♣ Characterization of β-Carotene Thermal Degradation Products in a Model Food System[1]

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A model system developed in our laboratory for the study of thermal degradation products (TDP) of carotenoids was employed. β-Carotene (10 g) in glycerol was heated at 210 C for 4 hr, 1 hr, 15 min and 5 min. The time and temperature chosen were similar to edible oil deodorization and deep fat frying. In this study, the TDP of β-carotene were quantified as influenced by time and temperature of heating. Results indicate that at 210 C, degradation is almost complete after 4 hr and most of the nonvolatile products are viscous, yellow-brownish material. Shorter times (1 hr, 15 min and 5 min) cause less degradation. TDP include nonpolar as well as oxidized derivatives of β-carotene. The results of this study provide information on the type, amount and mechanism of formation of compounds resulting from heating carotenoids.

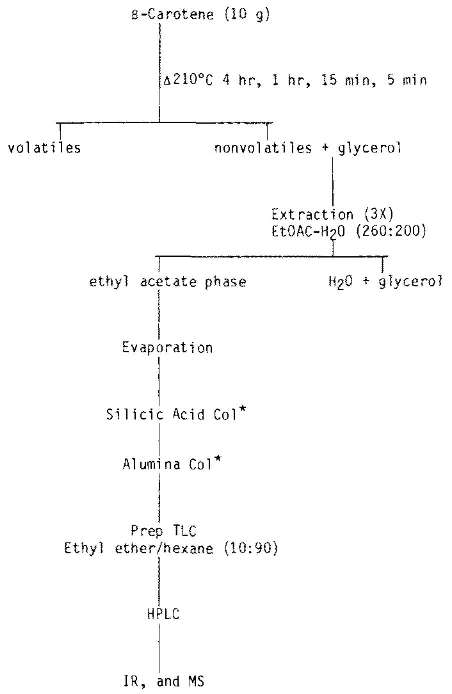
β-Carotene is the predominant carotenoid pigment in crude oil (0.05-0.5%) from palm fruit, *Elaeis guineensis* (1). In many countries in Africa, Asia and Latin America the oil is sold and consumed in the crude form. In the United States and Europe, the oil undergoes refining, bleaching and deodorization processes in order to be marketable for consumption. The pigments can be decolorized by bleaching alone or with high temperature treatment (110-149 F) (2-4).

Conventional deodorization is done at 360-455 F (182-218 C) (5). The carotenoid pigments can be degraded at deodorization temperatures of 260 C (3) and 210 C (6). Time and temperature conditions may differ for different edible oils, and also for different processes. According to Dudrow (3), the best deodorization conditions for refined and bleached soybean oil were observed to be 1-2 hr at 210 C and 0.5-1 hr at 230 C and 250 C. However, 0.5 hr was found to be too long at 270 C.

The deodorization step in edible oil processing is characterized by high temperature, high vacuum steam distillation of the volatile fraction (3). While odoriferous materials are removed, the thermal destruction of the carotenes takes place simultaneously. The thermal degradation products of β-carotene (TDP) formed in the oil as a result of the heat treatment are of interest with respect to nutrition and safety of foods. Most of the studies on TDP of β-carotene were in non-food systems and emphasized the volatiles (7-13). All of these investigators have reported aromatic hydrocarbons as thermal degradation products of β-carotene. Postulations have been made as to the probable formation of polycyclic aromatic hydrocarbon (PAH) as TDP of β-carotene (14). Minute amounts of PAH formed from β-carotene at 400 C and 700 C for a prolonged period of time have been reported (15). However, such temperatures do not represent normal food processing conditions. Only very few studies were done on the nonvolatile degradation products of β-carotene during deodorization and deep fat frying. Frying of fats and oils could lead to considerable losses of β-carotene and vitamin A (16,17). Mudambi and Rajagopol (17) estimated the amount of β-carotene in palm oil and the amount retained at deep fat frying temperatures. Ouyang et al. (6) reported β-apo-carotenone and β-apo-carotenals to be formed from β-carotene during simulated deodorization of palm oil at 210 C for 4 hr. In addition, we have reported (18) the formation of 3,7,10-trimethyl-1,12-bis(2,6,6,-trimethyl-cyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexaene and 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene as products of β-carotene degradation at 210 C for 4 hr. The objective of this study was to isolate and characterize the major nonvolatile products of β-carotene obtained during time and temperature conditions of oil deodorization and deep fat frying.

EXPERIMENTAL PROCEDURES

Materials. Crystalline β-carotene (lot #108101, Hoffman-



* Solvents: Hexane; Hexane/EtoAc (95:5, 75:25, 50:50, 25:75); EtoAc; MeoH

FIG. 1. Experimental scheme.

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LaRoche, Nutley, New Jersey); glycerol, alumina neutral grade I 80-200 mesh, silicic acid (Fisher Scientific Co., Fairlawn, New Jersey); precoated silica gel GF TLC plates 1000 μm 20 × 20, four coiled vacuum traps, drawing #12389-B (Lab Glass Inc., Vineland, New Jersey); four thermo-flasks or Dewar flasks; Lichroprep RP 18 25-40 μ Ace Scientific Co., East Brunswick, New Jersey); empty preparative HPLC column 7-8 mm × 30 cm, (Waters Associates, Framingham, Massachusetts).

Heating. A mixture of 10 g β-carotene and 50 ml glycerol were placed in a 3-necked flask in a high temperature oil bath and heated at 210 C for 4 hr, 1 hr, 15 min and 5 min, respectively. A continuous stream of nitrogen was swept through the reaction flask to remove the volatiles which were collected by being led through a series of cooler traps. After heating, the flask containing the nonvolatile TDP and glycerol was removed from the oil bath and allowed to cool before recovery of the TDP.

Extraction. The TDP of β-carotene were separated from glycerol by extracting with ethyl acetate-distilled water mixture. The glycerol product mixture was washed three times in a 1000 ml separatory funnel with the ethyl acetate water mixture, total volume 260:200. The ethyl acetate phase was kept for further analysis, and the water-glycerol phase was discarded. The ethyl acetate solution containing the nonvolatile TDP was dried with Na_2SO_4.

Evaporation. The solvent was evaporated using a Buchler rotary evaporator at room temperature and the residue weighed.

Chromatographic separation. The dried residue was redissolved in hexane before chromatographic separation. The initial separation of all TDP was accomplished on a silicic acid (SA) column. The low polar fractions from the SA column were then further separated on a partially deactivated alumina column. In both columns the sample was eluted with hexane, followed by mixtures of ethyl acetate (5%, 25%, 50%, 75%) and hexane, 100% ethyl acetate and finally 100% methanol.

Further separation of the samples was accomplished by preparative TLC. The solvent system for the TLC was ethyl ether (10%) in hexane.

Purification of two selected samples was done by reverse-phase HPLC. A Lichroprep RP-18 column was used on Model #440 Waters Associates. The mobile phase was acetonitrile-methanol (95/5).

Infrared (IR) and mass spectrometric (MS) analysis. Selected samples were subjected to IR and MS. IR spectrometric measurements were made by first evaporating the solvent in each sample with nitrogen at ambient temperature. Then about 2 μl of CCl_4 were added. Using a 10-μl syringe, the sample in CCl_4 was transferred to an ultramicro cavity cell (0.1 mm light path) in a Beckman Acculab 4 IR spectrophotometer.

Electron impact (EI) and chemical ionization (CI) mass spectrometric analysis were done using a Hewlett-Packard 5985 mass spectrometer. Ionization voltage was 70 ev, and the chamber temperature was increased gradually to 175-200 C. Samples were held 5-10 min to generate fragment ion spectra.

RESULTS AND DISCUSSION

Upon evaporation of solvent and after ethyl acetate-water extraction of the β-carotene TDP the respective residues from the four heat treatments were viscous glutinous materials of light yellowish-brown color. In this study, four experiments were conducted using different time and temperature conditions. The highest loss (97.8) of β-carotene was observed at 210 C after 4 hr of heating. The 91-97% loss of carotene observed in heat treatments at 210 C (Table 1) is analogous to the loss of carotene in deodorization of palm oil as well as other edible oils and deep fat frying of some foods. The β-carotene content and percent degradation in the nonvolatile fraction are shown in Table 1.

Over 70 nonvolatile compounds were observed in this study. However, due to time limitations only seven were identified. Selection of the seven was based on the large amount formed (Fig. 2a) and on the importance of the fraction. The least polar fraction was of prime importance because if any polycyclic aromatic hydrocarbons (PAH) were formed, they would be in the least polar, nonvolatile fraction of TDP. The more polar fraction was of interest because of the formation of compounds which may have some flavor attributes or nutritive value.

Separation. A total of seven groups of samples was obtained from heat treatment A nonvolatile fraction (Fig. 2a), four from B non-volatiles (Fig. 2b), and three groups from both C and D heat treatments nonvolatile fractions, respectively (Fig. 2c). The least polar group of each treatment was further separated on alumina. Compounds identified in this study were obtained from TLC and HPLC separation of the alumina column fractions (Figs. 2a, 2b, 2c).

Identification. The structures of the compounds identified in this study are shown in Figure 3.

Compound #1. [Fractions A(SA1-AL1-TLCI);

TABLE 1

β-Carotene Content (10 g) and Percent Degradation in the Nonvolatile TDP

i.d.	Heat Treatment: Time	Heat Treatment: Temp (°C)	Total Nonvolatile fraction (g)	Recovered Carotene amt (g) after	% Degradation
A	4 hr	210	3.60	0.08	97.8
B	1 hr	210	5.35	0.15	97.2
C	15 min	210	5.51	0.35	91.9
D	5 min	210	6.57	0.58	91.1
E	0 min	25	10.00	10.00	0.0

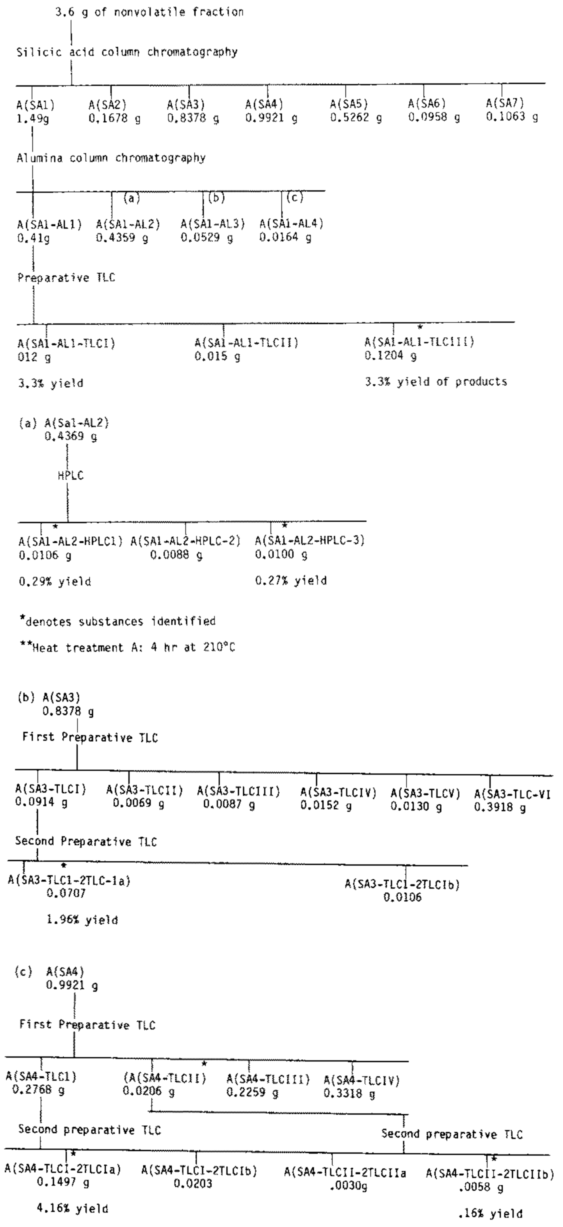


FIG. 2a. Nonvolatile fractions obtained from heat treatment.

A(SA3-TLCI-2TLC-Ia); B(SA2-AL1-2TLCIa]. The IR and mass spectrum of compound #1 are almost identical to the published spectra of 3,7,10-trimethy-1, 12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeca-1,3,5, 7,9,11-hexaene (18). This compound also has been reported by Ishiwatari (11) and Byers (13) to be formed from β-carotene.

Compound #2. A(SA4-TLCI-2TLCIa). The IR spectrum of this sample shows -CH-stretching at 3100-2800 cm^{-1}. Two weak absorption bands at 1650 and 1600 cm^{-1} are indicative of olefinic group. The MS fragmentation pattern of this compound is very similar to that of β-carotene. The major ion fragments include those at m/e 69 (100%), 119 (94.7%), 41 (90.7%), 105 (85.9%), 91 (66.3%), 55 (83.2%), 157 (29%), 169 (21.9%), M$^+$ 346 (4.8%). Compound #2 was identified as 3,7-dimethyl-8-toluenyl-1-(2,6,6-trimethylcyclohex-1-enyl)-octa-1,3,5,7-tetraene. This compound has been reported to be formed from β-carotene in geothermal reactions (12).

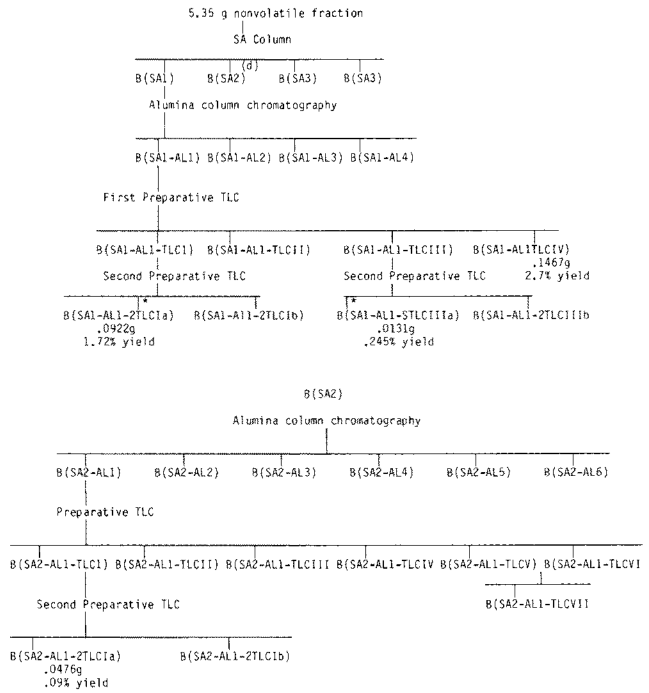


FIG. 2b. Nonvolatile fractions obtained from heat treatment B. (1 hr, 210 C).

Compound #3. [Fractions A(SA1-AL2-HPLC3); A(SA1-AL1-TLCIII); B(SA1-AL1-2TLCIIIa)]. The IR and mass spectra of this sample are identical to the published spectra of β-apo-13-carotenone (6).

Compound #4. A(SA1-AL1-2TLCIIIa). The IR spectrum showed absorption at 1760 cm^{-1} and 1680 cm^{-1} and is characteristic of a 5-membered ring α,β-unsaturated lactone. The molecular ion peak in the mass spectra is at m/e 180. The base peak is at m/e 111. Other major fragment ion peaks include those at m/e 137, 152 and 165. The fragmentation pattern is the same as the published spectrum of dihydroactinidiolide (19).

Compound #5. B(SA2-AL1-2TLCIa). The IR spectrum shows adsorption in the 3600-3000 cm^{-1} range, and it is characteristic of an alcohol group. The molecular ion of this compound was not resolved on the direct probe electron impact mass spectrometer. Chemical ionization was carried out in order to resolve the molecular ion, and the M + 1 ion fragment peak was observed at m/e 189. The compound was identified as 2-hydroxy-methyl-1,3,3-trimethyl-1,2-cyclohexandiol. This compound has been synthesized previously (19).

Compound #6. B(SA1-AL4). The IR spectrum of this compound shows absorption band at 3600-3200 cm^{-1} characteristic of the alcohol group. The mass spectrum parent ion, as in some alcohols, was difficult to detect. Chemical ionization was employed and the M + 1 ion was observed at m/e 275. The following fragment ion peaks and their respective intensities were obtained on direct probe EI-mass spectrometer: m/e 43 (100%), 55 (33.5%), 69 (30.7%), 91 (26.9%), 105 (23.7%), 119 (20.3%), 123 (9.5%), 145 (9.1%), 169 (4.2%), 165 (9.1%), 272 (0.9%), 273 (0.6%). The fragmentation pattern of this

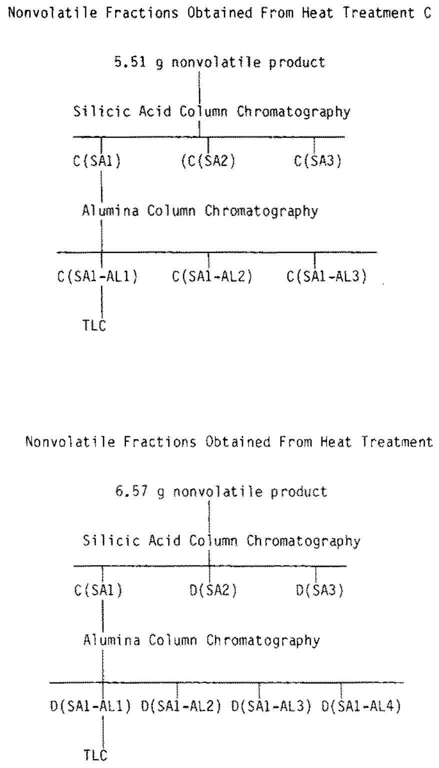
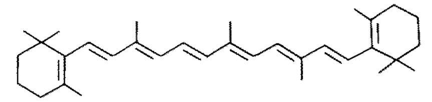


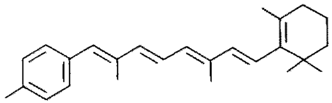
FIG. 2c. Nonvolatile fractions obtained from heat treatments C (15 min, 210 C) and D (5 min, 210 C).

Compound #1



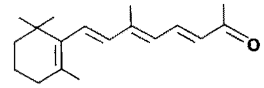
$C_{33}H_{48}$, Molecular weight 444
3,7,10-trimethyl-1,12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeco-1,3,5,7,9,11-hexaene

Compound #2



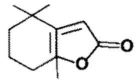
$C_{26}H_{34}$, Molecular weight 346
3,7-dimethyl-8-toluenyl-1-(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene

Compound #3



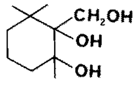
$C_{18}H_{26}O$, Molecular weight 258
β-apo-13-carotenone

Compound #4



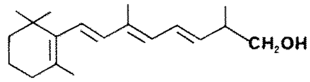
$C_{11}H_{16}O_2$, Molecular weight 180
dihydroactinidiolide

Compound #5



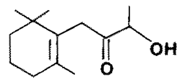
$C_{10}H_{20}O_3$, Molecular weight 188
2-hydroxymethyl-1,3,3-trimethyl-1,2-cyclohexandiol

Compound #6



$C_{19}H_{29}O$, Molecular weight 274
β-apo-14-carotenol

Compound #7



$C_{13}H_{22}O$, Molecular weight 210
1-(2,6,6-trimethylcyclohex-1-enyl)-3-hydroxy-2-butanone

FIG. 3. Compounds identified in this study.

compound is similar to the published spectra of an apo-carotene carbonyl compound (6). This compound has an alcohol substituent as seen in the IR. Loss of an allylic alcohol end group yields the fragment ion peak at m/e 59 which, upon loss of the methyl substituent and dehydrogenation, results in the fragment ion peak at m/e 43. The fragment ion peak at m/e 91 indicates formation and loss of a toluenyl ion from the parent ion. The ion peak at m/e 123 is indicative of the compound having at least one trimethylcyclohexenyl ring end group. This compound was identified as β-apo-14-carotenol.

Compound #7. A(SA4-TLCII-2TLCIIb). The IR spectrum of this compound, like the preceding compounds, shows alcohol absorption. CI mass spectrometry was used to confirm the parent ion. The major ion peaks on EI mass spectrum include those at m/e 43, 109, 111, 123, 137 and 163. The pattern of fragmentation is similar to the two alcohol compounds described earlier. Loss of the alcohol substituent gives rise to the ion peak at m/e 163 (Fig. 4). Ion peaks at m/e 137 resulted from cleavage, indicative of the presence of at least one trimethyl-cyclohexenyl ring structure. The compound was identified as 1-(2,6,6-trimethylcyclohex-1-enyl)-3-hydroxy-2-butanone.

The mechanism for the formation of compound #1 was reported earlier (18) to involve toluene expulsion from the polyene chain of the β-carotene molecule. Compound #2 has been postulated to be formed by cleaving of ionene moiety (11,12). This results in the formation of a radical, which undergoes a β-fission, then leads to the formation of the octatetraene compound. The mechanism for the formation of oxygenated compounds #3-7 can be said to follow the free radical mechanism. The free radical mechanism as seen in fatty acid autoxidation can be employed to explain thermal oxidation of β-carotene. β-Carotene, like unsaturated fatty acids, is susceptible to oxidation due to the presence of the polyene system. The β-carotene molecule is highly resonance-stabilized on a carbon centered radical, which upon reacting with oxygen can yield β-carotene-peroxy radical. The peroxy radical can then form a hydroperoxy derivative which leads to the formation of the oxygenated product found in this study. This can be applied to other oxygenated products. In this mechanism, given the conjugated polyene nature of β-carotene, the resonance-stabilized radical formation is favored over the non-resonance-stabilized. According to Burton and Ingold (20), the reaction of the radical with the peroxy group is very important at high temperatures, especially where resonance-stabilized radicals are involved.

Dihydroactinidiolide, a lactone which was formed in large amounts in our nonvolatile fraction, has been reported to be an important flavor compound in cooked

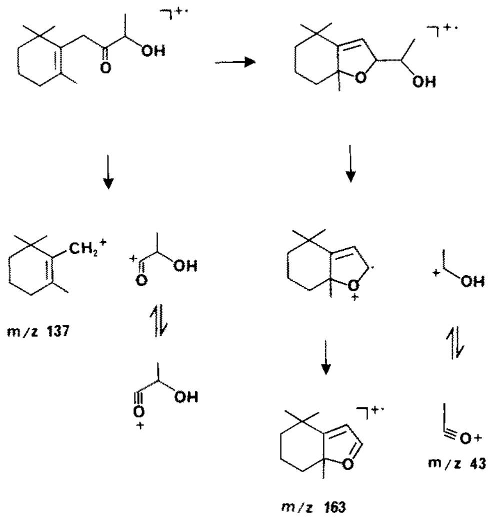


FIG. 4. Fragment ion formation from mass unit 210 compound #7[A(SA4-TLCII-2TLCIIb)].

corn and alcoholic beverages (21), in tea and tobacco (22-27), and in milk (28).

Significant amounts of hydroxylated compounds, ketones and aldehydes were also observed in our experiments. Among these are included compounds with molecular weights of 210, 274, 258 and 188 mass units. The compound with molecule weight 258 was reported by Ouyang and co-workers (6). The alcohol compounds with molecular weights of 210 and 274 were similar to ionone series compounds and vitamin A-type compounds. The compound with molecular weight 188 has been synthesized (19) but not reported as a degradation product of carotene.

The two prominent hydrocarbon compounds identified in this study were trimethyldodecahexaene (molecular weight 444) and the toluenyl-dimethyl-octatetraene (molecular weight 346). The formation of these two compounds took place at 210 C, which is comparable to both deodorization and deep fat frying conditions. Loss of toluene from the β-carotene molecule resulted in formation of the dodecahexaene compound. In this case, then, as toluene was expelled from the polyene chain of β-carotene molecule, the remaining parts of the carotene underwent polymerization. This led to the formation of a more stable compound. Details of its formation were reported by Onyewu et al. (18).

The compound with molecular weight 346 was formed because of losses of the terminal methyl substituent and ionene from the polyene chain of β-carotene. Ishiwatari first reported its formation in 1980 (12).

Based on molecular structure, it could be hypothesized that some of the compounds found in this study may have some nutritive value. Among those that could have some vitamin A activity are compounds with molecular weights 444, 346 and 274.

Another question that may be raised is whether there was evidence of formation of polynuclear aromatic hydrocarbons (PAH) in the TDP. Emphasis was placed on the analysis of the non-polar non-volatile thermal degradation products formed in large amounts, and no PAH were found among this group. This meant that if they were formed they would be present in minute amounts, and do not constitute major products of TDP of β-carotene under conditions of our experiments.

In summary, heat treatment of β-carotene under time and temperature conditions of deodorization as well as deep fat frying resulted in the formation of hydrocarbon type derivatives, products with a much shorter polyene system with either ketone, alcohol or a combination of two of these functional groups.

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