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Effect of illumination and chlorophylls on stability of tomato carotenoids

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Abstract

Lipidic extract from tomato peels, or tomato peels plus stalks, dissolved in ethanol were submitted to illumination. Lycopene, β-carotene, phytoene and phytofluene isomerisation and degradation, during storage at room temperature for 28 days, were studied. Degradation of chlorophylls a and b were analysed in lipidic extracts from stalks. Total lycopene and all-*E*-lycopene degradation was found to fit to a first-order model. The degradation rate constant was lower in extracts from peels −0.0137 (all-*E*-lycopene) and −0.0737 (total lycopene), than in those from peel plus stalk −0.0415 (all-*E*-lycopene) and −0.0854 (total lycopene). *Z*-lycopene isomers showed an inconsistence change during storage, in all analysed samples. Concentration of β-carotene from extracts of tomato peels plus stalks decreased slightly during storage. Phytoene and phytofluene degradation were not significantly affected by both storage conditions and chlorophylls. The obtained results showed that some compounds from stalks, such as chlorophylls, could favour lycopene and β-carotene degradation during storage under illumination.

Keywords: Tomato peels extracts; Tomato peels plus stalks extracts; Carotenoids degradation; Illumination

1. Introduction

Carotenoids had received great attention in recent years, because of their beneficial effect in some diseases. With an increasing understanding of the health benefits of carotenoids, how to preserve them during food processing and their storage, carotenoids have received much attention. Tomato is rich in some carotenoids, such as lycopene and β-carotene, that may to have beneficial health effects.

Carotenoids mostly exist in nature as the all-*E*-isomer form. Heat, light and oxygen among others, are factors that have an effect on carotenoid isomerisation and autooxidation. Isomerisation and degradation reduce the concentration of the all-*E*-isomer form.

The reduction of total carotenoids concentrations when samples like tomato powder (Anguelova & Warthesen, 2000), dehydrated tomato (Shi, Maguer, Kakuda, Liptay, & Niekamp, 1999), tomato puree (Shi, Maguer, Bryan, & Kakuda, 2003; Tamburini, Sandei, Aldini, Sio, & Leoni, 1999), tomato juice (Lin & Chen, 2005), tomato peel (Kaur, Sogi, & Wani, 2006), or lycopene (Lee & Chen, 2002) are stored in light or submitted to different temperatures for different times have been reported.

The studies of total lycopene degradation that have been performed fitted to a first-order, using model systems submitted to thermal treatments (Henry, Catignani, & Schwartz, 1998), to illumination and heating (Lee & Chen, 2002) and to a pseudo-first-order during heating of tomato pulp (Sharma & Maguer, 1996).

Carotenoids are consumed primarily as the all-*E*-isomer form but the majority of lycopene in blood and tissues exists as a variety of *E*- and *Z*-isomers. Frohlich, Kaurmann, Bisch, and Bohm (2006) and Clinton et al. (1996) observed that the ratio of lycopene *E Z*- geometrical isomers, in biological fluids differs from that in fresh tomato. Specific isomers may be involved in different biological

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reactions, and patterns of isomers may provide insight into the risk of pathogenesis of disease processes. Unlu, Bohn, Francis, Clinton, and Schwart (2007) have suggested that *Z*-isomers of lycopene may be better absorbed and thus more bioavailable than the all-*E*-isomers form. The isomeric forms of dietary carotenoids may possess different biological properties (Emenhiser, Sander, & Schwartz, 1995; Nguyen & Chen, 1999).

Chlorophylls can influence degradation of some carotenoids during exposure to visible light (Tregub, Schoch, Erazo, & Scheeder, 1996). Merzlyak and Solovchenko (2002) reported that carotenoids from a solution of apple fruit alone exhibited much higher light stability, under irradiation, than in the presence of chlorophyll.

Lycopene can be obtained from by-products from the tomato industry, such as peels and stalks, obtained when tomato sauce, canned tomatoes etc., are manufactured. Extraction of carotenoids from by-products containing peels and stalks could contain chlorophylls. It would be interesting to know if chlorophylls could influence carotenoid isomerisation and degradation during storage of extracts.

The influence of illumination during storage time and the presence of chlorophylls on isomerisation and degradation of carotenoids extracted from tomato by-products, containing peels or peels plus stalks, was studied.

2. Materials and methods

2.1. Reagents

All-*E*-lycopene 90–95% purity, chlorophyll a 96% purity and chlorophyll b 95% were obtained from Sigma Chemical (St. Louis, MO), all-*E*-β-carotene 97% purity and ethanol were purchased from Fluka Chemical (Buchs, Swizerland). All HPLC-grade solvents including methanol and methyl *t*-butyl ether (MTBE) were obtained from Labscan Ltd. (Dublin, Ireland).

2.2. Sample preparation

Tomatoes were bought in a local market. Stalks were removed and then the tomatoes were peeled. Stalks and peels were kept in glass bottles, protected from light at −30 °C until utilisation.

2.3. Carotenoids extraction

Lipidic extracts from: 10 g of tomato peels, 10 g of tomato peels plus 0.5 g of tomato stalks and from 0.5 g of tomato stalks were obtained. The extractions were performed in the three cases keeping the samples in 100 ml ethanol for 10 s and immediately mixing using an Ultraturrax macerator (Janke & Kaudel) for 3 min. After 3 min of stirring, the ethanol phase was passed through Whatman No. 1 filter paper and the filtrate was kept under refrigeration. After removal of the ethanol another 100 ml of the same solvent were added and the sample was mixed again in the Ultraturrax macerator and filtered as indicated. This process was repeated 3 more times. All the ethanol fractions were mixed and evaporated by rotavapor to final concentration of 100 ml, the 100 ml were divided (2 ml) in 4 ml vials. The extraction was performed in the dark.

The vials of the ethanol extracts of peels, peel plus stalks and stalks were kept among 22 and 25 °C, illuminated by one 20 W 55 cm fluorescent tube that was placed above the vials at a distance of 1 m. The illumination intensity was 4000 lux. The control samples were kept 0 days under illumination.

Three vials of the three samples were collected at different days, ethanol was evaporated with nitrogen and dry samples were stored at −30 °C in a nitrogen atmosphere prior to analysis by HPLC.

2.4. Carotenoids and chlorophylls quantification

The RP-HPLC separation was performed on a Beckman System Gold binary delivery system (module 126) equipped with a UV–vis photodiode array detector model 168 (Beckman Instruments, Fullerton, CA). Analytical separations were carried out on a stainless steel (250 × 4.6 mm i.d.) Develosil UG C_{30} (5 μm particle size) column (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, United Kindong) packed with ODS C_{18} Sample injection was performed by means of a valve (Rheodyne, Cotati, CA) with a 20 μl peek loop.

Elution was performed following a linear mobile phase gradient using methanol (4% H_2O):MTBE from 83:17 to 33:67 over 60 min at a flow rate of 1 ml/min. The column was thermostated at 22 °C on a Shimadzu CTO-10AS (Columbia, MD) column oven. The Gold Nouveau software data system was used to evaluate peak areas.

Commercial standards and spectral data were used to assign carotenoid peaks. All-*E*- and *Z*-lycopene isomers, β-carotene, phytoene, phytofluene, chlorophyll a and chlorophyll b were quantified by means of a calibration curve following the method described by Gomez-Prieto, Caja, and Santa-Maria (2002). A DU-70 spectrophotometer (Beckman, Instruments) was routinely used to check the concentration of the working standard solutions, these concentrations were calculated using the extinction 1% (1 cm) coefficient $E^{1\%}_{1\ cm}$ (Britton, 1995).

To prepare the samples to be injected, each dry sample was dissolved in a known volume of a mixture of methanol/MTBE 25:75 (v:v) and 50 μl were filtered through a 0.45 μm filter, a volume of 20 μl was injected.

2.5. Statistical analysis

Triplicate analyses were conduced for each illumination time; each sample was injected three times and the mean value was obtained. All the data were submitted to analysis of variance.

The degradation rate constant of the total amount of lycopene (all-*E*- plus *Z*-isomers forms) and of all-*E*-lycopene were calculated on lipidic extracts of tomato peels and on those of tomato peels plus stalks using the following formula

$$k = -Ln(CA/CA_0)/t$$

where CA is the total amount of lycopene after storage; CA_0 is the initial amount of lycopene; t: storage time.

3. Results and discussion

3.1. Influence of illumination on carotenoids stability

Fig. 1 shows the HPLC chromatograms of lipidic extract obtained from the tomato peel of the control (Fig. 1a) and samples after 28 days of storage (Fig. 1b). Certain chromatographic peaks were tentatively identified by comparison with previous separation on the polymeric C_{30} column (Ferruci, Nguyen, & Sander, 2001; Re, Fraser, & Long, 2001). The spectral characteristics and Q ratios were also used for tentative identification of the *Z*-isomers of lycopene. Therefore, peak 10 was all-*E*-lycopene, peak 11 was presumably 5-*Z*-lycopene, peak 7 was presumably 13-*Z*-lycopene and peaks 6, 8 and 9 were all identified as other *Z*-isomers of lycopene.

According to our investigation, during samples storage, total and all-*E*-lycopene concentration decreased. Fig. 2 shows the first-order plot for the degradation of total and all-*E*-lycopene during storage. A linear correlation

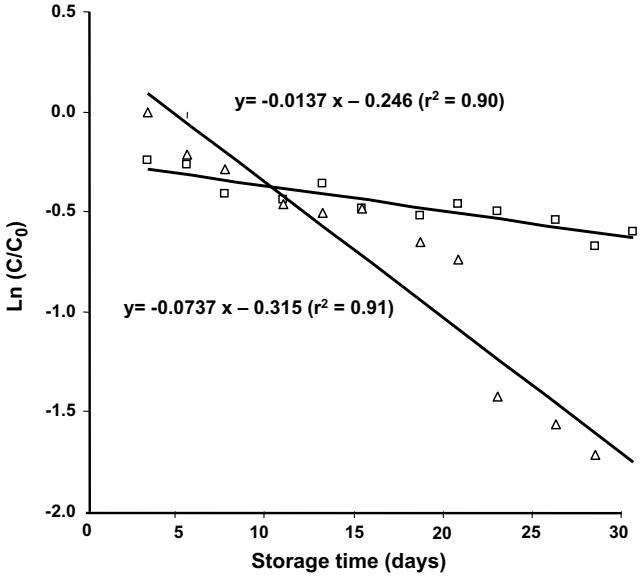

Fig. 2. First-order plot for the degradation of total (□) and all-*E*-lycopene (Δ) during illumination for 28 days of lipidic extract of tomato peels.

($r^2 = 0.90$), to total and ($r^2 = 0.91$) to all-*E*-lycopene, was found for the plot of the logarithm of the concentration of lycopene, vs. storage time.

The degradation rate constant was higher to all-*E*-lycopene (−0.0737) than to total lycopene (−0.0137). Similar order of reaction to total lycopene degradation was reported by Lee and Chen (2002) during illumination of lycopene in model systems.

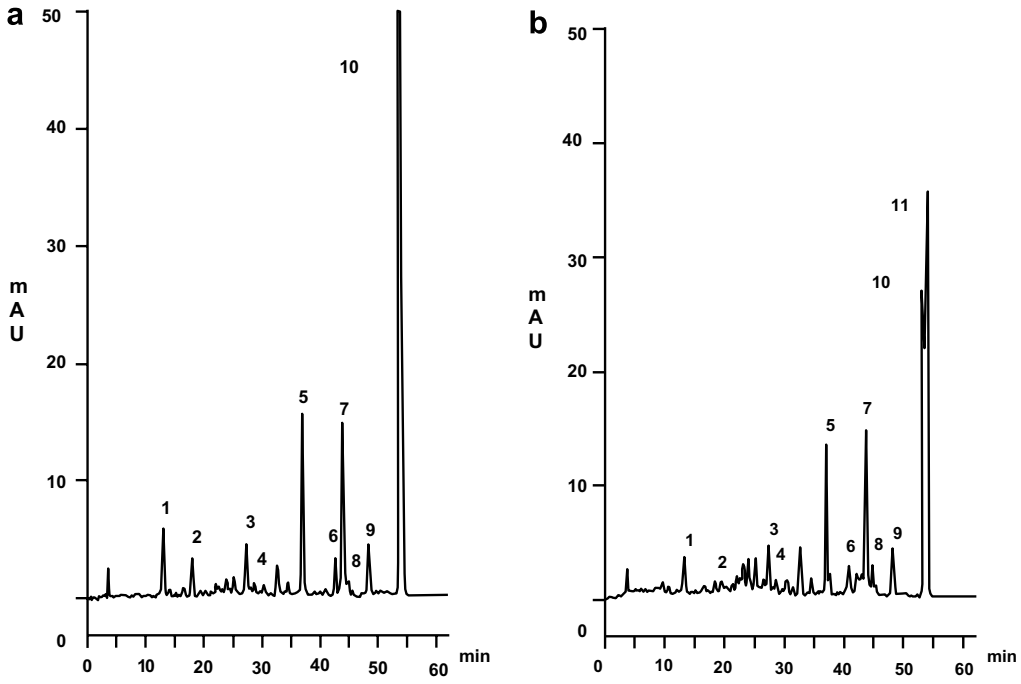

Fig. 1. HPLC chromatogram of carotenes of a lipidic extract obtained from tomato peels and kept under illumination (a) 0 or (b) 28 days. Chromatographic conditions are described in the text, chromatograms were obtained at 470 nm. Peak identification: 1-chlorophyll b, 2-chlorophyll a, 3-phytoene 4-phytofluene, 5-β-carotene, 6-*Z*-lycopene isomer 8-*Z*-lycopene isomer, tentatively identified as 13-*cis*-lycopene, 9-*Z*-lycopene isomer, 10-all-*E*-lycopene and 11-*Z*-lycopene isomer, tentatively identified as 5-*Z*-lycopene.

The concentration changes of the *Z*-isomers and total *Z*-lycopene isomers during storage are shown in Table 1. In general, the *Z*-isomers showed an inconsistence change. For instance, the level of peak 9 showed a slight increase during storage, whereas peaks 6, 7 and 8 did not suffer great variations. After 19 days of storage the peak tentatively identified as 5-*Z*-lycopene could be quantified; perhaps in the first days of storage this isomer is present, but in such low concentrations that its quantification is impeded by the relatively high concentrations of all-*E*-lycopene then present. Total *Z*-isomers showed an inconsistence change as individual isomers; no significant changes were detected between 0 and 14 days of storage, a slight increase was found between 14 and 19 days and after 19 days of storage no significant changes were detected.

The results obtained could indicate that isomerisation and degradation of all-*E*- and *Z*-lycopene isomers, during storage under illumination, could proceed simultaneously.

Lipidic extracts of tomato peels contained β-carotene, phytoene and phytofluene in significant concentrations. Concentration of the carotenoids was in the order β-carotene > phytoene > phytofluene. No significant changes in those carotenoids were observed during storage (see Figs. 3 and 4a). The β-carotene concentration was 4.78 ± 0.32 in control samples and 4.49 ± 0.82 mg/100 g after 28 days of storage. Phytoene concentration ranged from 2.04 ± 0.22 mg/100 g to 2.14 ± 0.62 from 0 to 28 days and phytofluene from 1.22 ± 0.36 to 1.37 ± 0.42 mg/100 g.

3.2. Influence of chlorophylls and illumination on carotenoids stability

Lipidic extracts from tomato stalks contained chlorophyll b, chlorophyll a and β-carotene in significant concentrations.

Concentration of chlorophylls and β-carotene decreased during the 28 days of storage under illumination. Chlorophyll b decreased from 5.44 ± 0.49 to 1.03 ± 0.34 mg/100 g from 0 to 28 days of storage, chlorophyll a from 2.71 ± 0.61 to 0.89 ± 0.41 and β-carotene from 1.97 ± 0.53 to 0.98 ± 0.22 mg/100 g. Some peaks appeared during storage, and their concentration increased with the storage time, those peaks were eluted between chlorophyll a and β-carotene peaks (see Fig. 1b). Those peaks could be degradation products of the chlorophylls and lycopene.

The concentration of total and all-*E*-lycopene on lipidic extracts from peels plus stalks decreased during storage. Fig. 5 shows the first-order plot for the degradation of total and all-*E*-lycopene during storage. A linear correlation ($r^2 = 0.98$) to total and ($r^2 = 0.93$) to all-*E*-lycopene was found for the plot of the logarithm of the concentration of lycopene vs. storage time. The degradation rate constant was higher for all-*E*-lycopene (0.0854) than for total lycopene (0.0415).

The degradation rate constants of total and all-*E*-lycopene from lipidic extracts of tomato peel plus stalks were higher than those from lipidic extracts of peel.

Table 1
Concentration (mg/100 g) of *Z*-lycopene isomers and total *Z*-lycopene isomers during storage of extracts from tomato peels and peels plus stalks stored at room temperature for 28 days

Time (days)	*Z*-lycopene isomers											
	Peak 6		Peak 7(13-*Z*-isomer)		Peak 8		Peak 9		Peak 11(5-*Z*-isomer)		Total *Z*-isomers	
	Peel	Peel plus stalks	Peel	Peel plus stalks	Peel	Peel plus stalks	Peel	Peel plus stalks	Peel	Peel plus stalks	Peel	Peel plus stalks
0	0.74^{a}	0.56^{a}	5.81^{a}	1.49^{a}	0.47^{a}	0.39^{a}	0.55^{a}	2.33^{a}	nd.	nd.	7.57^{a}	4.77^{a}
3	0.67^{a}	0.52^{a}	3.08^{b}	$1.38^{a,b}$	0.36^{b}	0.37^{a}	1.10^{b}	$2.14^{a,b}$	nd.	nd.	5.21^{b}	4.36^{b}
5	$0.80^{a,b}$	$0.49^{a,b}$	$3.62^{b,c}$	1.24^{b}	0.38^{b}	0.37^{a}	1.36^{c}	$2.01^{a,c}$	nd.	6.75^{a}	$6.17^{a,c}$	10.85^{c}
7	$0.98^{b,c}$	0.69^{c}	3.08^{b}	$1.10^{b,c}$	0.53^{a}	$0.42^{a,b}$	1.36^{c}	1.96^{c}	nd.	7.40^{b}	$5.96^{b,c}$	$11.6^{c,d}$
10	$0.96^{b,c}$	0.38^{d}	$2.98^{b,d}$	1.03^{c}	$0.50^{a,b}$	$0.36^{a,d}$	1.60^{d}	$1.68^{c,d}$	nd.	7.99^{e}	$6.05^{a,c}$	$11.4^{c,d}$
12	1.14^{c}	$0.48^{a,b}$	$3.19^{b,d}$	$1.20^{a,b}$	0.64^{c}	0.42^{b}	2.07^{e}	$1.62^{c,d}$	nd.	8.07^{c}	7.54^{a}	$11.8^{c,d}$
14	$1.00^{b,c}$	0.36^{d}	$3.69^{b,c}$	$0.92^{c,d}$	$0.61^{a,c}$	0.25^{c}	2.53^{e}	1.47^{d}	nd.	$7.84^{b,c}$	7.34^{a}	10.8^{c}
17	$0.96^{b,c}$	0.33^{d}	$3.10^{b,d}$	0.82^{e}	$0.75^{c,d}$	0.32^{d}	3.28^{f}	$1.24^{c,d}$	nd.	$6.70^{a,b}$	8.09^{d}	$9.41^{c,e}$
19	$0.89^{b,c}$	$0.48^{a,b}$	3.55^{c}	$0.88^{d,e}$	0.64^{c}	$0.33^{a,d}$	$3.61^{f,g}$	$1.27^{c,d}$	7.19^{a}	6.72^{a}	15.9^{e}	9.70^{c}
21	0.68^{a}	$0.43^{a,b}$	$3.33^{b,c}$	$0.84^{d,e}$	0.72^{d}	0.35^{d}	$3.85^{f,g}$	1.11^{h}	7.45^{b}	5.94^{d}	$16.0^{e,f}$	8.67^{f}
24	$0.68^{a,b}$	0.33^{d}	$3.16^{b,d}$	0.73^{f}	$0.65^{a,c,d}$	$0.33^{a,d}$	4.01^{g}	$0.96^{h,i}$	$7.31^{a,b}$	5.22^{d}	$15.8^{e,f}$	7.58^{g}
26	0.68^{a}	0.32^{d}	$2.92^{b,d}$	$0.74^{e,f}$	$0.74^{c,d}$	$0.26^{c,d}$	$3.73^{f,g}$	$0.84^{h,i}$	$6.95^{a,c}$	$4.43^{d,e}$	15.0^{f}	6.57^{h}
28	0.49^{d}	$0.42^{a,b}$	$2.79^{b,e}$	$0.65^{f,g}$	$0.68^{a,c,d}$	$0.29^{c,d}$	4.28^{h}	0.75^{i}	$6.99^{a,c}$	3.84^{e}	15.2^{f}	$5.96^{a,i}$

Values of means bearing different letters in the same column are significantly different ($P < 0.05$). nd: Not detected.

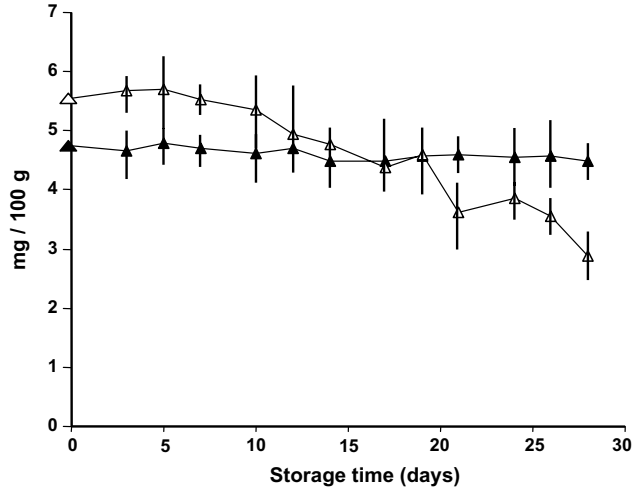

Fig. 3. Degradation of β-carotene during illumination for 28 days of lipidic extract of tomato peels (▲) and lipidic extract tomato peels plus stalks (Δ).

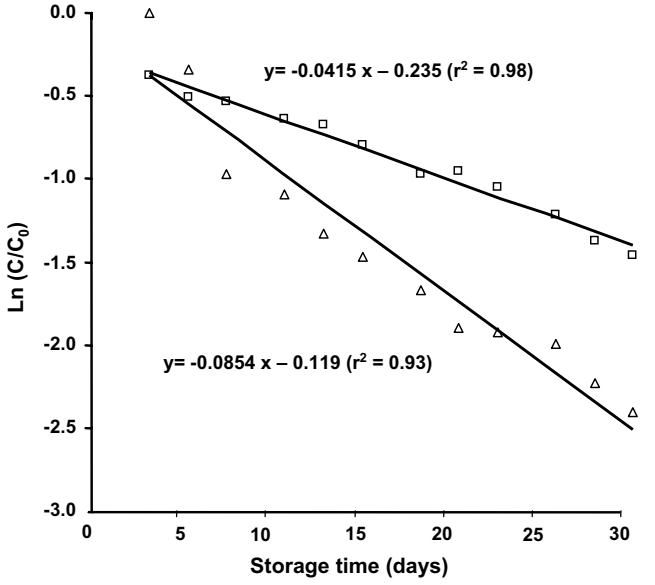

Fig. 5. First-order plot for the degradation of total (□) and all-*E*-lycopene (Δ) during illumination for 28 days of lipidic extract of tomato peels plus stalk.

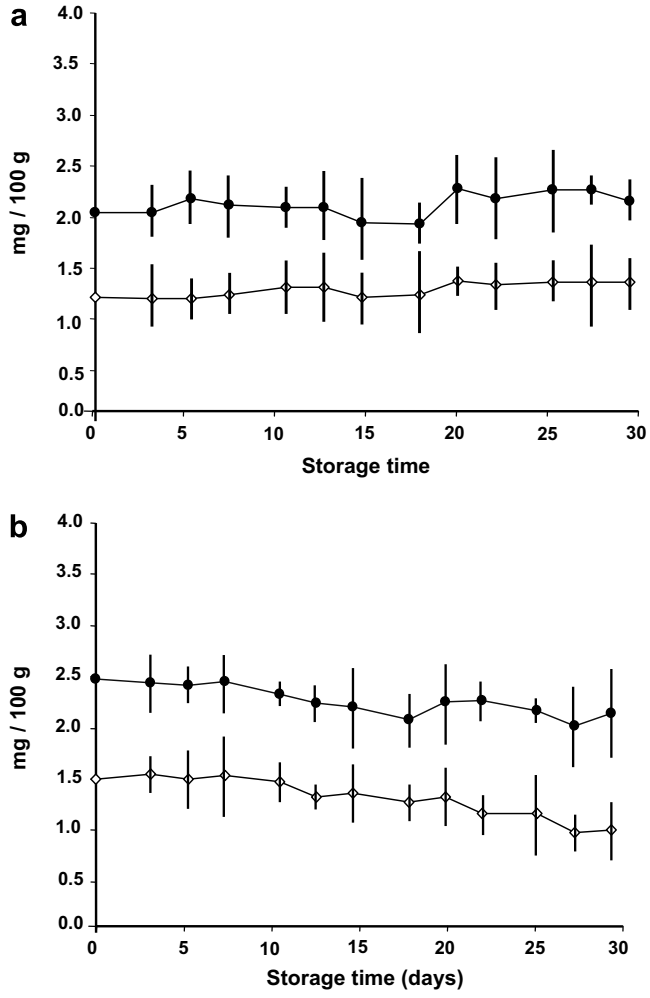

Fig. 4. Degradation of phytoene (●) and phytofluene (◇) during illumination for 28 days. Lipidic extract of tomato peels (a) and lipidic extract of tomato peels plus stalk (b).

After 28 days of storage the concentration of total lycopene was 19.7 ± 1.02 mg/100 g from the extracts of peels and 8.36 ± 0.82 mg/100 g in those of peels plus stalks; the concentration of all-*E*-lycopene was 4.46 ± 0.62 and 2.40 ± 0.42 mg/100 g in extracts of peels and peels plus stalks, respectively.

Concentration changes of the *Z*-isomers and total *Z*-lycopene isomers of the lipidic extracts of tomato peels plus stalks showed an inconsistence change during storage (see Table 1). The concentration of the peak identified tentatively as 13-*Z*-lycopene and peak 9 showed a slight decrease; peaks 6 and 8 did not suffer appreciable variation. After 5 days of storage the peak tentatively identified at 5-*Z*-lycopene could be quantified; its concentration increased slightly until 12 days of storage, after 14 days the concentration decreased slightly. Concentration of total *Z*-isomers did not change significantly between 5 and 17 days of storage and decreased slightly after 17 of storage.

It has been reported that the isomerisation of β-carotene at the central double bond was lower than in the other position (Qiu, Jiang, Wang, & Gao, 2006). This phenomenon could be applied to lycopene and a large percentage increase was thus found for peak 11 (tentatively identified at 5-*Z*-lycopene), followed by other *Z*-isomers. This behaviour was found in lipidic extracts of tomato peels and on those of peels plus stalks.

The concentration of the *Z*-lycopene isomers named as peak 6, 7, 8 and 9 was, in general, lower in the extracts of peels plus stalks than in those of peels. As was indicated, peak 11 was tentatively identified as 5-*Z*-lycopene and could be quantified in extracts containing peels plus stalks before those containing peels; however, between 19 and 28 days of storage, the concentration of this isomer was lower in extracts of peels plus stalks than in those of peels.

β-carotene concentration decreased slightly during storage (see Fig. 3). Concentration of β-carotene was 5.54 ± 0.34 mg/100 g in control samples and 2.89 ± 0.38 after 28 days of storage.

No appreciable variations in phytoene and phytofluene concentration were found during storage of extracts of tomato peels plus stalks (see Fig. 4b).

The obtained results could indicate that some stalks compounds, such as chlorophylls, could favour the isomerisation of *E*- lycopene, the degradation of both the *E*-isomer and the formed isomers and the β-carotene degradation.

The possible influence of chlorophylls on carotenoid stability has been previous described by Tregub et al. (1996) and Merzlyak and Solovchenko (2002).

Taking into account our results and the works reported in the bibliography, further studies will be performed in model systems to analyse the possible influence of the chlorophylls in the lycopene and β-carotene stability during light storage.

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