 ± 0.03 ^b	0.70 ± 0.01 ^c	0.62 ± 0.01 ^d	0.44 ± 0.01 ^a	0.39 ± 0.04 ^a	0.31 ± 0.00 ^b	0.26 ± 0.01 ^b	0.54 ± 0.01 ^a	0.45 ± 0.01 ^b	0.40 ± 0.02 ^c	0.31 ± 0.00 ^d
	20		0.73 ± 0.00 ^b	0.73 ± 0.01 ^b	0.78 ± 0.04 ^b		0.35 ± 0.00 ^b	0.37 ± 0.00 ^c	0.75 ± 0.05 ^c		0.40 ± 0.01 ^b	0.47 ± 0.01 ^c	0.46 ± 0.01 ^c
	37		0.95 ± 0.03 ^a	1.00 ± 0.05 ^a	0.93 ± 0.00 ^a		0.56 ± 0.06 ^a	0.56 ± 0.00 ^a	0.73 ± 0.07 ^b		0.63 ± 0.00 ^b	0.67 ± 0.03 ^b	0.75 ± 0.00 ^c
	50		0.95 ± 0.05 ^a	0.69 ± 0.04 ^b	0.50 ± 0.00 ^c		0.81 ± 0.07 ^b	0.65 ± 0.05 ^c	0.51 ± 0.01 ^a		1.02 ± 0.08 ^b	0.62 ± 0.02 ^a	0.51 ± 0.01 ^a
Lutein 3'- <i>O</i> -linoleate	4	0.18 ± 0.00 ^a	0.16 ± 0.01 ^b	0.12 ± 0.00 ^c	0.10 ± 0.00 ^d	0.08 ± 0.00 ^a	0.09 ± 0.01 ^a	0.07 ± 0.00 ^b	0.04 ± 0.00 ^c	0.10 ± 0.00 ^a	0.10 ± 0.00 ^a	0.07 ± 0.00 ^b	0.04 ± 0.00 ^c
	20		0.15 ± 0.00 ^b	0.14 ± 0.01 ^c	0.16 ± 0.01 ^b		0.09 ± 0.00 ^a	0.08 ± 0.00 ^a	0.15 ± 0.01 ^b		0.09 ± 0.00 ^a	0.10 ± 0.00 ^a	0.10 ± 0.00 ^a
	37		0.28 ± 0.02 ^b	0.28 ± 0.03 ^b	0.27 ± 0.01 ^b		0.18 ± 0.02 ^b	0.16 ± 0.00 ^b	0.24 ± 0.03 ^c		0.20 ± 0.01 ^b	0.18 ± 0.01 ^b	0.23 ± 0.01 ^c
	50		0.34 ± 0.02 ^b	0.21 ± 0.02 ^a	0.18 ± 0.01 ^a		0.34 ± 0.03 ^b	0.23 ± 0.03 ^c	0.17 ± 0.00 ^d		0.43 ± 0.05 ^b	0.19 ± 0.01 ^c	0.17 ± 0.00 ^c
Lutein 3- <i>O</i> -linoleate	4	0.84 ± 0.01 ^a	0.69 ± 0.02 ^b	0.59 ± 0.01 ^c	0.52 ± 0.01 ^d	0.36 ± 0.01 ^a	0.30 ± 0.03 ^b	0.25 ± 0.00 ^c	0.21 ± 0.01 ^c	0.44 ± 0.01 ^a	0.35 ± 0.00 ^b	0.32 ± 0.01 ^b	0.27 ± 0.00 ^c
	20		0.57 ± 0.00 ^b	0.59 ± 0.01 ^b	0.62 ± 0.03 ^b		0.27 ± 0.00 ^b	0.29 ± 0.00 ^b	0.59 ± 0.04 ^c	^a	0.31 ± 0.01 ^b	0.37 ± 0.00 ^c	0.36 ± 0.01 ^c
	37		0.67 ± 0.01 ^b	0.72 ± 0.02 ^c	0.66 ± 0.01 ^b		0.38 ± 0.03 ^a	0.40 ± 0.00 ^a	0.49 ± 0.04 ^b	^{ab}	0.43 ± 0.00 ^a	0.48 ± 0.02 ^{bc}	0.52 ± 0.01 ^c
	50		0.61 ± 0.03 ^b	0.48 ± 0.02 ^c	0.33 ± 0.01 ^d		0.47 ± 0.03 ^b	0.42 ± 0.02 ^b	0.33 ± 0.01 ^a	^a	0.58 ± 0.03 ^b	0.43 ± 0.01 ^a	0.33 ± 0.01 ^c
Lutein monopalmitate	4	1.56 ± 0.02 ^a	1.40 ± 0.04 ^b	1.12 ± 0.02 ^c	0.91 ± 0.01 ^d	1.01 ± 0.03 ^a	0.98 ± 0.09 ^a	0.71 ± 0.00 ^b	0.53 ± 0.02 ^c	1.23 ± 0.03 ^a	1.10 ± 0.01 ^b	0.89 ± 0.03 ^c	0.71 ± 0.02 ^d
	20		1.15 ± 0.00 ^b	1.03 ± 0.02 ^c	1.00 ± 0.04 ^c		0.78 ± 0.00 ^b	0.66 ± 0.01 ^a	0.96 ± 0.06 ^c		0.89 ± 0.03 ^b	0.88 ± 0.01 ^{bc}	0.78 ± 0.02 ^c
	37		1.23 ± 0.02 ^b	1.09 ± 0.01 ^c	0.93 ± 0.04 ^d		0.91 ± 0.05 ^a	0.76 ± 0.00 ^b	0.77 ± 0.03 ^b		1.04 ± 0.02 ^b	0.90 ± 0.02 ^c	0.87 ± 0.04 ^c

	50		1.00 ± 0.09 ^b	0.68 ± 0.00 ^c	0.43 ± 0.01 ^d		0.93 ± 0.03 ^a	0.60 ± 0.03 ^b	0.51 ± 0.02 ^b		1.22 ± 0.10 ^a	0.73 ± 0.01 ^b	0.52 ± 0.04 ^c
Lutein 3'-<i>O</i>-palmitate	4	0.48 ± 0.00 ^a	0.45 ± 0.01 ^b	0.33 ± 0.00 ^c	0.26 ± 0.01 ^d	0.31 ± 0.01 ^a	0.32 ± 0.03 ^a	0.21 ± 0.00 ^b	0.15 ± 0.01 ^c	0.38 ± 0.01 ^a	0.34 ± 0.00 ^b	0.25 ± 0.01 ^c	0.20 ± 0.01 ^d
	20		0.38 ± 0.00 ^b	0.30 ± 0.01 ^c	0.30 ± 0.01 ^c	^a	0.27 ± 0.00 ^b	0.19 ± 0.00 ^{ab}	0.29 ± 0.02 ^c		0.30 ± 0.01 ^b	0.25 ± 0.00 ^c	0.23 ± 0.00 ^c
	37		0.45 ± 0.02 ^a	0.37 ± 0.02 ^b	0.33 ± 0.00 ^b	^{ab}	0.35 ± 0.02 ^b	0.26 ± 0.00 ^c	0.29 ± 0.02 ^{ac}		0.38 ± 0.01 ^a	0.29 ± 0.01 ^b	0.31 ± 0.01 ^b
	50		0.41 ± 0.04 ^b	0.26 ± 0.01 ^c	0.19 ± 0.00 ^d	^a	0.39 ± 0.02 ^b	0.25 ± 0.02 ^c	0.21 ± 0.01 ^c		0.51 ± 0.06 ^b	0.26 ± 0.01 ^c	0.21 ± 0.01 ^c
Lutein 3-<i>O</i>-palmitate	4	1.07 ± 0.01 ^a	0.95 ± 0.03 ^b	0.79 ± 0.01 ^c	0.65 ± 0.00 ^d	0.69 ± 0.02 ^a	0.66 ± 0.06 ^a	0.50 ± 0.00 ^b	0.38 ± 0.02 ^c	0.86 ± 0.02 ^a	0.76 ± 0.00 ^b	0.64 ± 0.02 ^c	0.51 ± 0.01 ^d
	20		0.76 ± 0.01 ^b	0.73 ± 0.01 ^{bc}	0.70 ± 0.03 ^c		0.51 ± 0.00 ^b	0.47 ± 0.01 ^b	0.67 ± 0.04 ^a		0.60 ± 0.02 ^b	0.63 ± 0.01 ^b	0.55 ± 0.02 ^b
	37		0.77 ± 0.00 ^b	0.72 ± 0.01 ^b	0.59 ± 0.04 ^c		0.56 ± 0.03 ^b	0.50 ± 0.00 ^{bc}	0.48 ± 0.01 ^c		0.66 ± 0.01 ^b	0.61 ± 0.01 ^{bc}	0.55 ± 0.03 ^c
	50		0.59 ± 0.05 ^b	0.41 ± 0.00 ^c	0.24 ± 0.01 ^d		0.53 ± 0.01 ^b	0.36 ± 0.01 ^c	0.30 ± 0.01 ^c		0.71 ± 0.05 ^b	0.47 ± 0.00 ^c	0.31 ± 0.03 ^d
Lutein dilinoleate	4	0.05 ± 0.00 ^a	0.05 ± 0.00 ^b	0.04 ± 0.00 ^c	0.03 ± 0.00 ^d	0.01 ± 0.00 ^a	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^c	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b
	20		0.03 ± 0.00 ^b	0.05 ± 0.00 ^a	0.06 ± 0.00 ^c	^{ab}	0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^c		0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.02 ± 0.00 ^c
	37		0.07 ± 0.00 ^a	0.11 ± 0.01 ^b	0.16 ± 0.02 ^c	^a	0.02 ± 0.00 ^a	0.03 ± 0.00 ^a	0.07 ± 0.02 ^b		0.02 ± 0.00 ^a	0.04 ± 0.01 ^b	0.07 ± 0.01 ^c
	50		0.15 ± 0.00 ^b	0.14 ± 0.02 ^b	0.17 ± 0.01 ^d	^a	0.07 ± 0.02 ^{bc}	0.13 ± 0.04 ^c	0.07 ± 0.00 ^{ab}		0.09 ± 0.00 ^{bc}	0.06 ± 0.01 ^b	0.09 ± 0.02 ^c
Lutein dipalmitate	4	0.20 ± 0.00 ^a	0.17 ± 0.00 ^b	0.14 ± 0.00 ^c	0.12 ± 0.00 ^d	0.07 ± 0.00 ^a	0.06 ± 0.01 ^a	0.05 ± 0.00 ^b	0.04 ± 0.00 ^b	0.12 ± 0.00 ^a	0.10 ± 0.00 ^b	0.08 ± 0.00 ^c	0.07 ± 0.00 ^d
	20		0.13 ± 0.00 ^b	0.15 ± 0.00 ^b	0.13 ± 0.00 ^c		0.04 ± 0.00 ^b	0.05 ± 0.00 ^b	0.04 ± 0.00 ^b		0.07 ± 0.00 ^b	0.08 ± 0.00 ^c	0.07 ± 0.00 ^{bc}
	37		0.14 ± 0.00 ^b	0.16 ± 0.00 ^c	0.15 ± 0.00 ^d		0.06 ± 0.00 ^b	0.06 ± 0.00 ^{ab}	0.07 ± 0.00 ^{ab}		0.08 ± 0.00 ^b	0.10 ± 0.00 ^c	0.10 ± 0.00 ^c
	50		0.15 ± 0.01 ^b	0.12 ± 0.00 ^c	0.09 ± 0.00 ^d		0.07 ± 0.00 ^{ab}	0.07 ± 0.01 ^{ab}	0.05 ± 0.00 ^b		0.12 ± 0.00 ^a	0.10 ± 0.00 ^b	0.08 ± 0.00 ^c
Lutein 3'-<i>O</i>-linoleate-3-<i>O</i>-palmitate and Lutein 3'-<i>O</i>-palmitate-3-<i>O</i>-linoleate	4	0.21 ± 0.00 ^a	0.18 ± 0.00 ^b	0.15 ± 0.00 ^c	0.14 ± 0.01 ^d	0.05 ± 0.00 ^a	0.04 ± 0.00 ^a	0.03 ± 0.00 ^b	0.03 ± 0.00 ^b	0.08 ± 0.00 ^a	0.06 ± 0.00 ^b	0.06 ± 0.00 ^{bc}	0.05 ± 0.00 ^c
	20		0.15 ± 0.01 ^b	0.17 ± 0.00 ^{bc}	0.17 ± 0.01 ^c		0.03 ± 0.00 ^b	0.04 ± 0.00 ^c	0.04 ± 0.00 ^c		0.05 ± 0.00 ^b	0.07 ± 0.00 ^c	0.07 ± 0.00 ^c
	37		0.21 ± 0.00 ^a	0.25 ± 0.01 ^b	0.28 ± 0.00 ^c		0.06 ± 0.01 ^{ab}	0.08 ± 0.00 ^b	0.13 ± 0.02 ^c		0.09 ± 0.00 ^a	0.12 ± 0.01 ^b	0.15 ± 0.00 ^c
	50		0.25 ± 0.01 ^b	0.23 ± 0.02 ^{ab}	0.21 ± 0.01 ^a		0.12 ± 0.02 ^b	0.17 ± 0.04 ^b	0.12 ± 0.00 ^b		0.18 ± 0.00 ^b	0.16 ± 0.01 ^b	0.17 ± 0.01 ^b
(all-<i>E</i>)-β-Carotene	4	0.08 ± 0.00 ^a	0.09 ± 0.00 ^b	0.09 ± 0.00 ^b	0.05 ± 0.00 ^c	0.08 ± 0.00 ^a	0.10 ± 0.01 ^a	0.07 ± 0.00 ^b	0.04 ± 0.00 ^c	0.10 ± 0.00 ^a	0.11 ± 0.00 ^a	0.08 ± 0.00 ^b	0.05 ± 0.00 ^c
	20		0.08 ± 0.00 ^a	0.07 ± 0.00 ^b	0.05 ± 0.00 ^c		0.08 ± 0.00 ^a	0.05 ± 0.00 ^b	0.04 ± 0.00 ^c		0.09 ± 0.00 ^a	0.06 ± 0.00 ^b	0.05 ± 0.01 ^b
	37		0.07 ± 0.00 ^b	0.06 ± 0.00 ^c	0.03 ± 0.01 ^d		0.07 ± 0.00 ^b	0.04 ± 0.00 ^c	0.03 ± 0.00 ^d		0.08 ± 0.00 ^b	0.05 ± 0.00 ^c	0.04 ± 0.00 ^c
	50		0.05 ± 0.00 ^b	0.02 ± 0.01 ^c	0.01 ± 0.00 ^d		0.04 ± 0.00 ^b	0.02 ± 0.00 ^c	0.01 ± 0.00 ^c		0.07 ± 0.01 ^b	0.02 ± 0.00 ^c	0.01 ± 0.00 ^c

¹Data are the mean ± standard error (n=4 for the starting sample t=0 days; n=3 for the rest of samples). Different letters within the same line, for each pigment and cereal variety, indicate significant differences

($p < 0.05$) determined by the Duncan test.

Table 3. Reaction rate constant (k ; days⁻¹) and half-life time ($t_{1/2}$; days) for the total carotenoid content in durum wheat (Don Pedro, Simeto and Claudio varieties) and tritordeum (HT630, HT621 and HT609 lines) whole-grain flours during a short storage period (90 days) at 4, 20, 37 and 50 °C following a first-order kinetic model ($C=C_0\text{-exp}(kt)$).

Temp (°C)	Durum wheat									Tritordeum								
	Don Pedro			Simeto			Claudio			HT630			HT621			HT609		
	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)	$k \times 10^{-3}$ (days ⁻¹)	r^2	$t_{1/2}$ (days)
4	7	0.99	93	7	0.93	95	8	0.99	87	7	0.97	95	9	0.98	96	7	0.98	97
20	8	0.64	85	8	0.67	83	8	0.90	85	7	0.80	96	8	0.82	79	7	0.84	92
37	15	0.96	45	17	0.95	41	16	0.99	42	10	0.92	70	10	0.89	72	9	0.88	77
50	28	0.92	24	39	0.99	18	35	0.99	20	19	0.98	35	16	0.93	43	17	0.99	41

Durum wheat (Don Pedro)

Tritordeum (HT621)

Durum wheat (Don Pedro)

Tritordeum (HT621)

Highlights

Storage induces carotenoid losses for durum wheat and tritordeum whole-grain flours

Carotenoid content followed a temperature dependent first-order kinetic model

The occurrence of lutein esters explains the slower degradation in tritordeum flours

A storage temperature of 25-35 °C is proposed as optimal for whole-grain flours