Mechanism and Kinetics of Carotenoid Degradation during the Processing of Green Table Olives

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The only carotenoids that were transformed during lactic fermentation in green table olives were those whose molecular structure made them sensitive to the acid medium. The kinetic model that describes the transformation of these xanthophylls with 5,6-epoxide groups, violaxanthin and neoxanthin, into their corresponding 5,8-furanoid derivatives, auroxanthin and neochrome, fits first-order kinetics with respect to pigment concentration. The constancy of the overall carotenoid content throughout processing demonstrated the absence of other types of oxidative reactions that degrade them to colorless products.

Keywords: *Carotenoids, carotenoid degradation, kinetics, olives*

INTRODUCTION

The carotenoids constitute one of the most important groups of pigments and are present in all families of plants and animals. As colorants, they are well-tolerated as they are naturally present in foodstuffs, they are easily metabolized, and their metabolites are beneficial for health. The most notable property of these compounds at the physiological and dietary level in humans and other animals is that some of them have provitamin A activity (Bauernfeind et al., 1971). This important characteristic has led some authors to suggest a classification on the basis of their nutritional and biological activity (antiulcer, anticancer, immunological regulators, etc.) (Olson, 1989). It has been demonstrated in mammals, including humans, that a portion of the carotenoids present in the diet are absorbed and deposited in various tissues (adipose, plasma) and cells (erythrocytes and leucocytes) (Mathews-Roth, 1975, 1978).

Although the carotenoids are fairly stable in their natural environment, on heating, or when they are extracted with oil or organic solvents, they become much more labile. In this group of pigments, there are very few structural alterations that give rise to the formation of other colored compounds. Among degradations that do give rise to such compounds, the most significant is the cis–trans isomerization provoked chiefly by heat treatment in the absence of air. In addition, the formation of 5,8-furanoids from 5,6-epoxides may occur, this being mediated by an acid medium. Such a transformation only slightly affects the color intensity.

The most generalized alteration occurring in the carotenoids, however, is oxidative destruction, which has a considerable effect on the intensity of the color of the foodstuff (Goodwin, 1980). The high temperatures and/or low moisture levels reached during the processing of vegetables may provoke losses of up to 50% in the xanthophyll concentration. These losses have a greater effect on those carotenoids with epoxide groups than on lutein (Livingston et al., 1968). Nevertheless, neither thermal treatments nor bottling time significatively alters the total content of carotenes, although they do provoke a drop in the vitamin A content of between 15 and 30% as a result of cis–trans isomerization (Sweeney and Marsh, 1987). In general, those processing systems that retain a high degree of moisture during scalding protect the carotenoids from discoloration during thermal processing (Sian and Soleha, 1991).

Different model systems in which water activity (a_w) (Chou and Breene, 1972; Haralampu and Kard, 1983) and oxygenation (Goldman et al., 1983) are controlled have been developed to study discoloration of carotenoids. In model systems under conditions of illumination, oxidation of β-carotene has been found to obey first-order kinetics (Pesek and Warthesen, 1988; Pesek et al., 1990), as have oxidation of violaxanthin and neoxanthin (Wagner and Elstner, 1989), while the photoisomerization of *all-trans*-β-carotene into the 9-cis and 13-cis isomers has been found to follow apparent zero-order kinetics (Pesek and Warthesen, 1990).

The products of degradation of xanthophylls with 5,6-epoxide groups during processing of green table olives have been identified (Mínguez-Mosquera et al., 1989). The aim of the present paper is to establish a kinetic model to describe the transformation of the 5,6-epoxide groups into 5,8-furanoids.

MATERIALS AND METHODS

Apparatus. A Model R 110 Büchi rotavapor, a Waters 600 E multisolvent delivery system, a Waters 994 programmable photodiode array detector, and a Waters 5200 printer–plotter were used.

Reagents. All reagents were of analytical grade except those used for HPLC, which were of chromatographic grade. The water used was deionized and filtered through a 0.45-μm nylon membrane.

Procedure. *Processing of Green Table Olives.* Fruits (*Olea europaea* L.) of the Manzanilla variety (*pomiformis*) harvested in the optimal maturity degree (yellowish green) were processed as table olives in fermenters of 60-kg capacity. In general terms, processing of olives is performed in a similar way to that described by Mínguez-Mosquera et al. (1989). Fruits were treated with NaOH to eliminate bitter compounds, washed with water, and finally submerged in brine, where a heterolactic fermentation was developed. However, given the fact that in industry the NaOH concentration and the duration of the treatment can experience slight fluctuations, in the present study extreme conditions have been chosen to test if these fluctuations could influence pigment degradation. The systems employed are termed "long type" and "short type" processes, referring to the time for which fruits are in contact with the NaOH solution. Four fermenters were used (two for each type of process). The specific conditions were as follows: 2.22% NaOH for 7 h 15 min, washing for 6 h, and brining in 10.6% NaCl (long type process,

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0021-8561/94/1442-1551$04.50/0

Table 1. Evolution of Carotenoid Concentration (Milligrams per Kilogram) in Olives during the Fermentation Process as a Function of pH (Process Type Long)[a,b]

	PL1[c]							PL2						
time (days)	pH	β-c	Lut	Viol	Aurx	Neox	Ncr	pH	β-c	Lut	Viol	Aurx	Neox	Ncr
0		3.38	5.43	1.44		1.41			3.38	5.43	1.44		1.41	
4	8.44	3.15	5.63	1.30		1.27		7.41	3.47	5.12	1.24		1.29	
8	6.60	3.18	5.23	1.12	0.28	1.15		6.69	3.50	5.24	1.12		1.18	
14	5.40	3.11	5.37	1.03	0.45	0.97	0.34	5.47	3.41	5.41	0.83	0.67	0.98	0.41
19	5.41	3.02	5.67	0.86	0.57	0.94	0.45	5.39	3.58	5.26	0.76	0.68	0.80	0.53
20	5.08	2.93	5.87	0.72	0.65	0.92	0.53	4.90	3.07	6.05	0.63	0.76	0.76	0.82
26	4.83	3.31	5.31	0.59	0.89	0.74	0.55	4.81	3.32	5.76	0.46	1.01	0.62	0.84
33	4.55	3.19	5.93	0.52	1.12	0.52	0.78	4.69	3.40	5.83	0.38	1.12	0.51	0.97
54	4.44	3.47	5.22	0.38	1.20	0.29	1.12	4.47	3.19	6.44	0.23	1.17	0.13	1.08
89	4.33	3.27	6.05		1.30		1.23	4.38	3.35	6.38		1.50		1.36

[a] Destoned olive basis, average of duplicate analysis. [b] Key: β-c, β-carotene; Lut, lutein; Viol, violaxanthin; Aurx, auroxanthin; Neox, neoxanthin; Ncr, neochrome. [c] Fermentater code.

codes PL1 and PL2); 2.38% NaOH for 5 h 10 min, washing for 7 h, and brining in 10.6% NaCl (short type process, codes PS1 and PS2). Once the brine had reached neutral pH, analyses of the pigments was performed every 2–3 days during the first week of fermentation and subsequently every 8 days. Analyses were performed in duplicate using 10 g of pulp obtained from 15–20 stoned and triturated fruits.

In addition, Sevillian or Spanish-style green table olives of two different commercial trademarks (A and B) were purchased in a supermarket and their carotenoid content and provitamin A activity determined. Eight samples of each were purchased (glass containers of 163-g capacity), and each was analyzed in duplicate.

Extraction, Identification, Separation, and Quantification of Pigments. These operations were performed on the unsaponifiable fraction of the crude extract of total pigments obtained with acetone, according to the general method of Smith and Benitez (1955). The stoned olives were weighed (30 g) and triturated for 1 min with 50 mL of acetone. The extract was filtered and the solid residue treated several more times with acetone until all of the color was exhausted.

The filtrates were collected in a decanting flask and treated with ethyl ether. Distilled water was added until there was a clear separation of the two phases. The aqueous phase was discarded, while the ether phase, containing the dissolved pigments, was washed several times with water, until all remaining acetone had been completely eliminated. At this point, 300 mL of a 20% solution of KOH in methanol was added and the mixture was shaken vigorously. Shaking was repeated at 10-min intervals for 1 h. Once saponification was complete (2 h), the mixture was washed for various times with distilled water until the neutral point was reached, when it was treated with 2% sodium sulfate aqueous solution. Finally, the mixture was filtered through a bed of anhydrous sodium sulfate into a rotavapor flask for concentration at reduced pressure and at a temperature below 30 °C. The dry residue (0.07% carotenoid content) was dissolved in 5 mL of acetone and stored in a freezer at −30 °C until its use.

The carotenoid pigments in fresh olives, as well as the pigments arising during the processing of the fruits as table olives, have been described previously (Mínguez-Mosquera et al., 1989, 1991) and confirmed in the present paper. Pigments were identified by comparing their retention times and absorption spectra with those of authentic standards. β-Carotene, lutein, violaxanthin, and neoxanthin were obtained from a pigment extract of fresh olives saponified and separated by TLC (silica gel GF_{254} plates of 20 × 20 cm with a thickness of 0.7 mm) using petroleum ether (65–95 °C)–acetone–diethylamine (10:4:1). Auroxanthin and neochrome were obtained from violaxanthin and neoxanthin by acidification with HCl (0.1 M). All standards were purified by TLC using different eluents described in a previous publication (Mínguez-Mosquera et al., 1991). The separation and quantification of xanthophylls here was carried out using the reversed-phase HPLC method developed by Mínguez-Mosquera et al. (1991).

The provitamin A value was obtained from the content in carotenes and carotenoids with β-ionone rings of the olives, and the results are expressed in micrograms of retinol per 100 g of fruit, taking into account that 1 μg of β-carotene is equivalent to 0.167 μg of retinol or 0.56 IU of vitamin A (Bauernfeind et al., 1971).

RESULTS AND DISCUSSION

Transformation of Carotenoids during the Production of Table Olives. The initial treatment of fruits with NaOH did not affect the pigments under study at all, since none of the carotenoids present in the olive fruit is susceptible to structural changes as a result of alkaline treatment (Mínguez-Mosquera et al., 1991). This fact has allowed this study to be performed using an extract of saponified pigments and of a pigment concentrate free from chlorophylls and fatty substances. The identification of pigments during the processing of green table olives has allowed recognition of the fact that only those carotenoids whose structures are sensitive to the acid medium were affected, β-carotene and lutein (*3,3′-dihydroxy-α-carotene*) being unaltered. Violaxanthin (*3,3′-dihydroxytetrahydro-5,6:5′,6′-diepoxy-β-carotene*), with two 5,6-epoxide groups in its molecule, due to the acid pH developed in the fermentation medium, was transformed first into luteoxanthin (*3,3′-dihydroxy-5,6,5′,8′-tetrahydro- 5,6:5′,8′-diepoxy β-carotene*) with one 5,6-epoxide and one 5,8-furanoid group. Finally, both of these gave rise to the isomer auroxanthin (*3,3′-dihydroxy-5,8,5′,8′-tetrahydro-5,8:5′,8′-diepoxy-β-carotene*) with two 5,8-furanoid groups. The only 5,6-epoxide group that antheraxanthin (*3,3′-dihydroxy-5,6-dihydroxy-5,6-epoxy-β-carotene*) and neoxanthin (*3,3′,5′-trihydroxy-5,6,5′-trihydro-6′,7′-dihydro-5,6-epoxy-β-carotene*) possess also changed, for the same reason, into 5,8-furanoid, giving rise to mutatoxanthin (*3,3′-dihydroxy-5,8-dihydro-5,8-epoxy-β-carotene*) and neochrome (*3,3′,5′-trihydroxy-5′-hydro-5,8-dihydro-6′,7′-didehydro-5,8-epoxy-β-carotene*), respectively.

The qualitative and quantitative changes in these pigments during processing of fruits as table olives, as a function of the changes in the pH, are shown in Tables 1 and 2. Since they are minor components, the changes in luteoxanthin have been evaluated together with violaxanthin and those in antheraxanthin and mutatoxanthin with lutein. As can be seen from the data shown in these tables, the concentrations of β-carotene and lutein can be considered practically unchanged throughout the process within the normal limits for natural products. Violaxanthin and neoxanthin diminished in parallel with increases in the concentrations of auroxanthin and neochrome. The total carotenoid content of the fruits remained constant throughout the processing procedure studied. This indicates that there was no oxidative degradation of these compounds to colorless products.

Table 2. Evolution of Carotenoid Concentration (Milligrams per Kilogram) in Olives during the Fermentation Process as a Function of pH (Process Type Short)[a]

	PS1							PS2						
time (days)	pH	β-c	Lut	Viol	Aurx	Neox	Ncr	pH	β-c	Lut	Viol	Aurx	Neox	Ncr
0		3.98	5.89	2.01		1.55			3.98	5.89	2.01		1.55	
3	7.27	3.98	5.66	1.55		1.39		7.65	4.02	5.66	1.69		1.29	
6	6.12	3.91	6.10	1.10	0.81	1.25		6.18	4.17	6.10	1.44	0.61	1.20	0.31
10	5.31	3.73	6.29	0.87	1.24	1.14	0.40	5.13	3.81	6.29	0.85	1.17	0.97	0.52
12	5.17	3.83	5.97	0.79	1.30	0.97	0.51	5.09	3.96	5.97	0.76	1.21	0.82	0.66
19	4.94	3.96	6.14	0.61	1.42	0.66	0.85	4.90	4.02	6.14	0.62	1.15	0.46	0.91
26	4.84	3.86	6.16	0.42	1.69	0.42	1.08	4.83	3.93	5.97	0.20	1.90		1.15
33	4.77	3.92	6.02	0.24	1.81		1.45	4.71	3.98	5.97	0.20	2.01		1.44
47	4.71	3.70	6.44		1.94		1.49	4.66	4.00	6.02		2.12		1.42

[a] See Table 1 footnotes.

Consequently, the only transformation detected in the molecular structure of the carotenoids initially present in the fruits was the oxidation of the 5,6-epoxide groups to 5,8-furanoid groups, this being catalyzed by the presence of acids in the reaction medium.

The only carotenoid with provitamin A activity present in olives is β-carotene. As this pigment does not show any degradative losses during the processing procedure studied, it can be concluded that the provitamin A activity of the fruits is not altered, the mean value being 60.0 ± 2.4 μg of retinol/100 g.

Mechanism and Kinetics of Carotenoid Degradation. As has been mentioned in the previous section, the only carotenoids that were thus transformed were those whose molecular structures made them sensitive to the acid medium. The progressively increasing acidity of the fermentation medium catalyzed this degradation, and the kinetic equation describing these changes is as follows: $-dC/dt = k[H^+]^n[\text{pigment}]^n$, where C is the carotenoid concentration, t is time, and k is the apparent rate constant. Although physically the reaction occurs inside the chloroplasts, the medium in which it takes place is the fermenter, since it is diffusion across the membranes by osmosis that leads to fermentation. The intracellular pH is thus altered by the pH of the brine.

Considering the change in pH that takes place in the fermentation brine, it might at first seem that when the interior of the fruit reaches a pH of 8, the concentration of the carotenoid in the fruit is greater than that of hydrogen ions, in which case the kinetics of the transformation of the 5,6-epoxide groups into 5,8-furanoid groups, if it occurs, would be of second order. However, taking into account the fermenter as a whole, the concentration of hydrogen ions is not at all affected by this reaction, which as a result takes place without this reagent being limiting. As a result, the concentration of H^+ ions in the fermentation medium, compared with the existing carotenoids is, from the start of the process, in excess and can thus be considered to be constant. In this case the kinetic reaction can be expressed as $-dC/dt = k'[\text{pigment}]^n$, where $k' = k[H^+]^n$, which has been called a pseudo-order reaction (González, 1991).

The kinetic study was therefore based on violaxanthin and neoxanthin. These were the most common xanthophylls containing epoxide groups and progressively gave rise to their isomers auroxanthin and neochrome, respectively.

In Figures 1 and 2, the percent retention of violaxanthin and neoxanthin during processing is shown on a semilogarithmic scale. The data shown represent the changes in the carotenoids in fermenter PL2, which has been chosen for illustration. It can be seen that the changes fit a straight line, indicating that this type of degradation follows first-order kinetics ($y = e^{(kt+b)}$). The apparent rate constants

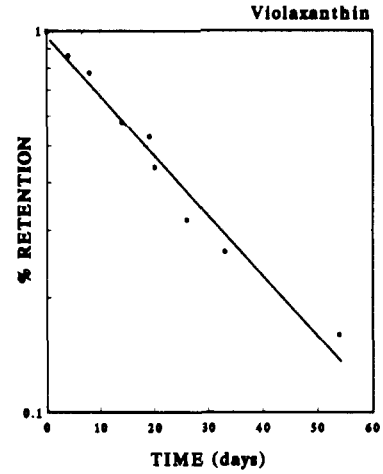

Figure 1. First-order degradation rate of violaxanthin in table olive processing (fermenter with code PL2) as a function of time.

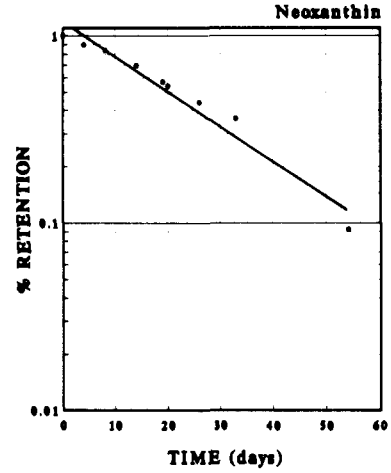

Figure 2. First-order degradation rate of neoxanthin in table olive processing (fermenter with code PL2) as a function of time.

and the corresponding correlation coefficients found are shown in Table 3. The rate constants obtained are of the same order for both violaxanthin and neoxanthin. It is noteworthy that for both pigments these values are somewhat higher in the short type processing system.

Carotenoid Composition and Provitamin A Value. In Table 4 the carotenoid composition and provitamin A value of a sample of green table olives purchased in a supermarket are shown. The mean provitamin A values were 35.5 ± 2.3 μg of retinol/100 g for trademark A and 27.5 ± 3.0 μg of retinol/100 g for trademark B.

It is noteworthy that large differences were found between the total carotenoid contents of the samples of commercially available olives and those of olives processed under the experimental conditions described here, even

Table 3. Apparent First-Order Rate Constants and Correlation Coefficients Obtained by Linear Regression Analysis for the Epoxide Xanthophyll Degradation during Fermentation Process of Green Table Olives

	violaxanthin		neoxanthin	
fermentater code	k^a	$r^{2\,b}$	k	r^2
PL1	−0.0257	93.71	−0.0303	97.86
PL2	−0.0353	96.54	−0.0451	96.46
PS1	−0.0563	98.06	−0.0528	97.97
PS2	−0.0651	96.03	−0.0685	98.54

[a] k, apparent rate constant (days^{-1}). [b] r^2, correlation coefficient × 100; $p < 0.001$.

Table 4. Carotenoid Composition (Milligrams per Kilogram) in Green Table Olives[a]

sample[b]	β-c	Lut	Aurx	Ncr	Provit A[c]
A1	2.45	4.50	0.63	0.71	40.84
A2	1.92	2.82	0.25	0.20	32.01
A3	2.07	2.12	0.14	0.07	34.51
A4	2.00	1.85	0.25	0.19	33.34
A5	2.12	2.53	0.44	0.38	35.34
A6	2.28	1.35	0.26	0.18	38.01
A7	2.09	3.35	0.33	0.36	34.84
A8	2.09	3.23	0.47	0.52	34.84
AM[d]	2.13 ± 0.14	2.60 ± 0.76	0.35 ± 0.13	0.33 ± 0.18	35.4 ± 2.3
B1	1.53	4.21	0.32	1.16	25.51
B2	1.48	3.44	0.27	0.38	24.67
B3	1.72	2.92	0.25	0.35	28.67
B4	1.54	3.79	0.29	0.51	25.61
B5	2.05	2.40	0.24	0.28	34.17
B6	1.60	2.97	0.24	0.24	26.67
B7	1.41	2.49	0.25	0.20	23.51
B8	1.88	2.82	0.28	0.35	31.34
BM	1.65 ± 0.18	2.89 ± 0.46	0.27 ± 0.03	0.43 ± 0.26	27.5 ± 3.0

[a] Key: β-c, β-carotene; Lut, lutein; Aurx, auroxanthin; Ncr, neochrome; Provit A, provitamin A. [b] A and B commercial trademarks. [c] μg of retinol/100 g (1 μg of β-carotene = 0.167 μg of retinol). [d] Mean values ± SD.

though all of the olives were of the same variety (Manzanilla). The concentrations found in olives processed experimentally are shown in Tables 1 and 2 of the present study and also in the previous study of Mínguez-Mosquera et al. (1990). For the same olive variety the overall pigment content in the fruits is directly related to the stage of maturity. As the fruits mature, their overall chlorophyll and carotenoid pigment content decreases progressively from 137.49 ± 10.36 mg/kg in the green stage, through 103.49 ± 9.74 mg/kg in the yellowish-green stage, to 69.68 ± 5.22 mg/kg in the small reddish spot stage (Mínguez-Mosquera and Garrido-Fernández, 1989). In this work, the experimental fermentation was performed using fruits in the optimal maturity degree (yellowish green) characteristic for this traditional processing system. The fact that the concentration of the carotenoid fraction in the commercial olives is considerably lower than that found in the olives processed under the experimental conditions described here could be due to diverse causes. On the one hand, the commercial product could have been elaborated from very ripe fruit in which the carotenoid content is too low. On the other hand, development of anomalous fermentations or exposure to light during storage could also perhaps have an effect. In summary, we believe that these differences indicate that an inadequate industrial practice was used for these samples.

ACKNOWLEDGMENT

We express our sincere gratitude to CICYT for supporting this research project, ALI 91-1166-C03-02.

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Received for review January 27, 1994. Accepted April 18, 1994.[⊛]

[⊛] Abstract published in *Advance ACS Abstracts*, June 15, 1994.