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Thermal Degradation Products Formed from Carotenoids during a Heat-Induced Degradation Process of Paprika Oleoresins (*Capsicum annuum* L.)

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The high-temperature treatment of paprika oleoresins (*Capsicum annuum* L.) modified the carotenoid profile, yielding several degradation products, which were analyzed by HPLC-APCI-MS. From the initial MS data, compounds were grouped in two sets. Set 1 grouped compounds with *m*/*z* 495, and set 2 included compounds with *m*/*z* 479, in both cases for the protonated molecular mass. Two compounds of the first set were tentatively identified as 9,10,11,12,13,14,19,20-octanor-capsorubin (compound **II**) and 9,10,11,12,13,14,19,20-octanor-5,6-epoxide-capsanthin (compound **IV**), after isolation by semipreparative HPLC and analysis by EI-MS. Compounds **VII**, **VIII**, and **IX** from set 2 were assigned as 9,10,11,12,13,14,19,20-octanor-capsanthin and isomers, respectively. As these compounds were the major products formed in the thermal process, it was possible to apply derivatization techniques (hydrogenation and silylation) to analyze them by EI-MS, before and after chemical derivatization. Taking into account structures of the degradation products, the cyclization of polyolefins could be considered as the general reaction pathway in thermally induced reactions, yielding in the present study xylene as byproduct and the corresponding *nor*-carotenoids.

KEYWORDS: Thermal degradation; carotenoids; *Capsicum annuum* L.; oleoresin; cyclization; polyolefins

INTRODUCTION

The molecular structure of carotenoids provides this group of natural pigments with their physicochemical properties, through which they exhibit essential functions as photosynthetic compounds and photoprotectors and as antioxidants toward reactive oxygen species and other free radicals (*1*). For mammals, which are reliant on diet for carotenoid absorption, the biological actions of carotenoids, such as their antioxidant ability, immune-enhancing properties, and vitamin A precursor function, make them valuable from the nutritional point of view. Therefore, plant sources such as fruits and vegetables and their processed products mainly contribute to carotenoid intake for humans.

Technical parameters applied during the processing of vegetable raw sources could decrease original carotenoid composition and affect their stability in the final product and even modify the bioavailability (*2*). Moreover, conditions reached in processing may generate carotenoid oxidation and some cleavage products, changing initial properties of the raw source, such as the antioxidant activity and other biological actions mentioned. Interest in characterizing the role of those oxidation products has recently increased. It is stated that processing could improve the antioxidant properties of natural antioxidants, changing their oxidation state or structure. There is a growing agreement that carotenoid oxidation products may be responsible of some biological activities (*1*), although to date studies have only focused on the oxidation products of two significant dietary carotenoids, lycopene and β-carotene.

Efforts made to identify oxidative compounds of lycopene were encouraged by their potential anticancer activity (*3*). Khachick et al. (*4*) described the structure of oxidative metabolites of lycopene, mainly epoxides, observed in tomato products. Mechanisms that produce such compounds were proposed in a prior publication (*5*). In the case of β-carotene, the literature describes several oxidation products, especially those formed during peroxide-mediated autoxidation. Thus, reaction between β-carotene and peroxyl radicals in model systems produces apocarotenals and epoxides (*6*−*8*). Identification of those compounds added to the understanding of mechanisms of antioxidant activity and clarified the possible role of intermediate products in it (*9*). Recent studies have dealt with retinoid-like cleavage compounds and the possible actions they could develop (*10*).

Techniques applied for the generation of carotenoid oxidation products mainly consist of model systems in which the carotenoid reacts with an oxidizing compound, commonly free radical initiators [azo compounds such as 2,2′-azobis(2,4-dimethylvaleronitrile) and 2,2′-azobis(isobutyronitrile)], hydrogen peroxide−osmium tetraoxide, or more recently $KMnO_4$

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(*11*−*13*). These procedures provide a plethora of compounds, which complicates the isolation and identification procedures. Despite advances made with this research strategy, the feasibility of these reaction processes in an in vivo system, or during food processing, should be taken into account. Some of them do not really resemble conditions used in the technical manufacture of food. In this sense, only a lycopene diol (2,6-cyclolycopene-1,5-diol) has been described in tomato paste and juice as a compound formed as a consequence of processing, and lately it was detected in human serum (*4*), showing that carotenoid oxidation products from processed foods are effectively incorporated in the human body. Therefore, efforts should be made in the identification of process-induced compounds and in the assessment of their activity.

High temperatures are often applied during the processing of food material that contains carotenoids. Some examples are extrusion cooking and deodorization in the refining of edible oils, when the product could be subjected to temperatures of 150−220 °C (*14*). Thermal degradation produces two fractions of products: volatile compounds of low molecular weight and a nonvolatile fraction. Marty and Berset (*15*) identified epoxy compounds formed from β-carotene that are later rearranged to furanoid ones, and in a subsequent work they described oxidative breakdown products, a series of aldehydes and a ketone (*16*).

Processing of red pepper fruits (*Capsicum annuum* L.) for producing paprika oleoresins, a highly concentrated carotenoids oil, involves some operation units where heating is the driving force to transform the raw material. The genus *Capsicum* biosynthesizes almost exclusively the carotenoids capsanthin and capsorubin. Several previous studies with paprika oleoresins have been performed to determine degradation kinetics and the effect of thermal processing in the carotenoid profile present in that processed product (*17*, *18*), and some possible degradation products were detected, although their contribution to the total carotenoid profile was not significant. The aim of the present work was to promote the production of those degradation products in order to identify them, using a heat-induced degradation process with temperatures similar to those applied in paprika oleoresin processing. Elucidation of the structure of the degradation compounds would add to the understanding of the mechanism of reaction through which carotenoids are degraded in the oily surrounding at high temperatures.

MATERIALS AND METHODS

Raw Material. The firm Extractos-Vegetales S.A. (La Línea de la Concepción, Cádiz, Spain) supplied the paprika oleoresin employed in this study.

Chemicals and Reagents. HPLC grade acetone and methanol were supplied by Romyl (Teknokroma, Barcelona, Spain). HPLC grade water was obtained with a Milli-Q water-purifying system from Millipore (Milford, MA). Diethyl ether, inhibitor free, HPLC grade, and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Sigma (St. Louis, MO). Solid-phase extraction (SFE) columns of octadecyl C18 (500 mg) were obtained from Baker (Philipsburg, NJ). Other reagents were all of analytical grade.

Work Plan. Aliquots of paprika oleoresins were placed separately in 2-mL vials, which were gas-flushed with nitrogen and airtight without headspace. Afterward, samples were subjected to a heat-induced degradation process. Previous experiments indicated 100 °C as a suitable temperature. Sampling was performed daily to determine the qualitative carotenoid composition and presence of new carotenoid degradation products, using one vial for analysis at each time point. The total heating time was 480 h.

Extraction and De-esterification of Carotenoid Profile. The procedure has been described in previous works (*19*, *20*). The sample (0.03 g) was dissolved directly in 100 mL of diethyl ether. De-esterification of the resulting solution was carried out in a 500-mL decanting funnel with 50 mL of 10% potassium hydroxide in methanol, shaking the mixture gently. After 1 h of reaction at room temperature, a portion of 200 mL of 10% sodium chloride in water was added, and the aqueous and organic phases were left to separate. The organic phase was washed with 200-mL portions of distilled water until the washings were neutral. Organic solution containing the carotenoids was filtered through a solid bed of anhydrous sodium sulfate, evaporating the filtrate to dryness in a rotary evaporator. The residue was dissolved in acetone to a volume of 10 mL and stored at −30 °C until its analysis by HPLC.

Isolation of the Carotenoid Degradation Products. To aid subsequent identification of new compounds detected, an isolation procedure was applied to the de-esterified extracts obtained previously. This methodology was developed by taking into account the polar characteristics of new compounds, which appear at the first stage of the chromatographic separation. Thus, 1 mL of the extract was placed in a SPE octadecyl cartridge, previously rinsed with methanol, and 3 mL of acetone/H_2O (75:25) was used as eluting solvent, discarding the initial 1 mL and recovering the next 1.5 mL of the elution, the fraction that contains new carotenoid degradation products, free of the original carotenoid profile of paprika oleoresin.

Cleanup of the Isolated Carotenoid Degradation Products. Isolated compounds were cleaned, before application of subsequent MS analysis, to discard contaminations that may complicate the spectra. The procedure was the following: the compounds, dissolved in acetone/H_2O (63:37), were placed in a SPE octadecyl cartridge, previously equilibrated with 6 mL of methanol followed by 6 mL of water. After passage of the solution containing the compounds, the cartridge was washed with 15 mL of H_2O and the compounds were recovered with 2 mL of acetone.

Separation of Carotenoid Profile by HPLC. For the isolation of each individual compound, a semipreparative HPLC technique was employed with the following chromatographic conditions: 500 μL of isolated fraction was injected in a 250 mm × 10 mm i.d., 5 μm, LiChrospher 100 C-18e reversed-phase column (Merck, Darmstadt, Germany) with an eluent composition of acetone/H_2O (63:37) (isocratic conditions) and a flow rate of 1 mL/min. Subsequent analyses by HPLC-MS or EI-MS were performed with each isolated compound. HPLC analyses were performed with a Waters 600E quaternary pump equipped with a Waters PDA 996 diode array detector (Waters, Milford, MA) and controlled with a Millenium data acquisition station. For analysis on the analytical scale of the carotenoid profile during the heat-induced degradation process, chromatographic separation was verified following a method described previously (*19*).

Mass Spectrometry. APCI-HPLC analyses of the sample extract were performed in a Beckman Gold system using a 126 pump with a 168 diode array detector (Beckman, Inc., Fullerton, CA) on-line with an MAT95 magnetic sector mass spectrometer (Finnigan Mat, Bremen, Germany) equipped with an APCI interface. A 250 mm × 4.6 mm i.d., 3 μm, Lichrospher 100RP-18 column (Merck), maintained at 30 °C, was used. Injections were performed with a Rheodyne injection valve (200-μL loop). Elution was performed at a flow rate of 1 mL/min, using as mobile phase a mixture of water/acetone/acetic acid (37:63:0.1, v/v) in isocratic mode. Detection was at 410 and 425 nm simultaneously. A split postcolumn of 25% of the column flow was introduced in the APCI interface. The APCI mass spectra, in the positive-ion mode, were obtained under the following conditions: vaporizer, 300 °C; capillary temperature, 250 °C; discharge corona, 5 kV; and lens, skimmer, and octapole voltages set to get optimal response for a reference solution of gramicidine. Nitrogen at 150 kPa was used as sheath gas. Afterward, partial defocusing of interface parameters was done to generate moderate collision-induced dissociation inside the ionic transport region. Under these conditions, the spectra show ionic fragmentation about structural information from the protonated molecular ion.

The EI-MS mass spectra were obtained at a 70-eV electron energy, 250 μA of ion current, and 3.5 kV of accelerating voltage. Pure isolated compounds were introduced in the ion source by using a temperature programmable direct insertion probe. The concentrate sample solutions (with a total amount of 25−50 ng of compound) were successively disposed of inside an inert crucible and evaporated to dryness inside of it prior to solids probe insertion on the ion source.

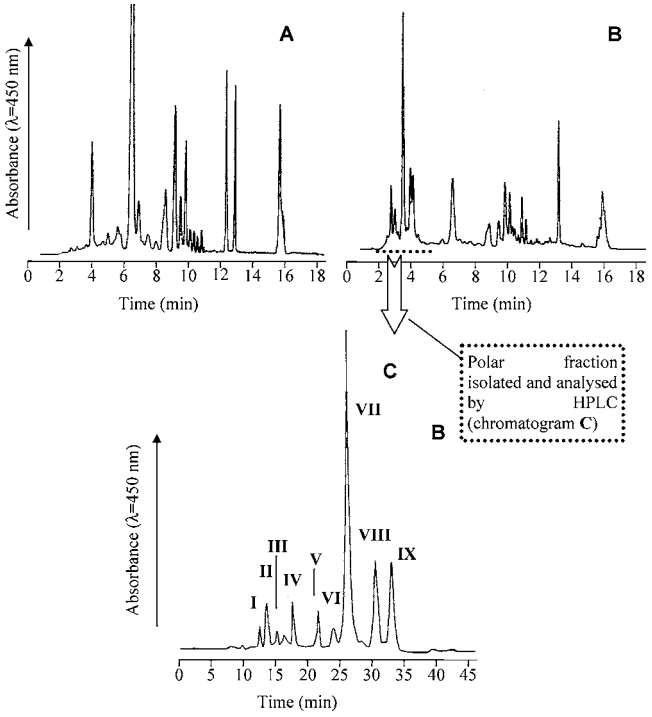

Figure 1. HPLC chromatograms corresponding to the analysis of a deesterified carotenoid extract from paprika oleoresin (*C. annuum* L.) at $t = 0$ (**A**) and after 384 h of thermal treatment at $t = 100$ °C (**B**). Sample depicted in panel **C** corresponds to the HPLC analysis of the isolated degradation products.

Chemical Derivatization of Isolated Compounds. Isolated compounds were subjected to chemical derivatization for identification of functional groups. For hydrogenation, the collected compound was subjected to a mild stream of nitrogen to remove the solvent and immediately redissolved in 5 mL of methanol (HPLC grade). Hydrogen was bubbled with PtO_2 as catalyst during 20 min. After filtering the solution, this was concentrated to 10 μL for analysis by EI-MS. For silylation, an aliquot of the isolated compound was dried with nitrogen and redissolved in 200 μL of bis(trimethylsilyl)trifluoroacetamide in pyridine, heating the solution at 90 °C for 20 min. Afterward, the final volume was evaporated with a nitrogen stream to 10 μL for analysis by EI-MS.

RESULTS AND DISCUSSION

The main effect of the thermal processing of food on carotenoids is isomerization, although this reaction pathway coexists with degradation, which generates different byproducts (*21*). The latter reaction route is preferential when the temperature of processing increases (*18*, *22*), as in the case of production of paprika oleoresins, for which the temperature−time regimes are severe. In the present work, the heat-induced degradation produced an unremitting decrease of carotenoid content, reproducing kinetics and reaction routes observed in previous publications (*17*, *23*). The interest of this study was to focus on the formation of carotenoid degradation products, during the heating of the oleoresin. **Figure 1** depicts two chromatograms, one corresponding to the analysis of the initial carotenoid pattern and one corresponding to the analysis of the carotenoid pattern after 384 h of thermal treatment. With respect to the starting sample (**Figure 1A**) two details can be observed in **Figure 1B**: the disappearance or relative decrease of the starting carotenoid profile and the appearance of five peaks, which were detected within the initial 5 min of the elution program. These compounds showed their higher concentration at $t = 384$ h. This new group of compounds is named herein the polar fraction (PF). The presence of the PF is denoted in the chromatogram corresponding to the initial sample, although the contribution of these compounds to the total carotenoid profile is minimal. The heating process to which the sample was subjected promoted reaction pathways that produced these kinds of degradation products, which are relatively the major contributors to the carotenoid pattern when the heating time increases.

To isolate the new peaks detected and discard the rest of the carotenoid profile, a SPE procedure was developed and successfully applied, because this procedure allowed the PF to be separated, completely free of carotenoids initially present in the paprika oleoresin. This provided an advantage for successive isolation and identification techniques. The collected PF was applied to positive ion APCI-HPLC-MS to determine the protonated molecular ion of compounds detected in paprika oleoresin after the thermal treatment. In this case, both mobile phase composition and elution mode were changed to check the content of the PF and to ensure the chromatographic separation, obtaining the type of chromatogram depicted in **Figure 1C**. Four new peaks appeared, which were coeluting with the previous ones obtained in **Figure 1B**. Nine compounds were detected. Compounds **II**, **III**, and **IV** (retention times of 12.48, 13.96, and 19.80 min, respectively) showed *m*/*z* 495 for the protonated molecular ion $[M + H]^+$, corresponding to a $C_{32}H_{47}O_4$ composition, and compounds **V**, **VII**, **VIII**, and **IX** (retention times of 23.72, 28.23, 30.15, and 31.52 min, respectively) showed the protonated molecular ion at *m*/*z* 479, corresponding to a $C_{32}H_{47}O_3$ composition. Compounds **I** and **VI** as well as relatively minor peaks in the HPLC profile shown in **Figure 1C** were not studied, as they did not show well-defined spectroscopic properties. Considering the values of protonated molecular ion of compounds in the study, they may be grouped in two sets, those with *m*/*z* 495 and those with *m*/*z* 479 for the protonated molecular ion (sets 1 and 2, respectively). Positive APCI mass spectra of the compounds of set 1 are shown in **Figure 2** and the corresponding spectra for compounds of set 2 in **Figure 3**. Application of derivatization techniques, like silylation and hydrogenation, was feasible with compounds of set 2, whereas the rest was directly analyzed by APCI or EI. In this case, only elemental composition is proposed, and in the case of structure assignment, this should be considered as tentative.

Mass spectra of the compounds of set 1 showed a fragmentation profile from the $[M + H^+]$ ion. All of them exhibit characteristic fragments corresponding to neutral losses of one or more molecules of water. Thus, the base peak of compound **IV** is at *m*/*z* 495, corresponding to $[M + H]^+$, whereas in compound **II** the base peak at *m*/*z* 477 corresponds to the $[M + H - H_2O]^+$ ion as a consequence of an intense water neutral loss elimination. The fragment ion at *m*/*z* 109 should correspond to a five-membered ring ion corresponding to a C_8H_{13} elemental composition for the protonated ion (**Figure 2**). The loss of an in-chain unit of 92 amu was denoted in the APCI mass spectra of this set of compounds. Isolation of each individual compound by preparative HPLC was possible only for compounds **II** and **IV**, whereas the amount of compound **III** was not enough to obtain quality mass spectra. Analysis by EI-MS confirmed the molecular mass as shown in **Figure 4**, which includes EI-MS spectra of compounds **II** and **IV**. The presence of fragments at *m*/*z* 476 and the peak at *m*/*z* 339 suggest the existence of hydroxyl and keto groups, respectively. The presence of an ion at *m*/*z* 368 could imply the elimination of a five-membered ring, as displayed in **Figure 4**, where the structural assignment for compounds **II** and **IV** is also presented. We propose the structure for compound **II** as 9,10,11,12,13,14,19,20-octanor-capsorubin and that of compound **IV** as 9,10,11,12,13,14,19,20-octanor-

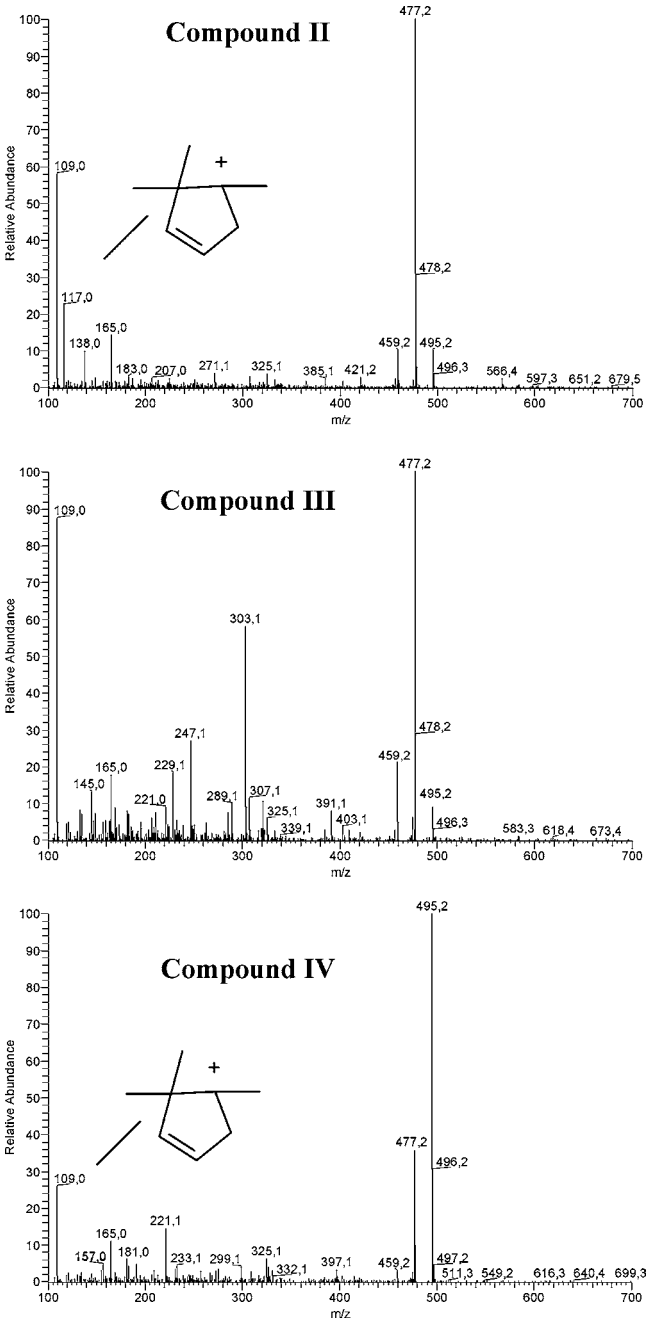

Figure 2. HPLC-APCI-MS analyses of degradation compounds of set 1. The structure of the fragment ion at *m*/*z* 109 corresponding to a five-membered ring is depicted.

5,6-epoxide-capsanthin. Both compounds have the same elemental composition ($C_{32}H_{46}O_4$), but one of the κ rings of compound **II** is changed to a 3-hydroxy-5,6-epoxide β ring in compound **IV**. The basis for that structural differentiation is the chromatographic behavior and the difference in the intensity of the ion at *m*/*z* 368. This ion is suggested to come from a rearrangement, with proton transfer, from the two five-membered rings in compound **II** (**Figure 4**). In contrast, compound **IV** presents one characteristic peak at *m*/*z* 221 corresponding to the successive neutral losses of two molecules of water, and a five-membered ring denoted by the even ion at *m*/*z* 368, but with lower intensity than the one observed for compound **II**. Additionally, it should include a six-membered ring with an epoxide group, which is denoted by the presence of the peak at *m*/*z* 414, characteristic for epoxy carotenoids (*24*, *25*).

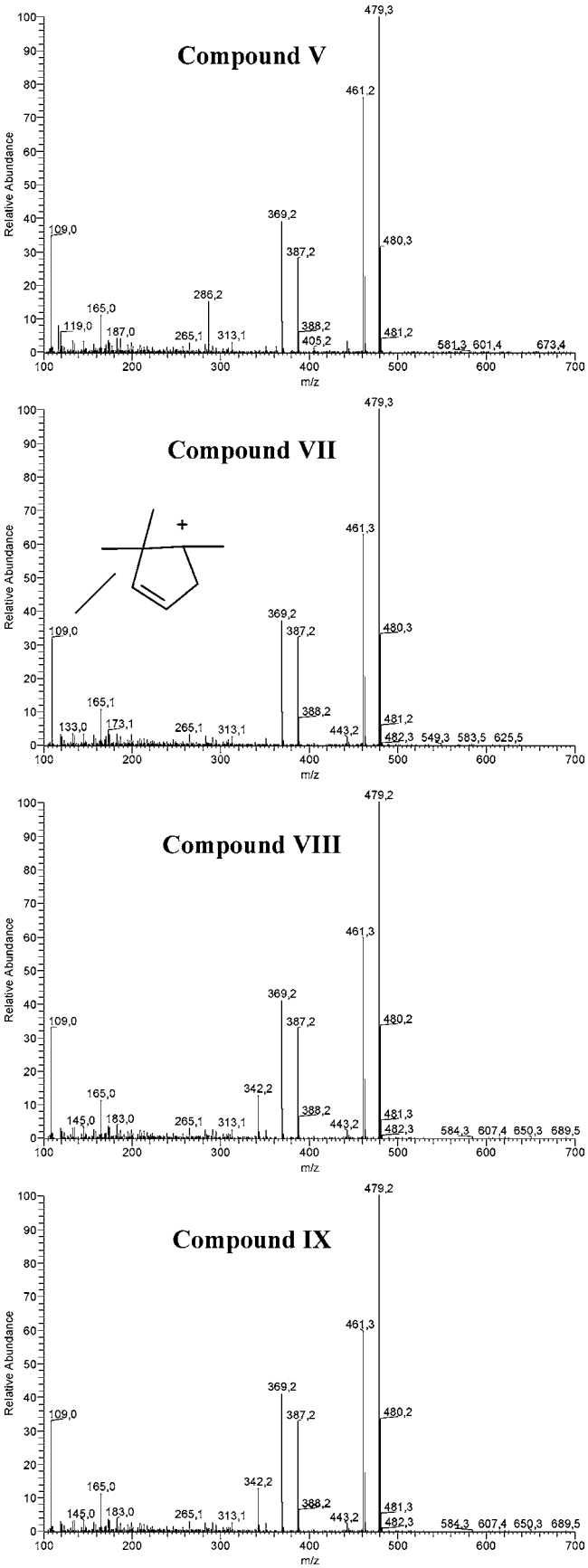

Figure 3. HPLC-APCI-MS analyses of degradation compounds of set 2. The structure of the fragment ion at *m*/*z* 109 corresponding to a five-membered ring is depicted.

Set 2 was formed by a group of four compounds characterized by showing a common protonated molecular ion at *m*/*z* 479 as

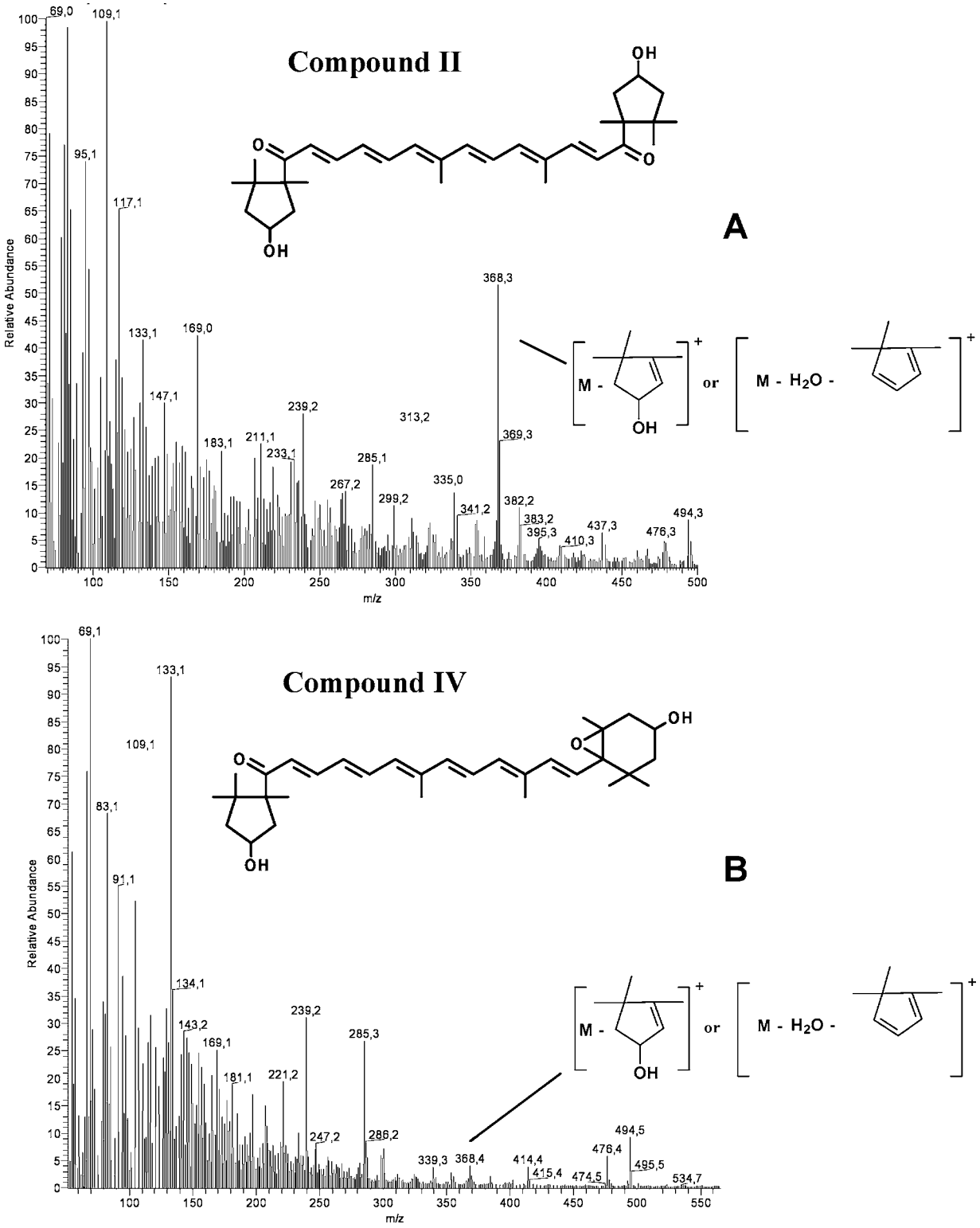

Figure 4. EI-MS analyses of compounds **II** and **IV** (**A** and **B**, respectively) including tentative structural assignment. The scheme for the formation of the fragment at *m*/*z* 368 is presented in panel **A**.

the base peak, two successive water neutral losses of 18 amu, and a typical elimination of an in-chain 92 amu fragment from carotenoids at *m*/*z* 461, 443, and 387, respectively. The fragment ion at *m*/*z* 369 should correspond to the successive elimination of one molecule of water and an in-chain loss of a 92 amu fragment. Additionally, the presence of a five-membered ring is denoted by the fragment at *m*/*z* 109 in the same way as compounds of set 1. This group is mainly represented by compound **VII**, which was the major degradation product formed during heating of paprika oleoresin, with *m*/*z* 479, for the protonated molecular ion. Analysis of the isolated compound, aided with preparative HPLC, using EI-MS, confirmed the molecular ion determined previously by APCI-HPLC-MS as shown in **Figure 5**, that includes the mass spectrum of compound **VII** by EI-MS (**Figure 5A**) corresponding to a $C_{32}H_{46}O_3$ composition. To reconfirm the estimated molecular ion and to establish the presence of hydroxyl groups, the isolated compound was silylated and analyzed by positive EI-MS. The

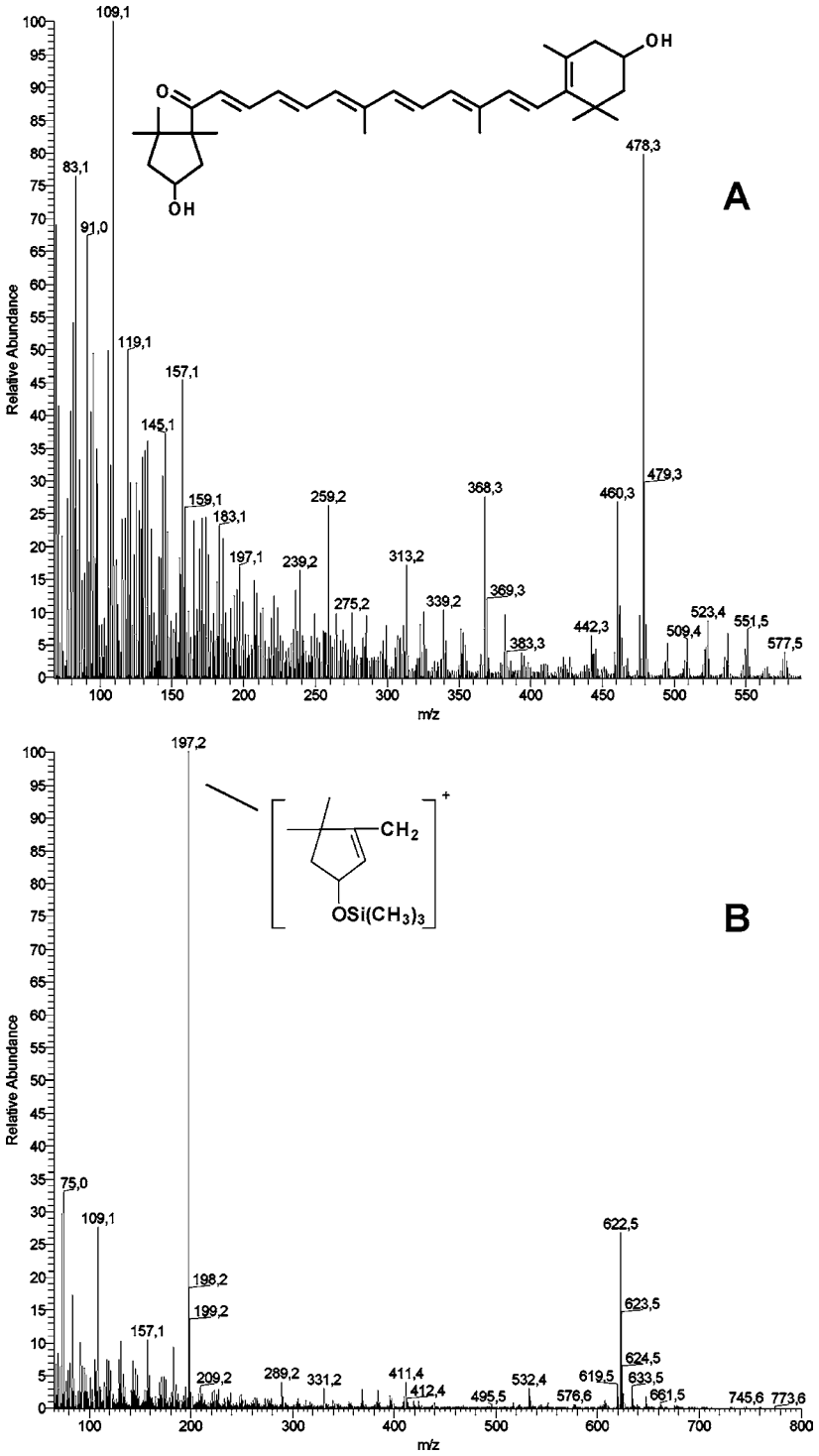

Figure 5. EI-MS analyses of compound **VII**, direct (**A**) and after silylation (**B**), including structural assignment. The structure of the base ion at *m*/*z* 197 in panel **B** is also depicted.

molecular ion increased in mass by 144 amu, corresponding to incorporation of two trimethylsilyl ether groups (**Figure 5B**). Additionally, the mass spectrum of the silylated compound showed a base peak at *m*/*z* 197 for $[M + H]^+$, which corresponds to the fragment presented in **Figure 5B**, confirming the five-membered ring with a hydroxyl group. Thus, compound **VII** is assigned as 9,10,11,12,13,14,19,20-octanor-capsanthin, and the proposed structure is presented in **Figure 5**. Compounds **VIII** and **IX** had similar UV−vis absorbance peaks and a mass identical to that of compound **VII**, but different retention times. Therefore, compounds **VIII** and **IX** are assigned as isomers of 9,10,11,12,13,14,19,20-octanor-capsanthin. Compound **V** was analyzed by only EI-MS, but it was not possible to apply chemical derivatization techniques, as not enough sample was available.

Isomerization, oxidation, and breakdown are the main degradation reaction pathways described for carotenoids, and products resulting from those routes are generally a mixture of epoxides and apocarotenals (*7*, *9*, *14*). However, in the present study the heat-induced degradation yielded a degradation profile formed by *nor*-carotenoids; that is, the original polyene skeleton has been shortened, producing a degradation product with end-groups identical to those in the parent compound, but eight carbon units have been eliminated. This explains the hypso-

chromic shift denoted in λ_{max} values of degradation products with respect to the one for their parent compounds. A similar, but not identical, degradation pathway was described by Onyewu et al. (*26*) when they studied the nonpolar, nonvolatile thermal degradation products formed by heating β-carotene at 210 °C for 4 h. In that case products were tentatively identified as 3,7,-10-trimethyl-11,12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexane, corresponding to an elemental composition of $C_{33}H_{48}$, and 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene, corresponding to an elemental composition of $C_{28}H_{42}$. A toluene molecule is eliminated from β-carotene to form the first degradation product, whereas a 12-carbon unit is removed to yield the second product, according to the mechanism proposed by Edmunds and Johnstone (*27*). The compounds found in the present study have been formed by the same pathway; that is, the elimination of the in-chain unit (in this case the parent compound, like capsanthin for compound **VII**, loses xylene), that occurs without hydrogen shifts involving scission of the double bonds, as proposed previously (*24*). The cyclization of polyolefins could be considered as the general reaction pathway in thermally induced reactions, yielding toluene, xylene, or dimethylcyclodecapentaene as byproducts.

LITERATURE CITED

(1) Britton, G. Biological actions of carotenoids: Do oxidation products and metabolites play a key role? In *Functionalities of Pigments in Food*; 2nd International Congress on Pigments in Food; Empis, J., Ed.; Sociedade Portuguesa de Química: Lisboa, Portugal, 2002; pp 33−40.

(2) Rock, C. L. Carotenoids: biology and treatment. *Pharmacol. Ther.* **1997**, *75*, 185−197.

(3) King, T. J.; Khachik, F.; Bortkiewicz, H.; Fukishima, L. H.; Morioka, S.; Bertram, J. S. Metabolites of dietary carotenoids as potential cancer preventive agents. *Pure Appl. Chem.* **1997**, *69*, 2135−2140.

(4) Khachick, F.; Steck, A.; Niggli, U.; Pfander, H. Partial synthesis and structural elucidation of the oxidative metabolites of lycopene identified in tomato paste, tomato juice and human serum. *J. Agric. Food Chem.* **1998**, *46*, 4878−4884.

(5) Khachick, F.; Pfander, H.; Traber, B. Proposed mechanisms for the formation of synthetic and naturally occurring metabolites of lycopene in tomato products and human serum. *J. Agric. Food Chem.* **1998**, *46*, 4885−4890.

(6) Kennedy, T. A.; Liebler, D. C. Peroxyl radical oxidation of β-carotene. *Chem. Res. Toxicol.* **1991**, *4*, 290−295.

(7) Handelman, G. J.; Van Kuijk, F. J. G. M.; Chatterjee, A.; Krinsky, N. I. Characterization of products formed during the autoxidation of β-carotene. *Free Radical Biol. Med.* **1991**, *10*, 427−437.

(8) Mordi, R. C.; Walton, J. C.; Burton, G. W.; Hughes, L.; Ingold, K. U.; Lindsay, D. A. Exploratory study of β-carotene autoxidation. *Tetrahedron Lett.* **1991**, *32*, 4203−4206.

(9) Yamauchi, R.; Miyake, N.; Inoue, I.; Kato, K. Products formed by peroxyl radical oxidation of β-carotene. *J. Agric. Food Chem.* **1993**, *41*, 708−713.

(10) Ben-Dor, A.; Nahum, A.; Danilenko, M.; Giat, Y.; Stahl, W.; Martin, H. D.; Emmerich, T.; Noy, N.; Levy, J.; Sharoni, Y. Effects of acyclo-retinoic acid and lycopene on activation of the retinoic acid receptor and proliferation of mammary cancer cells. *Arch. Biochem. Biophys.* **2001**, *391*, 295−302.

(11) Wendler, N. L.; Rosenblum, C.; Tishler, M. The oxidation of β-carotene. *J. Am. Chem. Soc.* **1950**, *72*, 234−239.

(12) Palozza, P.; Krinsky, N. I. Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview. In *Methods in Enzymology: Carotenoids*; Packer, L., Ed.; Academic Press: San Diego, CA, 1992; Vol. 213, pp 403−420.

(13) Caris-Veyrat, C.; Schmid, A.; Carail, M.; Böhm, V. Oxidative cleavage products of lycopene: production, characterization and mechanism of formation. In *Pigments in Food, More than Colours*; 3rd International Congress on Pigments in Food; Dufossé, L., Ed.; Pigments Publishing: Quimper, France, 2004; pp 306−308.

(14) Bonnie T. Y.; Choo, Y. Oxidation and thermal degradation of carotenoids. *J. Oil Palm Res.* **1999**, *2*, 62−78.

(15) Marty, C.; Berset, C. Degradation products of *trans*-β-carotene produced during heating in sealed glass tubes and extrusion cooking. *J. Food Sci.* **1986**, *51*, 698−702.

(16) Marty, C.; Berset, C. Factors affecting the thermal degradation of all *trans*-β-carotene produced during extrusion cooking. *J. Agric. Food Chem.* **1990**, *38*, 1063−1067.

(17) Pérez-Gálvez, A.; Jarén-Galán, M.; Mínguez-Mosquera, M. I. Effect of high-temperature processes on ketocarotenoids present in paprika oleoresins. *J. Agric. Food Chem.* **2000**, *48*, 2966−2971.

(18) Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Degradation, under non-oxygen-mediated autoxidation, of carotenoid profile present in paprika oleoresins with lipid substrates of different fatty acid composition. *J. Agric. Food Chem.* **2004**, *52*, 632−637.

(19) Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem.* **1993**, *41*, 1616−1620.

(20) Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Study of lability and kinetics of the main carotenoid pigments of red pepper in the de-esterification reaction. *J. Agric. Food Chem.* **1998**, *46*, 566−569.

(21) van den Berg, H.; Falks, R.; Granado, F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food Agric.* **2000**, *80*, 880−912.

(22) Chandler, L. A.; Schwartz, S. J. Isomerization and losses of *trans*-β-carotene in sweet potatoes as affected by processing treatments. *J. Agric. Food Chem.* **1988**, *36*, 129−133.

(23) Jarén-Galán, M.; Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Prediction of decoloration in paprika oleoresins. Application to studies of stability in thermodynamically compensated systems. *J. Agric. Food Chem.* **1999**, *47*, 945−951.

(24) Maoka, T.; Fujiwara, Y.; Hashimoto, K.; Akimoto, N. Characterization of epoxy carotenoids by fast atom bombardment collision-induced dissociation MS/MS. *Lipids* **2004**, *39*, 179−183.

(25) Johannes, B.; Brzezinka, H.; Budzikiewicz, H. Massenspektroskopische fragmentierungs-reaktionen. VII. Abbau der polyenkette bei carotinoiden. *Org. Mass Spectrom.* **1974**, *9*, 1095−1113.

(26) Onyewu, P. N.; Daun, H.; Ho, C. T. Formation of two degradation products of β-carotene. *J. Agric. Food Chem.* **1982**, *30*, 1147−1151.

(27) Edmunds, F. S.; Johnstone, R. A. W. Constituents of cigarette smoke. Part IX. The pyrolysis of polyenes and the formation of aromatic hydrocarbons. *J. Chem. Soc.* **1965**, *46*, 2892−2897.

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